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**EFFECTS OF TIDAL FLOW ON RIPARIAN ZONE HYDRAULICS AND  
NITROGEN DYNAMICS: IMPLICATIONS FOR NUTRIENT MANAGEMENT  
IN COASTAL CREEKS**

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## ABSTRACT

This study investigated the biogeochemical cycling within the tidal freshwater zone (TFZ) of a 3<sup>rd</sup> order blackwater stream in coastal North Carolina. Spatial patterns in biological and chemical parameters were investigated over one year of synoptic water quality sampling. The TFZ was found to have a nominal flushing time of 3-6 days. Phytoplankton biomass, as chlorophyll *a*, peaked during these times within the TFZ, averaging 6.3  $\mu\text{g L}^{-1}$  over a one year period. A significant decrease ( $p < 0.05$ ) in dissolved organic carbon concentration from 36 to 27  $\text{mg C L}^{-1}$  was observed between the non-tidal blackwater river and the TFZ, corresponding with a peak in bacterial respiration at 0.44  $\text{mg O}_2 \text{ day}^{-1}$ . Phytoplankton biomass was correlated with nitrogen (N) and phosphorus (P) concentrations. Therefore a series of nutrient addition bioassay experiments were performed to examine the effect of nutrient addition on phytoplankton and bacterioplankton. Relatively low concentrations of inorganic N and P were found to stimulate phytoplankton in the TFZ during spring and summer. Bacterial growth in the TFZ was stimulated by phosphorus in October. Given the sensitivity of TFZ phytoplankton to inorganic nitrogen, denitrification within the TFZ was evaluated for its ability to regulate N flux through the river. Monthly assays were conducted with laboratory-incubated sediment cores and a membrane-inlet mass spectrometer to measure  $\text{N}_2$  gas flux and subsequently denitrification rates. Rates averaged 2.2, 1.8, and 1.9  $\text{mg N m}^{-2} \text{ hr}^{-1}$  in forested, vegetated, and mudflat intertidal zones over a 10 month period. In-situ redox probes in intertidal sediments showed cycling between oxidized and reduced conditions that suggested coupled nitrification-denitrification may have occurred. When denitrification rates during this period were extrapolated across the intertidal area of the Newport River TFZ system-wide denitrification was estimated to be 102-427  $\text{mg N s}^{-1}$ , bracketing the observed riverine nitrate flux of 296  $\text{mg N s}^{-1}$ .

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## SUMMARY AND CONCLUSIONS

Nutrient over-enrichment is one of the most significant ecological problems in coastal ecosystems (NRC 2000). Effective management of N pollution requires knowledge of the rates and processes governing nitrogen transport and transformation in stream networks. Ecological function of the tidal freshwater zone (TFZ) of coastal rivers has been largely overlooked, but is a critical region of N transformations along the riverine to estuarine continuum. The purpose of this study was to 1) examine the effect of tidal hydrology on biological and chemical processes in the TFZ, 2) evaluate the effect of anthropogenic nutrient additions to phytoplankton and bacterial communities within the TFZ, and 3) quantify denitrification within intertidal sediments of the TFZ and the effect on riverine N loads.

Bi-weekly synoptic sampling of water quality was performed at 5 sites (1 riverine, 3 tidal freshwater, and 1 oligohaline) on the Newport River, a 3<sup>rd</sup> order blackwater river in Carteret County, North Carolina. The TFZ was found to have a nominal flushing time of 3-6 days, with longest residence times during spring low-flow periods. Phytoplankton biomass, as chlorophyll *a*, peaked during these times within the TFZ, and averaged  $6.3 \mu\text{g L}^{-1}$  with a peak value of  $48 \mu\text{g L}^{-1}$ . Average heterotrophic respiration on dissolved organic carbon ranged from  $0.22 - 0.31 \text{ mg O}_2 \text{ L}^{-1}$  along the longitudinal transect but no significant differences ( $p < 0.05$ ) were observed between sites. Whole-community respiration of particulate matter peaked at the lower end of the TFZ, averaging  $0.12 \text{ mg O}_2 \text{ L}^{-1} \text{ day}^{-1}$ , indicating an increase in the lability of the particulate matter or an increase in heterotrophic metabolism. A statistically significant decrease in DOC from  $36$  to  $29 \text{ mg L}^{-1}$  was observed between the non-tidal blackwater river and the TFZ, and from  $29$  to  $24 \text{ mg L}^{-1}$  from the TFZ to oligohaline estuary. There was a statistically significant decrease in specific UV absorbance at  $254 \text{ nm}$  from  $0.0555$  to  $0.0506 \text{ L mg}^{-1} \text{ cm}^{-1}$  between the non-tidal blackwater river site and the oligohaline estuary, suggesting an increase in DOC lability for heterotrophic consumption. These data indicate that the TFZ had significantly higher rates of primary production and greater bacterial respiration than the non-tidal river, likely due to a combination of increased residence time, nutrient concentrations, and in-situ conditioning of dissolved and particulate detrital material.

Phytoplankton biomass was correlated with nitrogen and phosphorus concentrations during one year of synoptic measurements, suggesting these nutrients may stimulate phytoplankton growth. To test this hypothesis, a series of nutrient addition bioassay experiments were performed to examine changes in nutrient limitation between non-tidal blackwater river, blackwater TFZ, and oligohaline estuary. Nitrogen was found to limit phytoplankton growth at the non-tidal blackwater site during spring, while N and P were found to be independently-limiting at the TFZ and oligohaline site during spring and summer. Nitrogen and phosphorus were frequently co-limiting at all sites. Light limitation was frequently observed at all sites, as well. Bacterial growth was stimulated by nitrogen at the nontidal blackwater stream in March, and N+P in June. At the TFZ bacterial growth was stimulated by phosphorus in October. At the oligohaline estuary bacterial growth was limited by nitrogen in June and October, by phosphorus in March and October, and was co-limited by N+P in March and October. While previous research has documented nutrient limitation of phytoplankton in blackwater rivers, this research demonstrated differential nutrient limitation along the non-tidal through TFZ river continuum.

Biogeochemical processes that affect N flux through the TFZ, particularly denitrification which results in a permanent loss of nitrogen from the system, may affect phytoplankton and bacteria since their growth was found to be limited by nitrogen. Denitrification was measured monthly at three intertidal sites during a 10 month period using laboratory incubations of sediment cores. A membrane inlet mass spectrometer was used to measure  $N_2$  flux from the cores and calculate denitrification rates. Rates averaged 2.2, 1.8, and 1.9  $mg\ N\ m^{-2}\ hr^{-1}$  in forested, vegetated, and mudflat intertidal zones, and no significant difference ( $p < 0.05$ ) was observed between these average rates. In-situ measurements of soil redox and porewater saturation in the riparian zone over a one month period revealed cycling between oxic and anoxic conditions in the upper 5 cm of sediment which corresponded with the tidal height and inversely with soil moisture. This suggested that coupled nitrification-denitrification may have occurred in these sediments, thereby increasing in-situ denitrification above the rates observed in fully-saturated laboratory cores. When denitrification rates during this period were extrapolated across the intertidal area of the Newport River TFZ, system-wide denitrification was estimated to be 102-427  $mg\ N\ s^{-1}$ . This range brackets the  $NO_3$  load at the lower end of the TFZ of 296  $mg\ s^{-1}$  during this period, indicating that a substantial fraction of the N load of the river may be denitrified during transport through the TFZ.

This study documented the importance of the distinctive geomorphology of the Newport River TFZ (a 3-fold increase in channel width, daily inundation of the riparian floodplain, and semi-diurnal tidal flow) in modulating riverine nutrient loading, phytoplankton, bacteria, and nitrogen cycling. These geomorphic features may be common throughout the blackwater rivers of the southeastern U.S. (SEUS), and future research is needed to corroborate these trends in other systems. The major implications of the current study are that phytoplankton in the TFZ of blackwater rivers may be especially susceptible to point-source nutrient loading of both nitrogen and phosphorus. Phytoplankton growth within the TFZ provides an important food resource for riverine and estuarine zooplankton, but in excess can be deleterious to oxygen concentrations and subsequently aquatic life. Further research on TFZ blackwater food webs is needed to understand the fate of phytoplankton biomass within the river, and at what level of production this biomass becomes detrimental to the ecosystem.

## RECOMMENDATIONS

This study documented the sensitivity of a blackwater, tidal freshwater river to anthropogenic nutrient pollution, while also demonstrating that the intertidal riparian zone had a substantial affect on nitrogen flux through the river system. Two fundamental management recommendations for tidal freshwater rivers in the North Carolina coastal plain resulting from this research are 1) develop nutrient input standards for tidal freshwater rivers, and 2) preserve the riparian zones surrounding them.

This research has advanced our understanding of resource limitation of phytoplankton and bacterial growth in blackwater rivers. Phytoplankton have previously been documented to be stimulated predominantly by nitrogen in blackwater rivers, and the current study corroborates this phenomenon. Within the TFZ, however, phytoplankton were as sensitive to phosphorus as nitrogen, and were more stimulated by a combination of nitrogen and phosphorus than in the river upstream. Even moderate concentrations of nitrogen ( $280 \mu\text{g N L}^{-1}$ ) and phosphorus ( $155 \mu\text{g P L}^{-1}$ ), combined with a moderate increase in irradiance, were found to stimulate algal growth above the NC water quality standard of  $40 \mu\text{g L}^{-1}$  chlorophyll *a*. The NC Division of Water Quality should recognize the sensitivity of TFZ blackwater rivers to N and P when issuing wastewater discharge permits in these systems. Further research is needed to evaluate the effect of elevated phytoplankton production on ecosystem functions within TFZ blackwater rivers.

A distinguishing characteristic of TFZ rivers in the SEUS is the hydrologic connection between the river and floodplain during each tidal cycle. This study found substantial denitrification within these intertidal habitats and rapid cycling between oxidized and reduced conditions in the surficial sediments over a tidal cycle. These data suggest that the riparian zone is a significant sink for nitrogen transported through the river and for remineralized nitrogen from intertidal sediments. Given the large aerial extent of the intertidal zone in the Newport River, denitrification was found to equal the magnitude of the river-borne nitrate load. Disturbance of this riparian zone by development, land clearing, or other hydrologic alterations may therefore reduce the capacity of the floodplain to act as a sink for nitrogen transiting the river network. In coastal environments where nitrogen is commonly the limiting resource for phytoplankton growth, disturbance of riparian floodplains may result in water quality degradation downstream.

## INTRODUCTION

Along the hydrologic continuum between upland rivers and downstream estuaries lies a unique ecotone where the river meets the estuary. This tidal freshwater zone (TFZ), where river flow becomes tidally-influenced but is upstream of saltwater intrusion, can comprise half the total length of rivers on the east coast of the U.S. (Anderson 1986). Biogeochemical processes within the TFZ affect riverine sediment, nutrient, and pollutant loads prior to export to estuaries downstream, and are in many ways unique to their non-tidal upstream counterparts (Schuchardt et al. 1993). The TFZ modulates the downstream transport of materials to estuaries, while estuaries serve as a source of biota and particulate matter transported upstream to TFZ in the form of anadromous fish. Knowledge of the processes regulating biogeochemical and ecological processes within the TFZ is critical to an integrated understanding of river networks, estuaries, and riparian ecosystems.

Only within the past several decades has attention been given to the ecological significance of the TFZ (Schuchardt et al. 1993). The pulsing of tidal energy into the TFZ makes this ecosystem ecologically unique (Odum et al. 1995) and drives the ontogeny of coastal landforms (Dame et al. 1992; Brinson et al. 1995). The geomorphology of the TFZ and the links to TFZ hydrology have not been thoroughly studied (except see Woldenberg 1972 and Ashley 1980), but TFZs are commonly assumed to have extended water residence times. Enhanced residence time and nutrient availability are commonly cited as reasons for phytoplankton blooms observed in many TFZ systems. A rapid increase in channel width along this transition zone may also provide increased irradiance to the stream channel that facilitates phytoplankton growth.

Freshwater marshes of the TFZ also host significant amounts of primary production and contribute to biogeochemical cycling within the river. These highly productive environments are generally a sink for riverine-derived N and P (Simpson and Whigham 1978; Bowden et al. 1991), and a source of organic carbon (Neubauer et al. 2000), inorganic carbon (Neubauer and Anderson 2003) and biogenic silica (Struyf et al. 2005) to the river. The lateral tidal excursion into riparian forests links the river with a wide terrestrial zone with are sinks for riverine N, P, and C (Brinson et al. 1981; Noe and Hupp 2005). Due to comparatively long residence time and extensive riparian connectivity, the TFZ is a hot-spot of biological production and nutrient cycling along the riverine-estuarine continuum.

Coinciding with the large drainage basins of TFZ rivers in the coastal plain is roughly half of the U.S. population now living within 100 miles of the coast. This figure is expected to reach 75% of the total population of the US by 2025 (Hinrichsen 1998). With this increase in human population comes the requirement for waste disposal and nutrient discharges, as well as incidental non-point source impacts to rivers. Nutrient enrichment from point and non-point sources is now the largest threat to coastal waters associated with human population growth in the coastal plain (Howarth et al. 2002). Nutrient enrichment frequently stimulates algal growth, which in excess can result in hypoxia and subsequent death of aquatic life (NRC 2000). The TFZ is situated at the interface of rivers and their estuaries, and thus it is critical to understand the biogeochemical processes within the TFZ and how they affect the downstream delivery of watershed-derived nutrient loads.

In the SEUS, the TFZ of coastal rivers is unique relative to those draining piedmont watersheds with more topographic relief. Blackwater rivers are the most common type of fluvial system in the SEUS coastal plain, and it is upon this ecological template that TFZs exist. High concentrations of dissolved organic carbon, low channel gradients, and wide riparian floodplains characterize these systems (Meyer 1990). Blackwater rivers have traditionally been thought to host low primary production due to the high attenuation of light, although during dry periods there can be net production (Edwards and Meyer 1987a). Despite low primary production, secondary production by invertebrates can be very high (Benke et al. 2001). Amorphous particulate seston, a combination of flocculated dissolved material, bacteria, and protozoans, supplies much of the energy needed for invertebrate production (Carlough 1994).

The affect of tidal flow regimes on the ecology and biogeochemistry of blackwater rivers has not been investigated. The abundance of these tidal blackwater systems in NC, and the lack of knowledge of their ecological function, warrants investigation of how their geomorphic structure affects biological production. Biological responses to nutrient pollution may have management implications for these systems. Biogeochemical processes, such as denitrification, may be profoundly different than non-tidal rivers, and these factors could affect how future efforts to model nitrogen flux through these systems. These are the research topics addressed in the current report.

#### SPECIFIC OBJECTIVES

The purpose of this study was to investigate spatial trends in biogeochemical and ecological processes along the non-tidal river through estuarine continuum of a coastal, blackwater river in N.C. Particular attention was devoted to nitrogen processing because it frequently stimulates algal growth in aquatic ecosystems and was hypothesized to be particularly biogeochemically reactive in the TFZ. The project addressed three closely-coupled questions:

1. Is materials processing (nitrogen, phosphorus, carbon, algal biomass, community respiration, light attenuation) in a blackwater TFZ distinctive relative to the non-tidal riverine and estuarine boundaries?
2. What resources (i.e. nutrients, irradiance, and residence time) limit growth of phytoplankton and bacteria in the TFZ, and is the TFZ distinctive relative to its upstream and downstream counterparts?
3. Given the distinctive hydrology of the TFZ, what affect does denitrification have on the downstream transport of nitrogen through the TFZ?

Results of this study are expected to advance the ecological understanding of tidal freshwater rivers and provide information that will be particularly relevant to environmental managers. As population growth within the NC coastal plain continues, point and nonpoint source nutrient pollution will continue to affect coastal rivers, and more accurate information will be required to make management decisions.

## **BIOCHEMICAL CHARACTERIZATION OF THE TIDAL FRESHWATER ZONE, NEWPORT RIVER, NC**

### **INTRODUCTION**

Assessing the sources of energy for aquatic food webs in rivers is a major research topic in river ecology. One of the most influential and debated concepts has been the River Continuum Concept (RCC) (Vannote 1980), which provided a theory of how longitudinal geomorphic changes throughout a river system affect the types of invertebrates in streams and the origin of their basal food source. The RCC proposed that invertebrate communities through a river system would become increasingly dependent on suspended particulate detritus and algae as tributaries became wider, irradiance to the channel increased, and allochthonous detritus was increasingly processed and recycled into finer material. Indeed, at the terminus of river networks lies the TFZ, wherein algal biomass commonly reaches a maximum prior to entering the estuary. Researchers in the U.S. (Filardo and Dunstan 1985; Anderson 1986), Germany (Schuchardt and Schirmer 1991; Rehbehn et al. 1993), Canada (De Seve 1993), and Belgium (Muylaert et al. 1997; Muylaert et al. 2000) have documented peaks in phytoplankton productivity within TFZs, and are often attributed to the increased hydrologic residence time of the TFZ.

Though only a small fraction of the total organic load, algal biomass is far more important to the riverine and estuarine secondary production than other detrital sources (Martineau et al. 2004; Sobzack et al. 2005). The extent to which zooplankton can utilize this productivity is limited by the residence time of the system (Pace et al. 1992). Microzooplankton, especially rotifers, can exert significant grazing pressure on phytoplankton during summer when larger zooplankton are absent (Park and Marshall 2000). Benthic filter feeders can also exert considerable influence over phytoplankton biomass within the TFZ (Cohen et al. 1984).

In blackwater rivers, bacteria are another significant component of the basal energy sources for protozoans and filter-feeding invertebrates (Edwards and Meyer 1986; Edwards and Meyer 1987b; Edwards et al. 1990). This microbial production is largely dependent on the massive concentration of dissolved organic carbon (DOC) in these systems. Flocculent material derived from DOC that is subsequently colonized by bacteria, fungi, and protozoans is also an important route for basal energy to enter the food web (Carlough and Meyer 1989; Carlough 1994). Physical characteristics of the TFZ may affect DOC quality and subsequent bacterial utilization. Rapidly increasing channel width in the longitudinal direction (Woldenberg 1972) allows greater solar irradiance to the water surface, enhancing the bioavailability of large molecular weight DOC molecules (Wetzel 2001). Longer water residence times allow for more bacterial processing of DOC and conversion to particulate organic matter. Understanding these processes is central to developing better understanding of the role of TFZs to processing of allochthonous carbon, basal energy sources to TFZ food webs, and export of nutrients and organic material from rivers to estuaries.

TFZ food webs in blackwater rivers are expected to have significantly elevated primary and secondary production. Algal and bacterial production are incorporated by invertebrate consumers, which in turn serve as food for anadromous fish and their larvae. The complexities of TFZ food webs is beginning to be understood through the use of stable isotope studies which show the transfer of marine-derived nutrients into the TFZ through anadromous fish migration

(Durbin et al. 1979; Garman et al. 1998), predation on this food source by riverine planktivores and piscivores (MacAvoy et al. 2000; Yako et al. 2000), and decomposition of anadromous fish biomass (Browder and Garmin 1994). A greater conceptual understanding of how chemical parameters of blackwater TFZs influence, and are influenced by, food web processes is needed given the requirement of environmental managers to address water quality issues in these systems.

This study provides a descriptive analysis of the longitudinal patterns in chemical and biological parameters over a one year period along a non-tidal blackwater river-TFZ-oligohaline estuarine river. Phytoplankton growth and bacterial respiration along this continuum are analyzed in light of their effects on chemical parameters as well as the effect of these resources on growth. These observational data will provide a framework to develop and refine mechanistic hypotheses subsequently addressed.

## MATERIALS AND METHODS

Synoptic water quality sampling was performed twice monthly at five sites on the Newport River in Carteret County, NC (Figure 1) between May 2006 and April 2007 to characterize water chemistry and biological parameters along the non-tidal through estuarine river continuum. The most upstream site (Southwest) was above the tidal influence and drained a 34 km<sup>2</sup> primary forested region of the Croatan National Forest. Three sites (Godwin, New Highway 70, Old Highway 70) were located along the TFZ with drainage areas of 100, 110, and 116 km<sup>2</sup>, respectively (Figure 1). The most downstream site, Dock, was located at the upper extent of the oligohaline estuary and drained a watershed of 162 km<sup>2</sup>.

A YSI 6610 multiparameter water quality sonde and 610 display/logger (Yellow Springs Instruments, Yellow Spring, OH) was used to measure temperature, salinity, and dissolved oxygen. A Li-Cor (Li-Cor Environmental, Lincoln, NB) 4-pi irradiance sensor and LI1400 display/logger was used to measure solar irradiance and light attenuation at 0.25 m intervals beneath the surface. Total suspended solids were measured by filtering up to 1 L of water through a 2.7 µm pre-combusted glass fiber filter (Whatman GF/D), drying at 105°C for 1 hr, and weighing. The organic carbon on this dried filter was determined by weight loss on combustion at 525°C for 3 hr. Chlorophyll *a* was quantified using the non-acidification method of Welschmeyer (1974) in which phytoplankton from a 50 mL sample were captured on a 0.7 µm glass fiber filter (Whatman GF/F), extracted in 90% acetone for 18-24 hr, and read on a Turner Trilogy fluorometer (Turner Designs, Inc, Sunnyvale, CA).

Community respiration was measured as the change in oxygen in closed bottles over a 24 hr period, following methods similar to Maranger et al. (2005) and Findlay et al. (1992). River water was incubated for 24 hr at ~23°C in 300 mL glass bottles. A YSI 5000 bench top dissolved oxygen meter and 5010BOD probe was used to measure dissolved oxygen at the beginning and end of the incubations. Duplicate samples of unfiltered water were used to measure respiration by heterotrophs on dissolved and particulate matter. Duplicate water samples filtered through 2.7 µm filters (Whatman GF/D), with an added bacterial “seed” of 3 mL of unfiltered water, was used to measure respiration on predominantly dissolved organic matter. The contribution of particulate matter >2.7 µm to total oxygen uptake was calculated by subtraction of oxygen uptake in dissolved material-only samples from the total oxygen uptake.

These data reflect not only the lability of organic matter present, but also the metabolic activity of the microbial community present. These differences in community structure and activity may be an inherent response to the substrate but also other resource limitations along the continuum such as inorganic nutrients. Relationships between oxygen uptake within particulate- and dissolved-constituent samples to nitrogen, phosphorus, dissolved organic carbon concentrations and specific UV absorbance were examined using Spearman's rank test for non-parametrically distributed samples.

Dissolved organic carbon (DOC) was determined on a Shimadzu 5000TOC analyzer (Shimadzu, Columbia, MD) after acidification and sparging with purified air for 6 minutes. The aromaticity of the DOC was measured using specific UV absorbance at 254 nm (Weishaar et al. 2003); this measurement provides a relative measure of the lability of DOC to bacteria, and has been used to partition sources of DOC in streams (Hood et al. 2006). A Shimadzu UVMini1240 (Shimadzu, Columbia, MD) with a 1 cm path-length quartz cell was used to measure UV absorbance at 254 nm of 0.7  $\mu\text{m}$  filtered water samples. Specific UV absorbance at 254 nm ( $\text{SUVA}_{254}$ ) was calculated as:  $\text{DOC} \div \text{UVabs}_{254}$  (Weishaar et al. 2003). Nitrate plus nitrite (referred to hereafter as just  $\text{NO}_3$ ), ammonium ( $\text{NH}_4$ ), total dissolved nitrogen TDN) and dissolved reactive phosphorus (referred to hereafter as just  $\text{PO}_4$ ) were measured using a Lachat Quikchem 8000 autoanalyzer (Lachat Instruments, Milwaukee, WI).

River discharge was measured monthly upstream of the limit of tidal influence at the confluence of the Northwest and Southwest prongs. This location drains a watershed area of 86  $\text{km}^2$ . A Sontek Flowtracker handheld acoustic doppler velocimeter (Sontek/YSI, San Diego, CA) was used to measure velocity at  $0.6 \times \text{depth}$  at 0.5 m intervals across the channel. Cross section bathymetry of the tidal freshwater zone was measured at 26 sites roughly 500 m apart from Dock to Confluence. Water depth was measured at one meter intervals using a survey rod. Channel volume between measurement sites was calculated as the sum of the average of the upstream and downstream cross-sectional area and the distance between sites. Bathymetry measurements were made in May-June 2007 when river discharge was the lowest measured over the study period. Therefore, river channel volumes during other periods of the year were estimated by first calculating the ratio of the cross-sectional area at Confluence (non-tidally influenced) during the period of interest to that of April 2007, and then adding that proportion to the channel volume during that that period.

Flushing time of the tidal freshwater zone during dates when discharge was measured was calculated using a modification of the tidal prism method in which the estuary is divided into segments corresponding with the tidal excursion (Ketchum 1951; Dyer 1997). Beginning at the upstream end of the tidal freshwater zone below the confluence, channel volumes between bathymetric survey sites were used to identify the location at which the intertidal volume (or local tidal prism,  $P$ ) was approximately equal to the river inflow during the flood tide. This location delimited the upstream boundary of the most upstream segment. Subsequent segments downstream were delimited consecutively such that the low tide volume ( $V$ ) of each segment equaled the high tide volume of the segment immediately upstream. The flushing time of each segment (in tidal cycles) was calculate as  $V+P/P$  and converted to days by dividing by 1.92. These calculations represent a nominal time for flushing since no consideration was given for incomplete mixing of water masses between segments, transient storage, and incomplete flushing

of water masses from the mouth of the river, which would all lengthen the actual residence time in the TFZ (Luketina 1998).

The contribution of the Town of Newport WWTP effluent on riverine  $\text{NH}_4$ , TDN, total phosphorus (TP), and biochemical oxygen demand (BOD5) constituent loads was estimated on a monthly basis at Old Highway 70, 250 m downstream from the treatment plant outfall. River discharge ( $Q_{confluence}$ ) measured at Confluence was scaled by watershed area to estimate discharge at Old Highway 70 ( $Q_{Old70}$ ) using the following equation:

$$Q_{Old70} = Q_{confluence} \times \frac{116\text{km}^2}{86\text{km}^2}. \quad \text{equation 1}$$

The parameter concentration at Old Highway 70 measured for the date closest to discharge measurement was used to calculate the riverine load. Reported discharge volume and concentrations from the WWTP were obtained from the NCDWQ (Thomas Moore, NCDWQ, pers. com., 2007) for the period 1 Jan 2006 – 30 Mar 2007. Discharge from the WWTP was available at daily intervals, but chemical constituents were reported less frequently. Therefore, constituent loads from the WWTP were calculated from reported concentrations for the period encompassed by riverine discharge measurement and New Highway 70 sample collection, or the most concurrent date available in the NCDWQ records. Measurement of riverine load and WWTP load were therefore not precisely contemporaneous, and are intended as an approximate comparison between chemical constituents to the river and the contribution by the WWTP.

## RESULTS

Channel cross-sectional area and width increased dramatically through the tidal freshwater zone (Figure 2). River discharge at the confluence of the Southwest and Northwest prongs of the Newport River varied between  $0.185 \text{ m}^3 \text{ s}^{-1}$  in April 2007 and  $4.117 \text{ m}^3 \text{ s}^{-1}$  in September 2006 (Figure 3). The low discharges during the spring and summer months led to long flushing times of the TFZ that peaked in April 2007 at 6.3 days (Figure 3). Nominal flushing times during winter and fall ranged from 1 – 3 days.

Temperature averaged between  $16.4 \text{ }^\circ\text{C}$  at the upstream sites and  $18.1 \text{ }^\circ\text{C}$  at the Dock (Table 1). Dissolved oxygen concentration was lower at the two downstream sites than at Southwest, ranging between an average value of  $7.8 \text{ mg L}^{-1}$  (56% saturation) at Southwest and  $6.2 \text{ mg L}^{-1}$  (39% saturation) at the Dock. Dissolved oxygen was below the NC state standard of  $4 \text{ mg L}^{-1}$  on two occasions at Dock and once at New Highway 70. Appreciable salinity was observed infrequently at Dock, averaging 0.25 with a peak of 2.6. Light attenuation did not exhibit statistically significant longitudinal trends; mean values at all sites was  $6\text{-}7 \text{ m}^{-1}$ . Specific UV absorbance was lower at Dock than Southwest, though the TFZ sites were not different than Southwest. Suspended organic matter was greater at Dock than the upper three sites.

Nitrate increased significantly along the river continuum, from  $14 \text{ } \mu\text{g N L}^{-1}$  at Southwest to  $218 \text{ } \mu\text{g N L}^{-1}$  at Old Highway 70 (Table 2). Likewise, total nitrogen was greater at the two most downstream sites than the upper TFZ site at Godwin. The Newport WWTP contributed approximately 22-100% of the total nitrogen load to the river during three periods during which comparison could be made (Figure 4). Ammonium showed no longitudinal trends along the non-tidal – oligohaline sites. Wastewater effluent accounted for 1-38% of the in-stream  $\text{NH}_4$  load. Dissolved reactive phosphate increased significantly between Southwest and New Highway 70,

and again between New and Old Highway 70 bridges. While TP was not analyzed as part of the synoptic sampling, the Newport WWTP contributed a large amount of phosphorus relative to the riverine PO<sub>4</sub> load. Dissolved organic carbon decreased significantly from 36.4 mg L<sup>-1</sup> at Southwest to 28.6 mg L<sup>-1</sup> at Godwin, and finally to 23.7 mg L<sup>-1</sup> at Dock (Table 2).

Chlorophyll *a* was significantly greatest at Old Highway 70 averaging 6.3 µg L<sup>-1</sup> (Table 3). An algal bloom occurred at this site on 1 May 2007 with chlorophyll *a* that measured 48.5 µg L<sup>-1</sup>, exceeding the NC state water quality standard of 40 µg L<sup>-1</sup> (Figure 5). Total oxygen uptake was greater at Dock than Southwest, and oxygen uptake from particulate matter only was greater at Old Highway 70 and the Dock than Southwest (Table 3). Chlorophyll *a* was significantly and strongly correlated with temperature, nitrogen, phosphorus, and suspended organic matter (Table 4). All oxygen uptake measurements were inversely correlated with SUVA<sub>254</sub>. Oxygen uptake with particulates was correlated with temperature, nitrogen, phosphorus, and suspended organic matter. Total oxygen uptake was correlated with temperature, nitrogen, and phosphorus. Five day BOD of the WWTP outfall ranged from 2 – 3.4 mg L<sup>-1</sup>. Extrapolating the 24 hr measurements made in this study to five days, and comparing these values to the WWTP BOD load, the WWTP contributed from 0.9 – 5.6% of the riverine load.

## DISCUSSION

Algal biomass, measured here as chlorophyll *a*, peaked within the lower portion of the Newport River TFZ. The positive correlation between chlorophyll *a* and inorganic nitrogen, total nitrogen, and DRP suggests that algal growth is stimulated by these nutrients. Also possible is that the hydrology around Old Highway 70 allows an accumulation of particulate material during low flow periods. Compared with other tidal freshwater rivers, the observed peak in chlorophyll *a* in the Newport River TFZ is not unique. However, such high concentrations are remarkable for blackwater rivers (Edwards and Meyer 1987), and demonstrates an important way in which the tidal influence affects these coastal rivers.

The tidal freshwater zone of rivers is often cited as having a higher residence time than its non-tidal counterparts, although this is rarely quantified in the studies citing this phenomenon. In the Newport River tidal freshwater zone nominal flushing times ranged from 4 – 6 days. The modification of the tidal prism method used here produced a minimum flushing time of ~3.6 days during a range of high flows. This is likely the result of higher channel volumes masking the effect of higher freshwater inflows. These flushing times show that there is adequate time during all periods of the year for phytoplankton populations to be sustained. These geomorphic features distinguish the TFZ from the non-tidal river above which hosts significantly less phytoplankton growth.

There was a significant decrease in DOC between the non-tidal and TFZ of the Newport River, suggesting microbial uptake or conversion of this material along the flow path was more rapid than tributary inputs. It is also possible that the two tributaries entering the Newport River in this region had significantly lower DOC concentrations and thus diluted the main-channel concentration. However, these tributaries drain forested regions of the Croatan National Forest and are expected to be similar in chemical characteristics as Southwest. While no differences were observed between SUVA<sub>254</sub> measurements along the river continuum until the oligohaline estuary, the inverse correlation between SUVA<sub>254</sub> and oxygen uptake suggests that a decrease in

aromaticity (lower  $SUVA_{254}$ ) allowed greater bacterial respiration. This evidence suggests that DOC may have become more labile along the river continuum, perhaps as a result of increased contribution of DOC or particulate carbon from algae, or photo-oxidation of DOC. Together, these data suggest that there was a net loss of DOC through the TFZ through bacterial respiration, attributable in part to long residence time and increased lability of this material. At the landscape-scale, the TFZ converted allochthonous carbon from the watershed to  $CO_2$ , thereby reducing the flux of carbon to coastal environments downstream.

There was an increase in ambient  $NO_3$  and  $PO_4$  concentrations along the Newport River continuum, peaking at Old Highway 70. The most parsimonious explanation for this is the contribution of the Town of Newport WWTP, but since neither of these constituents is reported to the NCDWQ this can not be confirmed. Regardless, a significant increase in these parameters occurs between Southwest and New Highway 70 that is not expected to be influenced by the Town of Newport WWTP since all sampling was conducted on an ebb tide. While non-point source contributions along this reach cannot be excluded as an explanation, in-situ remineralization processes in the water column and sediments may also be responsible. Future research is required to determine the contribution of photo-oxidation, remineralization, and nitrification on riverine loads through the TFZ.

Table 1. Summary statistics for water chemistry at five Newport River sampling locations, April 2006 - April 2007. The four rows for each parameter indicate the mean  $\pm$  stdev, min-max, n, and statistically significant ( $p < 0.05$ ) homogenous subset groupings (Tukey post-hoc analysis). Dashes indicate no significant difference ( $p < 0.05$ ) between sites (one-way ANOVA).

	Southwest	Godwin	New Highway 70	Old Highway 70	Dock
temperature ( $^{\circ}\text{C}$ )	16.4 $\pm$ 6.4	16.4 $\pm$ 6.0	17.1 $\pm$ 6.2	17.5 $\pm$ 6.5	18.1 $\pm$ 6.5
	6.7 - 26.8	6.6 - 25.9	6.6 - 26.5	7.4 - 27.2	6.8, 27.0
	28	23	27	23	28
	-	-	-	-	-
dissolved oxygen ( $\text{mg L}^{-1}$ )	7.8 $\pm$ 1.4	7.1 $\pm$ 1.6	6.5 $\pm$ 1.8	6.4 $\pm$ 2.0	6.2 $\pm$ 2.0
	5.5 - 10.6	4.6 - 10.4	4.4 - 10.5	3.8 - 10.5	3.1, 10.6
	28	23	28	23	28
	a	ab	ab	b	b
dissolved oxygen (% saturation)	79 $\pm$ 8	70 $\pm$ 11	66 $\pm$ 11	64 $\pm$ 16	63 $\pm$ 13
	56 - 92	41 - 85	49 - 91	40 - 96	39 - 87
	28	23	28	22	28
	a	ab	b	b	b
salinity	0.04 $\pm$ 0.02	0.05 $\pm$ 0.02	0.06 $\pm$ 0.03	0.07 $\pm$ 0.04	0.25 $\pm$ 0.54
	0.02 - 0.12	0.03 - 0.11	0.03 - 0.14	0.03 - 0.20	0.04 - 2.60
	28	23	28	23	28
	a	a	ab	ab	b
light attenuation ( $\text{m}^{-1}$ )	6.9 $\pm$ 1.1	6.9 $\pm$ 1.6	6.9 $\pm$ 0.9	6.8 $\pm$ 1.1	7.3 $\pm$ 1.4
	5.2 - 10.8	5.0 - 10.6	5.4 - 9.4	4.1 - 8.6	4.9 - 10.6
	25	10	22	21	25
	-	-	-	-	-
SUVA 254 ( $\text{L mg}^{-1} \text{cm}^{-1}$ )	0.0555 $\pm$ 0.0045	0.0524 $\pm$ 0.0027	0.0514 $\pm$ 0.0037	0.0523 $\pm$ 0.0032	0.0506 $\pm$ 0.0030
	0.0497 - 0.0667	0.0482 - 0.0562	0.0444 - 0.0560	0.0472 - 0.0565	0.0459 - 0.0543
	11	10	11	9	11
	a	ab	ab	ab	b
suspended organic ( $\text{mg L}^{-1}$ )	2 $\pm$ 3	2 $\pm$ 3	3 $\pm$ 3	3 $\pm$ 3	5 $\pm$ 3
	0 - 12	0 - 11	0 - 13	0 - 13	1 - 14
	24	23	25	23	28
	a	a	a	ab	b

Table 2. Summary statistics of elemental concentrations at five Newport River sampling locations, April 2006 - April 2007. The four rows for each parameter indicate the mean  $\pm$  stdev, min-max, n, and statistically significant ( $p < 0.05$ ) homogenous subset groupings (Tukey post-hoc analysis). Dashes indicate no significant difference ( $p < 0.05$ ) between sites (one-way ANOVA).

	Southwest	Godwin	New Highway 70	Old Highway 70	Dock
nitrate+nitrite ( $\mu\text{g N L}^{-1}$ )	14 $\pm$ 10	53 $\pm$ 23	119 $\pm$ 107	218 $\pm$ 155	216 $\pm$ 107
	3 - 59	5 - 120	5 - 452	20 - 582	27 - 493
	26	22	26	22	26
	a	ab	b	c	c
ammonium ( $\mu\text{g N L}^{-1}$ )	35 $\pm$ 10	46 $\pm$ 20	45 $\pm$ 18	43 $\pm$ 16	49 $\pm$ 22
	18 - 54	16 - 81	13 - 81	9 - 70	8 - 102
	26	22	26	22	26
	-	-	-	-	-
total nitrogen ( $\mu\text{g N L}^{-1}$ )	542 $\pm$ 122	498 $\pm$ 125	556 $\pm$ 130	681 $\pm$ 211	683 $\pm$ 214
	309 - 794	314 - 729	378 - 781	430 - 1070	428 - 1220
	22	19	22	19	22
	ab	a	ab	b	b
DRP ( $\mu\text{g P L}^{-1}$ )	8 $\pm$ 3	11 $\pm$ 4	17 $\pm$ 14	26 $\pm$ 14	25 $\pm$ 11
	4 - 17	6 - 20	3 - 60	6 - 54	10 - 42
	26	22	26	22	26
	a	ab	bc	d	cd
dissolved organic carbon ( $\text{mg C L}^{-1}$ )	36.4 $\pm$ 6.7	28.6 $\pm$ 6.0	26.7 $\pm$ 5.4	26.5 $\pm$ 6.3	23.7 - 5.5
	22.7 - 50.0	16.1 - 39.9	16.3 - 36.0	15.4 - 40.2	14.8 - 35.7
	25	21	24	20	25
	a	b	bc	bc	c

Table 3. Summary statistics of elemental concentrations at five Newport River sampling locations, April 2006 - April 2007. The four rows for each parameter indicate the mean  $\pm$  stdev, min-max, n, and statistically significant ( $p < 0.05$ ) homogenous subset groupings (Tukey post-hoc analysis). Dashes indicate no significant difference ( $p < 0.05$ ) between sites (one-way ANOVA).

	Southwest	Godwin	New Highway 70	Old Highway 70	Dock
chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	$0.6 \pm 0.5$	$1.5 \pm 1.7$	$3.6 \pm 4.7$	$6.3 \pm 11.0$	$4.8 \pm 5.8$
	0.2 - 2.6	0.3 - 7.2	0.3 - 22.0	0.3 - 48.5	0.2 - 27.2
	26	23	26	23	26
	a	a	ab	b	ab
total oxygen uptake ( $\text{mg O}_2 \text{ day}^{-1}$ )	$0.26 \pm 0.15$	$0.29 \pm 0.15$	$0.39 \pm 0.21$	$0.44 \pm 0.27$	$0.40 \pm 0.23$
	0.06 - 0.78	0.09 - 0.74	0.06 - 0.96	0.09 - 1.24	0.04 - 0.96
	25	23	25	23	25
	a	ab	ab	ab	b
oxygen uptake - dissolved material only ( $\text{mg O}_2 \text{ day}^{-1}$ )	$0.22 \pm 0.11$	$0.25 \pm 0.14$	$0.28 \pm 0.15$	$0.31 \pm 0.20$	$0.28 \pm 0.17$
	0.03 - 0.55	0.02 - 0.58	0.02 - 0.66	0.03 - 0.85	0.03 - 0.68
	23	23	23	23	23
	-	-	-	-	-
oxygen uptake - particulate matter only ( $\text{mg O}_2 \text{ day}^{-1}$ )	$0.03 \pm 0.02$	$0.04 \pm 0.05$	$0.08 \pm 0.07$	$0.12 \pm 0.11$	$0.09 \pm 0.08$
	0.00 - 0.07	0.00 - 0.16	0.00 - 0.26	0.00 - 0.39	0.00 - 0.28
	22	22	22	22	22
	a	ab	abc	c	bc

Table 4. Nonparametric correlation analysis between biologic variables and water chemistry parameters for all sites. Values are the Spearman rho, significance value, and sample size.

	total oxygen uptake	oxygen uptake - dissolved	oxygen uptake - particulate	chlorophyll <i>a</i>
temperature	0.21*	-0.050	0.423*	0.593*
	0.022	0.600	0.000	0.000
	120	114	109	123
light attenuation	0.029	-0.007	0.120	-0.019
	0.776	0.944	0.270	0.854
	97	91	86	100
nitrate+nitrite	0.222*	-0.007	0.385*	0.718*
	0.017	0.943	0.000	0.000
	116	110	110	119
ammonium	0.042	-0.076	0.117	.583*
	0.652	0.431	0.224	0.000
	116	110	110	119
total nitrogen	0.191	-0.052	0.380*	0.497*
	0.060	0.616	0.000	0.000
	98	95	95	101
dissolved reactive phosphorus	0.249*	0.040	0.391*	0.754*
	0.007	0.680	0.000	0.000
	116	110	110	119
dissolved organic carbon	0.068	0.130	-0.069	-0.454*
	0.482	0.189	0.490	0.000
	109	103	103	112
suva254	-0.465*	-0.435*	-0.322*	0.110
	0.001	0.002	0.024	0.438
	49	49	49	52
suspended organic matter	0.169	-0.100	0.353*	0.613*
	0.066	0.293	0.000	0.000
	119	113	108	119
chlorophyll <i>a</i>	0.270*	-0.037	0.464*	1
	0.003	0.695	0.000	.
	121	115	110	124

Figure 1. Map of the Newport River watershed and sampling sites.

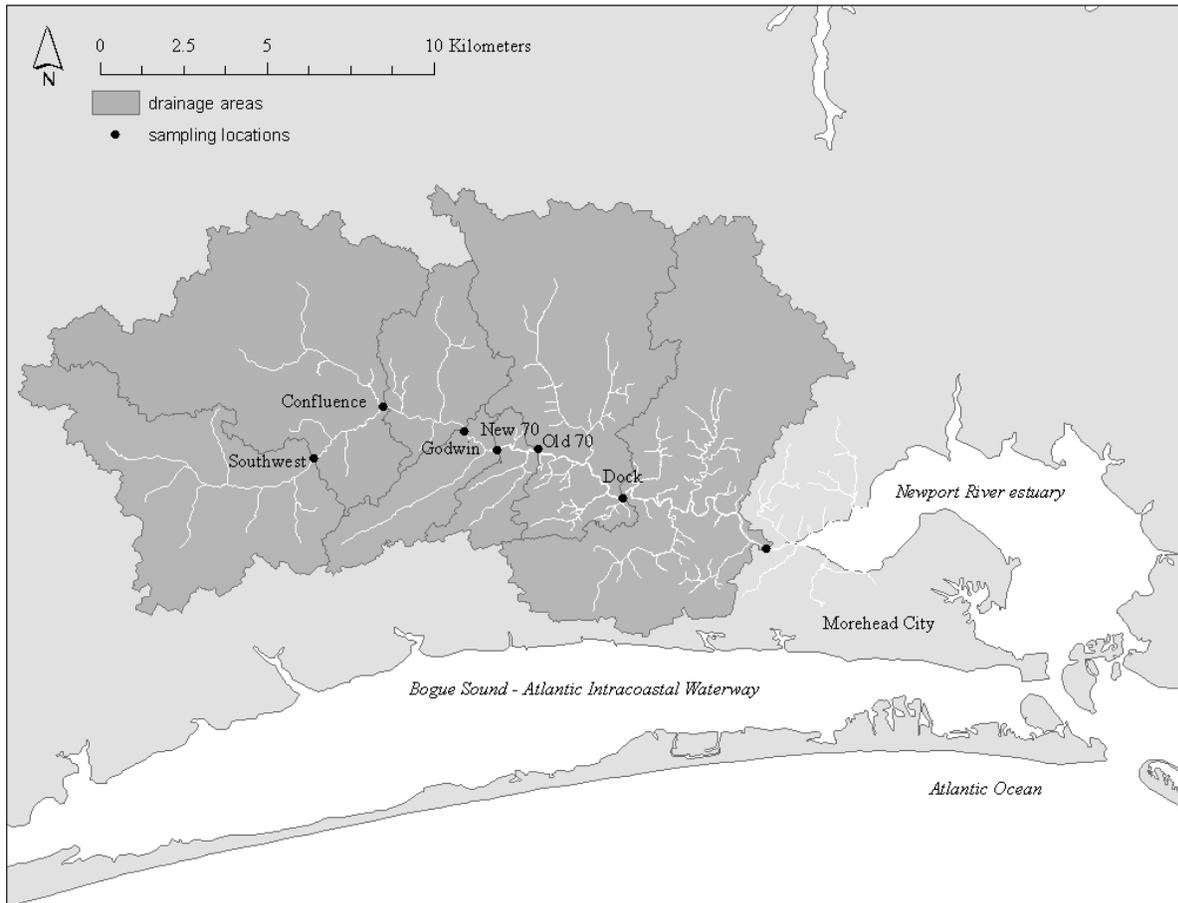


Figure 2. Width and channel cross-sectional area of the Newport River tidal freshwater zone; 8930 m is located 50 m upstream of the beginning of the tidal freshwater zone.

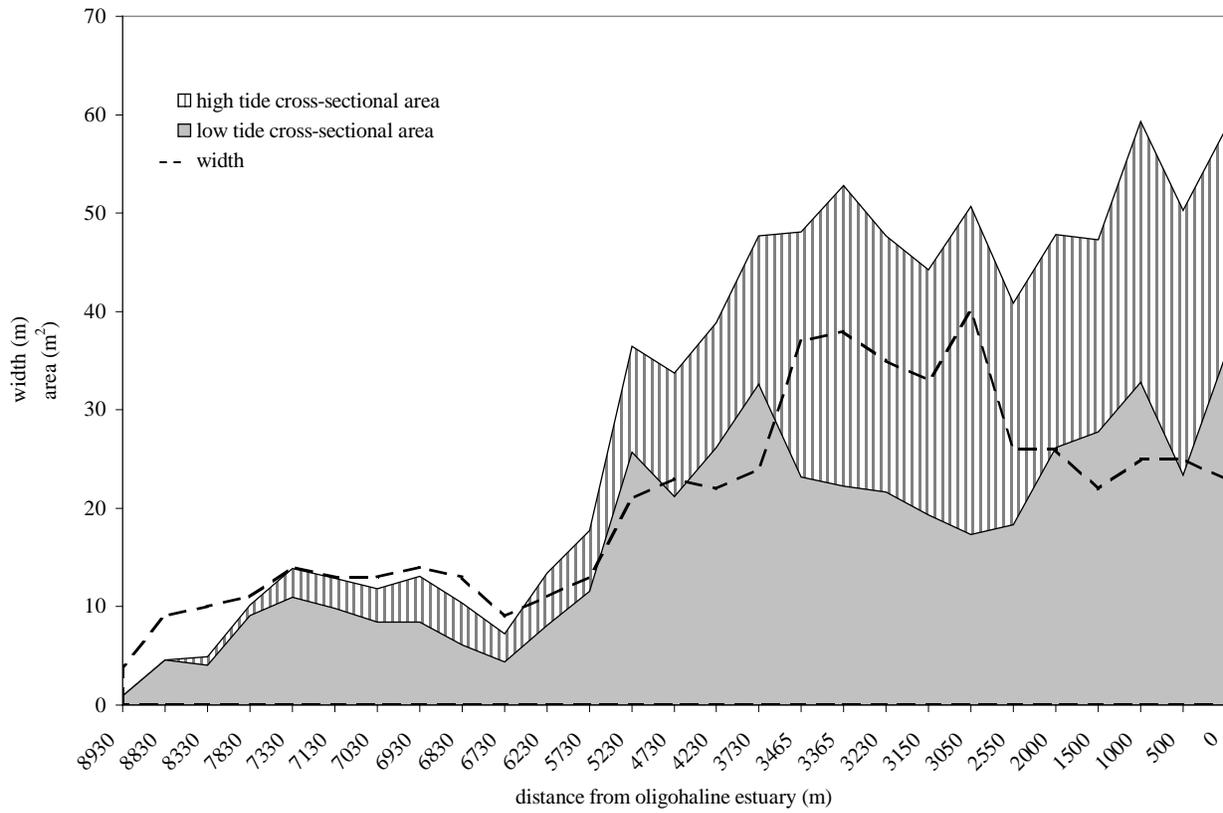


Figure 3. River discharge at Confluence and nominal flushing time of the TFZ of the Newport River.

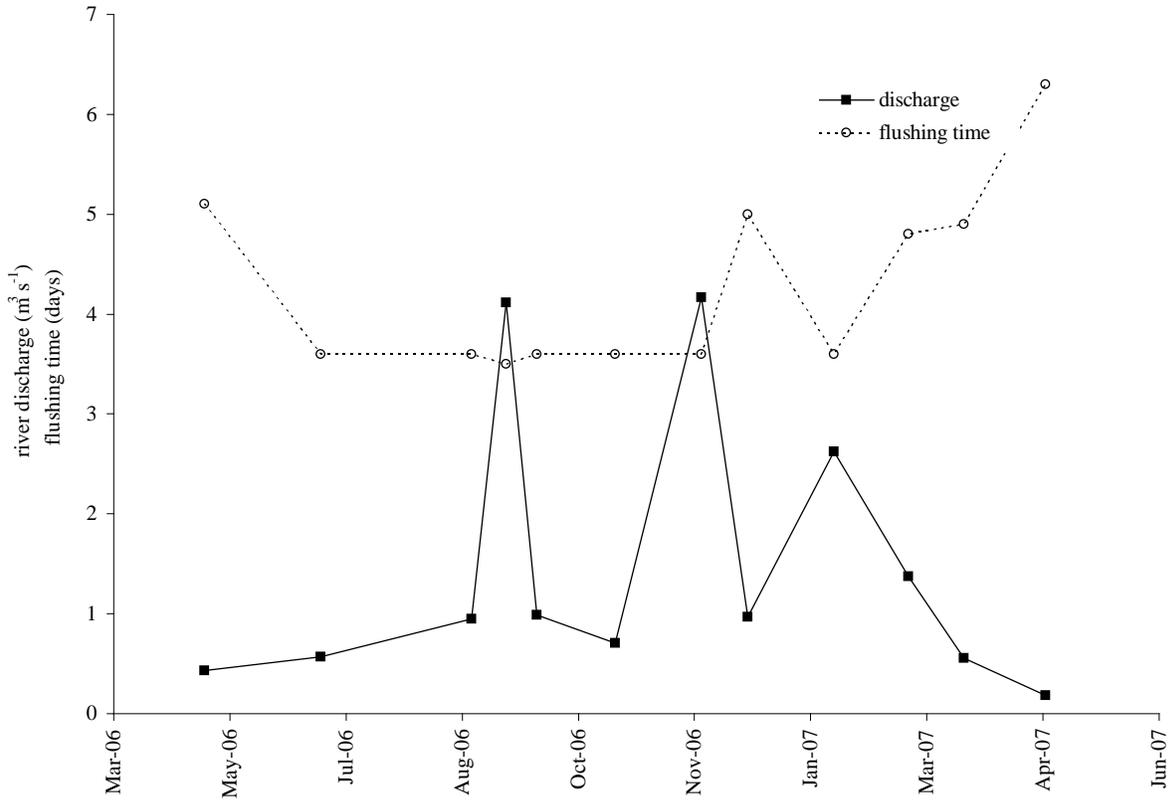


Figure 4. Constituent loads at New Highway 70 and the outfall of the Town of Newport WWTP.

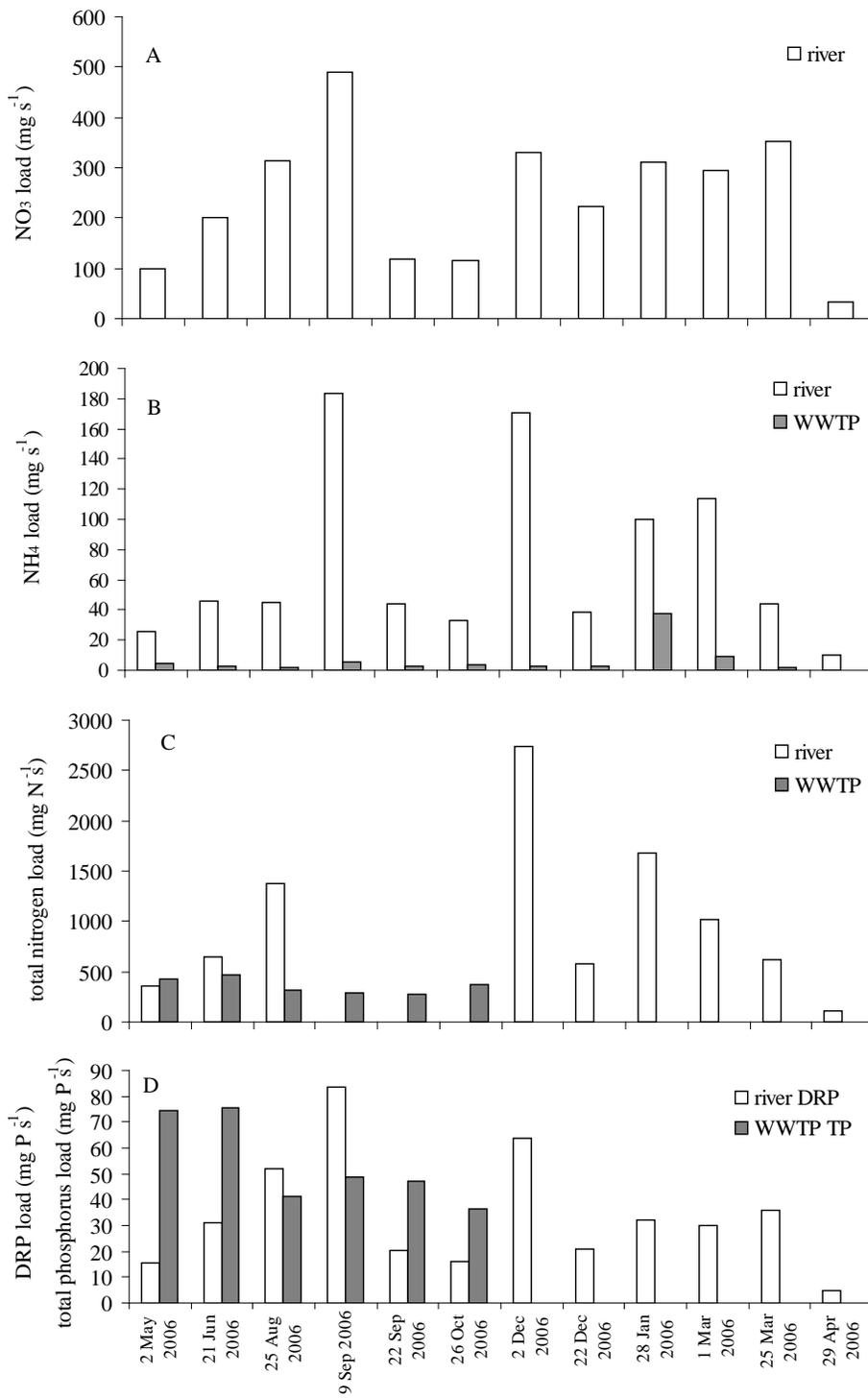
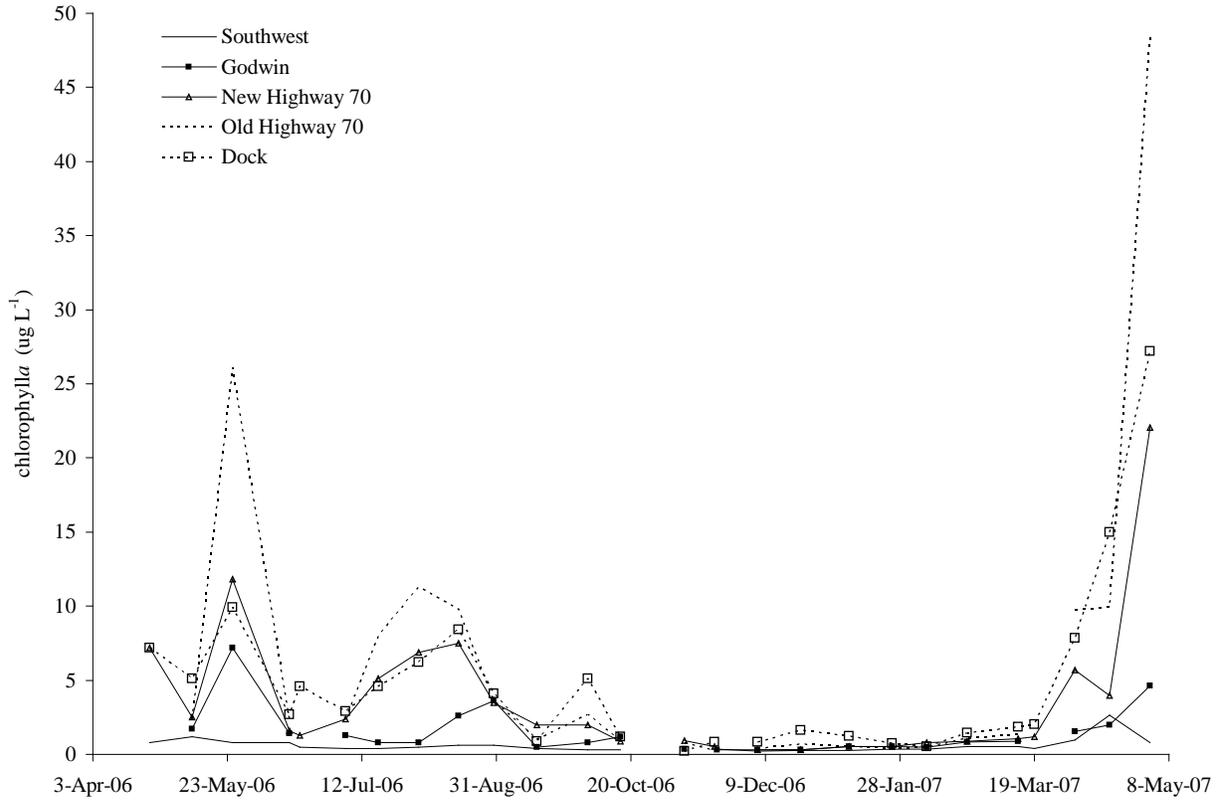


Figure 5. Chlorophyll *a* concentrations at the five Newport River sampling sites.



## RESOURCE LIMITATION OF PHYTOPLANKTON AND BACTERIAL GROWTH IN THE TIDAL FRESHWATER ZONE

### INTRODUCTION

The Newport River TFZ had significantly more phytoplankton biomass than the non-tidal river upstream, and the concentration of chlorophyll *a* exceeded the NC state water quality standard of 40  $\mu\text{g L}^{-1}$  during a period of long residence time. The decomposition of this algal bloom and subsequent dissolved oxygen uptake had the potential to degrade water quality in the river and estuary downstream through elevated biochemical oxygen demand (Mallin et al. 2004). Indeed, dissolved oxygen concentrations below the 4  $\text{mg L}^{-1}$  NC state water quality standard were observed in the TFZ during a low-flow period in August 2006. More moderate amounts of algal production within the TFZ are an important component of the riverine food web. The mechanisms and resources that limit phytoplankton growth are of interest to both environmental managers charged with preventing excessive algal growth and ecologists interested in riverine food webs.

Inorganic nutrients, residence time, irradiance, and zooplankton grazing are the predominant controls on phytoplankton growth in aquatic systems. Research presented above has shown that chlorophyll *a* is correlated with nutrient concentrations in the Newport River TFZ, suggesting limitation of phytoplankton growth by nutrients. Indeed, phytoplankton growth in the mesohaline Newport River estuary is limited by inorganic nitrogen (Thayer 1974), and nitrogen and phosphorus have repeatedly been found to stimulate phytoplankton in blackwater rivers of NC (Mallin et al. 2001; Mallin et al. 2004). Despite this fact, blackwater rivers, such as the Newport River, are not considered nutrient sensitive by the NC DWQ, thus discharge permits authorized under the NPDES system do not require consideration for nitrogen and phosphorus from these systems. Based on correlative data from the Newport River, and experimental evidence in other blackwater systems, it is likely that phytoplankton in this system are limited by nutrient availability, as well as residence time and irradiance.

In addition to phytoplankton, free-living and attached bacteria also provide an important basal energy source for aquatic invertebrates in blackwater rivers (Edwards et al. 1990). These systems have naturally high concentrations of bacteria, mostly derived from floodplain inputs. Given the importance of bacteria to the river ecosystem, previous research has examined the affect of nutrient concentrations on bacterial growth in these systems. Inorganic nitrogen and phosphorus have both been found to stimulate bacterial growth, increasing oxygen uptake from the water (Mallin et al. 2004). Thus, elevated nutrient concentrations may stimulate both excessive algal growth and heterotrophic respiration, compounding the adverse affect on dissolved oxygen in rivers and estuaries.

The current study was sought to explain how resource limitation of phytoplankton and bacterial growth within the TFZ of blackwater rivers compares to upstream non-tidal and estuarine areas. This information is essential for both environmental managers and ecologists working within this riverine-estuarine continuum. This study addressed this question through a series of mesocosm bioassay experiments with addition of nutrients from 3 sites along the non-tidal through tidal zone of the Newport River.

## MATERIALS AND METHODS

Nutrient limitation bioassay experiments were performed in April and October 2006, and February and March 2007. River water was collected from Southwest, New Highway 70, and Dock (Figure 1) and returned to the Institute of Marine Sciences (IMS) in Morehead City, NC. Five L of site water from each site was distributed into 16 10 L plastic cubitainers. Experimental nutrient additions (expressed as the concentration increase over background) were  $140 \mu\text{g N-NO}_3 \text{ L}^{-1} + 140 \mu\text{g N-NH}_4 \text{ L}^{-1}$ ,  $155 \mu\text{g P-PO}_4 \text{ L}^{-1}$ , and a combination of these treatments, with each treatment replicated 4 times. Cubitainers were floated in ponds at the UNC IMS at the ambient temperature of Bogue Sound and covered with 2 layers of neutral density screening to reduce irradiance 25%. The response of phytoplankton to these additions was measured as chlorophyll *a* after 1, 2, and 4 days after nutrient addition, and bacterial response was quantified by total bacterial cell counts after 2 days. Chlorophyll *a* analysis was performed as described on page 4 of this report. Differences between sites, treatments, and time of incubation were determined with a one-way ANOVA and Tukey post hoc test using SPSS software. All analyses were performed at the 0.05 significance level.

Sub samples from the bioassay experiment were taken for microscopy and direct counts of bacterial abundance. Twenty mL of unfiltered water from each of the bioassay samples was preserved with 1.0 mL of 47% formaldehyde and stored in the dark until analysis (Hobbie et al 1977). Slides were prepared using a  $0.45 \mu\text{m}$  mixed cellulose ester backing filter (Millipore Cat #HAWG02500) and  $0.2 \mu\text{m}$  black membrane filter (Whatman Nuclepore #110656). For each bacteria slide a 1 mL aliquot was stained with 1 mL of 0.01% acridine orange and allowed to stand for 5 minutes before filtration (Sherr et al. 2001). After filtration the membrane was transferred onto a microscope slide dampened with Immersion Oil B and a drop of oil was placed on top of the membrane followed by a glass slide cover (McMarth et al. 1998). For each set of filters a blank was prepared by filtering 1 mL of acridine orange solution without sample to obtain background counts. Slides were stored frozen until counted.

Following the procedure outlined by Sherr et al. (2001), a Nikon Eclipse E800 microscope fitted with a mercury lamp and a FITC filter optimized for blue light epifluorescence was used for direct counts of heterotrophic bacteria. Samples were viewed at a magnification of 1000x. Using a counting grip, a minimum of five randomly selected fields of view were counted over the membrane surface until a minimum of 500 bacteria had been counted (McMarth et al. 1998). Bacterial abundance was calculated as bacterial cells per mL with background counts subtracted (Sherr et al. 2001).

## RESULTS

Phytoplankton biomass at Southwest was stimulated by a nitrogen alone during March (day 4), and by a combination of N+P in March (days 2 and 4), April (day4), and June (days 2 and 4) (Figures 6-8). At the tidal freshwater New Highway 70, phytoplankton biomass was stimulated by nitrogen in April (days 1 and 2), phosphorus in April (day1), and a combination of N+P in April (days 1 and 2) and June (days 2 and 4) (Figures 7 and 8). The oligohaline estuary at Dock showed nitrogen limitation in March, April, and June on day 4, while colimitation by N+P was observed in March (day 4), April (day 4), and June (days 2 and 4) (Figures 6-8). No nutrient limitation was observed at any site in October (Figure 9).

Spatial patterns in N or P limitation along this longitudinal gradient differed during each month of investigation. In March, N limitation occurred at Southwest and Dock, while no limitation was observed in the TFZ. During April N and P were independently limiting in the TFZ while no limitation was observed during those instances upstream or downstream; N limitation at the Dock occurred when no limitation was observed at New 70 or Southwest. In June N limited growth at the Dock only. These data demonstrate distinct phytoplankton community responses along the upstream-downstream gradient.

Inorganic molar N:P ratios less than 23:1 in the control treatments at Southwest suggested phytoplankton would be nitrogen limited during all experiments (Wetzel 2001), but a significant nitrogen response was only observed in March (Table 5). At New Highway 70, bioassay results did support the expectation of nitrogen limitation (N:P ratio <23:1) during April. Stimulation of algal biomass at Dock by nitrogen in April and June complied with expectations of limitation, but nitrogen limitation was also observed during March when N:P ratios >23 suggested phosphorus limitation.

Phytoplankton biomass in the control treatment increased significantly over the 4 day incubation period at Southwest in April, and during all months at New Highway 70 and Dock (Figures 6-10). This suggests that increased irradiance in the bioassay experiment allowed for enhanced algal production. Light availability to the water column is highly reduced within the Newport River, with attenuation coefficients  $\sim 7 \text{ m}^{-1}$  (Table 1), producing a photic depth within the water column of 0.65 m. Given the average depth measured along the TFZ of 1.4 m, about 46% of the water column has sufficient irradiance to support photosynthesis.

Bacterial growth was stimulated by nitrogen at Southwest in March, and N+P in June (Figure 10). At New Highway 70 bacterial growth was stimulated by phosphorus in October (Figure 10). At the Dock bacterial growth was limited by nitrogen in June and October, by phosphorus in March and October, and was co-limited by N+P in March and October (Figure 10).

## DISCUSSION

Nitrogen, in concentrations similar to those used here, has been found to stimulate phytoplankton growth in blackwater rivers (Mallin et al. 2001; Mallin et al. 2004). Our results from Southwest also showed nitrogen alone stimulated algal growth during one of four months examined, and phosphorus never stimulated algal growth alone. In contrast to this non-tidal blackwater site, the TFZ was found to be limited by nitrogen and phosphorus independently and in combination. Based on the ambient N:P ratios, nitrogen limitation would be expected at both sites during April. Also, initial chlorophyll *a* was 9 times greater at New Highway 70 than Southwest, indicating a more substantial phytoplankton community which could take advantage of excess nutrients. The contrast between nutrient limitations observed between Southwest and New Highway 70 indicates fundamental differences in response between non-tidal and tidal freshwater rivers.

Bacteria and other microbial heterotrophs in blackwater rivers have been found to be stimulated by nitrogen and phosphorus independently (Mallin et al. 2001; Mallin et al. 2004). Our experiments revealed nitrogen-only limitation at Southwest, and phosphorus-only limitation at

New Highway 70. Again, this contrast between non-tidal and tidal blackwater rivers indicates a fundamental difference between these microbial communities and the resources limiting growth.

Nutrient limitation of phytoplankton growth was observed in the spring and summer months in the Newport River, but not in fall. This is similar to other blackwater rivers in North Carolina (Mallin et al. 2001; Mallin et al. 2004). It is possible that invertebrate grazers were exerting top-down control during these experiments. However, the duration of the experiments was designed to preclude significant grazer reproduction and thus reduce the potential for variable grazing among treatments.

Given the significant increase in the control treatment that was frequently observed, it appears that the experimental design allowed more irradiance to reach the cubitainers than was present in the field. While only 46% of the water column of the river can support photosynthesis, the experimental mesocosms were continually within the photic zone (<0.65 m). Clearly, this shallow photic zone, combined with turbulent vertical mixing, would be expected to curtail phytoplankton growth. As the river channel expands in width along the downstream continuum (Figure 2), a larger proportion of the channel volume is exposed to the photic zone. This increased light availability may facilitate phytoplankton growth in the TFZ more so than the river upstream. Phytoplankton blooms observed in a blackwater river whose riparian zone was clearcut were suspected to be caused by increased light availability and nutrient concentrations (Ensign and Mallin 2001).

The sensitivity of the phytoplankton community to increased irradiance, as demonstrated by the frequent increase in chlorophyll *a* in control treatments, has implications for management in blackwater rivers. Chlorophyll *a* was found to have a strong, inverse correlation with DOC at five Newport River sites. These two observations suggest that anthropogenic alteration of watershed characteristics that affect DOC loading may positively affect phytoplankton growth by reducing light attenuation. Activities which disconnect the river channel from sources of DOC in the floodplain, such as channelization, river dredging, and development, may decrease light attenuation to an extent beneficial to phytoplankton and stimulate growth.

An important effect of nutrient-stimulated phytoplankton production in blackwater rivers is the demonstrated increase in biochemical oxygen demand (Mallin et al. 2004). Dissolved oxygen in these river systems is commonly depressed by the heavy DOC load and subsequent bacterial growth (Howitt et al. 2007), and additional organic matter production could result in hypoxia. This effect may be manifest in the TFZ of blackwater rivers if residence time is adequate. Otherwise, this particulate material will be exported to the estuary where decomposition will occur.

Table 5. Initial nutrient concentrations in bioassay cubitainers following nutrient amendment.

month	site	treatment	NO <sub>3</sub>	NH <sub>4</sub>	PO <sub>4</sub>	molar N:P	chlorophyll <i>a</i>
March	Southwest	control	6	34	7	13	0.4
		N	146	174	7	101	
		P	6	34	162	1	
		N+P	146	174	162	4	
	New Highway 70	control	170	70	20	27	1.2
		N	310	210	20	58	
		P	170	70	175	3	
		N+P	310	210	175	7	
	Dock	control	207	47	19	30	4.1
		N	347	187	19	62	
		P	207	47	174	3	
		N+P	347	187	174	7	
April	Southwest	control	3	49	17	7	0.8
		N	143	189	17	43	
		P	3	49	172	1	
		N+P	143	189	172	4	
	New Highway 70	control	175	81	44	13	7.2
		N	315	221	44	27	
		P	175	81	199	3	
		N+P	315	221	199	6	
	Dock	control	226	92	32	22	7.2
		N	366	232	32	41	
		P	226	92	187	4	
		N+P	366	232	187	7	
June	Southwest	control	15	37	8	14	0.5
		N	209	185	8	109	
		P	15	37	163	1	
		N+P	209	185	163	5	
	New Highway 70	control	69	45	14	18	1.3
		N	209	185	14	62	
		P	69	45	169	1	
		N+P	209	185	169	5	
	Dock	control	188	50	36	15	4.6
		N	328	190	36	32	
		P	188	50	191	3	
		N+P	328	190	191	6	
October	Southwest	control	17	24	6	15	0.2
		N	157	164	6	118	
		P	17	24	161	1	
		N+P	157	164	161	4	
	New Highway 70	control	5	18	4	13	0.4
		N	145	158	4	168	
		P	5	18	159	0	
		N+P	145	158	159	4	
	Dock	control	174	20	12	36	0.6
		N	314	160	12	87	
		P	174	20	167	3	
		N+P	314	160	167	6	

Figure 6. Response of water column chlorophyll *a* to nutrient additions in March 2007. Letters indicate groupings of homogenous subsets of treatments during each day using a Tukey post-hoc analysis ( $p < 0.05$ ).

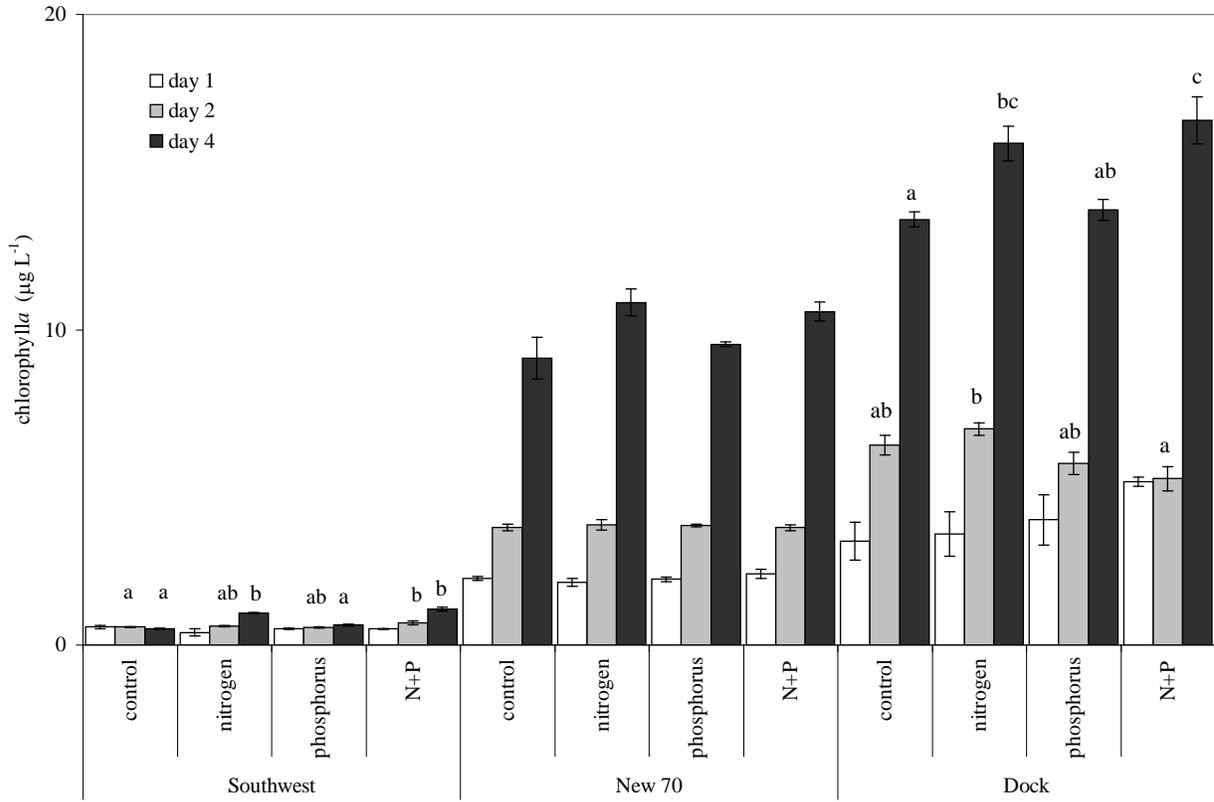


Figure 7. Response of water column chlorophyll *a* to nutrient additions in April 2006. Letters indicate groupings of homogenous subsets of treatments during each day using a Tukey post-hoc analysis ( $p < 0.05$ ).

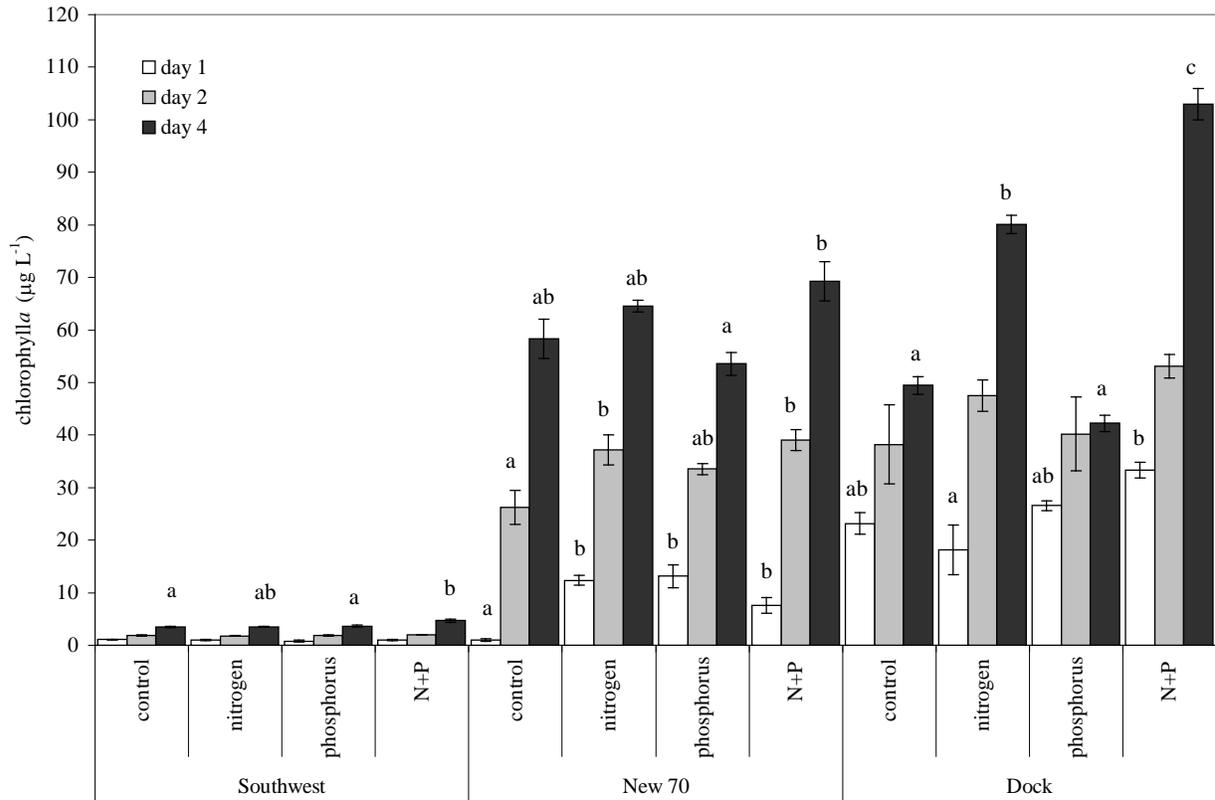


Figure 8. Response of water column chlorophyll *a* to nutrient additions in June 2006. Letters indicate groupings of homogenous subsets of treatments during each day using a Tukey post-hoc analysis ( $p < 0.05$ ).

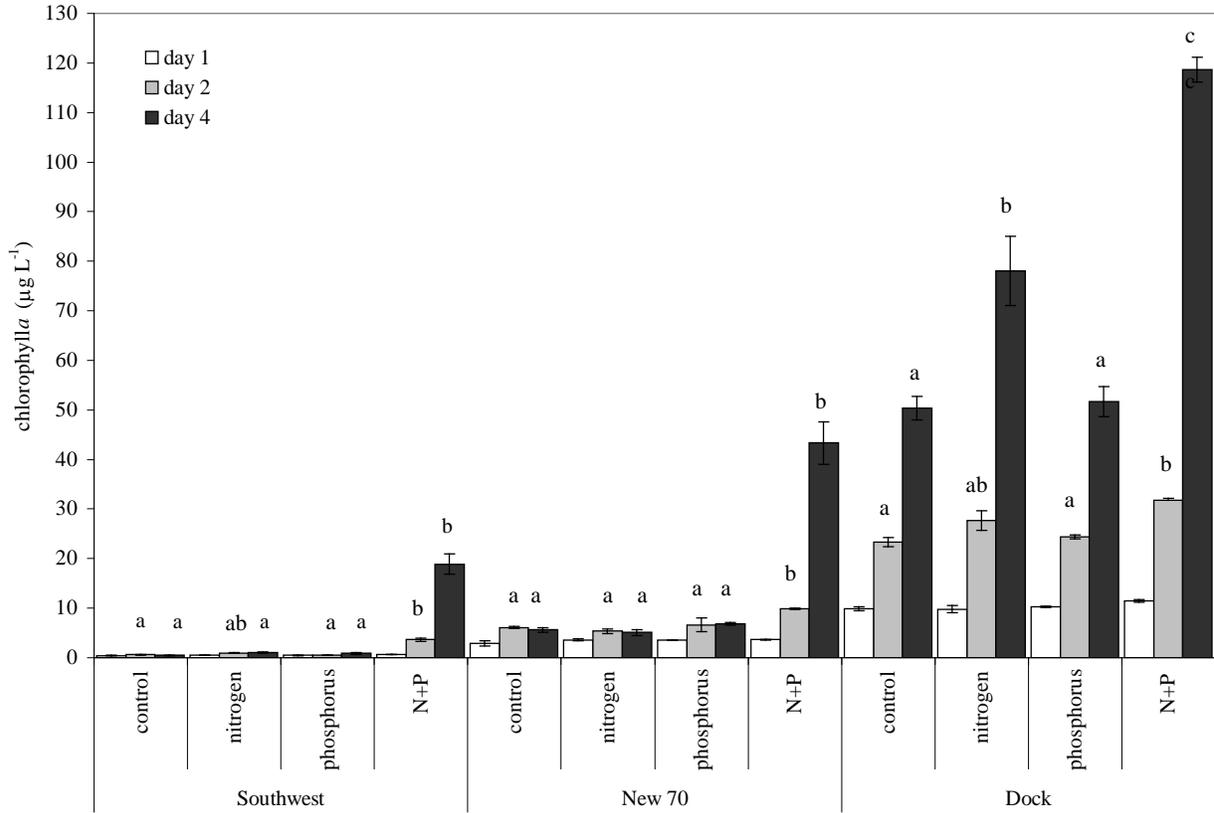


Figure 9. Response of water column chlorophyll *a* to nutrient additions in October 2006. No significant differences between treatments during any day at any site were found with a one-way ANOVA ( $p < 0.05$ ).

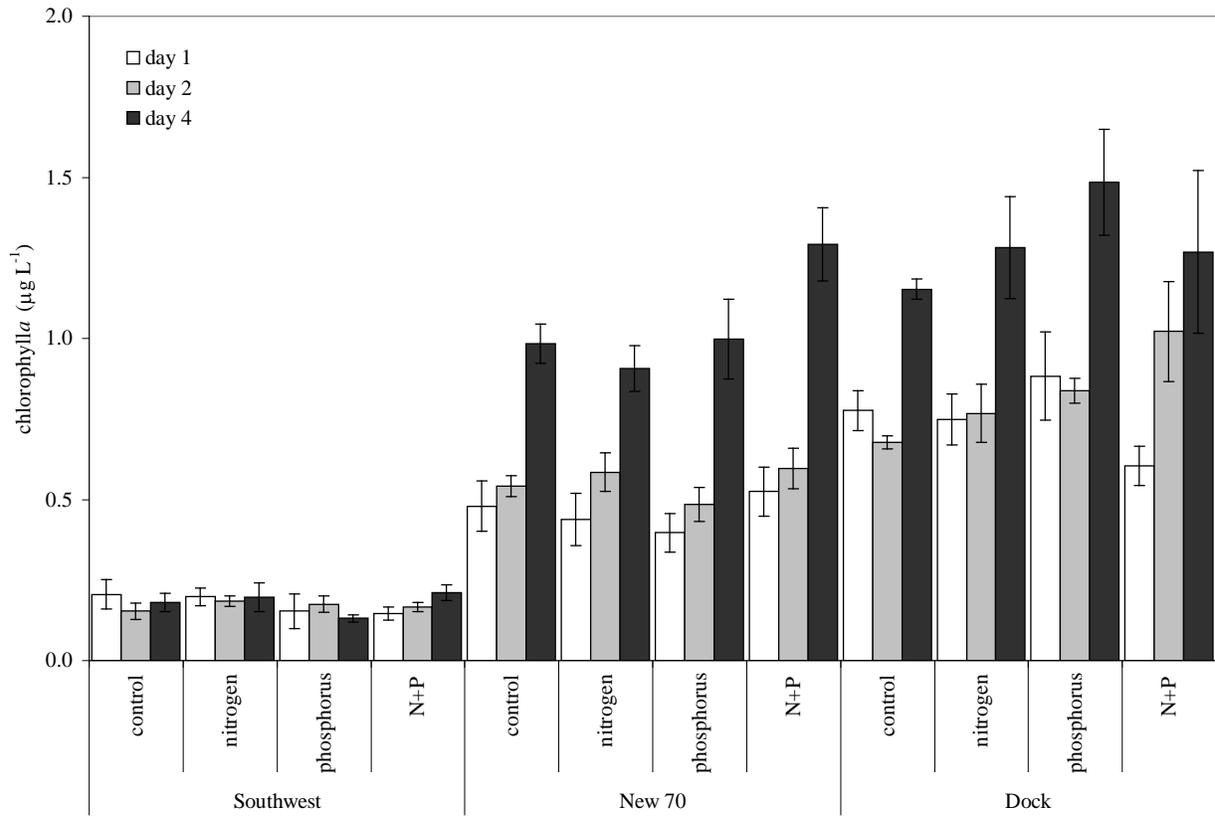
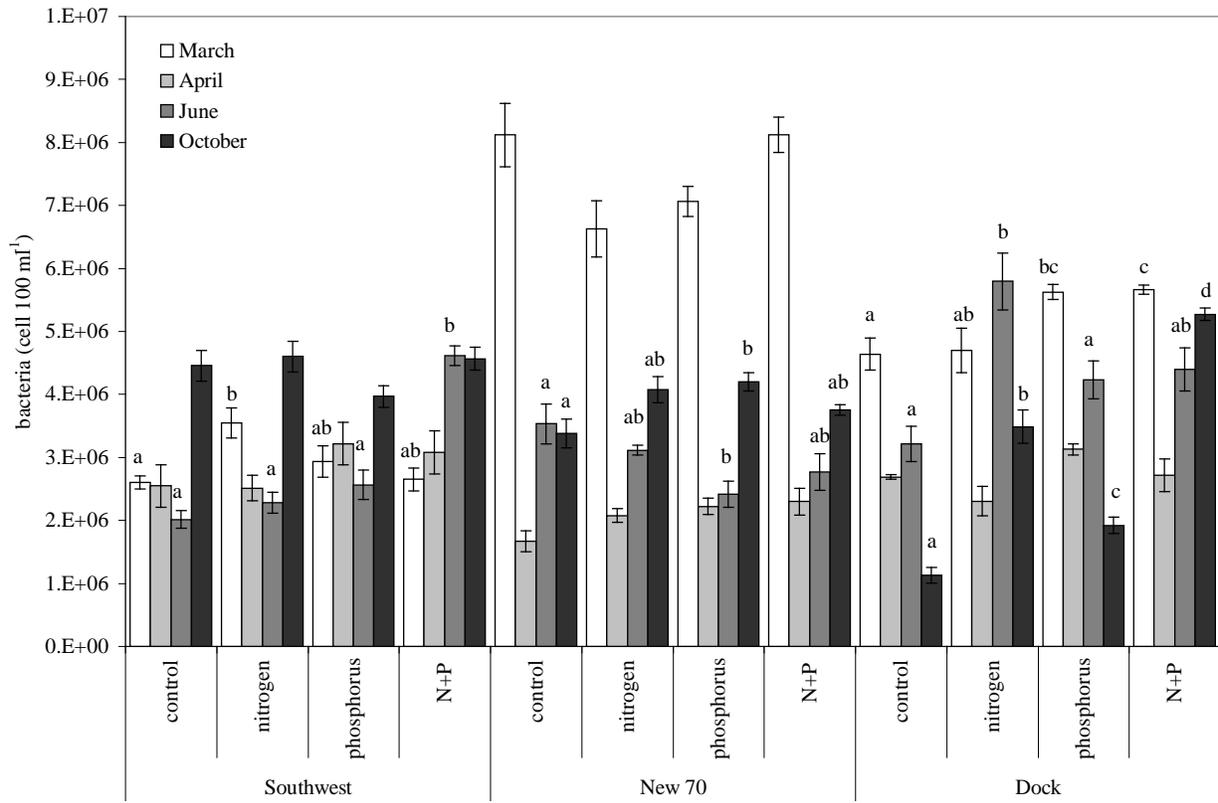


Figure 10. Response of total bacterioplankton to nutrient additions after 2 days of incubation. Letters indicate homogenous subsets of treatments during each month using a Tukey post-hoc analysis ( $p < 0.05$ ).



## DENITRIFICATION WITHIN THE TIDAL FRESHWATER RIPARIAN ZONE

### INTRODUCTION

Inorganic nitrogen was found to stimulate algal and bacterial growth throughout the non-tidal river through estuarine continuum of the Newport River, and previous work in the mesohaline estuary has also shown nitrogen to stimulate phytoplankton growth (Thayer 1974). In excess, this enhanced algal biomass can reduce dissolved oxygen to hypoxic levels and result in the death of aquatic organisms. This effect of nitrogen on coastal productivity is common in many coastal environments (NRC 2000), and management of anthropogenic nutrients has become a major research focus. A particular challenge in these efforts to understand nutrient loading has been the confounding effects of flow (Borsuk et al. 2004) and in-stream processes such as denitrification (Seitzinger 1988; Seitzinger et al. 2002) and other biochemical processes (Ensign and Doyle 2006).

Predicting nitrogen flux from river networks to estuaries requires understanding of transformations that occur through the TFZ, as it occupies a distinct ecotone between the river and estuary. The extensive floodplain wetlands of coastal rivers have been identified as an important location for denitrification (Seitzinger et al. 2006). In TFZ wetlands, fluctuation between anoxic to oxic conditions creates conditions for closely coupled nitrification-denitrification to occur in the upper sediment layer (Kerner et al. 1986), thereby enhancing nitrogen loss through denitrification. Nitrification in tidal freshwater marshes has been observed using isotopically-labeled  $\text{NH}_4$  additions (Gribsholt et al. 2005). Nitrate from nitrification in TFZ wetlands could provide a significant subsidy to the entrained river nitrate load available for denitrification.

The net effect of denitrification within tidal freshwater rivers has not been rigorously examined, although it could likely be a large sink for nitrogen along the river continuum. Given that residence time is much longer per unit length of channel in the TFZ than non-tidal rivers, the TFZ is expected to exhibit higher rates of denitrification than other zones of a river network (Seitzinger et al. 2006). The geomorphic shape of the river, tidal range, and river discharge are the predominant drivers of estuarine residence time. A generalized understanding of how these factors affect residence time, channel-riparian hydrologic exchange, and resultant riparian zone biogeochemistry of TFZs is necessary to incorporate this region of the river network into watershed-scale nitrogen budgets. Here, our goal was to evaluate denitrification rates in a variety of intertidal habitats, examine redox fluctuations in-situ, and extrapolate these values to potential denitrification rates occurring at the TFZ-scale.

### MATERIALS AND METHODS

Sediment cores (6.4 cm diameter by ~10 cm deep) were collected monthly from 3 intertidal habitats along the TFZ of the Newport River, NC nearest Old Highway 70 (Figure 1). Triplicate cores from each site and 50 L of river water were returned to the UNC IMS, capped with water-tight lids containing two ports. From August-November 2006 cores were incubated with a static volume of headspace for 6 hours and  $\text{N}_2$  was measured in the overlying water every 2 hours. From December-June 2007 cores were plumbed to multi-channel peristaltic pump that pumped river water through the headspace of the core in a flow-through design (McCarthy et al. 2007). A 50 L reservoir of aerated river water was pumped into the water-filled headspace above the

sediment at a rate of  $\sim 1 \text{ mL min}^{-1}$ . Cores were incubated at the temperature of the river during collection. Water samples from the cores were collected in 5 mL glass tubes from the inflow and outflow of the cores. Water blanks (site water without sediment) were run in triplicate.

A Balzers Prisma QME 200 quadrupole mass spectrometer (Pfeiffer Vacuum, Nashua, NH) was coupled to a silicon, gas-permeable membrane inlet as described by Kana et al. (1994). The mass spectrometer was tuned to monitor the ratio of  $\text{N}_2:\text{Ar}$  instead of  $\text{N}_2$  alone as the  $\text{N}_2:\text{Ar}$  signal is relatively unaffected by changes in oxygen above 50% saturation (Kana and Weiss 2004). A continually-mixed water bath containing tap water at  $16^\circ\text{C}$  was used to obtain an  $\text{N}_2:\text{Ar}$  signal ratio which served as a standard for calibration. The signal ratio of  $\text{N}_2:\text{Ar}$  ( $(\text{N}_2:\text{Ar})_{\text{standard\_signal}}$ ) of each sample was used to determine the  $\text{N}_2$  concentration of the sample using the following formula:

$$[\text{N}_2]_{\text{sample}} = (\text{N}_2 : \text{Ar})_{\text{sample\_signal}} \times \frac{([\text{N}_2] : [\text{Ar}])_{\text{equilibrium}}}{(\text{N}_2 : \text{Ar})_{\text{standard\_signal}}} \times [\text{Ar}]_{\text{sample}} \quad \text{equation 2}$$

Standard gas saturation tables for a range in temperature in freshwater were used to obtain the  $[\text{N}_2]:[\text{Ar}]_{\text{equilibrium}}$  and  $[\text{Ar}]_{\text{sample}}$  at the temperature of the water bath and incubation room, respectively.

Next, the denitrification rate (DEN) occurring within the sediment core was calculated as:

$$\text{DEN} = ([\text{N}_{\text{out}}] - [\text{N}_{\text{in}}]) \times \frac{F}{A} \quad \text{equation 3}$$

where  $F$  is the flow rate through the head space of the core and  $A$  is the surface area of the core. Differences between sites during each experiment and overall during the period of study were examined using an ANOVA and Tukey post-hoc analysis ( $p < 0.05$ ) with SPSS software.

Measuring  $\text{N}_2$  fluxes within sediment cores has a number of advantages over other methods of measuring denitrification such as the commonly employed acetylene block method (Cornwell et al. 1999; Groffman et al. 2006). Most importantly is that net denitrification rates are measured without interference of nitrification occurring in the cores, allowing coupled nitrification-denitrification to proceed at natural rates.

Denitrification measured using laboratory-based sediment core incubations described above reflect denitrification rates under continually saturated conditions. In reality, these intertidal sediments are continually exposed and inundated by the semi-diurnal tide. Since the laboratory cores were incubated for  $>48$  hours, redox condition of the sediments in the laboratory incubations may have been more reduced than conditions in the field. A critical question was whether the redox condition, and subsequently denitrification rate, in the permanently saturated lab cores is similar to redox conditions that occur in the field. To address this issue sediment redox and porewater saturation were measured in-situ.

The oxidation-reduction potential of sediment porewater and the soil moisture of the sediment was measured from 7 February 2007 – 7 March 2007. A 15 m wooden boardwalk was constructed perpendicular to the river between New Highway 70 and Old Highway 70 near the Town of Newport WWTP. Sensors were deployed along a 20 m transect from the river laterally into the floodplain. Nests of 2 redox probes (5 and 10 cm below the sediment/water interface) and one soil moisture probe (10 cm deep) were deployed at 2 m, 12 m, and 20 m from the low

tide channel margin. The platinum tipped redox probes were purchased from Dr. Wayne Hudnall at the Texas Tech University. Redox calibration was checked before and after deployment using a 220 mV redox solution; after 3 months of deployment all probes read  $220 \pm 10$  mV. Redox values were corrected to the standard hydrogen electrode by adding 200 mV; no temperature or pH correction was made to measured values. A Ag/AgCl reference electrode (Accumet 13-620-53) for the redox measurements was installed using a salt bridge (1 m of 2 cm diameter pvc pipe filled with a gelled solution of agar and KCl). Soil moisture probes (model CS616) were purchased from Campbell Scientific (Campbell Scientific, Incorporated, Logan, UT) and the manufacturer's calibration for loam soils with bulk density less than  $1.55 \text{ g cm}^{-3}$  and clay content less than 30% were used. Sensors were wired to a Campbell Scientific CR1000 data logger and measurements were recorded every 5 minutes from 7 February 2007 – 7 March 2007. Redox (Eh) values less than 300 mV were assumed to indicate reducing conditions at which denitrification was likely to occur (Faulkner and Patrick 1992).

In order to put measurements of denitrification and soil redox into context with nitrogen fluxes through the river, rate measurements, redox, and topographic data were combined in a spatio-temporal framework to estimate the total amount of denitrification occurring in the tidal freshwater riparian zone of the Newport River. The surface area of riparian floodplain inundated by high tide was estimated using spatial interpolation of elevation data collected by the North Carolina Floodplain Mapping Agency. The bare-earth Light Image Detection and Ranging (LIDAR) data was downloaded from [www.ncfloodmaps.com](http://www.ncfloodmaps.com). ArcMap (ESRI, Redlands, CA) geographic information system software was used to construct a triangulated irregular network (TIN) from the LIDAR data that were within 25 m of the river. A zero elevation hardbreak line was forced along the river channel. The 3-dimensional surface area of this TIN between 0 and 1.5 feet in elevation was calculated using ArcGIS. A system-wide denitrification rate for the Newport River tidal freshwater riparian zone was calculated as:

$$DEN_{\text{riparian}} = X \times A \times \alpha \quad \text{equation 4}$$

Where  $X$  is the denitrification rate,  $A$  is the area between 0 and 1.5 feet (the expected extent of high tide inundation given the 3 foot tidal range), and  $\alpha$  is the proportion of the time sediments were observed to be in reducing condition.

## RESULTS

Denitrification rates ranged from  $233 \mu\text{g m}^{-2} \text{ hr}^{-1}$  (mud flat in September 2006) to  $4418 \mu\text{g m}^{-2} \text{ hr}^{-1}$  (riparian forest in October 2006) (Figure 11). There was a significant difference between habitat types during September and October 2006, with rates from the forest higher than rates at the mudflat. There was no significant difference between the average rates in habitats over the study period. Denitrification over the 10 months of study averaged  $2161 \mu\text{g m}^{-2} \text{ hr}^{-1}$ ,  $1832 \mu\text{g m}^{-2} \text{ hr}^{-1}$ , and  $1883 \mu\text{g m}^{-2} \text{ hr}^{-1}$  at the forest, marsh, and mud flat, respectively.

Temperature of experiments ranged from 9.7 to 25.5 °C (Table 6). Nutrient concentrations were generally low to moderate, ranging from 93-450  $\mu\text{g N-NO}_3 \text{ L}^{-1}$ , 12-58  $\mu\text{g N-NH}_4 \text{ L}^{-1}$ , and 9-33  $\mu\text{g P-PO}_4 \text{ L}^{-1}$  (Table 6). The percent of organic matter in the top 5 cm of sediment of incubated cores was highest in the forest (19%), followed by the marsh (11%), and mudflat (10%) (Table 6).

Three rain events occurred during the period of redox measurements: 13-15 February (7 cm), 25-26 February (2 cm), and 1-3 March (4 cm) (<http://www.nc-climate.ncsu.edu/cronos/>). The effect of these storms on soil hydrology is evident from the rise in the saturated soil depth after each event (Figure 12). Following the 25-26 February event the riparian forest appears continually saturated with overlying water for the remainder of the study period.

Soil moisture at 5 cm soil depth increased markedly in response to the first precipitation event on 13-15 February, initially a result of overland flow following precipitation and later enhanced by increased river stage (Figures 13-15). The soil moisture probe at 24 m failed during the 1-3 March rain event (Figure 15). Large fluctuations in redox at 5 cm 2 m and 24 m from the channel occurred following the 25-26 February and 1-3 March rain events (Figures 13 and 15). The highly oxidized conditions observed closest to the channel during late February and early March may indicate percolation of rainwater or river water through this lateral hyporheic zone.

Redox potentials at both 5 and 10 cm decreased with distance from the channel (Figure 13-15). Oxidizing conditions ( $E_h > 300$  mV) occurred 76% of the time at 5 cm depth nearest the channel, but were always moderately reduced at 10 cm depth nearest the channel (Figure 13). Redox at the other two distances from the channel was always reduced (Figures 14 and 15). Redox fluctuated inversely with soil moisture nearest the channel at 5 cm depth, indicating that soil pore water and the overlying water column were closely coupled (Figure 13). All other redox measurements corresponded slightly to changes in soil moisture at those sites. Sediments were more reduced at 10 cm than 5 cm except at 14 m where fluctuations in redox at 10 cm exceeded those at 5 cm in both magnitude and mV (Figure 14).

From Confluence (the upper limit of tidally-influenced flow) to Dock (the downstream extent of the TFZ) there were 822,122 m<sup>2</sup> of surface area between 0 and 1.5 feet above sea level. A TFZ-wide estimate of denitrification occurring in the Newport River during February and March was calculated using the average denitrification rate for February and March of 1869  $\mu\text{g m}^{-2} \text{hr}^{-1}$ . The coefficient  $\alpha$  in equation 4 ranged from 24% at the channel site to 100% at the sites farther from the channel. Implementing equation 4 with this range of values yield a total denitrification rates of 369 g N hr<sup>-1</sup> (102 mg N s<sup>-1</sup>) to 1537 g N hr<sup>-1</sup> (427 mg N s<sup>-1</sup>).

## DISCUSSION

The denitrification rates observed in three intertidal habitats of the Newport River TFZ are similar to rates observed in other coastal environments (Seitzinger et al. 2006). Denitrification rates in a constructed wetland near the Newport River ranged from 1-4 mg m<sup>-2</sup> hr<sup>-1</sup> (Poe et al. 2003). The rate in a tidal freshwater marsh in Belgium was 2.7 mg m<sup>-2</sup> hr<sup>-1</sup>; despite this high rate measurement, the study did not find a significant difference between this and other denitrification rates in TFZ wetland sediments than non-tidal wetlands (Verhoeven et al. 2001). It should be noted, however, that the acetylene block technique was used, and thus the experiment was not able to measure denitrification during redox cycling.

Changes in sediment redox and soil moisture over tidal cycles in TFZ soils have been observed elsewhere in a similar range of fluctuations (Seybold et al. 2002). The volumetric water content of the Newport River parafluvial soils were all above the 60-80% threshold identified as corresponding with the greatest denitrification rates in a wide variety of ecosystems (Machefert

and Dise 2004). Soil moisture fluctuated up to 7% in response to tidal water level changes, but was always in the range expected to produce maximum  $N_2$  versus  $N_2O$  emissions (Machefert and Dise 2004). Redox values below 100 mV, as observed at the two sites farthest from the river channel, may indicate depletion of  $NO_3$  for denitrification, and a switch to iron, sulfate, or  $CO_2$  reduction, likely a result of low hydraulic connectivity with the overlying water column. Porewater movement, facilitated by slight topographic gradients along the river channel, can promote oxygen transport and subsequent nitrification several centimeters into the sediment, while denitrification has been found to occur up to 12 cm deep in intertidal sediments (Kerner et al. 1990).

The logging system employed here allowed stabilization of the differential voltage for 3000 ms. This has been demonstrated to be too short of a time for stabilization of the voltage from platinum tipped electrodes in reduced conditions (Bochove et al. 2002). Ideally, a resistor should be added to the measurement circuit that would keep the circuit closed and allow stabilization of the signals. The lack of this apparatus with electrodes in reduced soils (0-200 mV) can lead to under estimation of the true redox potential by up to 140 mV (Bochove et al. 2002). Therefore, the redox potentials measured at 10 cm depth in the current study, and at 5 cm depth at the two farther distances from the channel, are probably under-representing redox. However, most measurements, if corrected by adding 140 mV, would still be in a reduced state during the time of this experiment.

The complexity of solute transport between the river channel and parafluvial zone is a major factor in defining the role of the floodplain in nitrogen retention. It is ultimately the source of nitrate being denitrified that will most affect riverine nutrient loads, and how completely the parafluvial zone is flushed of porewater nutrients over each tidal cycle. The rapid cycling between oxidized and reduced conditions in the upper 5 cm of sediment suggests that tightly coupled nitrification-denitrification pathway for nitrogen. An internal source of nitrate within the sediments supplementing the nitrate within the water column would likely result in much higher denitrification rates than were measured. Since the laboratory core experiments did not replicate the cyclical oxidized-reduced conditions observed in the field, the rate measurements made in this study are likely to underestimate actual rates in the field.

The simple scaling model employed here was intended to provide an order-of-magnitude approximation of how the denitrification rates here compared to riverine nutrient loads. The range in TFZ-wide denitrification flux (102-427  $mg\ s^{-1}$ ) was based upon the period of time that redox values showed reducing conditions during the month of in-situ measurement. Nitrate load at Old Highway 70 on 22 Feb 2007 was estimated to be 296  $mg\ s^{-1}$ . Even acknowledging the potential error around these extrapolations, the overlap in these values suggests that intertidal denitrification could have a tremendous affect on nitrate loads through the Newport River. From a management perspective it is worth noting that TFZ denitrification is similar in magnitude as the TN discharge load (289 – 467  $mg\ s^{-1}$ ) from the Newport WWTP. This suggests the importance of an intact floodplain and unaltered riparian zones to buffer anthropogenic nitrogen loads in coastal rivers.

Sediment redox is a valuable parameter for understanding biogeochemical processes occurring at the landscape scale. For example, riparian zone redox condition has been tested in the Rhine

River valley as a way to monitor when denitrifying conditions occur and cease following controlled inundation (Brettar et al. 2002). In an effort to maximize denitrification within the riparian zone of this system during flood release, inundation could be sustained until redox values fell below a threshold value. Further research on denitrification in TFZ floodplains would benefit from a redox sensor network deployed across a larger spatial extent than was monitored in the current study.

Floodplains of the southeastern U.S. have been cited as critical in the permanent retention of nitrogen via denitrification (Lowrance et al. 1984). Non-tidal river floodplains are inundated during annual cycles of river discharge and precipitation, thus limiting their functional significance on nitrogen retention to select periods of the year. In contrast, tidal river floodplains are inundated to differing degrees on semi-diurnal cycles, which makes them instrumental in nitrogen retention on a daily basis. The TFZ also lacks appreciable sulfate concentrations that inhibit denitrification (Brundet and Garcia-Gil 1996). The combination of large surface area, rapid redox cycling, high frequency inundation cycles, and a lack of sulfate make the TFZ a critical buffer for upstream nitrogen loads. This geomorphic feature of TFZ requires explicit consideration in modeling nutrient flux through coastal river networks.

Table 6. Water quality parameters and sediment organic matter composition during denitrification experiments.

Date	Habitat	Temperature (°C)	NO <sub>3</sub> (µg N L <sup>-1</sup> )	NH <sub>4</sub> (µg N L <sup>-1</sup> )	PO <sub>4</sub> (µg P L <sup>-1</sup> )	% organic matter
31 Aug 2006	marsh	25	369	45	33	10.8
	mudflat					10.8
	forest					-
14 Sep 2006	marsh	22	93	37	15	8.7
	mudflat					7.7
	forest					14.3
24 Oct 2006	marsh	15.5	220	21	16	8.1
	mudflat					6.7
	forest					15.9
21 Nov 2006	marsh	14	178	30	9	-
	mudflat					-
	forest					-
14 Dec 2006	marsh	9.7	175	28	12	14.9
	mudflat					9.8
	forest					19.8
18 Jan 2007	marsh	12.2	124	24	10	17.9
	mudflat					21.1
	forest					25.9
14 Feb 2007	marsh	10	144	58	24	10.6
	mudflat					10.4
	forest					32.4
7 Mar 2007	marsh	11.9	450	23	32	12.9
	mudflat					4.1
	forest					8
16 May 2007	marsh	19.8	161	12	9	7
	mudflat					8.6
	forest					15.4
31 Jun 2007	marsh	25.5	294	19	29	-
	mudflat					-
	forest					-

Figure 11. Denitrification rates measured in three intertidal habitats of the TFZ Newport River, NC. Letters indicate homogenous subsets of treatments during each month using an ANOVA and Tukey post-hoc analysis ( $p < 0.05$ ).

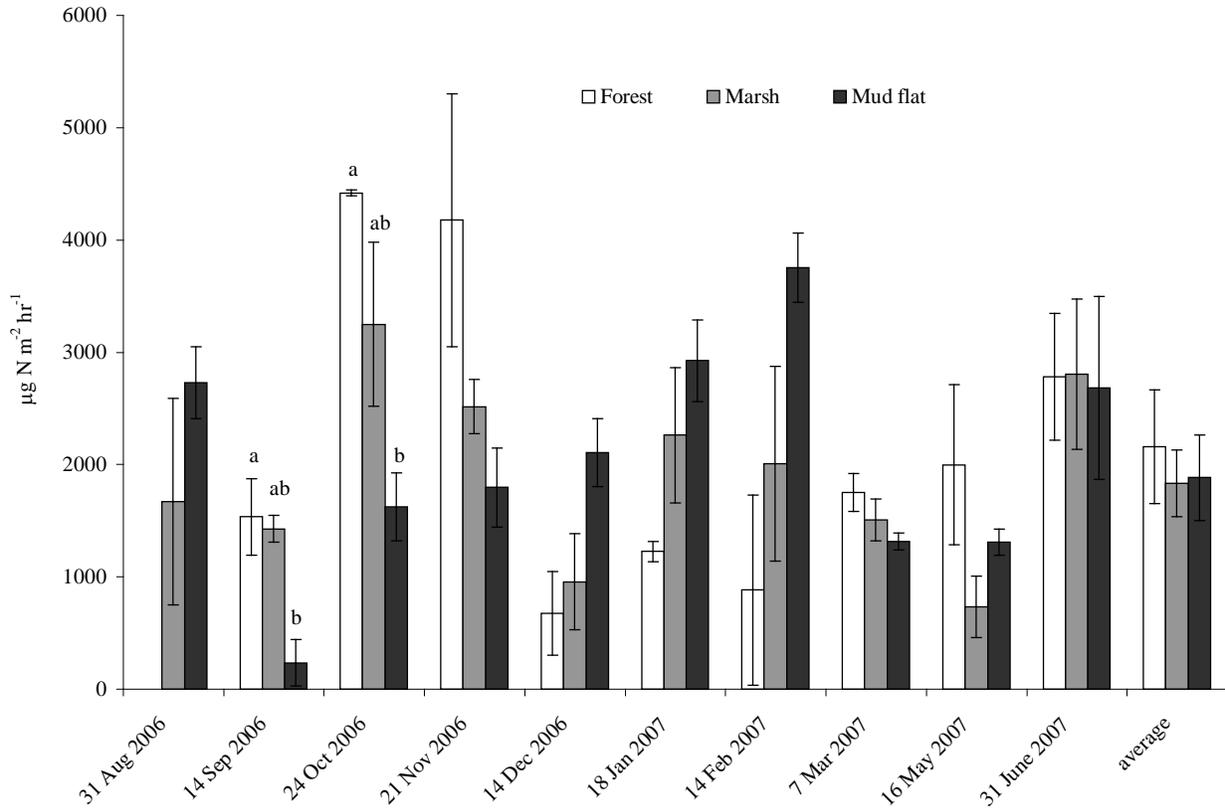


Figure 12. Ground water depth at 2 m and 24 m from the channel of the Newport River near Old Highway 70.

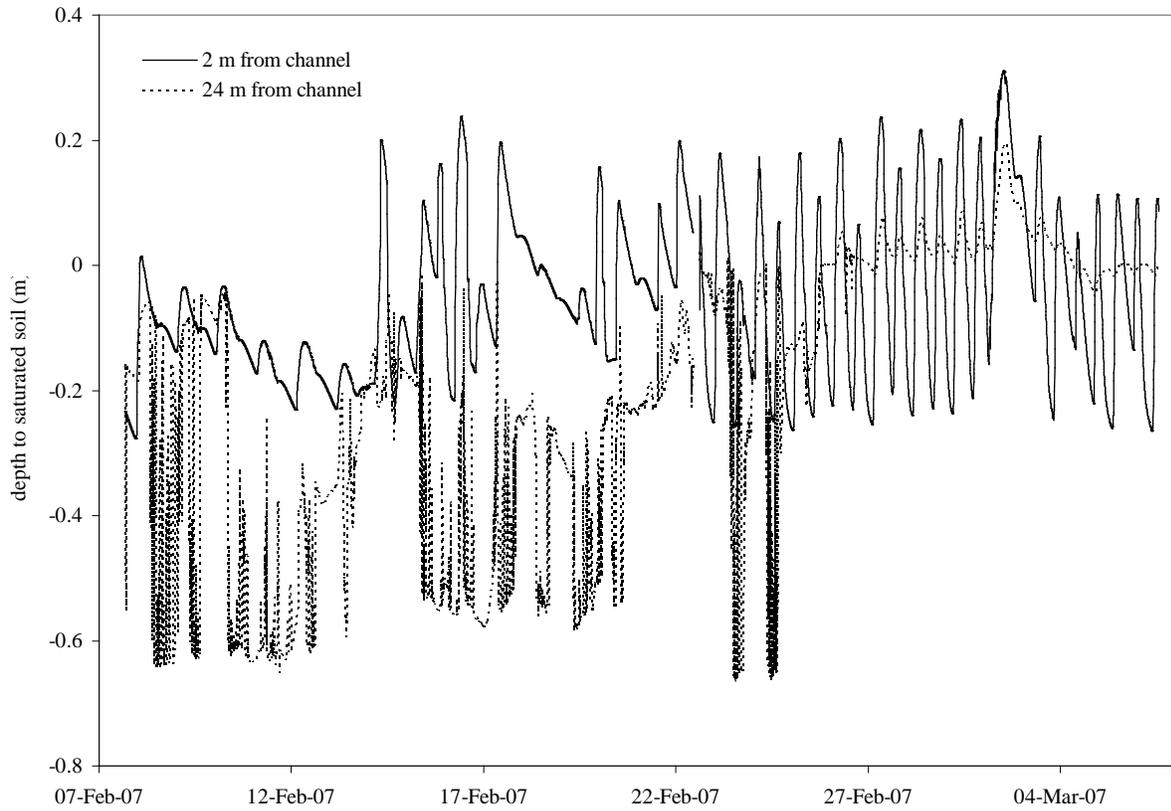


Figure 13. Redox potential and soil moisture 2 m from the channel of the Newport River near Old Highway 70.

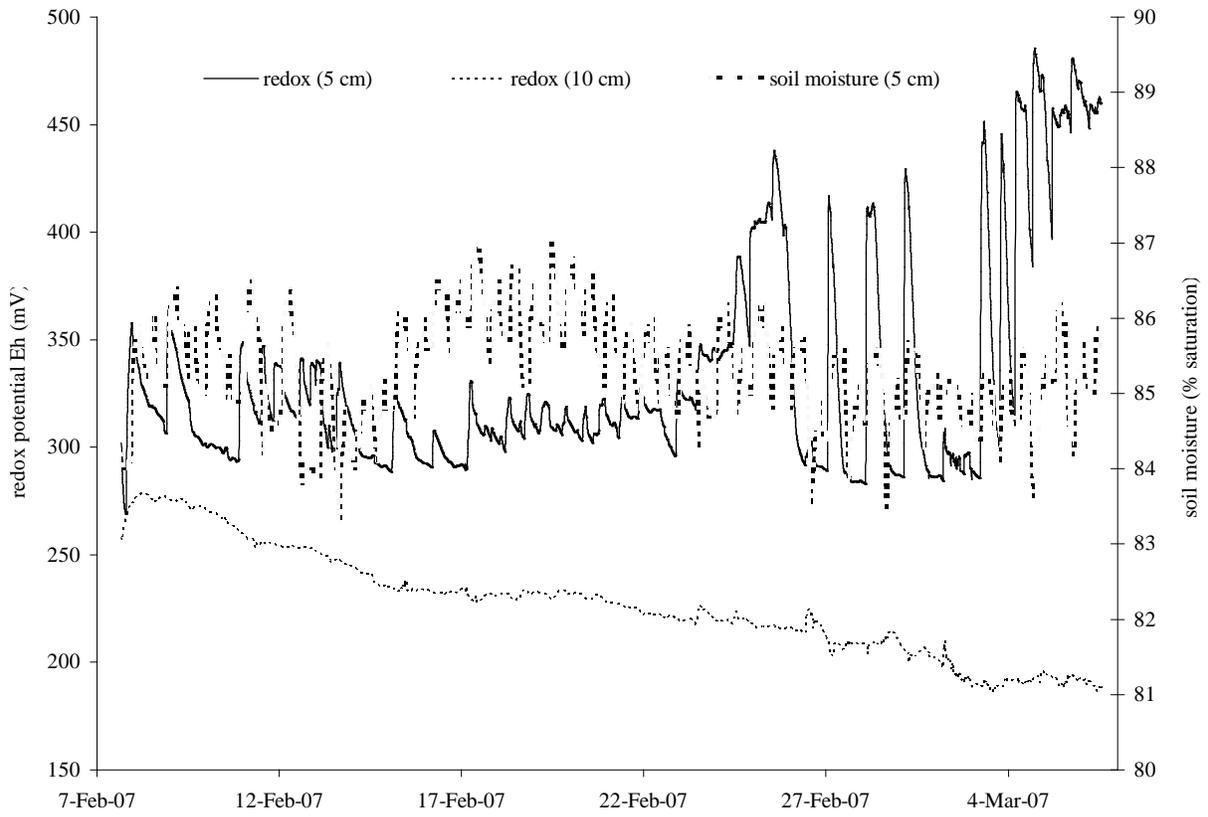


Figure 14. Redox potential and soil moisture 9 m from the channel of the Newport River near Old Highway 70.

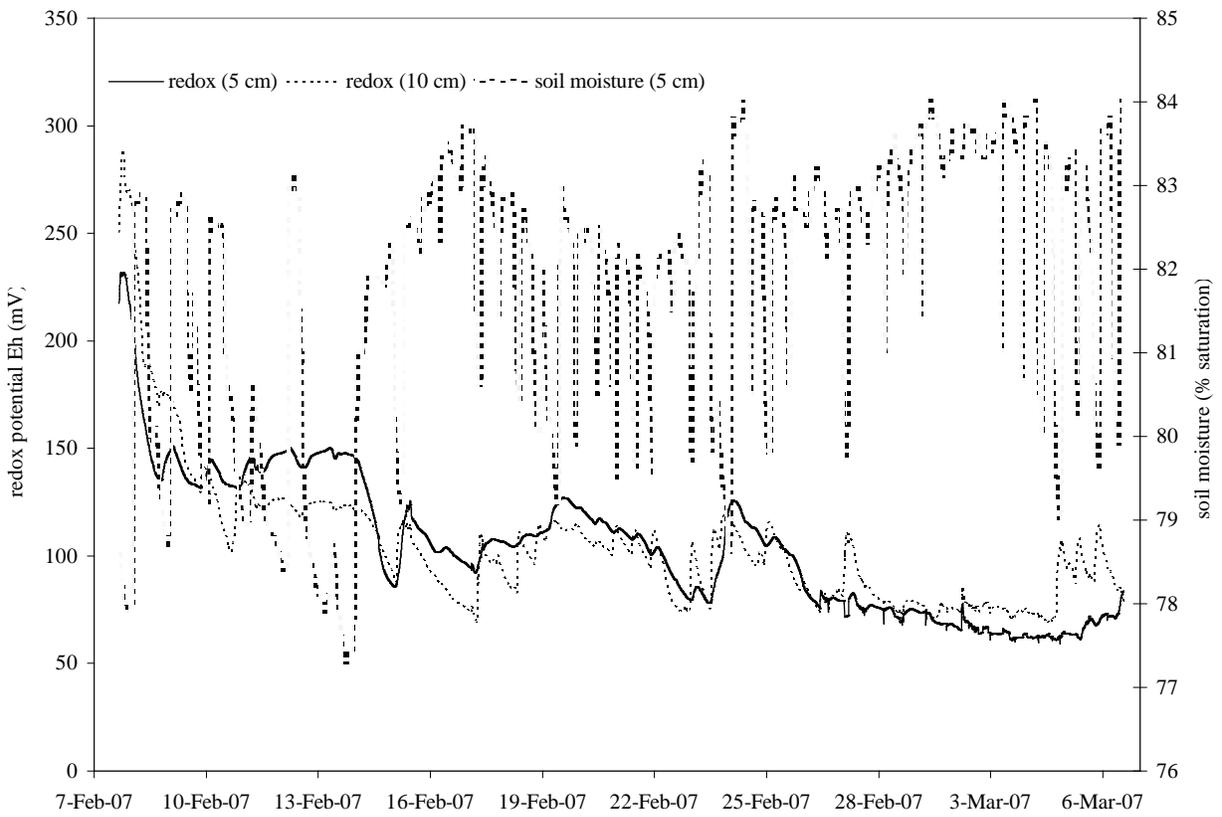
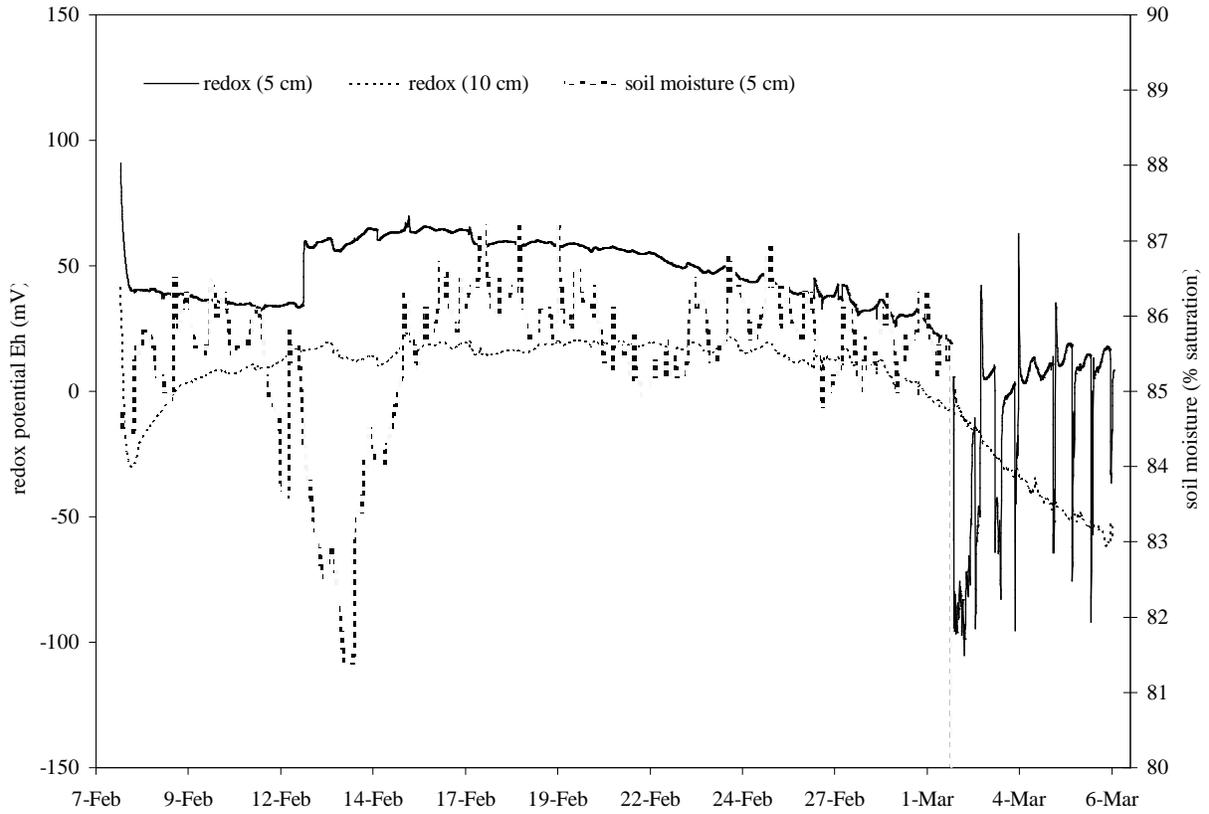


Figure 15. Redox potential and soil moisture 14 m from the channel of the Newport River near Old Highway 70.



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