

QUANTIFYING COASTAL STREAM METABOLISM ACROSS A GRADIENT OF HUMAN  
WATERSHED DEVELOPMENT

Caitlin Legene White

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Approved by:

Michael F. Piehler

Jaye E. Cable

Scott H. Ensign

Brent A. McKee

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## ABSTRACT

Caitlin Legene White: Quantifying Coastal Stream Metabolism across a Gradient of Human Watershed Development  
(Under the direction of Michael F. Piehler)

Coastal headwater streams are important conduits and processors of terrestrially derived materials, however they remain understudied compared to upland streams. In particular, little is known about the impact of watershed development on coastal stream metabolism although there are likely important implications for management decisions. Whole-stream metabolism was measured seasonally between July 2013 and April 2014 using single-and two-station methods in four streams of the New River Estuary, North Carolina, which represented a gradient in watershed development. Streams were heterotrophic year-round and net daily metabolism ranged from  $-58.2$  to  $-1.9 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . However, possible effects of watershed development including increased nitrogen concentrations, decreased dissolved organic carbon and chromophoric dissolved organic matter, and increased particulate organic carbon were correlated with decreased heterotrophy in impacted streams. These results provide a conceptual model of coastal streams in human dominated watersheds and will offer context for management at the scale of watershed decision-making.

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## LIST OF ABBREVIATIONS

CDOM	chromophoric dissolved organic matter
chl- <i>a</i>	chlorophyll- <i>a</i> (water column or sediment)
CHS	coastal headwater streams
COG-3	Cogdels Creek (3 <sup>rd</sup> most developed watershed)
DO	dissolved oxygen
DOC	dissolved organic carbon
ER	ecosystem respiration
FRN-1	French Creek (least developed watershed)
GPP	gross primary production
MCBCL	Marine Corps Base Camp Lejeune
NewRE	New River Estuary
NDM	net daily metabolism
NH <sub>4</sub> <sup>+</sup>	ammonium
NO <sub>x</sub>	nitrate + nitrite
PAR	photosynthetically active radiation
PO <sub>4</sub> <sup>3-</sup>	phosphate
POC	particulate organic carbon
S <sub>R</sub>	slope ratio: ratio of optical spectral slopes (S <sub>275-295</sub> : S <sub>350-400</sub> )

SUVA254	specific ultraviolet absorption at 254 nm
TAR-4	Tarawa Terrace Creek (4 <sup>th</sup> most developed watershed)
TDN	total dissolved nitrogen
TRP-2	Traps Creek (2 <sup>nd</sup> most developed watershed)
TSS	total suspended solids

## INTRODUCTION

Gross primary production (GPP) and ecosystem respiration (ER) are regulators of biogeochemical cycling in aquatic environments. The balance between GPP and ER is net daily metabolism (NDM). Metabolism in streams is a fundamental indicator of integrative ecosystem function that is sensitive to various stressors, including physical, chemical, and biological forcings, and can be measured discretely or continuously. Most other methods for assessing the ecological status of streams focus on structural characteristics such as water quality and community composition, while routine methods that assess stream function are scarce (Izagirre *et al.*, 2008). Measurements of metabolism in streams are sometimes made using experimental chambers, however it is difficult to incorporate realistic flow conditions and habitat complexity into chamber experiments (Bott, 2006; Marzolf *et al.*, 1994; Mulholland *et al.*, 2001). Therefore, open-system methods are the preferred metabolism approach for streams.

Whole-stream metabolism is an *in situ* method for measuring metabolism in flowing waters, which is based on the premise that the change in dissolved oxygen (DO) concentration can be attributed to photosynthesis, respiration, and gas exchange with the atmosphere (Odum, 1956). Stream measurements are good indicators not only of stream health, but also watershed-scale ecosystem health due to the integrating effect of runoff from the land entering as stream flow (Williamson *et al.*, 2008; Mulholland *et al.*, 2005). However, integrative effects from watershed development have been primarily studied in upland streams and, despite the fact that

the majority of the U.S. population lives within coastal watersheds (Beach, 2002), little is known about these effects on coastal streams.

Coastal headwater streams (CHS) connect terrestrial and estuarine ecosystems, however they remain understudied compared to their inland counterparts. These streams are the primary receiving waters for landscape derived runoff and associated materials and are conduits for and processors of nutrients and carbon. Flat topography of the coastal plain promotes material processing through increased residence times, which enables prolonged contact time between the water column and benthos (Alexander *et al.*, 2007). Because of their close proximity to dense human populations, CHS are susceptible to changes in watershed development, which can alter tributary loading and modify the biogeochemistry of streams and the greater estuarine ecosystem. CHS are often subject to loss of riparian habitat, channelization, ditching, and enhanced storm water flow that cause higher than normal material delivery from the land and reduce residence time (Corbett *et al.*, 1997; Kuenzler *et al.*, 1977; Shankman & Pugh, 1992). Although circumstantial evidence connects watershed development with changes in CHS metabolism, there are no previously published attempts to quantify these changes. Therefore, measurements of CHS whole-stream metabolism performed within the context of watershed development would advance understanding of how watershed development impacts stream structure and function in coastal regions.

The fuel for ER in streams is carbon, which can vary in quantity and quality (particulate versus dissolved, labile versus recalcitrant). Historically, CHS are blackwater with high concentrations of chromophoric dissolved organic matter (CDOM) and dissolved organic

carbon (DOC), both of which are derived from rain and groundwater that percolates through extensive adjacent floodplains and forested wetlands (Wainright *et al.*, 1992). These characteristics are unusual for upland headwater streams, but are characteristic of many streams and rivers in the coastal plain due to low topographic slope that allows for slow percolation of overland flow and overbanking to occur (Meyer, 1992; Meyer, 1990). Streams have been demonstrated to be efficient processors of allochthonous organic matter inputs, such as detrital matter and leaf litter, and are dependent on these inputs (Mulholland, 1981). Typically, in blackwater systems, primary production within the water column is not sufficient to support respiratory demands, suggesting the importance of allochthonous carbon sources (Edwards & Meyer, 1987). The quality and quantity of carbon is directly affected by land use and development (Findlay *et al.*, 2001; Wahl *et al.*, 1997) and optical properties (i.e. CDOM) may be altered, both of which have implications for stream metabolism.

Anthropogenic land use change can affect in-stream processes. Watershed development alters water quality, in part, by changing the composition and availability of materials (e.g. nutrients, carbon, sediment, fecal material) on land that can be transported to streams (Paul & Meyer, 2001; Mallin *et al.*, 2001a; Mallin *et al.*, 2001b). Headwater streams have been documented to respond to development in an array of chemical, physical, and biological factors associated with increases in population density and associated increases in percent of development and impervious cover within their watersheds (Beach, 2002). Land development can increase sources of nitrogen and phosphorous (Beaulac & Reckhow, 1982; Valiela *et al.*, 1992), deforestation and construction releases sediments that are transported to streams during rain events (Houser *et al.*, 2005), and fecal coliform bacteria has been positively related to

coastal development in tidal creeks (Holland *et al.*, 2004; Stumpf *et al.*, 2010; Mallin *et al.*, 2001*b*). Transitioning from a pristine ecosystem to a developed landscape increases the amount of impervious surface cover associated with residential, commercial and industrial land uses. Development can alter stream discharge regimes because impervious cover inhibits percolation of rainwater to groundwater recharge and diverts it directly to streams, creating periods of increased peak storm flows (Leopold, 1968) of diminished duration (Seaburn, 1969), which in turn may lead to decreased base flows (Barringer *et al.*, 1994). Wahl *et al.* (1997) documented specific impacts on coastal, blackwater streams of South Carolina including increased nutrient and decreased DOC loads and Corbett *et al.* (1997) noted increased storm flows and sediment loads in the same South Carolina streams. However, most previous work has been performed on upland systems, whereas little additional information is available for CHS.

It is vital to understand how coastal increasing population density and land alteration in coastal regions affect water quality due to the close proximity to downstream coastal aquatic habitats and potential for material processing in CHS. Therefore, feedbacks between material loading, metabolism, and carbon processing should be considered to fully understand the impacts of land use on CHS ecosystems and are essential for accurate projections of future estuarine function. There have been no previously published assessments of CHS metabolism, and therefore the degree to which human development affects stream metabolism is not known. Additionally, little information is available on the effects of watershed development on carbon delivery – quality and quantity – to CHS, as well as other potential drivers (nutrient and CDOM concentration) of stream structure and function.

The objectives of this study were to:

1. Measure stream metabolism in CHS during all four seasons.
2. Determine the degree to which human development affects metabolism.
3. Assess potential drivers of change in metabolism, including nutrients, carbon, and optical properties.
4. Provide a conceptual model of CHS in human dominated coastal watersheds.

To answer these questions, whole stream metabolism of four CHS of the New River Estuary, NC (NewRE) at Marine Corps Base Camp Lejeune (MCBCL) was measured during four seasons in combination with continuous monitoring of water quality parameters, carbon, and CDOM concentrations. Military reservations present an opportunity to study the effect of human activities on ecological processes in areas where land use can vary widely within a small geographic area. Stream watersheds selected for study represented a development gradient from 6% to 67% and had impervious cover that ranged from 1% to 23% of the watershed area. Whole stream metabolism was determined by monitoring dissolved oxygen concentration using single- and two-station methods and correcting for reaeration using direct measurement and empirical estimation of the oxygen reaeration coefficient ( $k$ ), an integral element of metabolism calculations. This research will assess a number of understudied ecological aspects of pristine and developed CHS.

## MATERIALS AND METHODS

### *Site Descriptions*

Stream metabolism and carbon processing were studied in four CHS of the NewRE, located within the coastal plain of North Carolina (Fig. 1). The sub-watersheds of these streams are encompassed by Marine Corps Base Camp Lejeune (MCBCL) and represent a gradient in land uses, ranging from 1% to 23% impervious cover. Land use within MCBCL includes residential areas, barracks, industrial parks, and impact zones. Relevant watershed development characteristics of the streams addressed in this study are listed in Table 1. The range in development and close proximity of these streams allowed for comparison of primarily un-impacted (FRN-1) to highly impacted (TAR-4) watersheds within a less than 600km<sup>2</sup> geographic



Figure 1. Location of Camp Lejeune Marine Corps Base (shaded green) in eastern North Carolina. Stream study sites within Camp Lejeune indicated by red dots in right-hand figure.



area, and enabled evaluation of across-site differences that were not complicated by disparities in temperature or precipitation patterns. Schwartz (2010) characterized additional information on stream watershed land use patterns in the NewRE.

Table 1. List of stream sites in order from least to most developed. Includes stream name (abbreviation), latitude and longitude, watershed development characteristics (percent development, impervious, and forested area), total area of the sub-watershed, stream length, and yearly average stream discharge between May 2013 – April 2014. 2001 National Land Cover Dataset (M. Brush).

<b>Stream Property</b>	<b>French (FRN-1)</b>	<b>Traps (TRP-2)</b>	<b>Cogdels (COG-3)</b>	<b>Tarawa (TAR-4)</b>
Location	34°38'10.7" N, 77°18'03.1' W	34°34'54.4" N, 77°19'59.2" W	34°39'18.4" N, 77°20'03.4" W	34°44'17.3" N, 77°22'54.7" W
% Development	5.7	29.5	34.2	66.8
% Impervious	1.06	4.13	13.79	23.2
% Forested	9.94	11.29	33.6	17.59
Watershed Area (ha)	807.3	51.0	835.8	139.1
Mean Slope (%)	7.4	7.2	9.4	7.3
Average Q (m <sup>3</sup> s <sup>-1</sup> )	0.082	0.006	0.112	0.0127

North Carolina CHS originate within the coastal plain, which has characteristically low elevation and shallow slopes. Therefore, these streams are low flow and can even be intermittent during portions of the year – particularly in summer when evaporation rates are high. Stream discharge of the four streams ranged from -0.12 to 1.19 m<sup>3</sup> s<sup>-1</sup> at the four streams sites – negative discharge values are attributed to tidal influence at FRN-1. All streams were freshwater and, with the exception of FRN-1, non-tidal.

Due to the low slope of the surrounding upland, many North Carolina CHS have broad floodplains that introduce humic acids and tannins, causing them – historically – to be blackwater. As water percolates through the surrounding soils or runs over floodplains, it picks up CDOM giving the stream water a black or brown appearance. The least developed study

stream, FRN-1, had a classic blackwater appearance whereas COG-3 and TAR-4 did not.

Floodplains are also a large source of DOC to CHS, meaning that CHS may be important for processing of watershed carbon and act as direct conduits for carbon and material loading to estuaries. Low stream slopes and low flows have profound implications for material processing along stream reaches, as well, allowing for increased contact time between stream benthos and water column materials.

### *Stream Monitoring & Water Quality Analysis*

All streams were equipped with a Teledyne ISCO automated water sampler (Model 6712), ultrasonic Doppler velocimeter and pressure transducer (ISCO 750 Area-Velocity Module), and YSI Inc. data sonde (Model 600XL). Streams were monitored continuously for water quality parameters and discharge from May 2013 through April 2014. Velocity, water level, temperature, conductivity, and DO were recorded at 30-minute intervals. YSI sonde DO and conductivity calibration was checked once every two weeks. Biweekly base-flow grab samples were taken from each stream and analyzed for water column chl-*a*, nutrients, carbon, and suspended sediments. ISCO samplers were programmed to perform targeted storm sampling when water velocity exceeded a threshold and were triggered to sample at flow-paced intervals once enabled. These automated grab samples were collected promptly after a storm event and returned to the lab for processing. Storm samples were selected to encompass periods before (base-flow), rising, peak, and falling limbs of the storm hydrograph at each site. At least one storm was characterized per month.

Base-flow grab samples and storm flow ISCO samples were analyzed for Nitrate+Nitrite (referred to as  $\text{NO}_x$ ), Ammonium ( $\text{NH}_4^+$ ), Phosphate ( $\text{PO}_4^{3-}$ ), total dissolved nitrogen (TDN), chlorophyll-*a* (chl-*a*), dissolved organic carbon (DOC), particulate organic carbon (POC), and chromophoric dissolved organic matter (CDOM). Water samples were filtered through Whatman GF/F filters (25mm diameter, 0.7  $\mu\text{m}$  nominal pore size) and frozen for later analysis. The filtrate was analyzed using a Lachat Quick-Chem 8000 automated ion analyzer for  $\text{NO}_x$ ,  $\text{NH}_4^+$ , and  $\text{PO}_4^{3-}$  concentrations using standard protocols (Lachat Instruments, Milwaukee, WI, USA:  $\text{NO}_2^-/\text{NO}_3^-$  Method 31-107-04-1-A,  $\text{NH}_4$  Method 31-107-06-1-A and  $\text{PO}_4^{3-}$ -P Method 31-115-01-3-G). Filters were stored in aluminum foil and frozen for subsequent water column chl-*a* analysis. Chl-*a* samples were extracted in 90% acetone at 0°C for 18 hours following 5 minutes of sonication. The extracted samples were then analyzed by fluorometry (Turner Designs Trilogy Model #7200-000). Samples for carbon analysis were filtered through 25mm Whatman GF/F filters pre-combusted at 525°C for 4 hours. Filters were frozen for subsequent particulate carbon and nitrogen analysis using a Perkin Elmer CHN analyzer (Model 2400 Series II) standardized with acetanilide. Filtrate was frozen and retained for DOC analysis. DOC concentrations were measured by high temperature combustion using Shimadzu TOC-5000 carbon analyzer calibrated with potassium biphthalate. All DOC data presented are the mean of three to five replicate injections with a <2% coefficient of variation. Remaining filtrate was retained for CDOM measurements, which were performed the day after sample collection. UV-Vis absorption spectra were obtained on a Shimadzu UV-VIS spectrophotometer (Mini 1240) using distilled water as a blank. Scans were run at room temperature between 250-800 nm in a 1 cm quartz cuvette. Samples were corrected for background noise associated with baseline drift, scattering, and refractive effects by subtracting average absorbance of deionized water between

from the spectrum between 250 – 700 nm (Green & Blough, 1994). Napierian absorption coefficients were calculated from corrected absorbance values using the equation,  $a_\lambda = 2.303 * A_\lambda / l$ , where  $a_\lambda$  is the absorption coefficient at wavelength  $\lambda$ ,  $A_\lambda$  is the absorbance at wavelength  $\lambda$ , and  $l$  is the path length of the cuvette in meters. Absorbance at 355 nm ( $a_{355}$ ) was used as an estimate of CDOM concentration. Other proxies were determined from optical scans in accordance with those performed by Leech *et al.* (In prep.).

Stream sediment chl-*a* was characterized once per month from each stream from December 2013 through August 2014. Sediments were collected for chl-*a* analysis from each stream at the upper, middle, and lower portions of the study reach utilized for the two-station whole-stream metabolism experiments. A 5cc syringe inserted to a depth of 1cm was used to obtain a small core of surface sediments, which was placed in a 15mL polystyrene test tube. Samples were transported back to the lab in a cooler on ice, wrapped in foil, and frozen until analysis. Sediment chl-*a* samples were extracted in 10mL of acetone:methanol:DI (45:45:10) at -20°C for 18 hours following 30 seconds of sonication. The extracted samples were then filtered through 25mm Whatman GF/F filters and analyzed using a Shimadzu UV-VIS spectrophotometer (Mini 1240). Samples were acidified with 100  $\mu$ L of 10% HCl and re-run on the spectrophotometer to correct for pheopigment concentrations (Lorenzen, 1967).

### *Whole-Stream Metabolism*

In order to determine the effect of land use on stream GPP and ER and implications for in-stream carbon processing, measurements of whole-stream metabolism were performed at each

of the four study streams. This occurred in conjunction with on-going water quality monitoring. RIVERMET<sup>®</sup>, an excel-based software, was used to calculate stream metabolism, using the single station method (Odum, 1956), from the diel DO data obtained through stream monitoring for a one-year period from May 2013 through April 2014 (Izagirre et al., 2007). Results from one-station calculations were grouped based on season – summer months were considered to be June, July, and August; fall months were September, October, and November; winter was December, January, and February; and spring was considered March, April, and May. Additionally, more focused measurements of metabolic rates were obtained using a two-station diel mass-balance DO approach and direct measurement of oxygen reaeration via tracer experiments (Marzolf et al., 1994). Two-station metabolism studies were performed at each of the four study streams during base-flow once per season from July 2013 through April 2014. Summer two-station experiments were performed in July and August 2013. Fall deployments occurred during September, October, and November 2013. Winter and spring experiments were performed during January 2014 and April 2014, respectively. Rates of GPP are expressed as positive values because this process produces oxygen and rates of ER as negative values because oxygen is consumed.

Data obtained from the single-station monitoring method at each stream was initially processed to remove periods when sondes were out of calibration or malfunctioned. Continuous records of DO, temperature, and discharge for each stream were then imported into the RIVERMET<sup>®</sup> software in order to perform calculations for GPP, ER, and NDM. Reaeration rate is a necessary component of stream metabolism calculations when utilizing an open-channel single or two-station method. Within the software, the Hornberger and Kelly (1975) method of

calculating the reaeration coefficient ( $k_{oxygen}$ ) was employed. This method analyzes the nighttime drop in oxygen concentration, which is driven primarily by respiration given that photosynthesis ceases from sunset to sunrise. Therefore, regressions of the nighttime decrease in oxygen concentration per unit time versus the oxygen saturation deficit are determined and from that linear relationship,  $k_{oxygen}$  is the slope and ER is the y-intercept. Within the software, the significance of the nighttime regression is determined. Days for which the statistical significance of this regression was less than 95% were excluded from analysis of one-station results. RIVERMET© results were also processed before final analysis to eliminate dates for which the GPP was incorrectly calculated as negative.

The two-station whole stream metabolism method utilized YSI 6600 sondes deployed at upstream and downstream stations established during summer 2013, which were 20 to 50m apart, depending on stream velocity along the study reach. Reach lengths at FRN-1, TRP-2, COG-3, and TAR-4 were 30, 20, 50, and 29m, respectively. Data sondes recorded temperature, conductivity, and DO at five-minute intervals and were deployed in each stream for at least 48 hours. Photosynthetically Active Radiation (PAR) was recorded over the same interval during two-station metabolism experiments at each site using a LI-COR  $2\pi$  light sensor (model: LI-1400 datalogger, Lincoln, NE, USA). Measured upstream-downstream changes in DO were corrected for reaeration flux using a reaeration coefficient determined empirically from tracer experiments performed to directly measure gas flux into and out of the stream along the study reach during each sonde deployment.

The reaeration coefficient,  $k_{oxygen}$  ( $\text{min}^{-1}$ ), was determined following the method of Genereux and Hemond (1992), which used simultaneous injection of a conservative solute tracer ( $\text{Cl}^-$  added as a NaCl solution) via a Mariotte bottle and a volatile gas tracer, argon (Ar), upstream of the study reach. A floating piece of plexiglass was placed over the gas injection site to increase diffusion of tracer gas into the stream. Conductivity was monitored on the upstream and downstream sondes using YSI 650MDS handheld data loggers to determine when tracer concentrations reached a plateau. Five replicate water samples for dissolved Ar analysis were taken at both stations over a 15–20 minute period during the plateau from the stream thalweg. Samples, collected in ground glass vials, were placed on ice and returned to the lab where they were stored submerged in water at 4°C until gas analysis was performed (typically within one week). Dissolved Ar gas samples were analyzed using Membrane Inlet Mass Spectrometry by means of a Balzers Prisma QME 200 quadrupole mass spectrometer (Pfeiffer Vacuum, Nashua, NH, USA).  $k_{argon}$  was determined using the equation  $k_{argon} (\text{min}^{-1}) = \tau^{-1} \times \ln(G_1 \times C_2 / G_2 \times C_1)$  where  $\tau$  is the stream water travel time between sites in minutes,  $G$  is the plateau concentration of Ar at upstream (1) and downstream (2) sites, and  $C$  is the plateau concentration of  $\text{Cl}^-$  at upstream (1) and downstream (2) sites.  $k_{oxygen}$  is proportional to  $k_{argon}$  (equal to  $0.89 \times k_{argon}$ ) and was used to calculate the actual instantaneous oxygen flux into or out of the stream. Oxygen reaeration flux was corrected for changes in stream temperature, but otherwise assumed to be constant during the course of a particular deployment.

In a few instances, direct measurement of the reaeration coefficient was not possible due to a rain event that interrupted the deployment or because upstream-downstream changes in argon concentration were too small to be accurately measured. In these cases,  $k_{oxygen}$  was

estimated using regressions of  $k_{oxygen}$  with stream velocity, discharge, and water depth. Regression equations for each site were used to predict  $k_{oxygen}$  based on velocity, discharge, and water depth measured during the particular deployment for which direct measurement failed. Predicted reaeration coefficients from the three regression equations were averaged to obtain a final estimated  $k_{oxygen}$ . This reaeration estimation method was used for TAR-4 spring, TRP-2 spring, FRN-1 fall, and FRN-1 spring deployments.

Two-station metabolism calculations were performed following computations for the upstream-downstream technique outlined by Bott (2006). Total daily ER was calculated as the average of hourly predawn and post-sunset respiration rates multiplied by 24. GPP was calculated as the sum of the absolute value of the hourly rates of net oxygen change during the photoperiod and the photoperiod respiration rate. Finally, NDM was calculated by subtracting the absolute value of ER from GPP.

### *Statistical Analysis*

All statistical analyses were performed using R (R Development Core Team 2011). Linear regressions were used to test for relationships between percent development or percent imperviousness and various water quality and metabolism parameters. Arcsine square root transformations were performed on percent development and percent imperviousness proportions, however the transformation did not consistently improve linear regressions and was not used. Linear regressions were also used to test for relationships between metabolism and water quality parameters. For all regressions, statistical significance was considered  $\alpha < 0.05$ .



## RESULTS

### *Discharge*

Monitoring of stream velocity and level resulted in continuous records of discharge from each stream and rain gauge data from MCBCL recorded daily rainfall (Fig. 2). Discharge thirty-minute intervals were averaged to obtain daily average stream discharge at each site from May 2013 through April 2014 (Fig. 3). The maximum daily discharge during the study period was  $0.70 \text{ m}^3 \text{ s}^{-1}$  at COG-3, which had minimum and average daily discharges of  $0.005$  and  $0.112 \text{ m}^3 \text{ s}^{-1}$ , respectively (Fig. 3C). Lowest observed daily discharge occurred at TRP-2 ( $< 0.001 \text{ m}^3 \text{ s}^{-1}$ ), which had an average of  $0.006 \text{ m}^3 \text{ s}^{-1}$  and maximum of  $0.092 \text{ m}^3 \text{ s}^{-1}$  (Fig. 3B). Low base discharge was also observed at TAR-4, which averaged  $0.0127 \text{ m}^3 \text{ s}^{-1}$  during the study period and ranged from  $0.002$  to  $0.168 \text{ m}^3 \text{ s}^{-1}$  (Fig. 3D). Average discharge at FRN-1 was  $0.082 \text{ m}^3 \text{ s}^{-1}$ , with minimum and maximum daily discharges of  $0.029$  and  $0.187 \text{ m}^3 \text{ s}^{-1}$ , respectively (Fig. 3A). FRN-1 was the only tidally influenced stream, though this impact was minimal and primarily evident in water level records during low-flow periods.

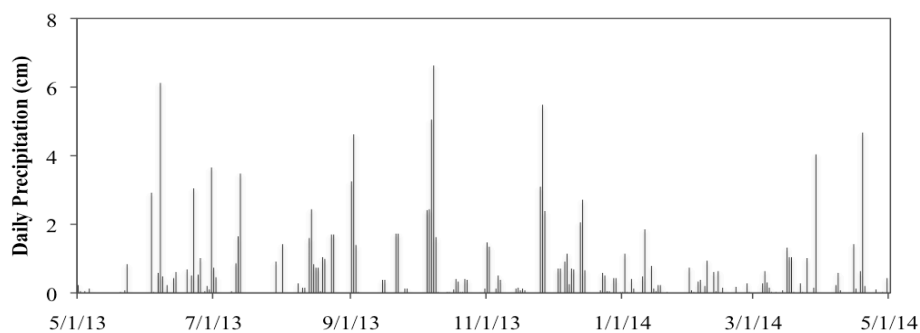


Figure 2. Total daily precipitation in centimeters at MCBCL from May 2013 through April 2014.

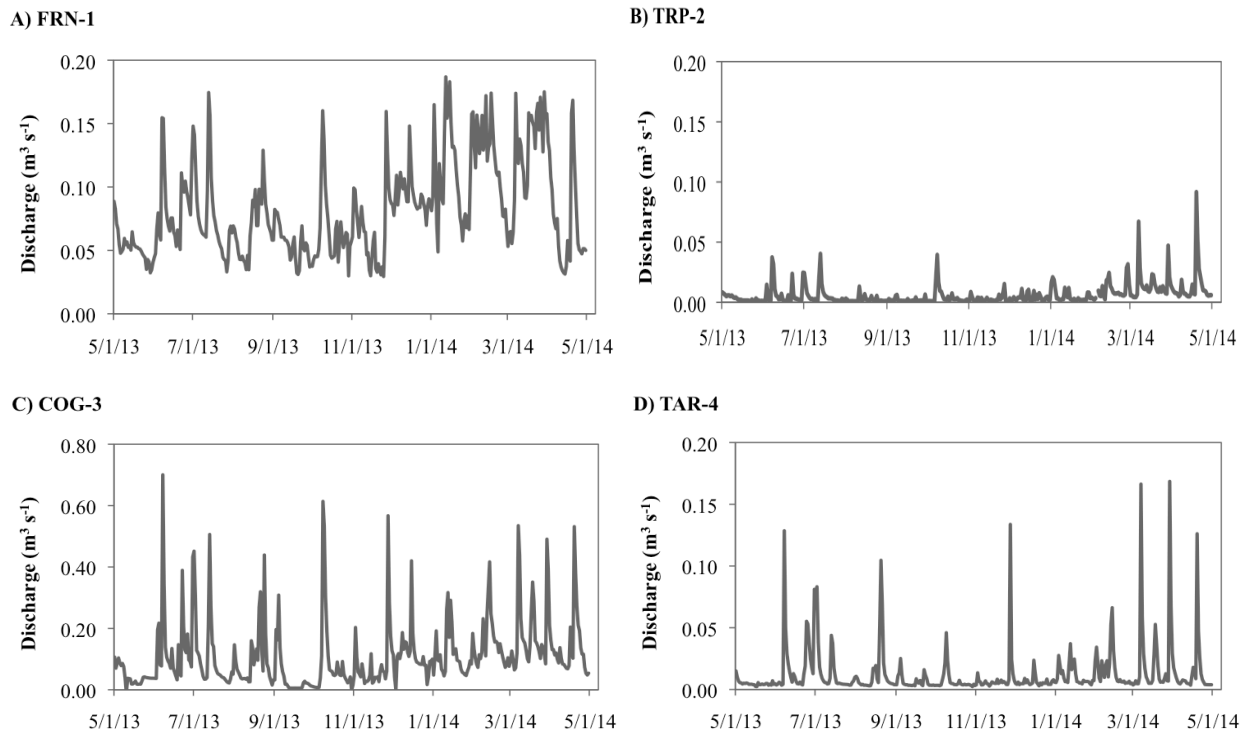


Figure 3. Daily average discharge records from May 2013 through April 2014 at French (A), Traps (B), Cogdels (C), and Tarawa (D). Streams presented in order from least to most developed. Note difference in scale of x-axis for Cogdels (C).

### *Stream Dissolved Oxygen*

Initial continuous monitoring of spring 2013 stream DO concentration revealed differences in diel patterns across sites. Streams in more impacted watersheds exhibited large diel excursions in DO (up to  $5 \text{ mg L}^{-1}$  change daily), whereas less impacted streams had less pronounced diel DO changes ( $< 0.5 \text{ mg L}^{-1}$ ) (Fig. 4). Rain events – indicated by peaks in discharge – appeared to disrupt or “reset” the DO cycle in more developed streams and had little impact on DO concentration or patterns in the least developed stream, FRN-1. In addition, FRN-1 had almost no discernable pattern in diel DO (Fig. 4A). TRP-2 had small, but measureable diel DO differences of less than  $1 \text{ mg L}^{-1}$  and storm events caused a peak in stream DO concentrations (Fig. 4B). The more developed streams, COG-3 and TAR-4, exhibited more

pronounced diel DO curves (Fig. 4C and D). COG-3 had daily DO excursions of 2 to 3 mg L<sup>-1</sup> and TAR-4 had the largest excursions (3 to 5 mg L<sup>-1</sup>). Rain events did not necessarily cause an overall peak in the DO concentration of the more developed streams, but seemed to raise the nighttime DO concentration and create a disruption in the diel cycle. The diel pattern was then reestablished over the following several days. This trend was particularly evident at TAR-4 (Fig. 4D).

The differences observed in diel DO excursions at the four stream sites suggested a difference in the magnitude of primary production and respiration, but the distinctions seemed to be confined to spring. Similar patterns were not observed during summer, fall, or winter. All streams were hypoxic (DO concentrations less than 2 mg L<sup>-1</sup>) during summer and fall; with the exception of FRN-1, which maintained higher DO concentrations (4 to 8 mg L<sup>-1</sup>) throughout the year. Streams with the lowest flows, TAR-4 and TRP-2, were particularly susceptible to hypoxic conditions. Winter DO concentrations and an variation observed during summer and fall at the four streams during June 2013 through February 2014 was caused by fluctuations in temperature, which alter gas solubility in water, or rain events that caused short term peaks in stream DO. As expected, diel DO patterns seen in April 2013 were observed again in spring 2014.

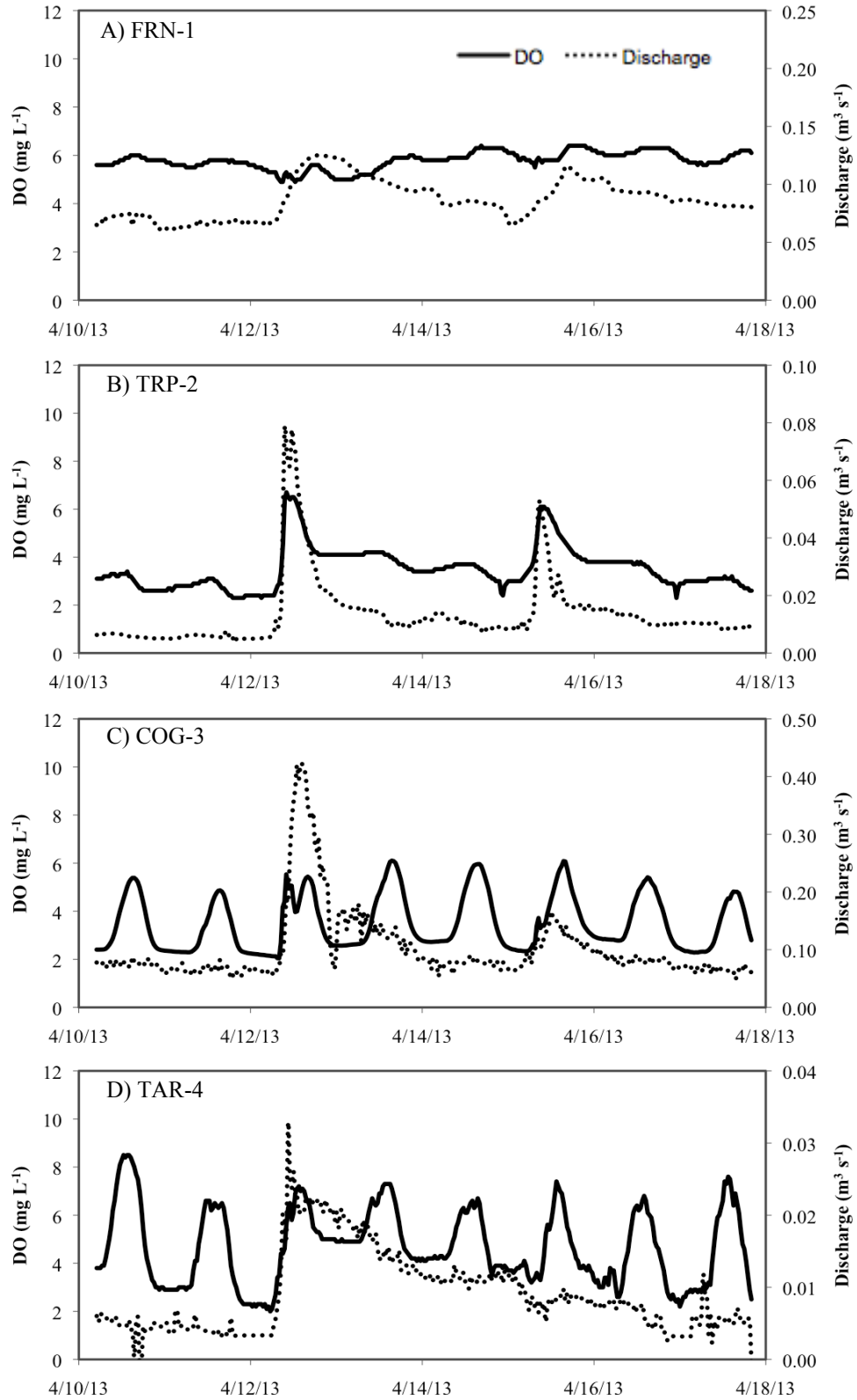


Figure 4. Dissolved oxygen (DO; solid line) and discharge (dotted line) records from April 2013 at French (A), Traps (B), Cogdels (C), and Tarawa (D). Streams presented in order from least to most developed. Note differences in scale for discharge axes.

### *Single-Station Metabolism*

Results from single-station metabolism analyses indicate that all study streams were heterotrophic year-round ( $\text{NDM} < 0$ ), though differences in the degree of heterotrophy were observed between streams and between seasons (Table 2). The maximum estimates of GPP were observed during spring at FRN-1, COG-3, and TAR-4, which measured  $2.20$ ,  $6.43$ , and  $3.49 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , respectively (Fig. 5A). Despite generally higher GPP during spring, ER exceeded GPP by about six to ten fold and spring NDM at these sites ranged from  $-30.26 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at COG-3 to  $-20.96$  and  $-20.98 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at FRN-1 and TAR-4. Spring ER at TRP-2 greatly exceeded the nearly non-existent GPP, though NDM in this site was less heterotrophic (mean  $-9.84 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) than the other three streams (Fig. 5A). Average NDM across all sites during spring was  $-20.51 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ .

Summer GPP decreased at FRN-1, COG-3, and TAR-4 compared to spring. Similar to spring, however, highest summer GPP was observed at COG-3 ( $3.41 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) (Fig. 5B). GPP at TRP-2 was generally low and did not vary significantly with season, however the highest average GPP at this site,  $0.51 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , was observed in summer (Fig. 5B). Although seasonal variation in ER was low at TRP-2 as well, respiration was lowest during summer, making NDM at that site least heterotrophic in summer. The degree of ER at FRN-1 and COG-3, on the other hand, was greatest during the summer ( $-71.18$  and  $-75.14 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , respectively) making summer the most heterotrophic season at those streams (Fig. 5B, note axis scale change). TAR-4 summer NDM remained similar to spring NDM at that site. Summer was the most heterotrophic

season, on average, and NDM across all sites averaged  $-43.69 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  although there was large variation.

Average fall GPP was lower than summer GPP and ER remained moderately high, though it was not as high as summer ER. COG-3 had the highest GPP ( $1.42 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ), with TAR-4 as the next greatest ( $0.94 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) (Fig. 5C). TRP-2 and TAR-4 had minimal GPP. Average fall ER at FRN-1, COG-3, and TAR-4 was similar across the three sites, greatly exceeding ER at TRP-2. Therefore, there was no significant difference between NDM at FRN-1, COG-3, or TAR-4, which ranged from  $-43.40$  to  $-40.24 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  for the fall season (Fig. 5C). Unlike the other streams, fall was the most heterotrophic season at TRP-2 and NDM was  $-13.29 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . Across all streams, however, fall was the second most heterotrophic season (NDM  $-34.76 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ).

Winter GPP was nearly absent at FRN-1 and TRP-2, which both averaged  $0.25 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . On the other hand, COG-3 and TAR-4 had slightly elevated GPP over fall ( $2.65$  and  $1.85 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , respectively) (Fig. 5D). Lowest ER was observed in winter at all sites, except TRP-2, which only decreased slightly from fall. COG-3 was significantly more heterotrophic than the other streams with average NDM equal to  $-21.97 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . FRN-1 was the least heterotrophic (NDM  $-10.08 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) and TRP-2 and TAR-4 had similar NDM ( $-12.26$  and  $-13.39 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , respectively) (Fig. 5D). On average, winter was the least heterotrophic season with NDM equal to  $-14.43 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , however this is likely attributable to lower levels of ER during the colder months.

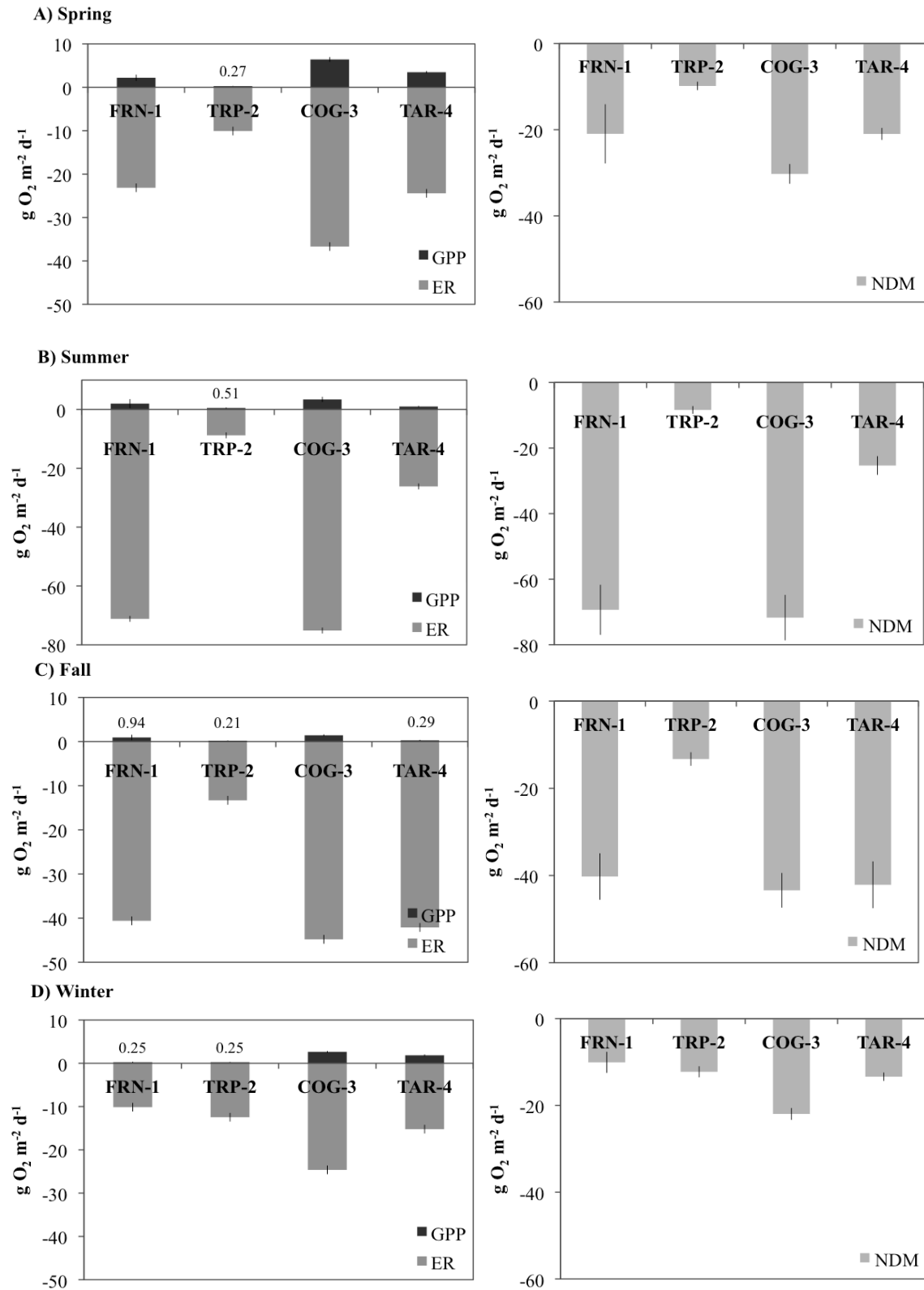


Figure 5. Average seasonal Gross Primary Production (GPP; black), Ecosystem Respiration (ER; dark gray), and Net Daily Metabolism (NDM; light gray) using the single-station method during Spring (A), Summer (B), Fall (C), and Winter (D). GPP values less than 1 g O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> are shown above the x-axis. Note difference in axis scale for summer (B). Bars indicate standard error.

Table 2. Average seasonal Gross Primary Production (GPP), Ecosystem Respiration (ER), and Net Daily Metabolism (NDM) in  $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at each site calculated using the single-station method. Sites listed in order from least to most developed and include spring, summer, fall, and winter seasons. Standard Error (SE) of each measurement is listed in parenthesis. Number of measurements (n) represents the number of days for each site and season that resulted in a statistically significant nighttime regression for calculation of reaeration.

Stream	Season	n	Average (SE) $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$		
			GPP	ER	NDM
FRN-1	Spring	10	2.20 (0.72)	-23.15 (7.15)	-20.96 (6.87)
	Summer	13	1.99 (1.52)	-71.18 (8.35)	-69.35 (7.64)
	Fall	13	0.94 (0.62)	-40.62 (5.25)	-40.24 (5.33)
	Winter	7	0.25 (0.11)	-10.14 (2.37)	-10.08 (2.43)
TRP-2	Spring	18	0.27 (0.05)	-10.08 (1.00)	-9.84 (0.98)
	Summer	17	0.51 (0.14)	-8.80 (1.20)	-8.38 (1.18)
	Fall	17	0.21 (0.06)	-13.32 (1.59)	-13.29 (1.55)
	Winter	33	0.25 (0.05)	-12.46 (1.28)	-12.26 (1.27)
COG-3	Spring	43	6.43 (0.55)	-36.69 (2.52)	-30.26 (2.30)
	Summer	14	3.41 (0.88)	-75.14 (6.65)	-71.73 (6.93)
	Fall	42	1.42 (0.20)	-44.82 (3.98)	-43.40 (3.98)
	Winter	76	2.65 (0.18)	-24.63 (1.46)	-21.97 (1.36)
TAR-4	Spring	54	3.49 (0.27)	-24.43 (1.56)	-20.98 (1.39)
	Summer	24	1.00 (0.21)	-26.17 (2.79)	-25.33 (2.86)
	Fall	24	0.29 (0.09)	-42.12 (5.30)	-42.13 (5.37)
	Winter	55	1.85 (0.21)	-15.22 (1.04)	-13.39 (0.97)

Some streams had low significance with the nighttime regression method used to calculate reaeration, which is reported within the RIVERMET© software. This was especially problematic at FRN-1, which only had 43 significant data points ( $n = 43$ ), and could have been caused by the minimal day-night change in DO observed in-stream that prevented accurate calculation of reaeration. TRP-2 also had relatively low significance of reaeration calculations and had only 85 useable dates. On the other hand, about half of the dates had acceptable significance with the nighttime regression at TAR-4 and COG-3, which had  $n$  equal to 157 and 175, respectively. The number of significant data points used for each site during each season is



listed in Table 2. Although use of the single-station metabolism method and RIVERMET© software provides a broad overview of seasonal patterns in these watersheds, it may not provide the most accurate measurement of metabolic parameters – indicated by low significance of the nighttime regression method. Therefore, the two-station method and direct measurement of stream reaeration were also employed at each stream.

### *Two-Station Metabolism*

Metabolism characteristics were successfully determined using the two-station method at three of the four study streams. This method failed at COG-3 because the reaeration coefficient could not be accurately determined, which obscured calculations of metabolism. Therefore, COG-3 has been excluded from two-station analyses and only results from FRN-1, TRP-2, and TAR-4 are considered in two-station results. Inaccurate characterization of reaeration was also problematic for metabolism calculations at FRN-1 during summer 2013, which resulted in the missing summer data for that site. It is suspected that these data gaps are the result of reach lengths that were not long enough to accurately determine reaeration, particularly during higher discharge. However, the two-station method was successful for all other sites and seasons. Similar to the one-station results, the two-station metabolism method also found all study streams to be net heterotrophic ( $NDM < 0$ ), particularly during summer and fall. Differences in the degree of heterotrophy across streams and seasons were also evident using this method and additionally indicate that, over specific time periods, watershed development and imperviousness may be an important factor in determining stream metabolism.

Summer two-station metabolism experiments were performed during July and August 2013. Two dates, July 9-10, were characterized at TAR-4, during which period GPP averaged  $1.44 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  (Fig. 6A). However, ER exceeded GPP on both dates by about four-times and average NDM at TAR-4 was  $-6.61 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . Metabolism at TRP-2 was characterized on July 18 and no replicate measurement dates were obtained. On that date, there was no in-stream GPP and ER measured  $-21.74 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  (Fig. 6A). Therefore, TRP-2 was much more heterotrophic than TAR-4 during the summer two-station metabolism experiments. As mentioned above, summer estimates are not available for FRN-1.

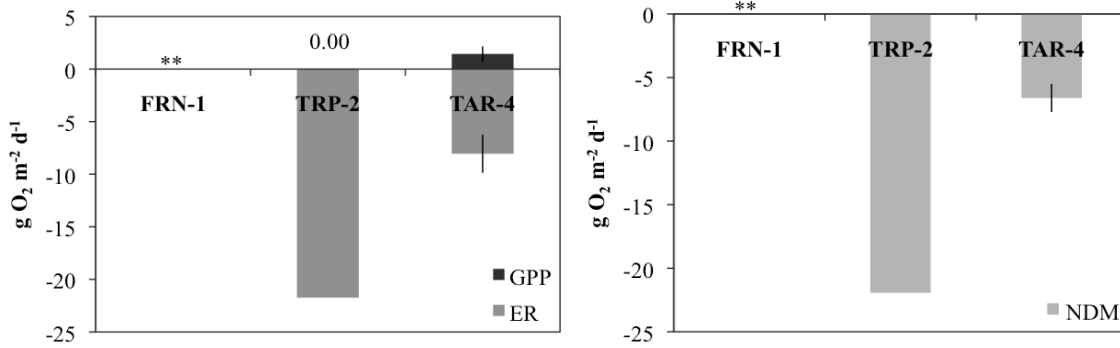
Fall experiments were conducted during September, October, and November 2013. During all fall two-station experiments, GPP was essentially absent from all study streams – FRN-1 and TRP-2 had no measurable GPP and TAR-4 GPP averaged  $0.08 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  (Fig. 6B). Metabolism at TRP-2 was characterized November 20-21 and again found to be the most heterotrophic stream across fall deployments (NDM  $-58.03 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ). TAR-4 was the second most heterotrophic stream, which had an average NDM of  $-23.31 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  from September 24-25. Fall estimates of ER at TRP-2 and TAR-4 were the largest measured at those sites (Fig. 6B; note axis scale change). Only one date (October 19) was characterized at FRN-1 due to instrument failure, but on that date NDM was  $-14.34 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ .

All winter two-station metabolism experiments occurred during January 2014. On average, study streams were less heterotrophic during winter than fall with measureable GPP occurring in all streams. The greatest GPP measured during winter deployments occurred at TAR-4 January 22-23, which averaged  $2.51 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  over two days (Fig. 6C). During January

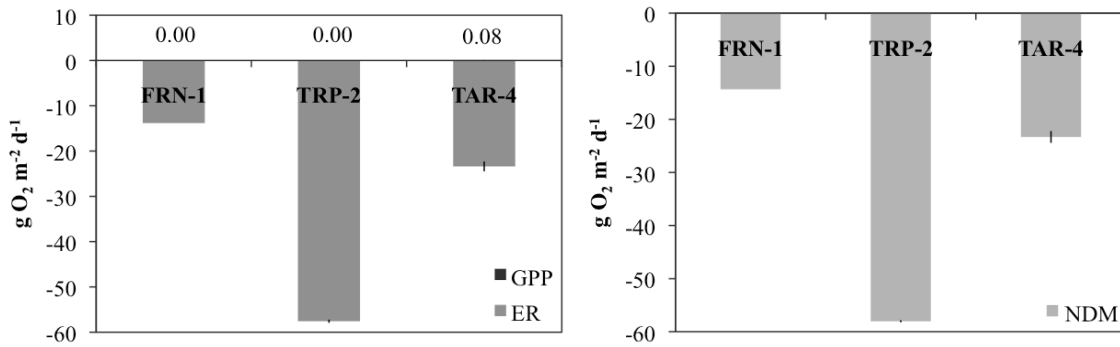
14-16, GPP at FRN-1 was about one-fifth of that measured at TAR-4 (GPP  $0.49 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ), although ER at both sites was approximately equal ( $-5.18 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at FRN-1 and  $-4.69$  at TAR-4). TRP-2 had minimal GPP from January 9-11, which averaged  $0.15 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , and similar to the previous seasons, was the most heterotrophic stream (NDM  $-18.98 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ). TAR-4 was the least heterotrophic during winter deployments, with NDM equal to  $-2.18 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , and also represented the least heterotrophic NDM calculated with the two-station method across all sites and seasons (Fig. 6C). NDM at FRN-1 was only slightly more heterotrophic than TAR-4 ( $-4.73 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ), making winter the least heterotrophic season measured for FRN-1.

Spring two-station deployments were performed in April 2014. Given the trends observed in spring 2013 diel DO curves at these streams (Fig. 4), it was hypothesized that trends in spring GPP and NDM with watershed imperviousness and development might be apparent using the two-station method. Measurements at TAR-4 occurred from April 2-3, at FRN-1 from April 11-13, and at TRP-2 from April 17-18. During those deployment periods, GPP ranged from nearly zero at FRN-1, the least developed site, to  $3.92 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at TAR-4, the most developed site, and was  $0.25 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  at TRP-2 (Fig. 6D). Additionally, ER was greatest at FRN-1 ( $-16.19 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) and lower at the more developed sites, TAR-4 and TRP-2 ( $-10.12$  and  $-8.30 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , respectively). Higher NDM values indicate that the degree of heterotrophy occurring during spring two-station experiments was inversely related to watershed development at these three streams (Fig. 6D).

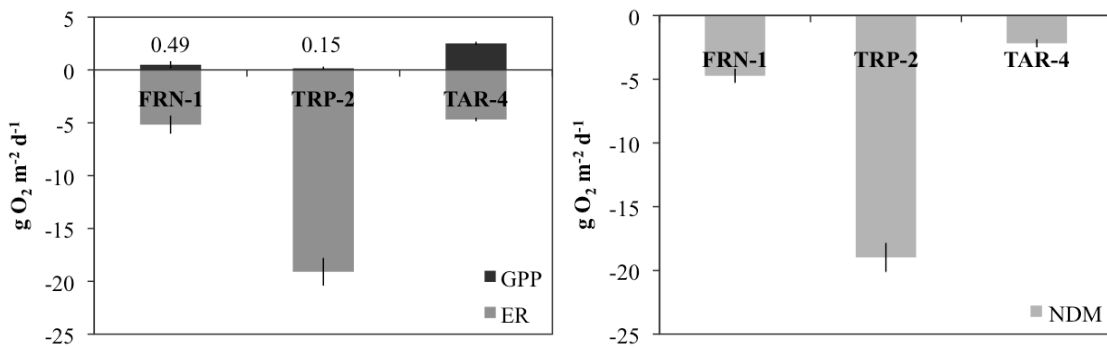
**A) Summer**



**B) Fall**



**C) Winter**



**D) Spring**

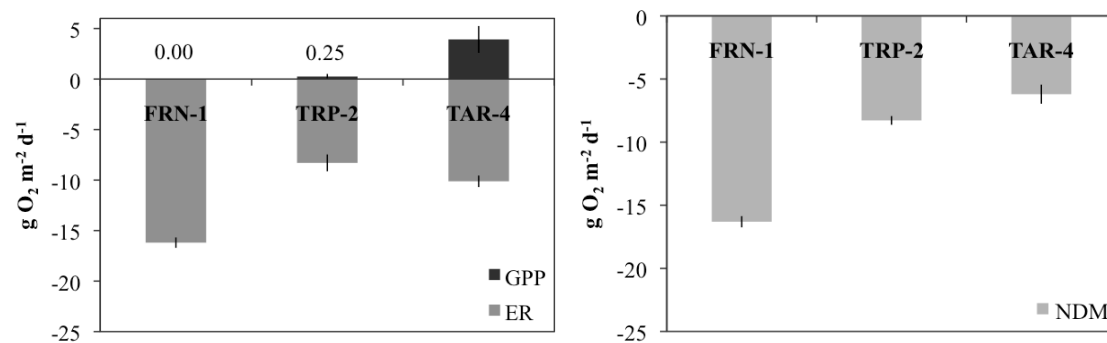


Figure 6. Average seasonal Gross Primary Production (GPP; black), Ecosystem Respiration (ER; dark gray), and Net Daily Metabolism (NDM; light gray) using the two-station method during Summer (A), Fall (B), Winter (C), and Spring (D). GPP values less than  $1 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$  are shown above the x-axis. Note difference in fall axis scale (B). Bars indicate standard error. Asterisks indicate missing data, explained in text.

### *Relationship Between Metabolism and Watershed Development*

Single-station metabolism was not correlated with watershed development during any season; therefore only results from two-station metabolism experiments are discussed. Linear regressions showed significant positive relationships between two-station GPP and both percent development and imperviousness during fall, winter, and spring two-station metabolism deployments (Table 3). Summer regressions were not significant likely due to missing data at FRN-1. Of the significant relationships, higher  $R^2$  values and lower p-values were found for regressions with percent imperviousness, indicating that watershed impervious cover may be a better predictor of stream GPP than development across seasons. The most significant relationship between GPP and percent imperviousness was observed during spring ( $R^2 = 0.85$ ,  $p < 0.01$ ) (Fig. 7A). Regressions with percent imperviousness and development were also significant when all dates were considered together for the entire year, however  $R^2$  values were lower for these regressions than when each season was considered individually (Table 3). Therefore, watershed development characteristics are not as effective of a predictor over broad temporal scales.

ER, on the other hand, was not significantly related to watershed development during any season or across the entire year (Table 3). Additionally, ER exceeded GPP at all sites causing streams to be highly heterotrophic regardless of watershed development characteristics (Fig. 6). Therefore, trends in NDM may be a better indication of whether watershed development alters trophic status and stream function.

Table 3. Results of regression analyses from two-station metabolism experiments. Values listed include the coefficient of variation ( $R^2$ ) for the linear relationship between Gross Primary Production (GPP), Ecosystem Respiration (ER), or Net Daily Metabolism (NDM) and watershed percent imperviousness or development during summer, fall, winter, spring seasons, and the entire year. The significance of each relationship, p-value, is shown in parentheses. Bolded values indicate significance below  $\alpha=0.05$  level. Number of measurements (n) represents the number of successful deployment dates for each season.

Season	n	Regressions with % Imperv.			Regressions with % Develop.		
		$R^2$ (p-value)			$R^2$ (p-value)		
		GPP	ER	NDM	GPP	ER	NDM
Summer	3	0.56 (0.47)	0.95 (0.15)	0.98 (0.079)	0.55 (0.47)	0.95 (0.15)	0.98 (0.079)
Fall	5	<b>0.89 (0.015)</b>	0.17 (0.49)	0.18 (0.47)	<b>0.78 (0.045)</b>	0.027 (0.79)	0.032 (0.77)
Winter	8	<b>0.82 (0.002)</b>	0.11 (0.42)	0.18 (0.29)	<b>0.60 (0.023)</b>	0.002 (0.93)	0.021 (0.73)
Spring	7	<b>0.85 (0.003)</b>	0.23 (0.27)	<b>0.59 (0.044)</b>	<b>0.76 (0.011)</b>	0.48 (0.086)	<b>0.82 (0.005)</b>
Year	23	<b>0.43 (0.001)</b>	0.041 (0.35)	0.067 (0.23)	<b>0.36 (0.002)</b>	0.005 (0.74)	0.016 (0.56)

The results of linear regressions of NDM and watershed development were similar to those of ER, with similar  $R^2$  values across ER and NDM regressions. No significant trend was observed in NDM with watershed development characteristics, except during spring when NDM was positively correlated to percent development and percent imperviousness of the watershed (Table 3). Unlike GPP, in this case, percent development was a better predictor of NDM ( $R^2 = 0.82$ ,  $p < 0.01$ ) than percent imperviousness ( $R^2 = 0.59$ ,  $p < 0.05$ ) (Fig. 7B). Therefore, watershed development does not alter overall stream trophic status except during spring when study streams in more developed watersheds became less heterotrophic.

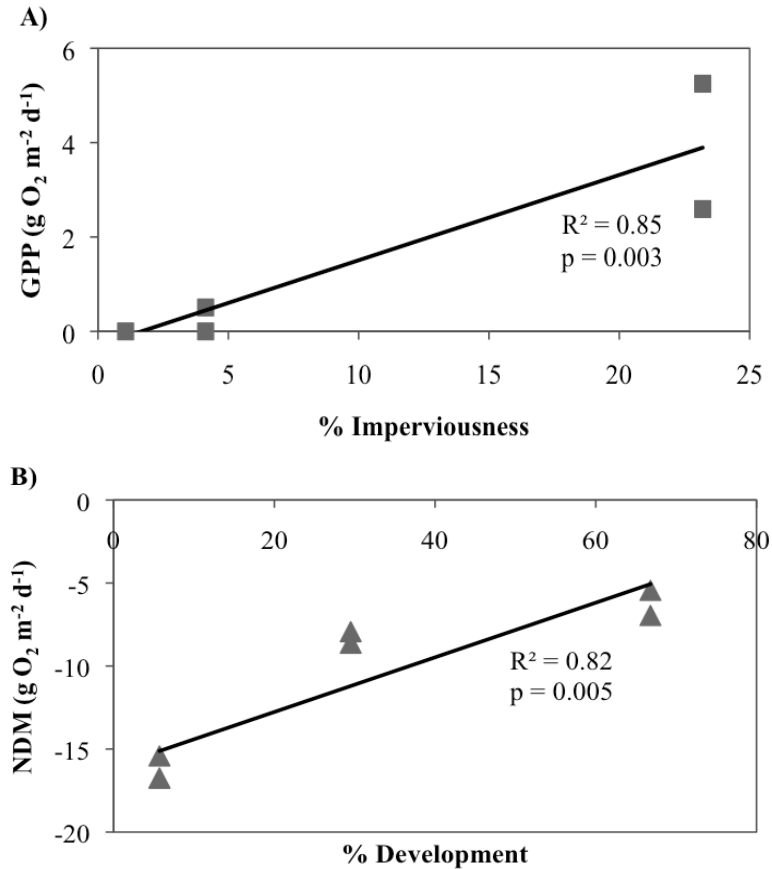


Figure 7. Linear regressions between spring Gross Primary Production (GPP) and watershed percent imperviousness (A) and spring Net Daily Metabolism (NDM) and watershed percent development (B). GPP and NDM units are  $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ .  $R^2$  and  $p$ -values for each relationship are shown below the regression line. Statistical significance was considered  $\alpha < 0.05$ .

### *Factors Affecting Stream Primary Production and Algal Biomass*

Stream nutrient and chl-*a* concentrations appear to be positively correlated with watershed development and imperviousness. Yearly average concentrations were calculated from storm and base-flow samples from continuous stream monitoring at all four study streams between May 2013 and April 2014 and correlations with watershed percent development and percent imperviousness were determined. Relationships with development and impervious surface were similar; therefore only regressions with percent development are shown (Fig. 8).

$\text{NO}_x$ ,  $\text{NH}_4^+$ , and chl-*a* concentrations were positively correlated with percent development with strong correlation coefficients (Fig. 8A, B, and D) and  $\text{PO}_4^{3-}$  showed a moderate positive correlation with development (Fig. 8C). However, these relationships were not significant at a 0.05 significance level. Although the nutrient and chl-*a* regressions were not significant across the streams considered in this analysis, previous evidence from continuous monitoring at MCBCL, which included additional study streams, indicated that a statistically significant relationship does exist, particularly for  $\text{NO}_x$  and chl-*a* concentrations.

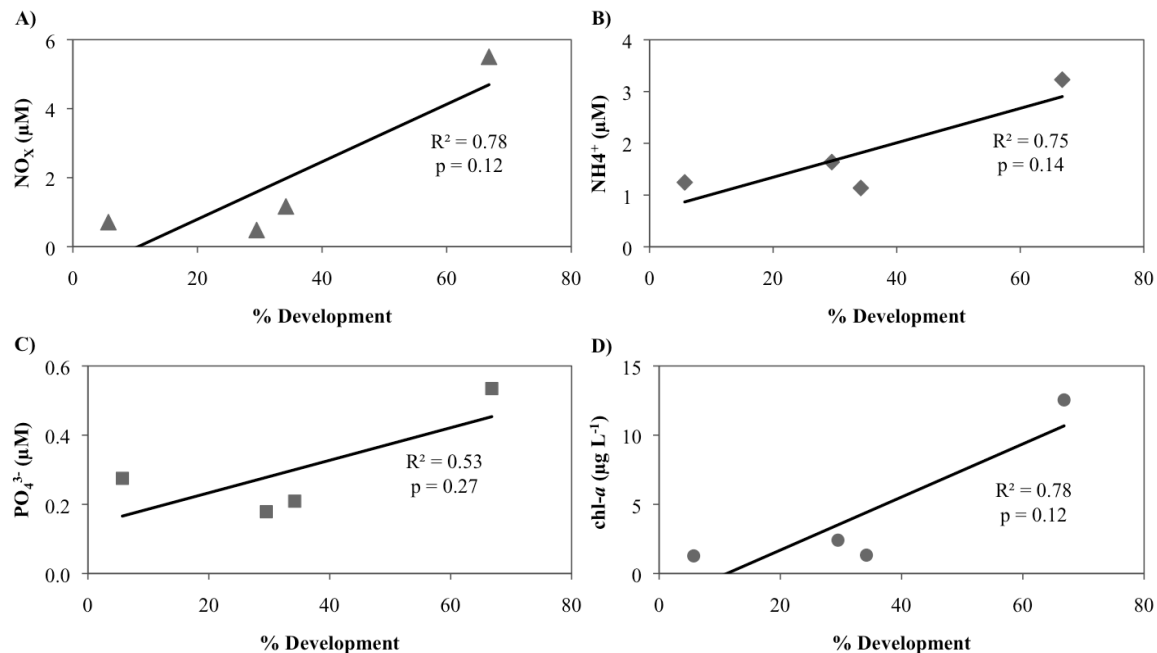


Figure 8. Linear regressions between average yearly Nitrate+Nitrite (A), Ammonium (B), Phosphate (C), and water column chlorophyll-*a* (D) concentrations and watershed percent development.  $\text{NO}_x$ ,  $\text{NH}_4^+$ , and  $\text{PO}_4^{3-}$  units are  $\mu\text{M}$  and chl-*a* units are  $\mu\text{g L}^{-1}$ .  $R^2$  and p-values for each relationship are shown below the regression line. Statistical significance was considered  $\alpha < 0.05$ .

Average seasonal nutrient and chl-*a* concentrations were also determined from grab and storm samples during May 2013 through April 2014. These averages were compared to average seasonal GPP and NDM from the single-station metabolism method to determine the impact of nutrients on primary production in each stream over a broad time frame. No significant



relationship was found between nutrients ( $\text{NO}_x$ ,  $\text{NH}_4^+$ , or  $\text{PO}_4^{3-}$ ) and GPP or NDM. Additionally, neither water column chl-*a* nor sediment chl-*a* were significantly related to GPP or NDM in any season. However, water column chl-*a* concentration was significantly positively correlated to nutrients in most seasons, although sediment chl-*a* did not show significant correlations to seasonal nutrients. Chl-*a* concentrations were most often significantly correlated with  $\text{NO}_x$  (spring, summer, and fall) and  $\text{NH}_4^+$  (spring, fall, and winter), however significant correlations with  $\text{PO}_4^{3-}$  were also found in summer and winter (Table 4). Correlation coefficients for these relationships were very strong (greater than 0.95) and p-values were significant (less than 0.02). Yearly average  $\text{NO}_x$ ,  $\text{NH}_4^+$ , and chl-*a* concentrations were significantly correlated, however the relationship with  $\text{PO}_4^{3-}$  was not significant across the entire year.

Table 4. Results of linear regression analyses between average seasonal water column chl-*a* and average seasonal nutrients for spring, summer, fall, winter, and the entire year from continuous monitoring. Values listed include the coefficient of variation ( $R^2$ ) for the linear relationship between Nitrate+Nitrite ( $\text{NO}_x$ ), Ammonium ( $\text{NH}_4^+$ ), and Phosphate ( $\text{PO}_4^{3-}$ ). The significance of each relationship, p-value, is shown in parentheses. Bolded values indicate significance below  $\alpha=0.05$  level.

Water Column chl- <i>a</i> Regressions with Nutrients			
	$R^2$ (p-value)		
Season	$\text{NO}_x$	$\text{NH}_4^+$	$\text{PO}_4^{3-}$
Spring	<b>0.98 (0.0088)</b>	<b>0.98 (0.012)</b>	0.47 (0.31)
Summer	<b>0.97 (0.013)</b>	0.74 (0.14)	<b>0.97 (0.015)</b>
Fall	0.80 (0.10)	<b>1.00 (&lt;&lt;0.0001)</b>	0.44 (0.34)
Winter	<b>0.98 (0.011)</b>	<b>0.98 (0.010)</b>	<b>0.96 (0.020)</b>
Year	<b>0.96 (0.020)</b>	<b>0.98 (0.0088)</b>	0.89 (0.054)

Results from the two-station metabolism experiments were also compared to nutrient and chl-*a* concentrations from the grab sample taken closest to the date of the metabolism experiment at each of the three streams considered. The strongest correlations occurred between  $\text{NO}_x$  and GPP, which exhibited significant positive correlations during fall, winter, and spring (Fig. 9B, C, and D). Summer regressions were not significant, likely due to missing data from FRN-1 during

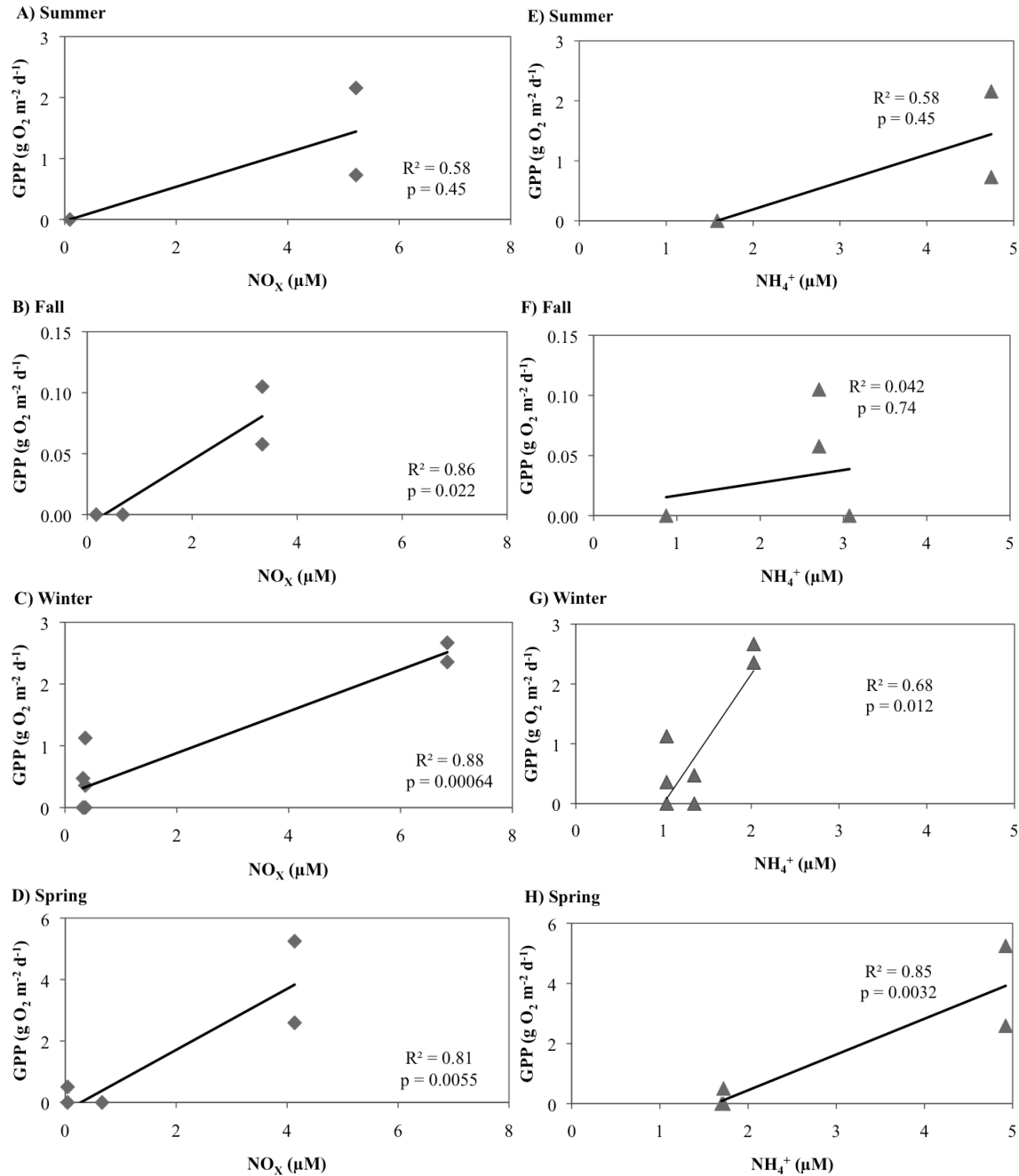


Figure 9. Linear regressions between seasonal Gross Primary Production from two-station metabolism experiments and grab sample nutrients (Nitrate+Nitrite and Ammonium) for summer (A and E), fall (B and F), winter (C and G), and spring (D and H). R<sup>2</sup> and p-values are shown for each regression line. Statistical significance was considered  $\alpha < 0.05$ .

that season (Fig. 9A). NH<sub>4</sub><sup>+</sup> was also significantly positively related to stream GPP during winter and spring (Fig. 9G and H), but not in the summer or fall seasons (Fig. 9E and F). No significant correlation was found between NO<sub>x</sub> or NH<sub>4</sub><sup>+</sup> and NDM, therefore the data is not presented here.

$\text{PO}_4^{3-}$  was significantly positively related to GPP and NDM in winter only ( $R^2 = 0.71$ ,  $p = 0.0089$  and  $R^2 = 0.87$ ,  $p = 0.00076$ , respectively); correlations with  $\text{PO}_4^{3-}$  during other months were not significant. Therefore,  $\text{NO}_x$  and  $\text{NH}_4^+$  are better predictors of seasonal GPP than  $\text{PO}_4^{3-}$  in these coastal streams. These results are in contrast to regressions performed with one-station metabolism results and nutrients from continuous monitoring, which found no relationship between GPP and stream nutrient concentrations.

Water column chl-*a* was significantly positively related to two-station GPP in fall ( $R^2 = 0.88$ ,  $p = 0.019$ ) and winter ( $R^2 = 0.85$ ,  $p = 0.0010$ ), but no significant relationship with NDM was found from the two-station metabolism results. Sediment chl-*a* was significantly positively correlated with GPP ( $R^2 = 0.78$ ,  $p = 0.0087$ ) and NDM ( $R^2 = 0.79$ ,  $p = 0.0073$ ) in spring, but no relationship was found during summer or winter seasons. Fall sediment chl-*a* was not collected; therefore analysis could not be performed for that season. Grab sample nutrients from two-station experiments were correlated with two-station water column and sediment chl-*a* during certain seasons, as well. Summer correlations could not be performed due to missing metabolism data from FRN-1, however fall, winter, and spring water column chl-*a* concentrations from grab samples showed a significant positive correlations with  $\text{NO}_x$  and  $\text{PO}_4^{3-}$  (Table 5). Water column chl-*a* and  $\text{NH}_4^+$  were also significantly correlated during winter and spring seasons. Sediment chl-*a* was correlated with  $\text{NO}_x$  and  $\text{NH}_4^+$  during winter and spring, but no relationship was found with  $\text{PO}_4^{3-}$ . Missing FRN-1 metabolism data during spring and missing sediment chl-*a* data during fall prevented analysis with nutrients during those seasons.

Table 5. Results of linear regression analyses between seasonal water column chl-*a*, sediment chl-*a*, and nutrients for two-station metabolism grab samples during summer, fall, winter, and spring. Values listed include the coefficient of variation ( $R^2$ ) for the linear relationship between Nitrate+Nitrite ( $\text{NO}_x$ ), Ammonium ( $\text{NH}_4^+$ ), and Phosphate ( $\text{PO}_4^{3-}$ ). The significance of each relationship, p-value, is shown in parentheses. Bolded values indicate significance below  $\alpha=0.05$  level. n/a indicates that missing data did not allow for a regression to be performed.

<b>Water Column chl-<i>a</i> Regressions with Nutrients</b>			
	<b><math>R^2</math> (p-value)</b>		
<b>Season</b>	<b><math>\text{NO}_x</math></b>	<b><math>\text{NH}_4^+</math></b>	<b><math>\text{PO}_4^{3-}</math></b>
Summer	n/a	n/a	n/a
Fall	<b>0.98 (0.0011)</b>	0.053 (0.71)	0.053 (0.71)
Winter	<b>1.0 (&lt;&lt;0.001)</b>	<b>0.91 (0.00021)</b>	<b>0.91 (&lt;&lt;0.001)</b>
Spring	<b>0.73 (0.014)</b>	<b>0.59 (0.045)</b>	<b>0.59 (0.045)</b>
<b>Sediment chl-<i>a</i> Regressions with Nutrients</b>			
	<b><math>R^2</math> (p-value)</b>		
<b>Season</b>	<b><math>\text{NO}_x</math></b>	<b><math>\text{NH}_4^+</math></b>	<b><math>\text{PO}_4^{3-}</math></b>
Summer	n/a	n/a	n/a
Fall	n/a	n/a	n/a
Winter	<b>0.53 (0.040)</b>	<b>0.86 (&lt;0.001)</b>	0.037 (0.65)
Spring	<b>0.76 (0.011)</b>	<b>0.88 (0.0018)</b>	0.13 (0.44)

### *Chromophoric Dissolved Organic Matter*

CDOM and other absorbance spectra properties appear to be correlated with watershed development and imperviousness. Yearly average CDOM concentration (estimated using  $a_{355}$ ), average DOC concentration (approximated by  $a_{254}$ ), SUVA<sub>254</sub> (proxy for CDOM aromaticity), and slope ratio ( $S_R$ , indicates CDOM source) were calculated from base-flow samples from continuous stream monitoring at all four study streams between May 2013 and April 2014. Correlations with watershed percent development and percent imperviousness were determined. Relationships between optical properties and development and impervious surface were similar; therefore only regressions with percent imperviousness are shown. CDOM concentration ( $a_{355}$ ) and DOC concentration ( $a_{254}$ ) proxies both showed negative trends with percent imperviousness

(Fig. 10B and A). SUVA<sub>254</sub>, which is related to CDOM aromaticity, also decreased with increasing imperviousness (Fig. 10C). High SUVA<sub>254</sub> indicates high terrestrial source; therefore, it appears that terrestrially sourced CDOM decreases with impervious watershed cover. However, the aforementioned trends were not statistically significant. Although regressions of CDOM concentration, DOC concentration, and SUVA<sub>254</sub> were not significant at the  $\alpha = 0.05$  level across the streams considered in this analysis,  $R^2$  values were strong and p-values were nearly significant for CDOM concentration and DOC concentration. Additionally, previous evidence from continuous monitoring at MCBCL, which included an additional study stream, indicated that a statistically significant relationship does exist for most CDOM and optical proxies addressed here.

A significant correlation with percent imperviousness was found for one optical property: slope ratio ( $S_R$ ) (Fig. 10D).  $S_R$  is used to characterize changes in CDOM source by indicating changes in molecular weight and thus the level of photodegradation of the CDOM. Low  $S_R$  at lower percent impervious sites indicates a high molecular weight, which means that the CDOM had been less photodegraded and was therefore allochthonous in nature. However, as imperviousness of the watershed increased, the CDOM had increasingly lower molecular weight and was less allochthonous in origin.  $S_R$  less than one at all sites indicates a significant terrestrial source in all streams, however the extent of the terrestrial source decreases with impervious and development – indicated by increasing  $S_R$ .

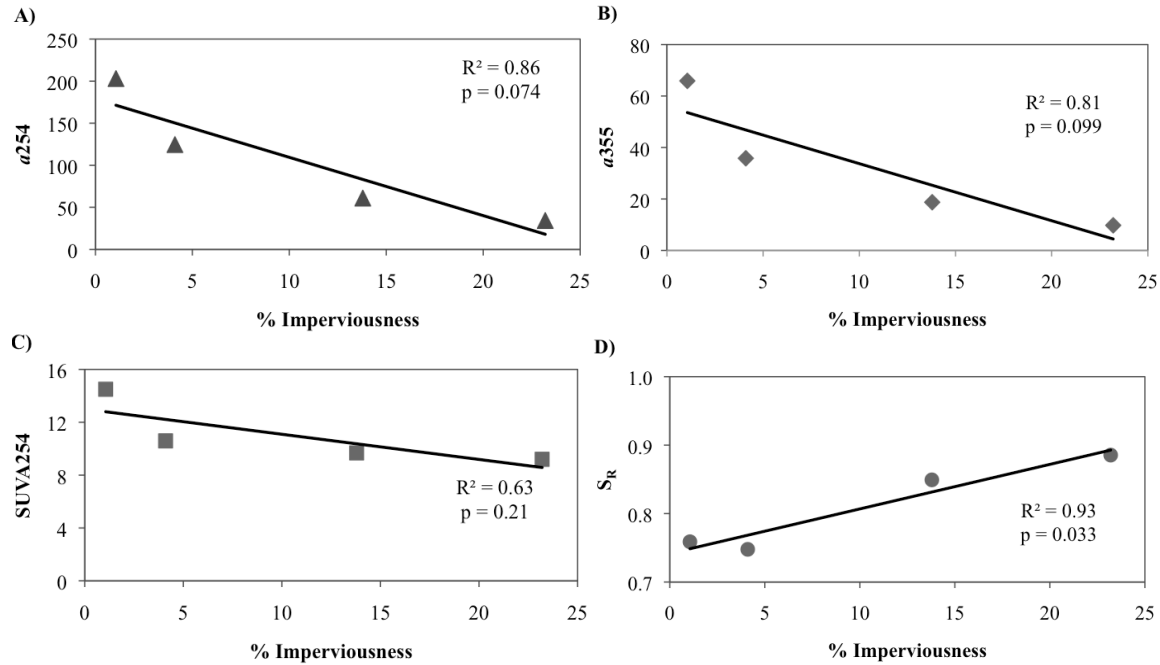


Figure 10. Linear regressions between percent watershed imperviousness and yearly average optical properties, including  $a_{254}$  (A),  $a_{355}$  (B), SUVA254 (C) and slope ratio (D). Significance of optical properties discussed in text.  $R^2$  and p-values are shown for each regression line. Statistical significance was considered  $\alpha < 0.05$ .

Correlations between CDOM and one-station metabolism estimates were weak for all seasons. The strongest correlation coefficient was found between average winter GPP and average winter CDOM ( $R^2 = 0.63$ ), however the negative relationship was not significant ( $p = 0.21$ ). This indicated that GPP might increase with decreasing CDOM but that seasonal variability may be difficult to characterize. Results from the two-station metabolism experiments found nearly significant negative correlations between GPP and daily CDOM during summer, fall, and spring deployments ( $R^2 = 0.58, 0.68$ , and  $0.55$ , respectively). Additionally, highly significant negative correlations were found between CDOM and two-station ER ( $R^2 = 0.74$ ,  $p = 0.012$ ) and NDM ( $R^2 = 0.97$ ,  $p = 6.5 \times 10^{-5}$ ) during spring deployments and nearly significant negative correlations were found between summer CDOM and summer ER ( $R^2 = 0.95$ ,  $p = 0.14$ ) and NDM ( $R^2 = 0.98$ ,  $p = 0.079$ ). These findings from two-station metabolism experiments also

indicate that decreasing CDOM concentration or correlated carbon concentrations in developed streams might cause them to become less heterotrophic.

### *Dissolved and Particulate Organic Carbon*

DOC concentration was moderately negatively correlated to percent imperviousness of the watershed indicated by a strong correlation coefficient and nearly significant p-value ( $R^2 = 0.89$ ,  $p = 0.054$ ). The correlation with development showed a similar trend, but the correlation was not as strong ( $R^2 = 0.75$ ,  $p = 0.13$ ). This trend was expected given decreasing CDOM and  $a_{254}$  with development as those optical properties are typically correlated with DOC concentrations. POC concentration was moderately positively correlated to percent development ( $R^2 = 0.63$ ,  $p = 0.21$ ) and weakly correlated with percent impervious ( $R^2 = 0.32$ ,  $p = 0.43$ ).

Average seasonal DOC was not related to one-station metabolism parameters in any season. Two-station metabolism parameters were compared to DOC samples from the nearest grab sample collection date. Regressions between seasonal metabolism and DOC concentration resulted in only a few significant correlations. Spring NDM was negatively correlated with DOC concentration ( $R^2 = 0.71$ ,  $p = 0.018$ ), indicating that streams became more heterotrophic as DOC increased (Fig. 11G). Summer NDM showed a similar trend ( $R^2 = 0.98$ ), however the p-value was not significant for that regression due to a limited number of data points (Fig. 11A). Fall and winter NDM showed no correlation with DOC concentration (Fig 11C and E). Greatest DOC concentrations during the two-station experiments were observed in winter at FRN-1 ( $20.1 \text{ mg L}^{-1}$ ) and lowest DOC was found at TAR-4 during summer ( $3.1 \text{ mg L}^{-1}$ ). However, seasonal

averages from continuous monitoring of DOC indicated that greatest seasonal DOC occurred at FRN-1 during summer (20.8 mg L<sup>-1</sup>) and TAR-4 had lowest DOC during fall (4.1 mg L<sup>-1</sup>).

Average seasonal POC was also not correlated with one-station metabolism. On the other hand, average seasonal POC concentration was negatively correlated with two-station metabolism ER and NDM during the fall season. These regression between fall POC and NDM had a very strong R<sup>2</sup> value (0.98) and was significant with a p-value 0.0015 (Fig. 11D). Summer POC correlation with NDM also had a R<sup>2</sup> = 0.98, however the trend was not significant (p = 0.079) likely due to missing summer data points (Fig. 11B). No trend was detected during winter and POC concentrations were quite low (Fig. 11F). Spring NDM, on the other hand, was positively correlated with POC concentration (R<sup>2</sup> = 0.60, p = 0.04) (Fig. 11H). Overall, POC concentrations were much lower than DOC concentrations during all seasons and at all sites. The greatest POC concentrations during two-station experiments were observed in fall at TRP-2 (5.3 mg L<sup>-1</sup>) and lowest POC was found at TAR-4 during summer (0.6 mg L<sup>-1</sup>). However, seasonal averages from continuous monitoring indicated that greatest seasonal POC occurred at TAR-4 during spring (4.5 mg L<sup>-1</sup>) and COG-3 had lowest POC during summer (0.43 mg L<sup>-1</sup>).



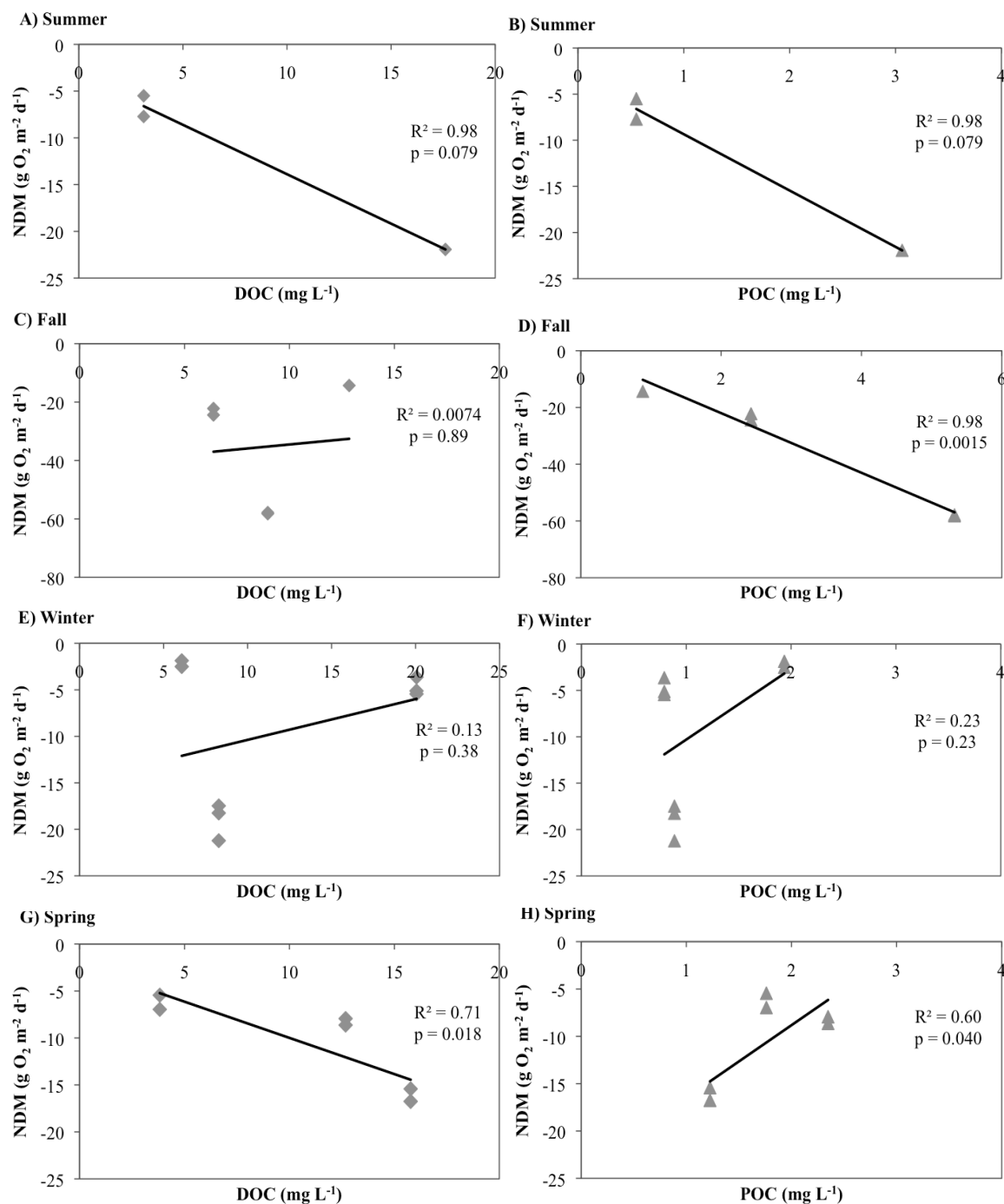


Figure 11. Linear regressions between seasonal Net Daily Metabolism from two-station metabolism experiments and grab sample dissolved organic carbon (DOC) and particulate organic carbon (POC) for summer (A and B), fall (C and D), winter (E and F), and spring (G and H). Note difference in NDM axis scale for fall graphs, difference in DOC scale for winter, and difference in POC scale for fall.  $R^2$  and  $p$ -values are shown for each regression line. Statistical significance was considered  $\alpha < 0.05$ .

## DISCUSSION

### *Coastal Streams and Metabolism*

Coastal headwater streams (CHS) are conduits of allochthonous materials that directly connect terrestrial ecosystems and estuaries. Streams are susceptible to changes in watershed development that may elevate stream temperatures, increase nutrient and carbon loading, and drive changes in community structure. Understanding and quantifying the role that CHS play in exporting materials to downstream estuaries under impacts from watershed development is essential for accurate projections of future estuarine function. Rates of whole-stream metabolism are fundamental indicators of ecosystem structure and function, including nutrient and organic matter cycling, and a number of studies have indicated that metabolism parameters have potential as indicators of the ecological status of stream ecosystems (Gessner & Chauvet, 2000; Mulholland et al., 2005; Young et al., 2008). However, there are no previously published attempts to characterize metabolism in CHS and there is limited understanding of how watershed development may alter variability in ecosystem metabolism of streams (Bernot *et al.*, 2010). Therefore, measurements of whole-stream metabolism were performed within the context of watershed development in order to assess impacts on stream structure and function. This study found that possible effects of watershed development, including increased nitrogen concentrations, decreased DOC and CDOM, and increased POC were correlated with less heterotrophic conditions in NewRE CHS. Further analysis of these findings is presented below.

## *Stream Discharge Regimes*

The magnitudes of discharge seen in the four CHS were low compared to other headwater streams found in the literature. A review of twelve upland headwater streams across North America observed a range in discharge from 0.005 to 1.2 m<sup>3</sup> s<sup>-1</sup> (Peterson *et al.*, 2001). Average flows in study streams of the NewRE ranged from 0.006 to 0.112 m<sup>3</sup> s<sup>-1</sup>, which fall on the low end of the range recorded for upland headwater streams. Based on this study, it appears likely that coastal headwater streams exhibit lower discharges, on average, than upland headwater streams and perhaps even other small streams in the coastal plain. In another study of North Carolina coastal streams, a coastal swamp stream measured by Mulholland (1981) exhibited storm flow discharges that often exceeded 2 m<sup>3</sup> s<sup>-1</sup>, whereas average daily storm discharge in the four study streams did not exceed 1 m<sup>3</sup> s<sup>-1</sup>. However, base-flows in the swamp stream were very low and even became stagnant at times, similar to coastal streams of the NewRE. Mulholland (1981) also noted that streams tended to become hypoxic during low-flow periods. Hypoxia was particularly evident at TRP-2, which typically had discharge less than 0.01 m<sup>3</sup> s<sup>-1</sup>. Peaks in discharge of all study streams were often coincident with peaks in DO concentration, however this trend was most pronounced in low-flow streams such as TRP-2 and TAR-4. Discharge had consistent and often profound influences on CHS oxygen dynamics. Discharge regimes are also important in context of the NewRE because the cumulative importance of coastal streams as a freshwater source to the estuary is likely minimal at base-flow, but may become significant during storm-flow conditions.

### *Dissolved Oxygen and Diel Excursion*

DO concentrations are the currency of metabolism calculations but are also used as an indicator of habitat quality for aerobic aquatic organisms. NewRE study streams were frequently hypoxic (DO less than 2 mg L<sup>-1</sup>) and most had DO concentrations below 5 mg L<sup>-1</sup> throughout the year. FRN-1, however, exhibited hypoxia less than 0.1% of the year. It is possible that a source of DO, such as oxic groundwater, was present. FRN-1 had DO concentrations less than 5 mg L<sup>-1</sup> only 33% of the time. On the other hand, TRP-2 was hypoxic 50% of the year and had low DO concentrations (< 5 mg L<sup>-1</sup>) nearly 92% of that time. COG-3 and TAR-4 were hypoxic 29-30% of the study period and exhibited DO concentrations below 5 mg L<sup>-1</sup> 64% and 70% of the year, respectively. Under-saturation of oxygen in coastal streams is likely caused by low discharge, which limits turbulence and mixing of oxygenated surface waters, and the predominance of heterotrophic conditions consuming oxygen throughout the year (Baker *et al.*, 2000). Based on this study, CHS may not be consistently high-value habitat for organisms due to frequently low DO concentrations. The impact of watershed development on CHS habitat quality is difficult to isolate. The least developed stream, FRN-1, had relatively high DO concentrations through the year, but the second least developed stream, TRP-2, was the most hypoxic stream. Therefore, other factors such as groundwater impacts on stream oxygen budgets, magnitude of discharge, and specific land use may have obscured effects of watershed development on stream DO concentration regimes.

Diel DO excursion is an important indicator of in-stream metabolism (Mulholland *et al.* 2005). Daytime maximums and nighttime minimums in DO concentration indicate the presence

of primary production that produces DO through photosynthesis during the day, which is then drawn down through respiration at night (Odum 1956). The cycle of daytime DO production and nighttime DO consumption typically creates a symmetrical curve, which can be analyzed to determine the component rates of GPP, ER, and diffusion (Odum, 1956). Observed differences in the magnitude of spring diel DO excursion at the four stream sites indicated differences in GPP and ER, which may be attributable to impacts of watershed development that altered stream metabolism. The absence of daily DO variation at FRN-1, for instance, is likely due to negligible primary productivity in the stream as well as indicating possible oxic groundwater inputs, which would also explain the oxic conditions that prevailed throughout the year in that stream. TAR-4 exhibited the largest diel DO excursion, indicating greater GPP than other less developed streams. Given these preliminary observations, we hypothesized that streams with more watershed development and impervious cover would have greater rates of GPP and perhaps be autotrophic due to factors such as increased nutrient loading, decreased carbon concentrations, and reduced CDOM light limitation (i.e. TAR-4). Pristine streams with little watershed development (i.e. FRN-1), on the other hand, would be characteristically heterotrophic due to low nutrient concentrations, high DOC, and high CDOM.

### *Stream Metabolism and Watershed Development*

This study is the first published measurement of metabolism in CHS. Two approaches for measuring metabolism were used – single- and two-station whole stream metabolism. A more in-depth comparison of method performance is presented below, however both methods had reasonable success in CHS. Metabolism calculations for both methods indicated that all four

streams were highly heterotrophic ( $ER \gg GPP$ ) during the entire year. GPP in NewRE streams was negligible throughout much of the year, with maximum GPP in most streams occurring in spring as has been seen in other studies (e.g. Houser *et al.*, 2005; Acuña *et al.*, 2004). This result is intuitive because spring conditions are particularly favorable for photosynthesis, i.e., light levels are relatively high because leafout has not yet occurred, day length is increasing, and temperatures are warming. However, rates of ER exceeded GPP in every instance and NDM calculated for CHS of the NewRE using the two-station method span a broad range from slightly heterotrophic ( $-1.9 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) to highly heterotrophic ( $-58.2 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ). Other small regional streams reported in the literature also tend to be heterotrophic. However, it appears that CHS have more heterotrophic conditions than other streams of similar size (Table 6). A headwater creek in northern Indiana, Shatto Creek, exhibited a broad range of NDM as well (Griffiths *et al.*, 2013). However, the most heterotrophic NDM observed,  $-31.7 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ , was less

Table 6. Comparison of Net Daily Metabolism (NDM) ranges found during two-station experiments in this study to other small United States regional streams reported in literature. Stream type and stream order (HW – headwater; 2<sup>nd</sup> – second order), location, watershed size(s), and source for each stream estimate are listed. n/a indicates data not available.

Stream Type (Order)	Location	Watershed Area (ha)	NDM Range ( $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ )	Source
Coastal Streams (HW)	New River, NC	51.0 – 807.3	-58.2 – -1.9	<i>This Study</i>
Shatto Creek (HW)	Northern IN	n/a	-31.7 – +1.3	Griffiths <i>et al.</i> , 2013
Hugh White Creek (2 <sup>nd</sup> )	Southwest NC	61.1	-9.89	Mulholland <i>et al.</i> 1997
Ft. Benning Creeks (2 <sup>nd</sup> )	Western GA	33.1 – 369.0	-10.0 – -0.3	Mulholland <i>et al.</i> , 2005
Urban Streams (n/a)	Central NC	90 - 760	-1.09 – +0.16	Sudduth <i>et al.</i> , 2011

heterotrophic than the maximum observed in this study. Shatto Creek was also autotrophic on several occasions, unlike NewRE coastal streams that were heterotrophic year-round. Second order streams in North Carolina and Georgia assessed by Mulholland *et al.* (1997; 2005) were only moderately heterotrophic and urban streams in central North Carolina ranged from slightly

heterotrophic and autotrophic with only small variation in NDM (Sudduth *et al.*, 2011).

Therefore, metabolism calculated for CHS in this study seems reasonable within the context of other small streams, but indicate that CHS are more heterotrophic compared to the range of streams previously assessed.

No significant relationship between single-station metabolism parameters and watershed development or imperviousness was discovered. Therefore, over broad temporal scales, it appears that there were not differences in NewRE CHS metabolism across the range of watershed development considered in this study. In contrast, findings of Houser *et al.* (2005) identified catchment disturbance (defined as percent of bare ground on slopes >5%) as a significant factor causing decreased ER rates in heterotrophic second- and third-order streams in central western Georgia, although disturbance had no impact on GPP. Houser *et al.* concluded that burial of coarse woody debris due to increased sedimentation in disturbed catchments was responsible for observed decreases in ER. However, low slopes of the North Carolina coastal plain are unlikely to cause extensive delivery of terrestrial sediments to streams, thus burial of coarse woody debris may not be as prevalent in NewRE streams. Additionally, percent disturbance and the development indices considered here (percent development and imperviousness) encompass different impacts of land use and, although related, likely produce different correlations with stream metabolism. Simply considering one development index at a time may be overly simplistic, as indicated by Riva-Murray *et al.* (2010). Other factors, such as canopy cover may have been more important over broad temporal scales and have been found to override the effects of land use on stream metabolism in other studies (e.g. Sudduth *et al.*, 2011; Bott *et al.*, 2006). Therefore, seasonal scale metabolism may not be the most appropriate scale

for effective determination of land use and watershed development impacts because other driving factors of shorter time scales may obscure trends.

Results from two-station metabolism experiments did indicate that watershed development was an important factor in determining stream metabolism on short temporal scales. Correlations between GPP and percent imperviousness of the watershed were significant during fall, winter, and spring. Increased watershed imperviousness led to increased GPP rates in streams. A similar trend was observed in summer, but was not significant during summer due to missing FRN-1 summer data. Sudduth *et al.* (2011) found that summer GPP in small urban streams was positively correlated to impervious watershed cover, though they did not observe this trend in the winter. NDM was not correlated with development during summer, fall, or winter, however, spring NDM increased with development. Therefore, it seems that watershed development does not alter the overall trophic status except during spring when highest rates of GPP occur and impacts of increased watershed development caused streams to become less heterotrophic. This result was anticipated given differences in daily DO variation observed during spring 2013, where the degree of diel DO excursion increased with watershed development and imperviousness. Few other studies have investigated correlations between percent impervious cover or development and stream metabolism, but have instead focused on nutrient loads, light availability (i.e. PAR, canopy cover), temperature, and availability of organic matter (e.g. Acuña *et al.*, 2004; Bott *et al.*, 2006; Griffiths *et al.*, 2013; Houser *et al.*, 2005). Stream temperature and PAR were not correlated with two-station metabolism parameters in this study. Other effects of watershed development that were likely to have driven observed



differences in metabolism of NewRE CHS, such as algal biomass, nutrient, CDOM, and carbon concentrations, are discussed below.

### *Performance and Comparison of Methods*

Although it was not within the primary scope of this project to perform a comparison of methods for measuring whole-stream metabolism, it is difficult not to consider how these two different methodologies performed when implemented in new systems. No previous studies of metabolism in coastal headwater streams were found. Therefore, it is important to comment on the effectiveness of each method. In general, both methods provided reasonable estimates of whole-stream metabolism in highly heterotrophic systems. However, minor issues and limitations for each method are discussed below.

Complications with the single-station method and significance of the nighttime regression method in streams with very little diel DO change may preclude the use of this method in streams similar to FRN-1. A possible oxic groundwater source at this site led to conditions that were not ideal for modeling reaeration flux using the nighttime regression method. Additionally, placement of the sondes for continuous monitoring may not have been ideal for characterization of overall stream DO. For example, sondes at TAR-4 and FRN-1 were each located at the outflow of a culvert pipe, which may have caused altered stream DO conditions. Additionally, the sonde at COG-3 was located at the entrance to a culvert pipe in an area of greater stream depth than the mean depth upstream of the sonde. This difference in depth may have altered reaeration and DO conditions from those upstream due to a stratified water column.

Effectiveness of the two-station method was primarily influenced by characterization of oxygen reaeration, as well. Placement of the sondes in constrained and well-mixed upstream and downstream locations was considered, however stream reaches might have been too short to adequately characterize metabolism and reaeration coefficients in certain streams and during specific deployments. In particular, longer reaches were needed in higher flow streams, including COG-3 and FRN-1. COG-3 had to be excluded from final analyses due to the difficulty of calculating reaeration along the stream reach. Reach lengths were established in summer 2013 when stream discharges were low and travel time was long. When stream velocities increased during wetter and cooler months, however, travel times became shorter and it became difficult to discern a detectable difference in upstream-downstream DO during deployments and dissolved Ar during gas tracer experiments given sonde and MIMS instrument limitations. These issues could be avoided in future deployments by using longer reaches or adjusting reach length based on measured stream velocity at the time of each experiment.

In the case of this study, it is difficult to compare daily metabolism results between specific dates because single-station method results were not always available for the dates on which two-station metabolism experiments were performed. Generalizing two-station estimates to compare with seasonal averages from the one-station method is questionable, as well. Two-station metabolism only looked at base-flow conditions on constrained dates, whereas the single-station method considered all dates for which a reliable DO record was obtained (irrespective of base- or storm-flow) and for which the nighttime regression method was significant. Additionally, both methods were utilized in this study in order to provide both a coarse overview

of broad temporal scale observations (single-station method), as well as more precise, targeted measurements (two-station method with direct measurement of reaeration). The purpose was not to compare these methods or results directly. However, a few general observations can be made.

Estimates of GPP and ER obtained by both methods at FRN-1 were comparable for winter and spring seasons. A reliable measurement of metabolism from the two-station method was not obtained for summer. In fall, however, ER estimates from the one-station method exceeded those obtained using the two-station method by over three-fold. A similar difference in ER estimates from the two methods was found at TAR-4 during all seasons. Measurements of GPP were similar between the two methods, but ER obtained from the single-station method was two to three times greater. Therefore, results from the single-station method during most seasons at FRN-1 and TAR-4 calculated that those streams are more heterotrophic than results from the two-station method. At TRP-2, metabolism estimates were very close during summer, winter, and spring. During fall, however, ER results from the two-station method exceeded those obtained from the one-station method by about three-fold, the opposite of what was observed at FRN-1 and TAR-4. Two-station experiments at COG-3 were not successful and therefore comparisons cannot be made.

Overall, calculations of GPP were similar between the two methods. Differences in the magnitude of estimated ER, however, indicate that the effectiveness of determining NDM in these streams is reliant on the precision of ER measurements. ER calculations are, in turn, dependent on estimates of reaeration, which is known for being a source of error in metabolism calculations (Aristegi *et al.*, 2009; Marzolf *et al.*, 1994; McCutchan *et al.*, 1998). Therefore, the

key difference between the two methods used in this study is likely the way by which reaeration was determined. The single-station metabolism method utilized the RIVERMET© software to estimate reaeration based on the nighttime regression method and two-station metabolism directly calculated reaeration using a tracer method. Direct measurement is thought to be the more precise method (Marzolf *et al.*, 1994), but is time consuming and – in the case of this study – longer stream reaches were needed at certain sites to more accurately measure reaeration. Therefore, either method can feasibly be used to measure whole-stream metabolism in CHS but study sites and stream reaches should be assigned with care and direct measurement of reaeration is favorable.

#### *Nutrient Limitation and Algal Biomass in Coastal Headwater Streams*

Human development in coastal watersheds has extensively been linked to increasing nutrient loads and eutrophication along the U.S. east coast (Nixon, 1995; Kaushal *et al.*, 2008; etc). Although the response of larger aquatic systems, such as the Chesapeake Bay, to human-driven nutrient loading has been well documented, fewer studies have been performed on these effects in small blackwater rivers and streams of the coastal plain, which may respond differently to the same pressures (Mallin *et al.*, 2001a; Mallin *et al.*, 2004). Nutrient and chl-*a* concentrations of the four study streams were not significantly related to development indices, however these correlations were significant during previous studies that included additional NewRE CHS. In particular, NO<sub>x</sub> and water column chl-*a* concentrations significantly increased with percent development and imperviousness when examined over 5 and 10 watersheds. Phytoplankton communities are not common in most small streams due to flow conditions that

preclude significant water column chl-*a* concentrations, however flow conditions of NewRE CHS allow for development of phytoplankton populations. Though not tidal, CHS streams have long residence times and therefore have the ability to develop phytoplankton populations similar to that observed by Ensign *et al.* (2012) in North Carolina tidal freshwater creeks.

Nutrients and water column chl-*a* were significantly positively correlated to each other within the four study streams, however sediment chl-*a* was not correlated to nutrient concentrations. Nutrient correlations with water column chl-*a* were strongest with NO<sub>x</sub> and NH<sub>4</sub><sup>+</sup> compared to PO<sub>4</sub><sup>3-</sup>, indicating that streams are nitrogen limited instead of phosphorous limited, as is common in most upland freshwater streams (Hecky & Kilham, 1998). Mallin *et al.* (2001*a*) found that freshwater blackwater rivers in the North Carolina coastal plain were nitrogen limited, therefore similar factors may also cause historically blackwater CHS to be nitrogen limited.

No correlation was detected between single-station GPP and chl-*a* or nutrients. However, this study did find a significant positive correlation between two-station GPP with water column chl-*a* during fall, winter, and spring. Sediment chl-*a* exhibited large variability between sampling location and dates, but was positively correlated with GPP in spring. Therefore, changes in chl-*a* concentration (particularly water column chl-*a*) seem to predict daily changes in stream GPP, particularly during spring; however broad season-scale relationships were not discernable due to large variations in chl-*a*.

Along with chl-*a*, two-station GPP was positively correlated with NO<sub>x</sub> during fall, winter, and spring. GPP correlations with NH<sub>4</sub><sup>+</sup> were also significant during winter and spring, whereas the PO<sub>4</sub><sup>3-</sup> regression was only significant during winter. These results further indicate that freshwater CHS are primarily nitrogen limited, in contrast to findings of other whole-stream metabolism experiments that have found phosphorous to be the important nutrient factor controlling GPP in small, freshwater streams (i.e. Mulholland *et al.*, 2001; Griffiths *et al.*, 2013). Bioassay experiments performed during Summer 2013 with water from FRN-1 and TAR-4 also concluded that when streams were nutrient limited, nitrogen additions stimulated phytoplankton growth, whereas phosphorous additions did not (Couper, 2014). These findings verify correlations described above between GPP, water column chl-*a* and nitrogen concentrations and indicate that nutrient loading from human activities can alter the function of CHS.

#### *Chromophoric Dissolved Organic Matter and Carbon*

Another factor that could impact stream primary production is optical properties of the water (i.e. CDOM concentration). Phytoplankton growth in blackwater rivers is often primarily light limited due to light attenuation by CDOM, which includes dissolved organic humic and fulvic substances that preferentially absorb PAR in the critical blue-green region on the visible spectrum (Phlips *et al.*, 2000; Gallegos, 2005), however bioassay experiments including light treatments found that light limitation in two CHS, FRN-1 and TAR-4, is of secondary importance to nitrogen limitation (Couper, 2014). Therefore, stream CDOM is likely not a limiting factor of primary productivity in NewRE CHS as was originally hypothesized. Streams were likely shallow enough that light attenuation from CDOM even in the most blackwater

stream, FRN-1, is usually insufficient to limit water column or sediment chl-*a* production. Additionally, canopy cover and suspended particulates (including phytoplankton) both contribute to light attenuation in streams and determine the amount of irradiance reaching stream and benthic primary producers in blackwater rivers and streams (Vähätalo *et al.*, 2005). Results from light limitation bioassays were confirmed by the lack of correlation between daily PAR, which accounts for differences in stream canopy cover, and GPP during two-station experiments. Only one significant correlation was detected between CDOM and NDM and ER – spring NDM and ER decreased as CDOM concentration ( $a_{355}$ ) increased. This could indicate slight light limitation during spring, but is probably driven by carbon availability and lability. Because of the nature of CDOM entry into streams while runoff percolates through soils, CDOM is typically positively correlated with DOC concentration. Therefore, a correlation between CDOM and NDM could alternatively indicate that a change in carbon source or concentration was stimulating ER in less developed, more blackwater CHS.

Historically blackwater streams are characterized by a large DOC pool (Meyer 1990). Highest seasonal DOC concentrations were observed at three of the four streams during summer, likely because of low flows (Muholland, 1981). Seasonal DOC lows occurred during fall at TAR-4 and FRN-1 and during winter at COG-3 and TRP-2. Development of NewRE CHS watersheds may be linked to decreasing DOC concentrations in streams, a trend also observed in South Carolina coastal streams (Wahl *et al.*, 1997), though NDM was correlated with DOC only in spring. Therefore, DOC availability in all streams is ample and not a limiting factor of respiration during much of the year. However, POC – although not a significant fraction of the overall carbon pool – was significantly related to two-station net daily metabolism and may drive

differences in NDM in these streams during portions of the year, particularly during fall when litterfall likely contributes large inputs of POC to streams (directly and via overland flow). POC concentrations were also high during spring. Winter NDM was not correlated with POC when litterfall inputs were probably minimal and POC concentrations were low. Spring two-station metabolism results indicated that NDM was positively correlated with POC concentration and the highest average seasonal POC concentration was observed in spring at TAR-4. This anomalous finding can be explained by increased phytoplankton growth during spring, which would contribute to increased POC in streams – particularly at TAR-4, which had high GPP during spring. Although regressions were not significant, POC concentration appears to increase with watershed development and significant negative correlations observed between POC and NDM indicate that POC coming from developed watersheds is not entirely recalcitrant, but likely labile and available for heterotrophic respiration, which leads to decreased NDM.

#### *Coastal Streams in a Human Dominated Landscape*

The contemporary landscape of coastal watersheds includes many varied types of land development, which can have identifiable influences on the function of aquatic systems. In the case of the NewRE watershed, the presence of MCBCL controls land development within certain regions of the watershed. FRN-1 served as the reference stream because developed land and impervious surface cover were minimal. This reference stream has a number of defining factors characteristic of blackwater coastal streams – high CDOM, high DOC, and low nutrient concentrations, and highly heterotrophic metabolism. Other study streams exhibited an increasing gradient of land use, which could be compared back to the baseline CHS, FRN-1.



Watershed development causes various alterations, which appeared to modify the function of CHS, indicated by differences in metabolism. Impacts such as increased nutrient concentrations, decreased CDOM, and decreased DOC with percent development and percent imperviousness lead to streams that were less heterotrophic overall (Fig. 12). These impacts were particularly evident during spring. However, one-station metabolism results provided context of entire year and, despite detectable differences in metabolism parameters, GPP only increased slightly and

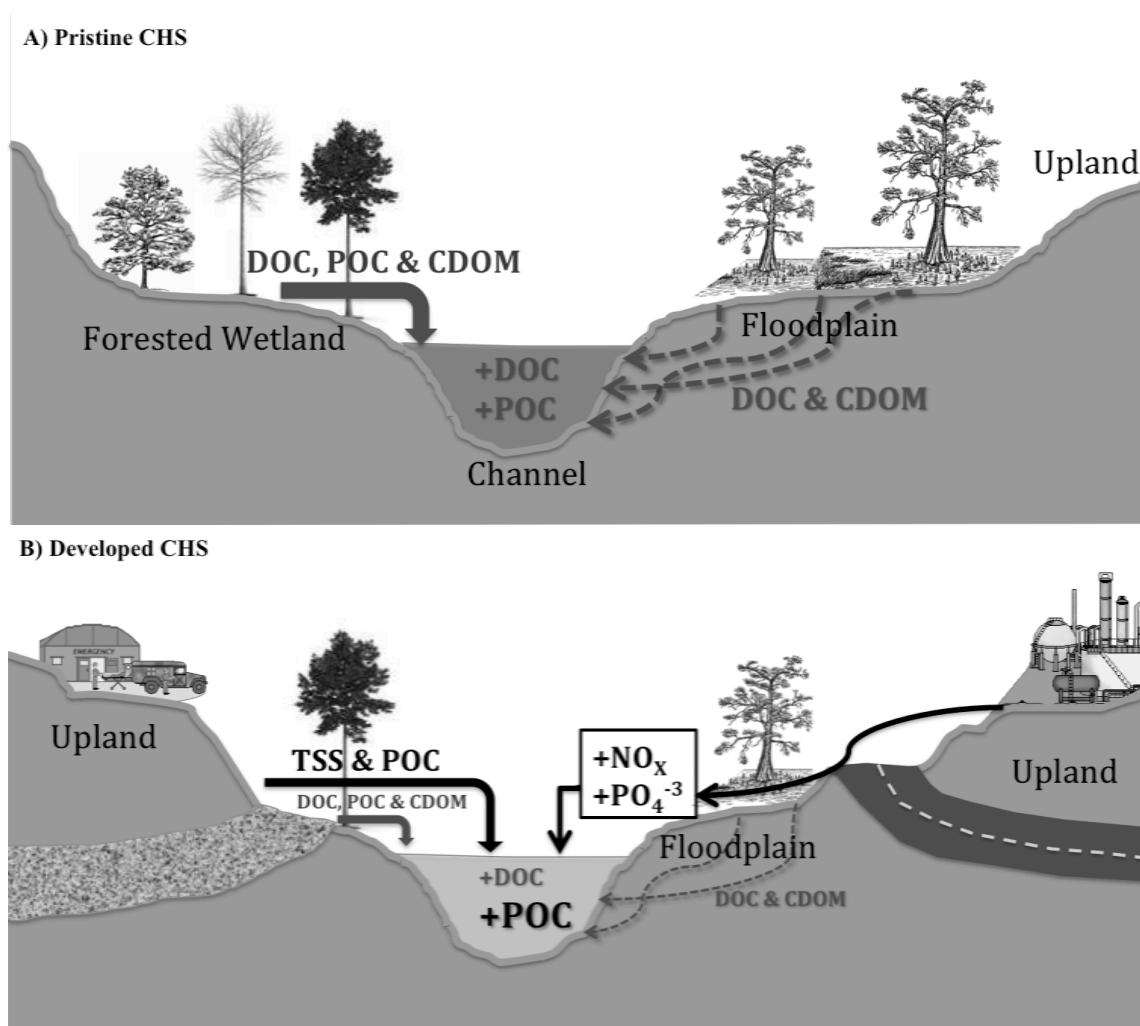


Figure 12. Conceptual diagram illustrating differences between inputs to pristine (A) and developed (B) CHS given findings of this study. Diagram shows natural overland (solid arrows) and groundwater (dashed arrows) inputs of dissolved organic carbon (DOC), particulate organic carbon (POC), and chromophoric dissolved organic matter (CDOM) in gray. Development driven inputs of nitrogen ( $\text{NO}_x$ ), phosphorous ( $\text{PO}_4^{-3}$ ), total suspended solids (TSS), and POC are shown in black.

developed streams never exhibited net autotrophy. Therefore, the influence of watershed development on autochthonous production was minimal – though continued anthropogenic pressures or loss of stream canopy cover could trigger a change in net metabolism.

Watershed development may alter coastal headwater stream function in the context of the carbon cycle, however. NDM was relatively constant across streams considered in this study and streams were heterotrophic during all portions of the year. Despite a slight decrease in heterotrophy in developed streams, when considered within the context of other upland streams, heterotrophy in coastal headwater streams still greatly exceeds that of other small streams. Carbon is the fuel for respiration and watershed development driven changes in POC were associated with changes in respiration in NewRE CHS. This new carbon delivered to the streams in particulate form is fixed using new nutrients brought in from watershed development.

## CONCLUSIONS

Whole-stream metabolism was successfully measured in coastal headwater streams (CHS) using both single- and two-station methods and found that streams were highly heterotrophic year-round, exhibiting more heterotrophy than is typically seen in other small streams (Objective 1). Both whole stream-metabolism methods were applicable in CHS, but considerations need to be taken for future work, including appropriate stream reach length for detectable DO change and accurate tracer experiments, quantifying groundwater inputs when applicable, and sonde placement within the stream. The degree of stream heterotrophy decreased with increasing watershed development, particularly during spring and was likely driven by changes in carbon and nutrient concentrations coincident with development trends (Objective 2). This trend was initially evident in spring diel DO excursions, which were minimal in pristine streams and more pronounced in developed streams. Compared to whole-stream metabolism, information about diel DO regimes is easy to obtain and could be used as an indicator of human impact in CHS that are experiencing a shift towards decreased overall heterotrophy.

Optical properties (CDOM) had minimal impact on stream metabolism, but regressions with nutrients indicated nitrogen limitation in freshwater CHS (Objective 3). Carbon analyses indicate that DOC dominated in-stream carbon concentrations and was not a limiting factor of respiration except during spring. POC inputs were labile and stimulated respiration during the fall and possibly summer seasons. Additionally, DOC concentrations seemed to decrease with

watershed development, whereas POC concentrations may have increased. Very little terrestrial carbon accumulates in the ocean, therefore decomposition must occur within the coastal zone (Cole & Caraco, 2001; Cole *et al.*, 2007). High respiration rates in CHS indicate that these systems may be important for processing of terrestrial carbon and as sources of CO<sub>2</sub>. Therefore, further research in these systems may help elucidate portions of the global carbon cycle. Additionally, feedbacks likely to occur because of development and climate change induced warming of streams could alter heterotrophy in coastal streams and further increase their importance within the carbon cycle. In general, the quantity of carbon exported to downstream estuaries from developed CHS will likely decrease, but the carbon will be more labile in quality.

Results of this work also have implications for coastal land management. Estuarine and coastal water quality can be greatly impacted by development upstream (Valiela *et al.*, 1992, for example), however decisions that affect coastal streams structure and function are more often made locally. This work is performed at the scale of for which management decisions are made and which would allow for educated development decisions to be made. We present a conceptual model of CHS in human dominated coastal watersheds (Objective 4; Fig. 12) that will hopefully begin to provide context for watershed development decisions at the scale of local impacts on coastal streams.

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**Software**

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