

STREAM NUTRIENT MONITORING VIA IN SITU WET-CHEMISTRY
INSTRUMENTATION

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ABSTRACT

An in situ wet chemistry autoanalyzer was used to simultaneously monitor nitrate, phosphate and ammonium concentrations in arctic tundra streams. The objectives of this work were to assess whether the specific instrument, an Autonomous Profiling Nutrient Analyzer (APNA) can be reliably used for stream ecosystem research in remote field environments and to evaluate whether this type of instrument will be useful for long-term stream monitoring programs.

Field work was conducted over two summers (2010 and 2011) near Toolik Field Station, Alaska. Two sampling regimes were used with the instrument: first, time-interval sampling was conducted where hourly measurements of nitrate, phosphate, and ammonium were made over deployments of up to two weeks. Second, continuous monitoring (one second data return for all analytes) of slug nutrient additions was performed to test the instrument utility in stream nutrient uptake experiments.

Inverse diurnal oscillations of nitrate and ammonium were observed during a time-interval deployment during baseflow conditions. Nitrification during ammonium slug injections was also seen. Based on these results it is clear that in stream processing of nutrients on a short time-scale is of major importance in these systems. Validation of nitrate and phosphate concentrations based on comparisons between in situ measurements and laboratory analyzed grab samples showed a close relationship. In situ ammonium measurements were imprecise, likely due to the deterioration of photo-sensitive reagents.

For future use in stream research it is recommended that the OPA analytical method for ammonium be substituted for the nitroprusside method, which will be less sensitive and less likely to degrade over a deployment of several weeks. Despite this, the APNA proved to be robust with several uses in stream ecological research. This instrument has proven to be reliable in a challenging field environment, useful for long term monitoring programs, and has shown potential to advance our general knowledge of fine time-scale stream nutrient cycling.

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CHAPTER 1: INTRODUCTION

Biogeochemical research on streams has greatly advanced our understanding of complex in-stream processes and the relationships and connectivity between fluvial systems and their surrounding landscapes. New methods of approaching stream water research that take advantage of new technologies are constantly emerging. In situ analysis of hydrochemical constituents, including inorganic nutrients such as nitrate and phosphate, is becoming more frequently used in basic monitoring and to examine in-stream transport and processing dynamics.

In the following thesis project an in situ autoanalyzer was used to simultaneously measure concentrations of nitrate, phosphate, and ammonium in remote arctic tundra streams on Alaska's North Slope. The specific instrument used in this investigation was an Autonomous Profiling Nutrient Analyzer (APNA) by SubChem, Inc. The APNA is a field-ready, wet chemistry autoanalyzer capable of continuous or programmed time-interval sampling. This type of instrument is more commonly used in oceanographic studies and is rarely used in limnological studies. However, there is a growing realization that this type of instrumentation could provide valuable new information about biogeochemical dynamics in stream ecosystems. For example, diurnal oscillations of stream nitrate concentration have been reported in several recent studies, a pattern which is more easily captured using in situ nutrient analyzers with the capability of high temporal frequency measurements. Discovery of these patterns sheds new light on short time-scale nutrient processing in streams.

Our field sites were in the vicinity of the Arctic Long-Term Ecological Research (ArcLTER) site at Toolik Field Station, Alaska. Our rationale for exploring the use of this instrument at the ArcLTER site was to evaluate whether this type of instrument could be used reliably in remote field environments. Secondly, we wanted to evaluate whether this type of instrument was useful for long-term monitoring programs such as the National Environmental Observatory Network (NEON). The ArcLTER site is the arctic domain site for NEON and so there is direct interest in the utility of this type of instrument there.

Two field seasons were included in this thesis project. The first season, 2010 (Chapter 3), served as an opportunity to learn how the instrument would function outside of the laboratory and ultimately yielded valuable insight into how the instrument could be better operated in later deployments to produce accurate and useful data. In the 2011 field season (Chapter 4), the APNA was used both in nutrient injection monitoring and hourly time-interval sampling, each producing notable findings. Examples included observation of nearly instantaneous nitrification during ammonium nutrient additions and the detection of inverse diurnal oscillations of nitrate and ammonium for several days during baseflow conditions.

CHAPTER 2: COMPREHENSIVE LITERATURE REVIEW

2.1. Introduction

Stream and river systems transport terrestrially-derived constituents (sediments, dissolved nutrients, etc.) to larger rivers, lakes and eventually coastal systems (Wollheim et al, 2006). While advancing through these systems, inorganic nutrients in dissolved form are affected by an assortment of in-stream biological and abiological processes. Autotrophs and heterotrophs assimilate and release nutrients within the biotic compartment. Adsorption and desorption to/from sediments occurs as chemical constituents move downstream. Transformations also occur through the process of mineralization by microbial activity.

In situ nutrient analyzers are being used more often in stream research. These instruments are capable of making high temporal resolution measurements without substantial human labor. Attaining data on this scale, as opposed to less frequent sampling (hourly vs. daily sampling, for example), can allow for the description of fine-scale hydrochemical patterning for extended durations. The ability to recognize such patterns can allow researchers to better understand stream nutrient cycling and more accurately assess the dynamics controlling stream ecosystem function.

This chapter will introduce concepts in stream ecosystem nutrient cycling and review the current extent of in situ nutrient analysis in aquatic studies. Currently, there is not substantial information pertaining to the use of in situ instruments capable of simultaneously measuring nitrate, phosphate and ammonium in freshwater stream systems. The study which follows will examine whether the Autonomous Profiling

Nutrient Analyzer (APNA) can reliably be used to monitor nutrient concentrations in a remote arctic headwater stream system.

2.2 Nutrient Cycling in Stream Systems

2.2.1 Major Stream Nutrients

Rates of primary production and heterotrophic microbial activity are greatly influenced by nitrogen (N) and phosphorus (P) availability. The supply of these two nutrients can vary substantially in and among stream ecosystems, both in space and time. Nutrient concentrations vary due to a variety of extrinsic factors including differences in watershed characteristics such as land-use history, terrestrial vegetative composition, watershed geology and hydrology. In addition, nutrient concentrations vary due to intrinsic stream characteristics like geomorphology, in-stream nutrient processing ability or sorption/transformation efficiency (Bernhardt et al., 2002). Seasonal (or shorter timescale) changes in nutrient supply and concentration can be due to changes in discharge, temperature, evaporation, and biological transformations, among others, adding a temporal component to nutrient variation (McNamara et al., 2008).

2.2.2 Nitrogen

Nitrogen occurs in many chemical states in freshwater ecosystems. Ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) together make up a portion called dissolved inorganic nitrogen (DIN). Autotrophs and heterotrophs are able to incorporate nitrogen in this form either through uptake or assimilation, whereupon it is transformed into organic nitrogen. Dissolved organic nitrogen (DON) consists of amino-nitrogen

compounds (polypeptides, free amino compounds) and other organic molecules, whereas most particulate organic nitrogen (PON) is associated with bacteria, algae, zooplankton and detritus (Allen and Castillo, 2009). Total N includes the sum of dissolved organic and inorganic nitrogen, as well as particulate nitrogen.

Transformations of nitrogen can occur either as organisms obtain N for structural synthesis (assimilatory uptake) or during energy-yielding reactions (dissimilatory transformations) such as nitrification and denitrification. Autotrophs and heterotrophs remove nitrogen from solution in the water column, a process referred to as immobilization, for biological uptake and incorporation of nutrients into new tissue for growth and development. Ammonium is taken up more readily than nitrate, which requires additional energy to convert (or reduce) to ammonium before assimilation can occur (Allen and Castillo, 2009; Kemp and Dodds, 2002).

In the process of nitrification, specialized bacteria obtain energy by using ammonium as a fuel to produce nitrite and nitrate. In denitrification, bacteria use nitrate as an oxidizing agent, eventually forming atmospheric nitrogen (N_2) (Allen and Castillo, 2009). Nitrifiers fix carbon dioxide (CO_2) from the energy gained by oxidizing the cation NH_4^+ to the anion NO_3^- . The production of NO_3^- is important in cases where nitrification and denitrification are closely linked (Cooke and White, 1987). Stream sediments can become the dominant source of NO_3^- to denitrifiers, especially when inputs of NO_3^- to the stream ecosystems are low (Duff and Triska, 2000). This may play an especially large role in Arctic headwater streams, where nutrient concentrations can be extremely low.

Bernhardt et al. (2002) examined the degree to which in-stream nitrification controls variation among streams in ambient nitrogen levels as well as stream transport and watershed export of inorganic nitrogen in 13 stream reaches in Hubbard Brook Experimental Forest. Nitrification rates were measured by monitoring the NO_3^- produced during short-term constant rate injections of $\text{NH}_4^+ + \text{Cl}$. They found a great deal of variability among stream reaches for rates of nitrogen uptake, the relative demand for NH_4^+ versus NO_3^- , and the potential for nitrification within stream sediments. Results from the study suggested that in-stream nitrification in the Hubbard Brook streams could not sufficiently explain the variation among streams in ambient NO_3^- concentration. The authors suggested that the low NH_4^+ concentrations present could not produce enough NO_3^- to dramatically alter NO_3^- concentrations. Rather, the ambient NO_3^- concentration, itself, may indirectly influence nitrification rates by facilitating a competitive demand for NH_4^+ between heterotrophs and nitrifiers (Bernhardt et al., 2002).

Although it was not the case in Hubbard Brook streams, it is possible that with high NH_4^+ availability, nitrification may produce sufficient NO_3^- to drive stream water NO_3^- concentration. While uptake rates are typically higher for NH_4^+ than NO_3^- , NH_4^+ is usually a small fraction of DIN as compared to NO_3^- in streams (Allen and Castillo, 2009).

In arctic tundra streams this may not be the case. McNamara (2008) explains that tundra vegetation in these watersheds is extremely nutrient limited and retains inorganic N efficiently. Nitrate mobility is limited because N that is added to the tundra by nitrification, deposition, or mineralization is quickly taken up by plants.

Tundra soils tend to be easily waterlogged and are often anoxic due to shallow active layers underlain by permafrost and to low hydrologic gradients (Gebauer *et al.*, 1995). Because ammonium cannot be oxidized to nitrate in such conditions, nitrification is strongly inhibited. Therefore, the ratio of ammonium to nitrate should remain high in the tundra soil water.

When these soil waters reach stream banks, however, there is greater potential for exposure to oxygen and the ratio of ammonium to nitrate decreases (McNamara *et al.*, 2008). If ambient concentrations of nitrate in these streams are low, then, lateral inputs from soil water may contain a high enough ammonium load for nitrification to strongly influence nitrate concentration in the oxic stream environment.

2.2.3 Phosphorus

As with the case of nitrogen, phosphorus is present in stream water in several forms, both dissolved and particulate. Dissolved forms consist of inorganic phosphate readily available for uptake and assimilation as well as various organic compounds. Particulate forms consist of complexes with inorganic substances such as clays, iron hydroxides, hydroxides, and detritus as well as cellular components such as enzymes and vitamins (Hendricks and White, 2000). Total phosphorus (TP) encompasses all of these forms, dissolved and particulate, organic and inorganic.

In contrast to nitrogen, the principal reservoir for P is rocks and sediments. It is released slowly by weathering and in unpolluted waters often in short supply relative to metabolic demand (Allen and Castillo, 2009). Phosphorus generated from plant

breakdown and stored in the soil organic layer is an important input to streams, entering through surface runoff and subsurface pathways (McDowell et al., 2001).

Phosphorus in stream water is influenced by biological, physical and chemical and processes. Biotic processes include assimilation by vegetation, plankton, periphyton, and microorganisms. Abiotic processes include sedimentation, adsorption/desorption to and from charged clays and organic particles, precipitation and exchange processes between soil and overlying water column (Reddy et al., 2005). In addition, chemical processes influence phosphorus under aerobic conditions. Dissolved inorganic and organic P may combine with metal oxides and hydroxides to form insoluble precipitates (Allen and Castillo, 2009).

2.3 Solute Characteristics

A solute is a substance dispersed within another substance, often water. Knowledge of solute dynamics is essential to interpret physical and chemical processes in stream systems. In the context of flowing streams (lotic systems) solute dynamics refer to the spatial and temporal patterns of dissolved materials transported and transformed in water (Stream Solute Workshop, 1990). Studies of solute dynamics provide information on the rates of transport and transformation of solutes and can help quantify specific hydrodynamic properties of streams such as hyporheic flow (Webster and Valett, 2006). Quantification of such properties can be achieved through the use of model equations relating solute concentrations to characteristics such as advection, dispersion,

groundwater and tributary inputs, etc., which help to illustrate solute transport and exchange processes (Stream Solute Workshop 1990).

2.3.1 Conservative vs. Non-Conservative Solutes

Two types of solutes will be discussed in this review. First, dissolved nutrients such as common inorganic forms of nitrogen and phosphorus are referred to as non-conservative solutes. Non-conservative solutes are readily altered by biological and abiological processes within streams. In contrast, solutes such as chloride and bromide are conservative. Conservative solutes are not altered by biological means, or, as in the case of chloride, exist in concentrations that far exceed biological need (Webster and Valett, 2006). They also are not chemically reactive, and are not changed by physical processes. The terms conservative and non-conservative are widely used in literature pertaining to stream solute dynamics (eg., Gooseff and McGlynn, 2005; Gooseff et al, 2005; Payn et al, 2005; Runkel, 2007; Stream Solute Workshop, 1990; Webster and Valett, 2006; Zarnetske et al, 2007).

Conservative solute dynamics are slightly less complex than non-conservative dynamics in that there is neither consumption nor production of the solutes by in-stream processes (Webster and Valett, 2006). Although they affect all solutes, advection and dispersion are the two fundamental processes that drive conservative solute dynamics. Advection is the transport of a solute mass by the actively flowing water body. A solute mass is carried downstream by advective transport at a rate determined by the average velocity of the stream. Dispersion, on the other hand, is the spreading of a solute mass, which results from molecular diffusion or from a shear stress such as turbulence in

streams (Runkel, 2007). This is the major mechanism responsible for the enlargement of a solute cloud while the center of that cloud is moved downstream by advective transport (Ng, 2000).

The dynamics of non-conservative solutes, on the other hand, include both biotic and abiotic processes. Biotic processes that affect non-conservative solute dynamics include heterotrophic uptake, plant uptake, and mineralization (Webster and Valett, 2006). Heterotrophic uptake of solutes occurs as organisms such as bacteria incorporate them into biomass. Uptake of solutes by plants, likewise, occurs as these organisms take in solutes for use in their own biotic processes of growth and development. Mineralization is the conversion of organic substances to inorganic substances. Nitrogen mineralization, for example, occurs when an organic nitrogen compound is converted to an inorganic form such as ammonia by microbial activity.

Examples of abiotic processes that affect non-conservative solutes include adsorption, desorption, precipitation, and dissolution (Webster and Valett, 2006). Adsorption is the accumulation of molecules onto some solid surface whereas desorption is the release of molecules from a surface. Precipitation describes the formation of a solid from dissolved molecules, while dissolution is the passing of a solid into the solute form. In summary, adsorption and precipitation represent abiotic removals of solute, while desorption and dissolution contribute to solute addition in the water column.

2.3.2 Instream vs. Hyporheic Transient Storage

Transient zones of streams are those areas in which the flow experiences delayed downstream transport when compared with the open flowing channel, or thalweg

(Zarnetske et al, 2007). The water located within a transient zone in a stream is said to be in transient storage. Transient storage zones occur either in the form of in-stream storage such as immobile pools and eddies in the active stream (also known as in-channel dead zones), or as hyporheic zones within streambed sediments (Gooseff et al, 2005). In-stream and hyporheic storage zones are controlled by the prevailing physical conditions of the stream, such as discharge, channel structure, and bed composition (Zarnetske et al, 2008).

Hyporheic zones lie below and/or lateral to a stream channel and serve as an exchange site between ground and stream waters. Consequently, the hyporheic zone is an important location for nutrient exchange between ground and surface waters. Advective flow into and out of the hyporheic zone takes place in three dimensions, comprised of a longitudinal, vertical, and lateral component (Jones and Holmes, 1996). Longitudinal hyporheic flow occurs as an overall downstream movement of water which transfers between the surface and subsurface of the stream bed. The vertical component of hyporheic flow is driven by the vertical distribution of hydraulic head, which is often influenced by stream bed topography (Harvey and Bencala, 1993). Lateral flow in hyporheic zones occurs as water flows beneath channel bars in meandering streams.

2.4 Stream Nutrient Uptake

2.4.1 Nutrient Spiraling Concept, Uptake Measures and Quantification

Assessing biological uptake of nutrients in streams is important to basic ecological research and management issues concerning transport of nutrients by streams.

Studies of nutrient uptake and retention, therefore, are not only essential to understanding the impact and possible remediation of human activity on global nutrient cycles, but are also needed to assess stream ecosystem function and allow for comparisons of response to management actions (Payn et al, 2005).

Studies of solute dynamics coupled with mathematical models can be used to estimate stream hydrologic and solute retention properties (Stream Solutes Workshop 1990). Solute is added to streams to examine their physical and biological dynamics such as discharge, storage properties and nutrient uptake. In this approach, a solute of a known concentration is added at the top of a stream reach and is monitored at one or several locations downstream. The addition of a solute can take place either as a sudden pulse (slug addition) or at a slow and continuous rate over a period of time (constant rate addition).

Constant Rate Addition (CRA) experiments require that a solute of a known concentration is added to the stream at a fixed volume per unit time. This is performed while concentration is monitored at downstream sampling locations with the objective of raising the concentration to a stable level. This plateau in solute concentration indicates that the total mass of tracer residing in the reach is at a “steady state” (Payn et al. 2008). That is, the plateau indicates that uniform mixing and even distribution of the solute has occurred within the stream reach (Stream Solutes Workshop, 1990). At this point, stream water samples are extracted at predetermined downstream sample locations for later analysis.

Instantaneous Addition (IA) experiments, on the other hand, consist of introducing a single volume or “slug” of a known mass of dissolved tracer to the stream in a very short amount of time (within seconds). The length of time required for this addition is considered instantaneous because it is negligible relative to the time of advective transport through the stream reach (Payn et al. 2008).

In studies of nutrient uptake in streams, solute addition experiments have traditionally involved the CRA technique, where a non-conservative solute is coupled with the injection of a conservative tracer (Runkel, 2007). The concentration of conservative and non-conservative solute, and therefore the ratio of one to the other, is known at the point of injection. A comparison can then be made between the concentration of the injectate upstream and that detected downstream for the two tracers over time. Decreases in the conservative solute concentration with distance downstream are used to correct for the effects of dilution by groundwater or lateral inputs. Accounting for the changes in concentration of the non-conservative tracer relative to the conservative tracer in this way corrects for the effects of physical dilution and allows the observer to assess the biological, chemical, and/or physical processes acting on the non-conservative tracer alone.

In these experiments, an important objective is to determine the average distance traveled by dissolved nutrients before uptake, or uptake length (S_w). Uptake length is a core component of the nutrient spiraling concept (Newbold, 1981). Nutrient spiraling is a cycling model in which dissolved nutrients are transported downstream until the point of their removal from the water column by a process such as biological uptake, and later

return to solution in the stream waters. As some nutrient molecules travel farther downstream than others before uptake, S_w is reported as an average distance. Upon release of the nutrient from the biotic compartment, the cycle continues with that molecule now being available for uptake or assimilation downstream. Uptake length is also a measure of nutrient use efficiency. That is, uptake length is a measure of uptake relative to supply, and is measured as the inverse of the fractional rate of nutrient uptake from water per unit stream length, giving S_w units of distance (Mulholland et al. 2002).

Other components of nutrient spiraling include the measures of uptake velocity (v_f) and areal uptake rate (U). Uptake velocity corrects uptake length for effects of discharge (stream velocity and depth), and is calculated by:

$$v_f = \frac{u * z}{S_w}$$

where u is stream velocity and z is depth (Webster and Valett, 2006). Since uptake velocity standardizes uptake length for effects of discharge, it provides a more appropriate variable for comparing solute dynamics among different streams. Areal uptake rate (U) represents the immobilization of a nutrient on a per area per time basis and is calculated by the equation:

$$U = v_f * C$$

where C is the ambient nutrient concentration. Uptake rate reflects the magnitude of the flux of inorganic element from the water column to the biota (Webster and Valett, 2006).

Each component of nutrient spiraling mentioned above (S_w , v_f and U) has a unique purpose in studies focused on describing nutrient dynamics in stream systems.

Areal uptake provides valuable information on biotic consumption, but no information on the spatial characteristics of nutrients. Uptake length gives a reach-scale estimate of nutrient retention, while uptake velocity is a more practical measure for cross-system comparisons, as it corrects for the effects of discharge (Webster and Valett, 2006).

Measuring nutrient uptake by artificially elevating nutrient concentrations, as in the case of the CRA and IA injections mentioned above, causes an increase in uptake rate (Payn et al, 2005). Thus, a problem arises with nutrient addition experimentation in that a measurement may not actually describe the ambient rate of uptake, but an elevated rate instead. This can partly be explained by the concept of limiting nutrients. For example, if one essential nutrient for primary production is in short supply relative to other nutrients, it will be exhausted first. Organisms will thereafter be unable to increase rate of production. Once more of this limiting nutrient becomes available, however, organisms will increase rates of production, using larger amounts of this nutrient than before, until again exhausted or another nutrient becomes limiting. Thus, the measured rate of uptake during an experiment which enriches stream nutrient concentration has the potential to be an overestimate and therefore not a true representation of the ambient rate of uptake in the stream.

To compensate for this overestimation, Payn et al (2005) developed a method in which several nutrient additions are performed with increasing levels of solute concentration and the net uptake length for each level is determined. Ambient uptake length can then be estimated by using a regression of the measured values of uptake

length against the elevated nutrient concentration. An extrapolation is then performed to assume an uptake rate at the ambient nutrient concentration.

Another approach which compensates for overestimation of uptake due to nutrient enrichment, but requires only a single pulse addition, was presented by Covino et al. (2010). The Tracer Additions for Spiraling Curve Characterization (TASCC) methodology allows for characterization of nutrient spiraling across a wide range of concentrations from a single nutrient addition experiment. The TASCC method offers several benefits compared to continuous rate additions and breakthrough curve (BTC) integration, including improved confidence in estimates of ambient-spiraling metrics determined from nutrient additions, enhanced characterization of spiraling response curves, better assessment of stream nutrient saturation state and inner-system comparisons, as well as its applicability to large river systems (Covino et al., 2010). In this method, uptake rate (k_w) for each grab sample though a BTC is calculated by plotting the natural log of the nutrient : conservative solute ratio of injectate and each background corrected grab sample collected at the base of the reach against stream distance (Figure 2.1). The respective slopes of the lines derived from these data pairs are the (k_w) values for each grab sample. Uptake length (S_w) metrics are then calculated as the negative inverse of the k values (Figure 2.1).

The high data density used in the TASCC approach improves extrapolations to estimate ambient spiraling metrics. Covino et al. (2010) performed a comparison of nutrient uptake parameters measured by TASCC, BTC integration and plateau approaches which yielded no significant differences across methods.

2.4.2 Conservative Solutes in Uptake Experiments

Conservative solutes used in uptake experiments include various types of salts, fluorescent dyes or isotopic tracers. Salts used include chloride, bromide, lithium, potassium and magnesium, chloride being generally accepted as the most conservative of the commonly available solutes (Stream Solute Workshop, 1990). In many streams, however, chloride cannot be used due to relatively high ambient concentrations, partially due to winter road salting or geographic setting. Bromide can be used as a replacement conservative solute in such locations.

Fluorescent dyes such as Rhodamine WT (RWT) are also used as conservative solutes where detection at extremely low concentration is desired. Detection is possible by the use of a fluorometer, many of which have low-end detection limits in the hundredths of parts per billion. RWT is non-toxic at low concentrations, and under natural environmental conditions it will not react to form toxic contaminants. However, under forced laboratory conditions of low pH and high temperature (90°C), Rhodamine will react with high nitrite concentrations to form nitrosamine, a carcinogenic/mutagenic substance (Abidi et al., 1986). The conditions required for the formation of nitrosamines are unlikely to occur in nature because high temperature and low pH combined with high nitrite concentrations are not likely to be present in natural water systems. Furthermore, nitrite is unstable in natural water systems and is readily oxidized to nitrate by nitrifying bacteria (Abidi et al 1986).

Of greater practical importance, however, Laenen and Bencala (2001) noted that losses of mass of Rhodamine WT dye are typically observed in tracer studies. These

losses were attributed to photodegradation and sorption of RWT to streambed sediments. RWT decay rates were determined in their study and the time scales of RWT decay and storage process time were compared. It was found that time scales for decay were typically slower than transient storage times for the streams in question. With the notion that Rhodamine dye moves into and out of storage more quickly than the rate at which it decays, it can be suitable for use as a tracer for transient storage studies in streams and rivers.

Deuterium (2H) and tritium (3H) are isotopic tracers that have been used as conservative solutes in some studies. These are ideal hydrologic tracers in that they behave almost identically to water and demonstrate truly conservative behavior (Stream Solute Workshop, 1990). Analysis of samples is tedious and materials are expensive and as a consequence these isotopes are employed less frequently in stream solute addition experiments.

2.4.3 Fine Temporal Scale Nutrient Dynamics

Recently several investigators have noted that nutrient concentrations in some streams have regular, diurnal patterns that can provide useful insight into stream nutrient cycling (Hessen et al., 1997; Roberts and Mulholland, 2007; Heffernan and Cohen, 2010; Rusjan and Mikos, 2010). Diurnal nutrient oscillations are especially evident during times of high stream productivity, and can therefore vary seasonally with metabolism (Hessen et al., 1997). Detection of nutrient patterns at high temporal resolution can also provide information on nutrient processing and transport in the watershed as a whole and introduces the possibility of studying a wide range of environmental, physical, and

biogeochemical factors known to play an important, yet a highly changeable role in nutrient processing and transport (Rusjan and Mikos, 2010).

For example, Heffernan and Cohen (2010) showed how fine temporal scale patterns in nutrient concentration could be used to quantify autotrophic assimilation within stream ecosystems. In their study of a spring-fed stream in Florida, assimilatory N demand was calculated from diel nitrate oscillations based on the integrated difference between an estimated nitrate baseline (estimated using two different approaches) and the daily observed nitrate oscillation (Figure 2.2).

Heffernan and Cohen (2010) showed a strong relationship between GPP and N assimilation and suggested a link between stream metabolism and N uptake. In their system, N removal occurred predominantly through denitrification. The study demonstrated that high temporal resolution stream nutrient data can be used to quantify autotrophic assimilation and discriminate among N removal mechanisms as well as evaluate the dynamics of the processes.

2.5 High Temporal Resolution In Situ Data

In situ nutrient sensors are gaining popularity as a tool to capture high temporal resolution (hourly, for example) stream nutrient data, and for general nutrient monitoring in both freshwater and marine applications (Gardolinski et al. 2002; Thouron et al., 2003; Gilbert et al., 2008; Heffernan and Cohen, 2008; Pellerin et al., 2009; Rusjan and Mikos, 2010). These sensors can provide a wealth of information about short-term trends of which researchers may have been previously unaware. They have the potential to be

used in TMDL assessment, pollutant monitoring, and to help expand our knowledge of day-to-day and longer term nutrient dynamics in aquatic systems.

2.5.1 Current Instruments and Descriptions

The two main categories of state-of-the-art in-situ nutrient analyzers are optical and wet-chemistry sensors. Optical (reagent-free) sensors measure concentrations of dissolved chemicals based on characteristics of their absorbance spectra, often in the ultra-violet (UV) range (Johnson and Coletti, 2002). A variety of chemicals absorb UV light, each with a unique absorbance spectrum that allows for the direct determination of concentrations of these chemicals. Examples of nitrate-specific UV sensors include the In Situ Ultraviolet Spectrophotometer (ISUS, Satlantic, Inc.) utilized in Heffernan and Cohen (2010), the Submersible Ultraviolet Nitrate Analyzer (SUNA, Satlantic, Inc.), and the NITRAX sc UV Nitrate Sensor line (HACH, Inc.). Although UV analyzers require no reagents and do not produce chemical waste, their detection limits are not as low as those of wet-chemistry techniques (Adornato et al, 2007). Limits of detection for the sensors listed above are around $0.5 \mu\text{M NO}_3^-$ for both the ISUS and SUNA (manufacturer specification sheet) and $7.1 \mu\text{M NO}_3^-$ for the NITRAX sc UV Nitrate Sensor (manufacturer specification sheet).

In situ, wet-chemistry analyzers utilize techniques traditionally used in the laboratory to detect nutrient concentrations at very low levels, on the order of tenths or hundredths of μM . Nitrate can be assessed by cadmium reduction, soluble reactive phosphorus by molybdate and ascorbic acid colorimetric method, and ammonium by OPA fluorescence or nitroprusside colorimetric methods. Wet-chemistry analyzers

contain pumps for drawing sample water and adding reagents, have heating elements for temperature specific colorimetric reactions, and optical cells for determining light attenuation or fluorescence. In instruments currently on the market, reagents are kept separated in medical intravenous (IV) bags or cartridges to ensure longevity. The reagents remain stable for deployments of up to 3 months according to specifications provided by manufacturers. Examples of models of these instruments include the Autonomous Profiling Nutrient Analyzer (APNA) by SubChem Instruments, the MicroLab and other models by Envirotech, and the Cycle-PO₄ by WetLabs. These three instruments are similar in terms of the mechanical and electronic components within the units and the analytical methods used, but have different software and graphical user/interfaces. Detection limits for the APNA (which was used in this study) are around 0.11 μM NO₃⁻, 0.03 μM PO₄³⁻, and 0.18 μM NH₄⁺ (from manufacturer specifications and first-hand calibrations, see Chapters 2 and 3), around 0.15 μM NO₃⁻, 0.06 μM PO₄³⁻, and 0.15 μM NH₄⁺ for the MicroLab (manufacturer specification sheet) and as low as 75 nM PO₄³⁻ for the Cycle-PO₄ (manufacturer specification sheet).

2.6 Gap Analysis

Although frequency of their use continues to grow, in situ nutrient analyzers such as those mentioned above remain underutilized in freshwater stream and river research. There is currently a lack of studies that have utilized the Autonomous Profiling Nutrient Analyzer (APNA) in freshwater stream systems. Likewise, there is a lack of studies focused on monitoring high temporal resolution nitrogen dynamics in streams that

separate ammonium and nitrate trends. This study addresses these gaps by using an APNA to characterize nutrient patterns in six arctic tundra streams over two field seasons.

High temporal resolution nutrient patterns were measured at two temporal scales: first, hourly analysis for nitrate, phosphate, and ammonium was conducted over periods up to two weeks, and second, in an original approach to monitor short-term nutrient addition experiments to quantify nutrient uptake. In the latter, slug injections and constant rate additions were monitored at one second intervals for several hours for all analytes.

The purpose of this research is to enhance our understanding of stream water nutrient cycling by advancing the methodology by which stream nutrients are monitored and nutrient processing measures are quantified.

2.7 Literature Cited

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2.8 Figures

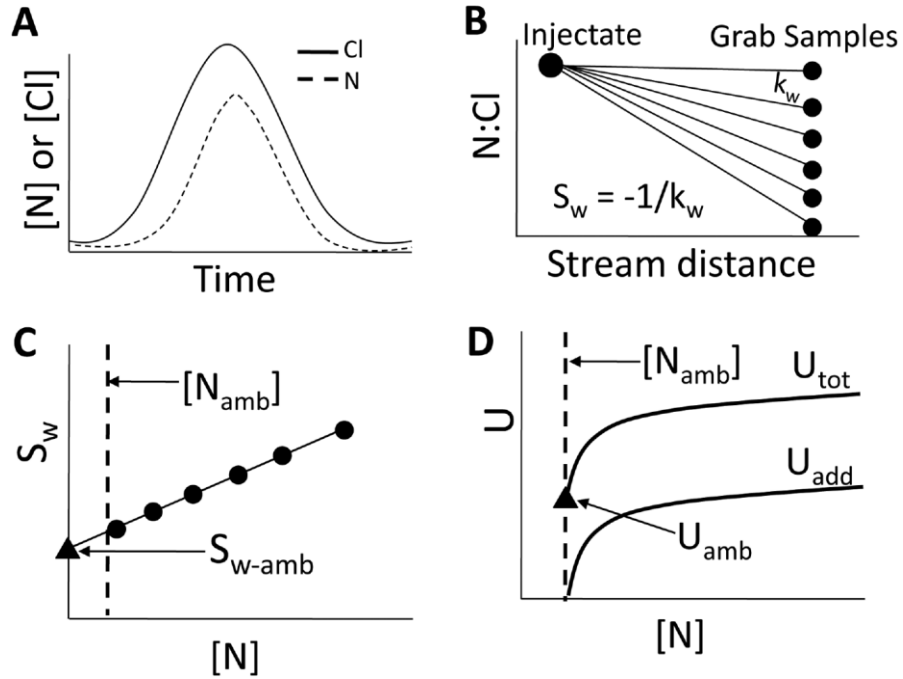


Figure 2.1: Conceptual: Diagram illustrating TASC approach from Covino et al, 2010

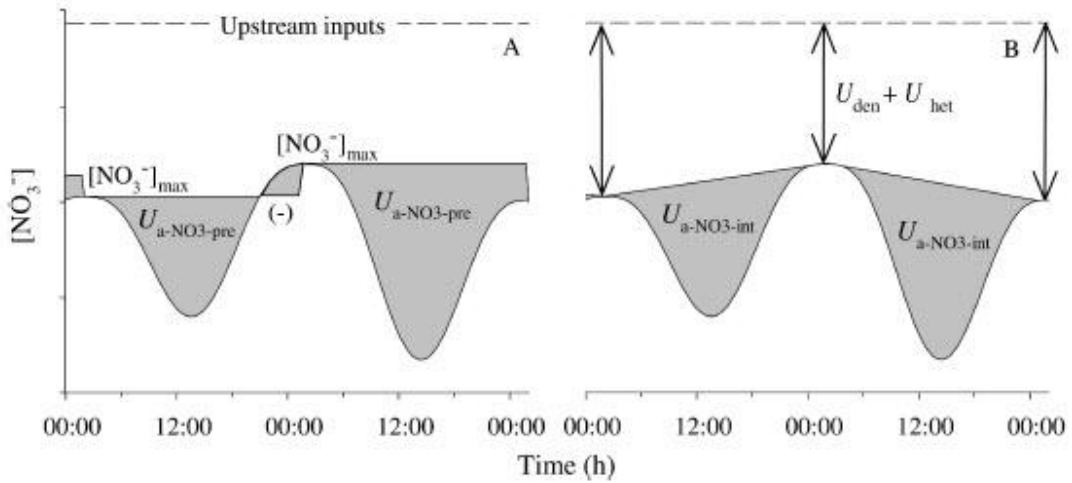


Figure 2.2: Estimating N assimilation and denitrification based on area integration of diel NO_3^- oscillations using (A) baseline set at previous day's maxima and (B) interpolated baseline between peaks. Arrows in (B) illustrate that the sum of denitrification (U_{den}) and heterotrophic assimilation (U_{het}) is calculated from the difference between inputs and NO_3^- max, (Heffernan and Cohen, 2010)

CHAPTER 3: IN-SITU NUTRIENT ANALYSIS IN FRESHWATER ARCTIC TUNDRA STREAMS: FIELD SEASON 1

3.1 Introduction

The summer 2010 field season was the first attempt to deploy the APNA in a remote field environment. The first season was intended to develop standard operating procedures for APNA field deployments and test whether the instrument would reliably function for an extended duration in a remote stream environment.

The APNA was deployed in 6 arctic tundra streams in 2010 with trial duration ranging from 3 to 8 days. Different instrument housings were tested and deployments spanned conditions of base flow and increased flow due to storm-events. Although some positive results such as diurnal oscillations of nitrate were observed, several malfunctions occurred during the first field season. Examples included a battery failure, clogged sample inlet filters due to suspended sediments and improper priming of reagent lines. These challenges yielded valuable insight into how to best operate the instrument in later deployments to produce accurate and valuable data.

3.2 Methods

3.2.1 Field Sites

The APNA was deployed 6 times in northern Alaska near Toolik Lake during the summer of 2010 (Table 3.1 and Figure 3.1.1). Sampling locations in the Toolik Lake Inlet-Series (I-Series) were used in this study because other related research projects were

also focused on these streams during the period of deployment and could provide valuable supplementary data. The I-Series catchment is 66.9 km² consisting of mainly of tussock-tundra and elevated areas of heath. Terrestrial vegetation includes sedges and grasses mixed with dwarfed birch, low willows and various forbs (Kling et al., 2000). Toolik Inlet, I-8 Outlet and I-8 Inlet have similar stream morphologies, largely consisting of cobble-bedded pool-riffle features. These differ from the Peat reach, which is beaded with deep pools (12 m wide and up to 3 m deep) connected by relatively narrow but deep (up to 1.5 m deep) runs. The reach is primarily peat-bedded with large macrophytes abundant in both pools and runs (Brosten et al., 2009).

Kuparuk Reference and Fertilized sites were chosen to examine differences in nutrient dynamics between the reaches as a result of an ongoing fertilization experiment. The Kuparuk is a clear-water tundra river with a drainage area of 143 km² above the intersection with the Dalton Highway (Kriet and Peterson, 1992). Terrestrial vegetation near the study reach is similar to that of I-series study sights with sedges and grasses mixed with dwarfed birch, low willow and forbs. As part of the Arctic Long Term Ecological Research (LTER) project, phosphoric acid has been added to the Kuparuk River throughout each summer since 1983 to evaluate the potential eutrophication of the arctic stream ecosystem (Slavik et al. 2004). The addition of phosphorus to the system has resulted in a drastic shift in ecosystem biota. Slavik et al. (2004) noted the positive response to fertilization at all trophic levels, including increases in epilithic algal stocks, some insect densities, and even fish growth rates. Furthermore, bryophytes, especially

Hygrohypnum spp., grew in the fertilized reach where they were absent before fertilization (Bowden et al., 1994).

3.2.2 Data Processing

A few hours (4-6 h) prior to each field deployment, a standard curve trial was performed using “internal” standard additions. By using the concentration of a known calibration standard (CAL) and measured injection flow rates (4 possible injection “set-points”), the concentration at each of the 4 injection set-points can be determined as:

$$(Q_{CAL} * C) / Q_{total}$$

where Q_{CAL} is the flow rate of calibration standard at a given set-point (liters per minute), C is the concentration of the standard solution (moles), and Q_{total} is the combined flow (liters per minute) of the sample inlet, reagents, and standard through the channel in question. Inferred concentration from each injection rate is plotted against its respective level of absorbance or fluorescence to create a standard curve for each analyte (Example, Figure). The analytical slope and y-intercept are used to in determining concentrations in “unknown” samples.

In addition to the stream samples taken each hour, an in-situ standard addition was performed every fourth hour. This was performed in a 3-step sequence;

1. stream sample + reagent
2. stream sample + reagent + low rate CAL addition
3. stream sample + reagent + high rate CAL addition

Analytical slope was calculated from each in-situ standard addition throughout the deployment. Taking into account all analytical slope calculations throughout the deployment, drift in analytical slope could be examined.

Grab samples were taken throughout the Kuparuk River deployments to compare results of nutrient analyses run in situ by the APNA to those run the laboratory. Grab samples were taken at the same time and location as samples being drawn by the APNA. Grab samples were filtered in the field using a 0.45 μM filter and were frozen until analysis.

3.2.3 Instrument Housings

The APNA was installed in stream or river channels using two types of housings in the 2010 field season. The first housing used was a rebar frame in which the APNA was placed beneath a separate reagent container. Though this would be a reasonable housing for a lake or marine profile, it was not well suited for long-term deployments in a stream channel. This housing offered relatively little protection from stream debris and the reagent reservoir was unable to sufficiently block light from reaching reagents and delivery tubing, possibly causing reagents to degrade rapidly.

The second housing was an 8 inch inner-diameter PVC pipe. With the housing upright, the APNA was seated in the bottom with the reagent IV bags held directly above it. This housing offered more protection to the APNA unit and was able to block light more efficiently and extend reagent life.

3.3 Results

The Kuparuk reference and fertilized reach deployments showed diurnal oscillations in nitrate and ammonium (Figure and Figure). During the reference reach deployment nitrate concentration fluctuated from 0 to 3 μM . Ammonium concentration fluctuated between 0.6 μM and 6 μM during this deployment and oscillations were less organized in terms of patterning than those of nitrate. Of concern in this data set is the rise in nitrate concentration over the first few hours of the deployment, which could indicate insufficient priming of the reagent lines. In this situation, reagents do not fully mix with sample for the first several hours of the deployment.

During the deployment in the fertilized reach of the Kuparuk River nitrate concentration fluctuated from below detection levels up to around 1.5 μM , ammonium concentration from below levels of detection up to around 6 μM . As was the case during the reference reach deployment, oscillation patterns in ammonium concentration were less smooth than those of nitrate. Noteworthy features in this data set are the scattered concentrations occurring during the first three days of the deployment and the gap in data around August 22. These noisy data occurred because the inlet filter rapidly clogged with suspended sediment mobilized by a high flow event. The extended rain event that caused high flows in the Kuparuk also caused the eventual depletion of the APNAs power source, resulting in the gap in nutrient data on August 22. Limited daylight over the span of several days prohibited the solar panel from charging the APNA's 12-volt battery. When the battery was completely depleted on August 22 the APNA was forced to shut down until the battery was recharged and measurements restarted on August 23.

A comparison of nitrate concentrations between the two methods shows that measurements made by the APNA were consistently lower than those in grab samples (Table 3.2 and Figure), on average by 0.68 μM (SD = 0.43). By contrast, ammonium measurements were typically an order of magnitude higher than those measured through grab sample analyses (Table 3.3 and Figure).

3.4 Discussion

Although no significant nutrient trends were observed during the first I-Series deployments, the deployments helped to assess the most effective ways to run the APNA in a field environment. After the first deployment in Toolik Inlet the housing for the APNA was upgraded. The newer housing protected the instrument better in the stream and prevented light from reaching the reagents. This is of particular concern for the ammonium OPA fluorescence reagent, which readily photo-degrades. At the beginning of the I-Series deployments and the Kuparuk reference reach deployment nutrient concentrations seemed to increase rapidly. This was probably not a “real” trend, and was most likely an issue resulting from insufficient priming of the reagent lines. Nutrient data from the two Kuparuk River deployments were useful, nevertheless, showing diurnal oscillations during periods not affected by high sediment load or failure of the power supply.

The 2010 field season provided valuable lessons for the study that followed in 2011. Specifically, we learned that it is critical to protect reagents from light, properly prime all lines immediately prior to deployments, and be mindful of flow and weather

conditions that could cause inlet filters to rapidly degrade and exhaust the instrument's power supply, respectively. We also learned how best to conduct the post-processing required to calculate nutrient concentrations. Estimates proved most accurate when based on "external" standard curves, which eliminate errors associated with the uncertainty of pump or flow rates through sample and reagent channels within the APNA. The experiences gained from the first field season were heavily drawn upon to produce valuable results in the second field season in 2011.

3.5 Literature Cited

Brosten, T. R., J.H. Bradford, J.P. McNamara, M.N. Gooseff, J.P. Zarnetske, W.B. Bowden, and M.E. Johnston. "Estimating 3D variation in active-layer thickness beneath arctic streams using ground-penetrating radar." *Journal of Hydrology*, 373(3-4), (2009). 479-486.

3.6 Tables

Table 3.1: 2010 field season APNA deployments sites and dates

Deployment #	Location Name	Dates (2010)
1	Toolik Inlet	July 9 – July 16
2	I-8 Outlet	July 21 – July 24
3	I-8 Inlet	July 24 – July 27
4	Peat	July 27 – July 30
5	Kuparuk Reference	August 12 – August 17
6	Kuparuk Fertilized	August 17 – August 25

Table 3.2: Comparison nitrate concentration measured in situ by the APNA and grab samples analyzed in the laboratory from the Kuparuk River deployments

Date/Time	APNA NO ₃ ⁻ (μM)	Grab Sample NO ₃ ⁻ (μM)
8/16/2010 20:17	0.45	2.00
8/17/2010 10:20	2.35	2.78
8/17/2010 17:05	1.11	1.71
8/18/2010 20:15	0.81	0.98
8/20/2010 16:20	0.32	1.06
8/23/2010 11:50	1.32	1.96
8/25/2010 11:18	1.77	2.37

Table 3.3: Comparison of ammonium concentration measured in situ by the APNA and grab samples analyzed in the laboratory from the Kuparuk River deployments

Date/Time	APNA NH ₄ ⁺ (μM)	Grab Sample NH ₄ ⁺ (μM)
8/16/10 20:17	5.18	0.58
8/17/10 10:20	4.16	0.38
8/17/10 17:05	8.29	0.84
8/18/10 20:15	2.21	0.39
8/20/10 16:20	3.17	0.78
8/23/10 11:50	1.99	0.42
8/25/10 11:18	3.65	0.32

3.7 Figures

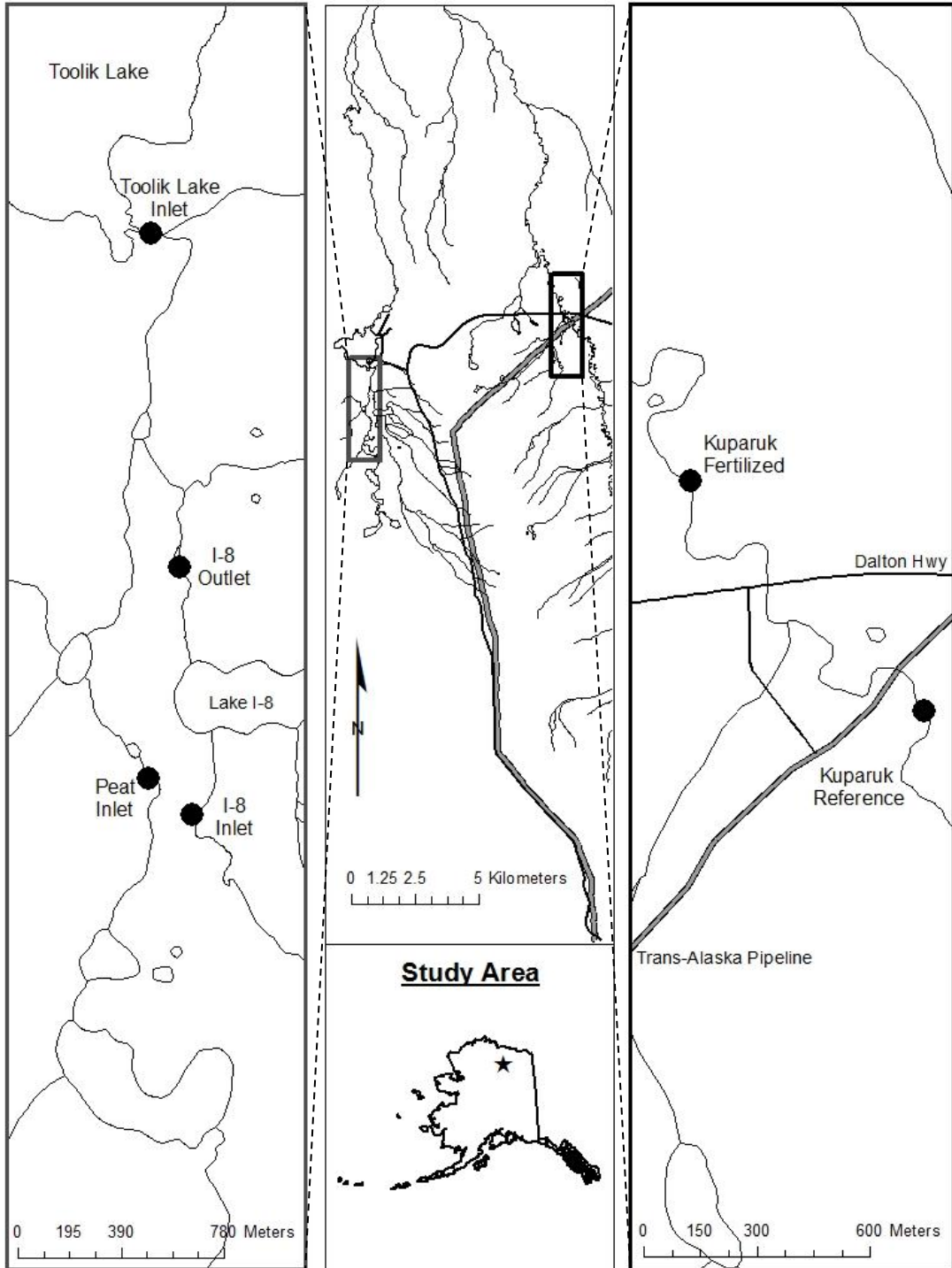


Figure 3.1: Summer 2010 APNA Field Deployment Locations on Alaska's North Slope

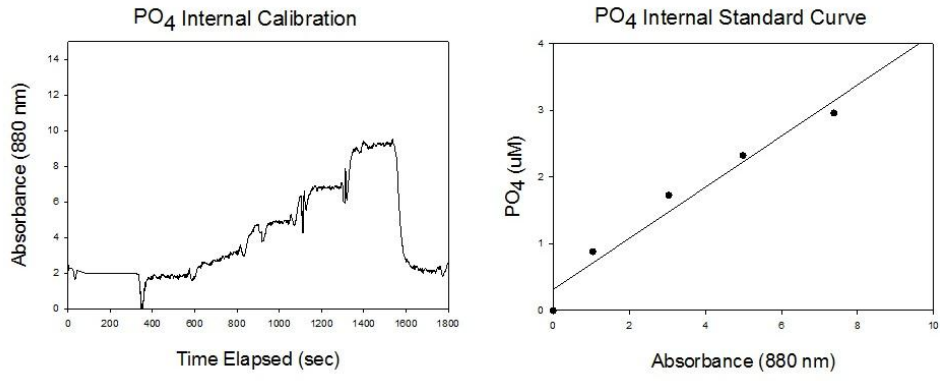


Figure 3.2: Example of a standard curve based on internal standard additions using the APNA

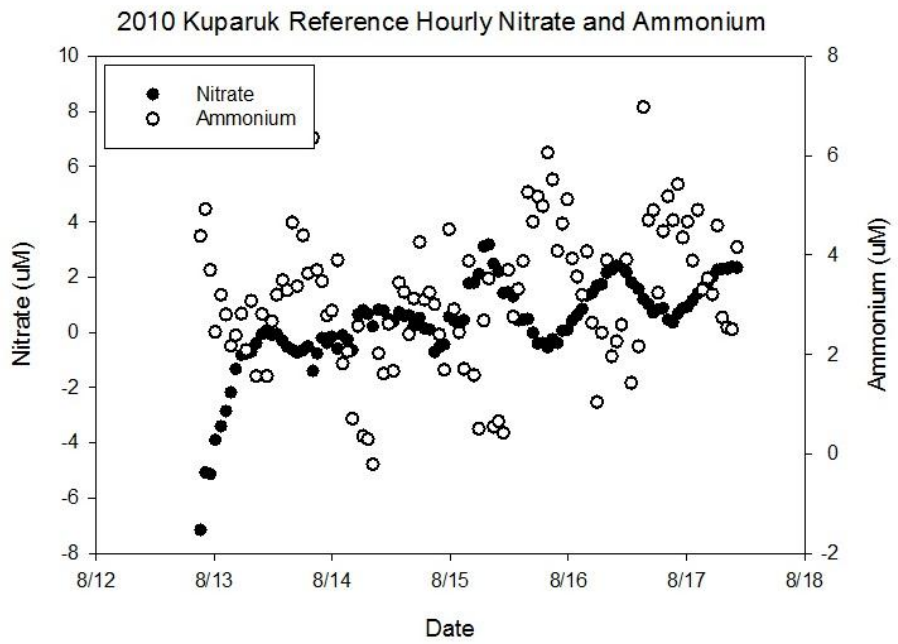


Figure 3.3: Concentration of nitrate and ammonium throughout the Kuparuk reference reach deployment

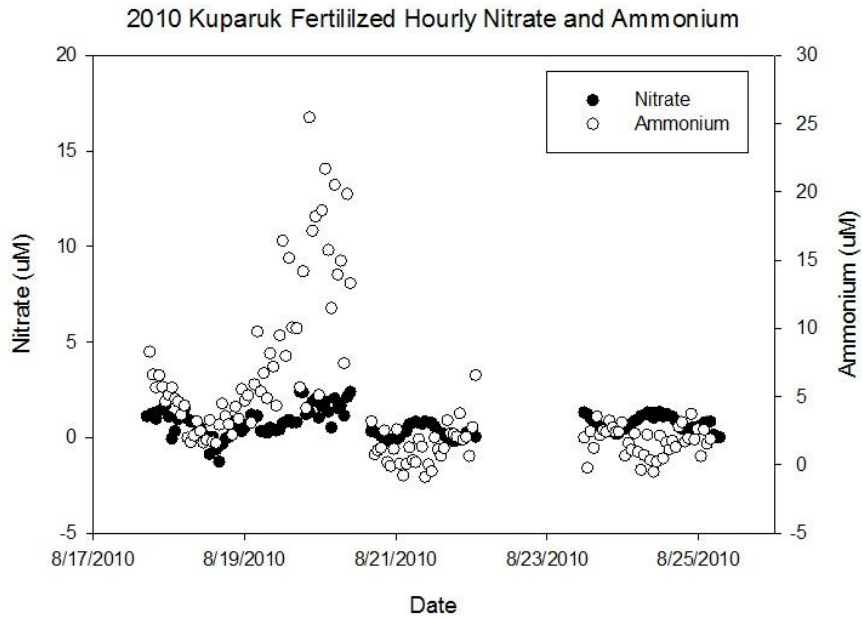


Figure 3.4: Concentration of nitrate and ammonium throughout the Kuparuk fertilized reach deployment

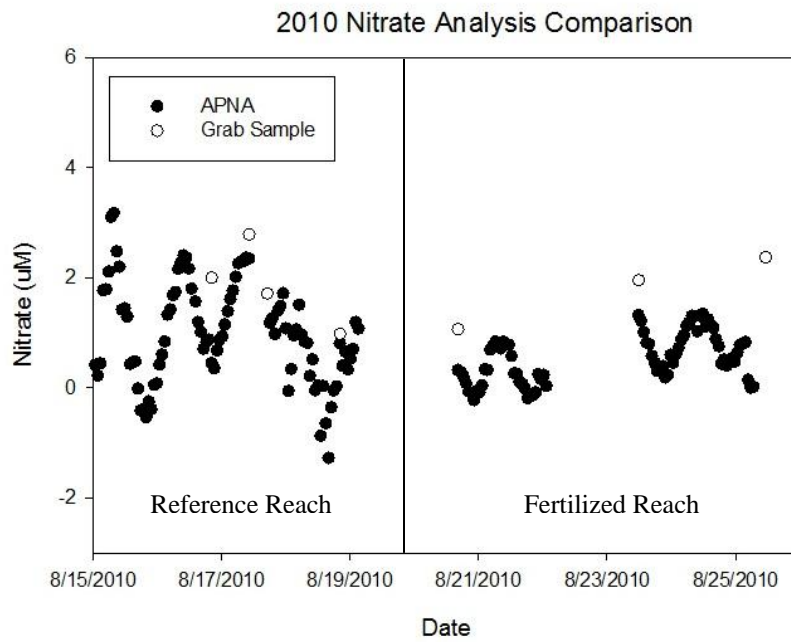


Figure 3.5: Comparison of nitrate concentrations measured in situ by the APNA and grab samples analyzed in the laboratory from the combined Kuparuk River deployments

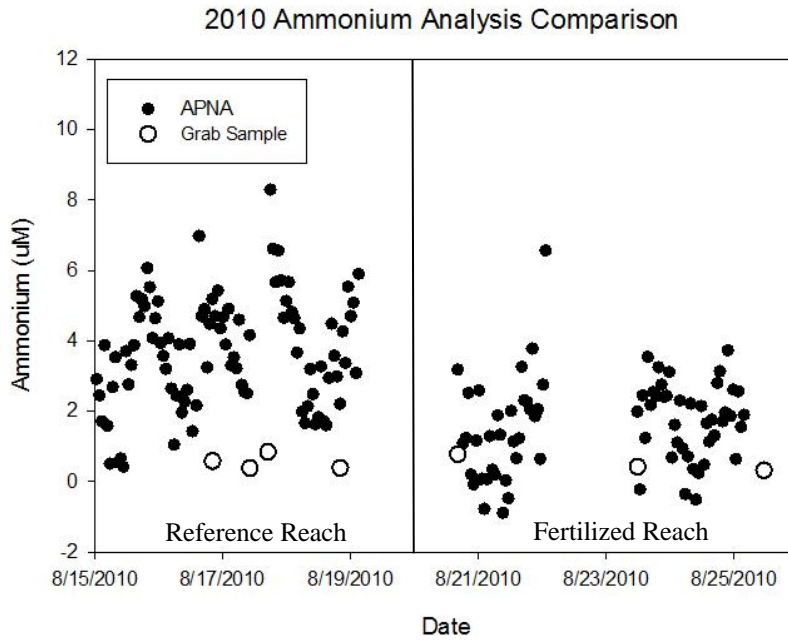


Figure 3.6: Comparison of ammonium concentrations measured in situ by the APNA and grab samples analyzed in the laboratory from the combined Kugaruk River deployments

CHAPTER 4: IN-SITU NUTRIENT ANALYSIS IN FRESHWATER ARCTIC TUNDRA STREAMS: FIELD SEASON 2

4.1 Introduction

The importance of in situ nutrient analyzers capable of high temporal resolution measurements is becoming recognized in stream ecological research. Attaining data at a higher frequency as opposed to intermittent sampling (hourly instead of daily or weekly sampling, for example), can allow for the description of hydrochemical trends, such as diurnal oscillations, that would not be recognized otherwise. The ability to identify these trends can allow researchers to better understand stream nutrient cycling and more accurately assess the dynamics controlling stream ecosystem functions.

There is currently a lack of studies focused on monitoring ambient trends in ammonium and nitrate concentration at the high temporal resolution required to assess short-term nutrient dynamics in streams. An Autonomous Profiling Nutrient Analyzer (APNA) was used to simultaneously measure concentrations of nitrate, phosphate, and ammonium in an arctic tundra stream. The instrument was deployed using two sampling regimes: first, to sampling at an hourly interval in a deployment lasting ten days and second, continuously sampling (one second data for all analytes) to monitor short-term nutrient addition experiments. The latter sampling regime was performed during a separate but related project to determine stream nutrient uptake metrics.

4.2 Methods

4.2.1 Field Site

The APNA was deployed twice in an arctic tundra stream in northern Alaska near Toolik Lake during the summer of 2011 (Table .1 and Figure). The field site location in the Toolik Lake inlet-series (I-Series) stream was chosen due to supplementary research projects further characterizing aspects of the stream ecosystem ranging from whole stream metabolism to hydrologic flow paths during the period of deployment. The I-Series catchment is 66.9 km² and consists mainly of tussock-tundra and elevated areas of heath. Terrestrial vegetation includes sedges and grasses mixed with dwarfed birch, low willows and various forbs (Kling et al., 2000). The morphology of the study reach below Lake I-8 is cobble-bedded with step-riffle and pool features. Dominant aquatic vegetation consists of *Didymosphenia*, bryophytes including *Hygrohypnum* and *Schistidium*, and algae such as *Spirogyra*.

4.2.2 Instrumentation

An Autonomous Profiling Nutrient Analyzer (APNA) by SubChem Systems, Inc. was used to examine high temporal resolution (hourly and continuous monitoring) nutrient characteristics. The instrument was installed in the actively flowing stream and performed in situ wet chemistry analyses for nitrate, phosphate and ammonium. The nutrient analyzer was powered by a solar charged deep-cycle 12 volt battery.

Wet chemistry methodology for total nitrate + nitrite was based on the reduction of nitrate to nitrite, which is then determined colorimetrically at 540nm (USEPA, 1993). Reduction of nitrate to nitrite is accomplished by running sample through a reduction

column containing copper coated cadmium. A diazo compound (compound with two linked N molecules as its terminal group) is then formed after combination with sulphanilamide and N-(1-naphtyl)-ethylenediamine.

Ammonium analysis utilized an OPA fluorescence reagent combined with an EDTA conditioning reagent (Holmes, 1999). Ammonium ions in the sample react with OPA and sulfite to yield a molecule that can be detected fluorometrically. EDTA was used as the buffering reagent in place of the typical borate buffer due to the low solubility of borate at the expected cold-water conditions (temperature < 5 °C). This lowered solubility would result in loss of buffering capability as well as clogging within the instrument lines. The rationale for using OPA fluorescence with the APNA as opposed to colorimetric methods was to be consistent with the methods currently used by the streams component of the Arctic Long Term Ecological Research (ArcLTER) project for determining NH_4^+ at our study site.

Analysis of phosphate involved the ascorbic acid and molybdate methodology (USEPA, 1971). In this, molybdate and orthophosphate react in the presence of antimony to form a phosphor-molybdate complex. The complex is reduced with ascorbic acid to form a blue compound detected spectrophotometrically at 885nm.

During field deployments individual reagents were kept separate in sterile intravenous (IV) bags. If reagents remain cool and in the dark they will remain active for several months (information from manufacturer).

Photosynthetically Active Radiation (PAR) data were acquired using a Li-Cor, Quantum Model LI-190SB sensor on a meteorological station at Toolik Field Station

(Lat. 68° 38' N, Long. 149° 36' W), about 1.6 kilometers from the field site. Hourly PAR data are averages of 60 readings measured over the previous hour and are reported as $\mu\text{mole of quanta (photons) per meter squared per second}$.

4.2.3 Data Processing

Within 4-6 hours of each field deployment, we developed standard curves based on external standards as well as internal standard additions. In the case of internal standard additions, we calculated the concentration at each of the 4 standard addition set-points as:

$$(Q_{CAL} * C) / Q_{total}$$

where Q_{CAL} is the flow rate of standard solution at a given set-point (liters per minute), C is the concentration of the standard solution (moles), and Q_{total} is the combined flow of the sample inlet, reagents, and standard solution through the channel in question (liters per minute). Inferred concentrations from each injection rate were plotted against the respective level of absorbance or fluorescence to create a standard curve for each analyte (e.g., Figure).

During the discrete sampling deployments, we programmed the APNA to perform an in-situ standard addition every fourth hour. This was performed in a 3-step sequence;

1. stream sample + reagent
2. stream sample + reagent + low rate CAL addition
3. stream sample + reagent + high rate CAL addition

Analytical slope was calculated from each in-situ standard addition throughout the deployment. We used the standard addition slope values throughout the deployment to account for analytical drift in the instrument. The slope of the regression line fit to

standard addition analytical slope values over the course of the deployment was analyzed by a two-tailed t-test to determine whether analytical drift was different from zero at a significance level of 0.05.

An example of an external standard curve is shown in Figure . At least 5 standards were prepared and each run individually in order of increasing concentration. The concentration of each standard then was plotted against its respective absorbance values to yield an analytical slope and y-intercept, which later was used to determine the nutrient concentration in “unknown” samples.

Calculated nutrient concentrations for 2011 were based on external standard curves. This was due to the higher expected accuracy in the external standard curves. Internal standard additions have higher uncertainty stemming from dependence upon multiple flow rates within each analyte channel, which could slightly change during an extended deployment of several weeks and easily go undetected. Despite exclusive use of externally derived standard curves for sample analyses, the internal standard additions described above were performed regularly during the extended deployment to examine any possible analytical slope drift.

Concentrations of nitrate or phosphate in an “unknown” sample were calculated as:

$$A_s \times m + b$$

where A_s is sample absorbance (sample plus reagents), m is the analytical slope and b is the y-intercept.

Ammonium concentrations were calculated as:

$$F_s \times m + b$$

where F_s is sample fluorescence (sample plus reagents), m is the analytical slope and b is the y-intercept.

The method detection limit (MDL) was estimated for all analytes as:

$$MDL = s \times t_{(n-1, 1-\alpha=0.99)}$$

where n is the number of replicate samples, s is the standard deviation of the measured concentrations of n replicates, t is the Student's t value at $n-1$ degrees of freedom and $1-\alpha$ (99 percent) confidence level (when $n=7$ and $\alpha=0.01$, $t=3.14$), and α is the level of significance (USEPA MDL). A measurement of the lowest concentrated standard ($0.25 \mu\text{M PO}_4^{3-}$, NO_3^- , NH_4^+) was repeated 7 times to calculate the MDL.

Slug injection analysis involved calculating “observed” (background corrected) and “expected” breakthrough curves (BTCs). Observed nutrient concentrations (C_{obs}) were calculated as:

$$C_{obs} = C_{measured} - C_{amb}$$

where $C_{measured}$ is the actual nutrient concentration measured by the instrument at any point in the BTC and C_{amb} is the ambient nutrient concentration before the arrival of the BTC. Expected BTCs were calculated as:

$$C_{exp} = C_{conservative} \times \left(\frac{i_{nutrient}}{i_{conservative}} \right)$$

where C_{exp} is the concentration of nutrient expected in the BTC should uptake and transformation of the nutrient be equal to zero, $C_{conservative}$ is the concentration of conservative tracer (chloride) at any point in the BTC, $i_{nutrient}$ is the mass of nutrient in the injectate and $i_{conservative}$ is the mass of conservative tracer (chloride) in the injectate.

Nitrification was seen to occur in APNA monitored ammonium slug injections (section 3.3.2). Conversion of ammonium to nitrate was quantified in terms of conversion (or nitrification) efficiency using:

$$E = \frac{(NH_4 Inj - NH_4 Rec)}{NO_3 Rec} \times 100$$

where E is conversion efficiency (%), $NH_4 Inj$ is the mass of ammonium (as nitrogen) added to the stream (g), $NH_4 Rec$ is the mass of ammonium (as nitrogen) recovered (g) and $NO_3 Rec$ is the mass of nitrate (as nitrogen) recovered (g). In effect, conversion efficiency is represented by the fraction of immobilized ammonium that is transformed to nitrate.

Validation of nutrient concentration for all analytes was performed by comparing grab samples taken simultaneously with the APNA sampling. Grab samples were taken over a wide range of concentrations during slug injections on July 16 and September 2, 2011. Grab samples also were taken for ambient samples during the hourly-sampling deployment. All grab samples were filtered through 0.45 μ m filters and frozen within 24 hours for later analysis. Samples were analyzed within 6 months in the laboratory using a Lachat autoanalyzer. The analytical methods for PO_4^{3-} and NO_3^- were the same as those used by the APNA. The analysis for NH_4^+ in the laboratory utilized the nitroprusside colorimetric method (USEPA, 1993), as opposed to the OPA method used by the APNA.

4.3 Results

4.3.1 August 29 – September 9 Hourly Nutrient Analysis

Hourly analysis of nitrate, phosphate and ammonium was performed from August 29 to September 9, 2011. Detection limits for these analytes were determined in the

laboratory prior to instrument deployment and are displayed in Table . This deployment encompassed a period of base flow and a period of rising discharge due to precipitation events mid-deployment (Figure and Figure). Base-flow occurred from the beginning of the deployment on August 29 through mid-day September 1, followed by a period of steadily increasing discharge through the end of the deployment on September 9.

Diurnal oscillations in discharge were observed from 29 August through 1 September (Figure - A). Maximum discharge during this time was about 27.2 L/sec just after midnight. Minimum discharge was about 23.4 L/sec and occurred in early afternoon. Average daily fluctuation in discharge was 3.8 L/sec or about 16% change in discharge during the period of base flow.

Oscillations in ammonium concentration occurred during the base flow period, trending concurrently with discharge and conductivity (Figure - B). High concentrations of ammonium occurred in early morning at around 4.4 μM and lows in late afternoon around 2.2 μM . The average fluctuation of around 2.2 μM was a doubling in concentration from day to night and a decrease of 50% by the next afternoon.

Oscillations in nitrate concentration occurred during the base flow period, with trends inversely related to those of discharge, conductivity and ammonium concentration (Figure - B). The maximum concentration of nitrate (about 1.54 μM) occurred in late afternoon, while the minimum (0.97 μM) was in the early morning. The average amplitude of the nitrate concentration oscillation was about 0.57 μM , which was a 37% daily change in nitrate concentration during the period of base flow.

Diurnal conductivity oscillations were synchronous with those of discharge from 29 August through 1 September (Figure – C). Maximum specific conductance (120.9 $\mu\text{S}/\text{cm}$) was measured after midnight, whereas the minimum specific conductance (119.0 $\mu\text{S}/\text{cm}$) was in early afternoon. The average daily fluctuation in specific conductance, around 1.9 $\mu\text{S}/\text{cm}$, accounted for only a 1.6% change in conductivity during the period of base flow.

After discharge began to increase on September 1 due to precipitation events, oscillations in ammonium concentration ceased and concentration slowly trended upward, increasing by about 2.5 μM over a 6 day period (Figure - B). A gap in data for all nutrients occurred on September 2 and 3 when the APNA was temporarily re-deployed for the nutrient injection experiment described in section 3.3.2, below.

Similar to ammonium, oscillations in nitrate concentration ceased after discharge began to increase on September 1 (Figure - B). Nitrate remained relatively stable at about 0.8 μM through September 4, then slowly increased by about 0.5 μM over a 4 day period.

Phosphate concentration did not exhibit diurnal oscillations during the deployment (Figure - C and Figure - C). Concentrations of phosphate remained between 0.03 and 0.08 μM during the base flow period (below limit of detection). After the rise in discharge, phosphate remained stable at around 0.10 μM through September 5, and then slowly increased to about 0.20 μM over 4 days.

4.3.2 Nutrient Addition Monitoring

Four slug injections were monitored using the APNA's "continuous" mode during the summer of 2011. Two injections contained nitrate and phosphate plus a chloride as a conservative tracer, and two injections contained only ammonium and chloride.

The first round of slug injections was conducted on July 16, 2011. Flow rate during the time of injection was 146 L/sec as measured by dilution gauging (Day, 1977). A discharge measurement, again by dilution gauging, was taken the day prior to the experiment to inform the amount of nutrient and conservative tracer to be added. It was intended that the peak of the nutrient breakthrough curve (BTC) would fall near the top of the APNA's linear range of detection for all analytes. Expected and observed BTCs (above background) for nitrate + phosphate and ammonium additions on July 16 are shown in Figure (A-C).

The ambient concentration of NO_3^- at the time of injection was 0.23 μM , while PO_4^{3-} concentration was below the detection limit. Nutrient BTCs suggest peak concentrations of 17.0 μM and 4.5 μM for NO_3^- and PO_4^{3-} , respectively.

The ambient concentration of NH_4^+ at the time of injection was 1.7 μM and peak concentration of the BTC was 6.5 μM . The injection of NH_4^+ produced an immediate and pronounced rise in concentration of NO_3^- despite the fact that no NO_3^- was added. Ambient NO_3^- concentration before addition of NH_4^+ addition was 0.3 μM and rose to around 1.4 μM 48 minutes after injection, then slowly declined to 0.6 μM in the hour following the peak. The APNA was shut down before NO_3^- returned to ambient concentration.

A larger discrepancy in observed vs. expected BTCs for PO_4^{3-} than for NO_3^- may indicate a higher relative demand for PO_4^{3-} in this system. This is supported by mass balance which showed a higher percent recovery for NO_3^- (72.9%) than PO_4^{3-} (31.5%) (Table). However, PO_4^{3-} has a much higher likelihood of adsorbing to sediments than NO_3^- . Conversion efficiency from NH_4^+ to NO_3^- for the NH_4^+ slug injection was 19.4%.

The second round of APNA monitored TASCSC injections was conducted on September 2, 2011. Discharge at the time of injection was 96 L/sec as measured by dilution gauging. Again, a discharge measurement was taken the day prior to the experiment to inform the amount of nutrient and conservative tracer to be added. Observed and expected BTCs for nitrate + phosphate and ammonium additions are shown in Figure (A-C).

Ambient concentrations of NO_3^- and PO_4^{3-} at the time of the September 2 injection were 1.0 μM and 0.1 μM , respectively. Peak BTC concentration of NO_3^- was 4.7 μM while the PO_4^{3-} peak concentration was 0.8 μM .

Ambient concentration of NH_4^+ at the time of injection was around 2.2 μM . The NH_4^+ BTC was not well defined and produced no definite peak, but a well-defined rise in NO_3^- concentration was again observed. Ambient NO_3^- concentration before addition of NH_4^+ was around 1.0 μM and rose to around 2.2 μM 53 minutes after injection, then declined to 1.6 μM in the following hour. The APNA was again shut down before NO_3^- had returned to ambient concentration.

Similar to the July 16 addition, PO_4^{3-} showed a larger difference between observed and expected BTCs than NO_3^- and lower percent recovery (8.6% vs. 76.8%).

As before, this suggested higher relative demand for PO_4^{3-} , or greater adsorption onto sediment. Conversion efficiency from NH_4^+ to NO_3^- for the NH_4^+ slug injection was 19.9%.

4.3.3 Constant Rate Injection Monitoring

On September 3, 2011 a constant rate addition (CRA) of nitrate, phosphate and chloride as a conservative tracer was monitored using the APNA. Chloride concentrations were inferred from continuous field measurements of specific conductance. Ambient NO_3^- concentration was $0.9 \mu\text{M}$. Plateau was achieved at $2.5 \mu\text{M}$ for the first 2 hours, then quickly increased to around $3 \mu\text{M}$ for the remaining 2 hours (Figure).

Ambient PO_4^{3-} concentration was $0.3 \mu\text{M}$. Throughout the 4 hours of injection, phosphate concentration increased steadily, reaching a $0.9 \mu\text{M}$ plateau at the end of the experiment (Figure).

4.3.4 Validation of Nutrient Measurements

Instrument analytical drift was examined by in situ standard additions of nitrate phosphate and ammonium every fourth hour during the extended deployment. The linear regression of the analytical slope values through time is used to determine significance of instrument drift. At a 0.05 significance level, no analytical drift in nitrate was detected ($p=0.06$, $m = 0.0052$, $SE = 0.0022$, $DF = 52$), though there was a significant relationship seen in phosphate analytical slope ($p=0.00003$, $m = 0.0324$, $SE = 0.0070$, $DF = 52$) (Figure) over the 10 day deployment. No usable data for ammonia slope drift were obtained for the extended hourly deployment.

APNA nutrient analyses for the slug injections were verified by comparison with grab samples which were later analyzed in the laboratory on a Lachat autoanalyzer. The concentrations reported by the APNA and the laboratory analyses for NO_3^- and PO_4^{3-} from the July 16, 2011 slug injection were similar (Figure). There was an especially good fit for NO_3^- below 5 μM , above which APNA measured concentration was slightly lower than that of grab samples. Phosphate data fit well below 3 μM , but above this threshold APNA measured concentrations were slightly higher than those measured in the laboratory. Ammonium baseline concentrations between APNA and laboratory analyzed samples were similar, but diverged immediately above concentrations of 3 μM , where APNA measured concentration was consistently 50-60% lower than that measured in grab samples. Results were similar for September 2, 2011 TASC injections (Figure). Nitrate fit was tighter at high concentrations near 4 μM , below which APNA measurements tended to be slightly higher than grab sample measurements. All PO_4^{3-} measurements resulted in concentrations below 1 μM for the September 2 injection. Comparison of data showed a similar trend for the breakthrough curve except for the peak, where APNA measured concentration was about 0.2 μM higher than that measured from grab samples. No discernible ammonium breakthrough curve was detected during the ammonium slug injection by the APNA. A breakthrough curve was seen from grab sample analysis, which resulted in a lack of fit between APNA and grab sample comparison.

4.4 Discussion

4.4.1 Nutrient Analysis Validation

Because no significant shift in analytical slope was seen in nitrate during the hourly deployment, it was not necessary to make corrections to calculation of nitrate concentration through time. Phosphate did show a significant relationship through time, though there was much more variance around the data than that of nitrate (Figure). However, the “external” standard curves for phosphate generated before and after the deployment were extremely similar, with analytical slope differing by only 0.0019 between them. Based on the similarity between the pre- and post-deployment standard curves, it was decided unnecessary to make corrections to phosphate calculations. The original slope value measured in the laboratory immediately prior to the deployment was used in all concentration calculations, both for the slug and hourly sampling.

Unlike the concentrations of nitrate and phosphate, the concentration of ammonium at the highest rate of internal standard addition was lower than the measured ambient stream water ammonium concentration. Upon the addition of nutrient standard solution and its mixing with stream water within the instrument a diluting effect occurred and analysis of standard additions could not be performed. Therefore, the original slope value measured in the laboratory prior to the deployment was used in concentration calculations for ammonium.

Results from nutrient validations for nitrate and phosphate for the July 16 TASCC injections indicated accurate in situ measurements by the APNA across a broad range of concentrations. Ammonium comparison, however, displayed a poor fit with increasing

concentration during the slug injection. The APNA utilizes OPA fluorescence to analyze ammonium, compared to the nitroprusside colorimetric method used in the laboratory for grab samples. The difference in methods can result in different estimates of ammonium concentrations (e.g. Holmes, 1999). One explanation for the discrepancies observed between methods may be the tendency of chromophoric dissolved organic matter (CDOM) to auto-fluoresce in varying amounts under certain environmental conditions (Watras et al., 2011). Using OPA fluorescence to measure ammonium in the presence of CDOM, then, could erroneously raise estimates of ammonium. However, since APNA measurements at the peak of the BTC were lower than those of grab samples measured in the laboratory, this potential error can be dismissed. Another explanation for the contrast in measured values across the range of concentrations could be exposure of grab samples to ambient ammonia in the atmosphere or entrained in laboratory air (Richardson et al., 1991). It should be noted that the lack of potential for sample contamination is one major advantage of in situ analysis over grab samples that are preserved and later analyzed in the laboratory. Another explanation for the lower concentrations measured by the APNA compared to concentrations measured in the laboratory is the deterioration of OPA fluorescence reagent in the field. Exposure of the reagent to incoming light or excessively warm temperatures within the instrument housing would likely reduce its fluorescing effect when interacting with ammonium and result in inaccurately measuring lower concentrations.

Nutrient validations for the September 2 TASC injections again indicated good in situ measurements by the APNA for nitrate and phosphate across a range of

concentrations, but not for ammonium. APNA analysis showed no discernible ammonium BTC during the September 2 ammonium addition, whereas grab sample analysis did show an ammonium BTC, though somewhat disorganized. The lack of BTC detection by the APNA resulted in the stray from a 1:1 relationship seen in the APNA and grab sample comparison (Figure). This result is further evidence that the OPA fluorescence reagent is likely to deteriorate during the course of a deployment. This outcome is troubling in that with no ammonium slope drift data, the date or time at which deterioration becomes evident cannot be identified. Due to the strong diurnal oscillations observed before the September 2 TASC injections, ammonium patterns are expected to be genuine, even if precision is lost at some point in time.

4.4.2 Diurnal Trends in Extended Deployment

Diurnal oscillations were observed in discharge, conductivity, nitrate and ammonium during a base flow period from August 29 through September 1, 2011. Figure shows that high discharge levels were seen after midnight, whereas low discharge levels occurred in early afternoon. Average daily fluctuation in discharge was 16%, specific conductance 1.6%, ammonium 49.7% and nitrate 37%. The daily fluctuation in discharge of 3.8 L/sec and may be attributed to increased lateral inputs or through-flow from the active (thawed) soil layer during the evening. It should be noted that this watershed is not influenced by glacial activity and is unlikely to exhibit the typical arctic hydrologic fluctuation of higher discharge during the day from glacial melt water. During the daylight hours, soil water uptake and transpiration by terrestrial vegetation could explain the reduction of lateral inputs, which began around 7:00AM as PAR

increased in the morning. The loading of ammonium within the soil water added to the stream at night may have been substantial. With a daily average increase of 3.8 L/sec at night, ammonium increased from 2.2 μM to 4.4 μM , doubling in concentration. Nitrate concentration, on the other hand, decreased by 37% at night, to some extent explained by dilution, provided that soil water contained little to no NO_3^- . The soil water, then, was most likely high in ammonium and extremely low in nitrate. Such conditions could be explained by anoxia in the soil water, inhibiting nitrification and keeping ammonium concentrations elevated in comparison to nitrate. When soil waters reach stream banks and enter the well-oxygenated stream environment there is greater potential nitrification, which lowers the ratio of ammonium to nitrate in stream water. As PAR begins to increase again in the morning, terrestrial vegetation begins to draw soil water and transpire, decreasing lateral inputs to the stream and cutting off the additional load of ammonium. Nitrification occurring in the water column and/or hyporheic zone acts to decrease ammonium concentration and increases nitrate concentration throughout the day.

The slug additions of ammonium provide evidence in support of the argument that these streams have a high capacity for nitrification. July 16 and September 2 NH_4^+ injections resulted in 19.4% and 19.9% conversion efficiency, respectively. That is, of the ammonium added but not recovered from the injection (immobilized ammonium), 19.65% on average was converted to nitrate.

4.4.3 Nutrient Injections

Both slug injections of $\text{NO}_3^- + \text{PO}_4^{3-}$ showed a higher percent mass recovery of nitrate than phosphate. The July 16 and September 2 injections had a recovery of 72.9% and 76.8% nitrate, respectively. Phosphate recovery for the injections was 31.5% and 8.6%, respectively. The lower recovery of phosphate is likely due to a combination of higher biological demand and adsorption than is the case with nitrate. The high demand of phosphorus by biota observed in this stream is consistent with the extremely low measured ambient PO_4^{3-} concentration (often hundredths of a micromole of phosphorus or below levels of detection) and the findings of others studies concerning ambient stream nutrient levels and uptake in the surrounding landscape (Peterson et al., 1992; Kling et al., 2000; Slavik et al., 2004). Constant Rate Addition (CRA) data from September 3, 2011 may support both the notion of a higher demand for PO_4^{3-} and some portion of PO_4^{3-} immobilization occurring as adsorption. Both NO_3^- and conductivity (NaCl was used as the conservative tracer) are seen to nearly reach “steady-state” at an elevated level in the first hour of the CRA breakthrough curve, whereas PO_4^{3-} takes nearly the entire 4 hour injection period to reach a stable plateau (Figure and Figure). The extended time required for PO_4^{3-} to achieve a steady-state as compared to conductivity and NO_3^- suggests a combination of higher biological demand for PO_4^{3-} and an extended time requirement for adsorption sites to be filled within a variety of flow paths, likely including the hyporheic zone.

Hyporheic or other transient storage zone nutrient processing also was evident during both NH_4^+ slug injections. Most distinct in the July 16 injection, production of

NO_3^- extended at least an hour beyond the tail of the NH_4^+ breakthrough curve (Figure - C). NO_3^- production in this time frame suggests that immobilized NH_4^+ continued to be nitrified, possibly in large part, within transient zones with longer residence times. This also speaks to the legitimacy of using the slug approach. A common question concerning this method is whether biota has time to adjust to rapidly changing nutrient concentrations throughout a breakthrough curve. The ability for this system to rapidly nitrify ammonium supports the notion that stream biota can promptly “activate” and process nutrients as a pulse of nutrient passes through a stream reach. Conversion efficiency from NH_4^+ to NO_3^- for the July 16 and September 2 NH_4^+ injections was 19.4% and 19.9%, respectively. That is, of the ammonium-N immobilized, on average 19.65% was converted to nitrate-N. Considering the differences in mass of ammonium injected, discharge, and season between the two injections, the similarity of these estimates is notable.

4.5 Conclusions

Several recent studies have shown the prevalence of diurnal oscillation in nitrate concentration using in situ nutrient analyzers (Roberts and Mulholland, 2007; Heffernan and Cohen, 2010; Rusjan and Mikos, 2010). This investigation, however, may be the first to reveal concurrent (and inverse) ammonium and nitrate diurnal oscillations. Nitrogen processing in this system may be strongly influenced by nocturnal inputs of ammonium from soil water with increased through-flow. Nitrification was seen to be a major driver in ambient nitrate and ammonium, changing concentrations in both analytes

throughout the day. The recognition of such fine temporal scale nutrient dynamics may have important implications for how researchers interpret ecosystem nutrient transport and processing from the terrestrial landscape to headwater streams and their export to lacustrine and coastal systems, at least in the arctic.

The work presented above supports the suitability of the slug methodology for measuring stream nutrient uptake dynamics. As shown with nitrification during ammonium slug additions, stream biota is capable of promptly reacting to rapidly changing concentration of nutrient in the stream.

Regarding the specific instrument used in this study, a possible improvement may be realized if the OPA fluorescence could be changed to the nitroprusside colorimetric method for analysis of ammonium. As the nitroprusside reagents are not as photo-sensitive, ammonium reagent degradation may be minimized over an instrument deployment of several weeks. Reagent degradation, however, could also be caused by wide temperature fluctuations within the instrument/reagent housing. This problem would be very difficult to resolve in non-shaded and shallow arctic headwater streams.

Though obstacles do exist, the Autonomous Profiling Nutrient Analyzer (APNA) can produce accurate nutrient analyses in remote freshwater stream environments. The further advancement of in situ nutrient analyzers with multiple analyte capabilities should prove to be important in future aquatic ecological research. These instruments have the capability to yield valuable information on fine temporal scale biogeochemical dynamics in stream ecosystems, broadening our understanding of in-stream and broader ecosystem nutrient cycling.

4.6 Literature Cited

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4.7 Tables

Table 4.1: List of APNA Deployments in Summer 2011

Deployment #	Location Name	Dates
1	I-8 Outlet	July 16 – July 18, 2011
2	I-8 Outlet	August 29 – September 9, 2011

Table 4.2: MDLs for September APNA Deployment

Analyte	MDL (μM)	Stand. Dev.
Nitrate	0.241	0.077
Phosphate	0.222	0.071
Ammonium	0.951	0.303

Table 4.3: Mass recovery from July 16, 2011 TASCSC injections

Analyte	Mass Injected (g)	Mass Recovered (g)	Percent Recovery	NO_3^- Generated (g)	Conversion Efficiency (%)
NO_3^- N	44.7	32.6	72.9	-	-
PO_4^{3-} P	52.4	16.5	31.5	-	-
NH_4^- N	45.9	8.7	19.0	7.2	19.4

Table 4.4: Mass recovery from September 2, 2011 TASC injections

Analyte	Mass Injected (g)	Mass Recovered (g)	Percent Recovery	NO ₃ ⁻ Generated (g)	Conversion Efficiency (%)
NO ₃ ⁻ N	7.5	5.8	76.8	-	-
PO ₄ ³⁻ P	17.8	1.5	8.6	-	-
NH ₄ ⁻ N	20.8	NA	NA	4.15	19.9

4.8 Figures

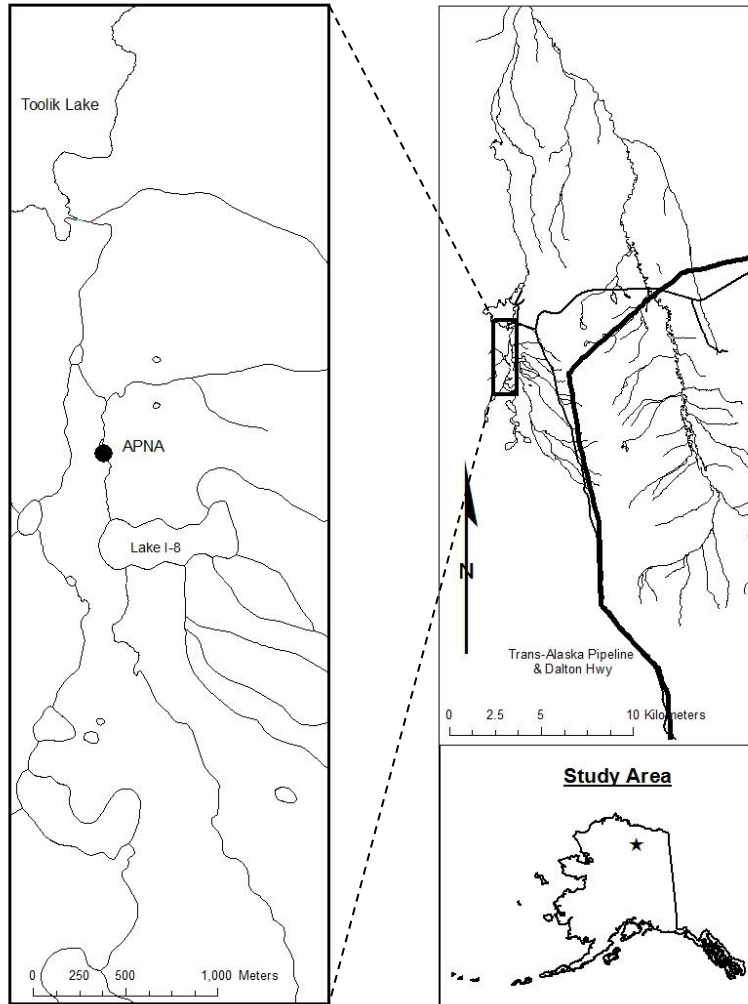


Figure 4.1: Summer 2011 APNA Deployment Site

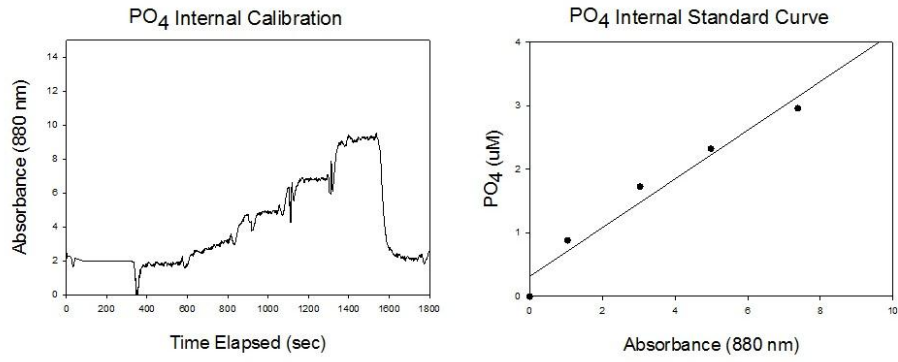


Figure 4.2: Example of “Internal” standard curve trial using the APNA

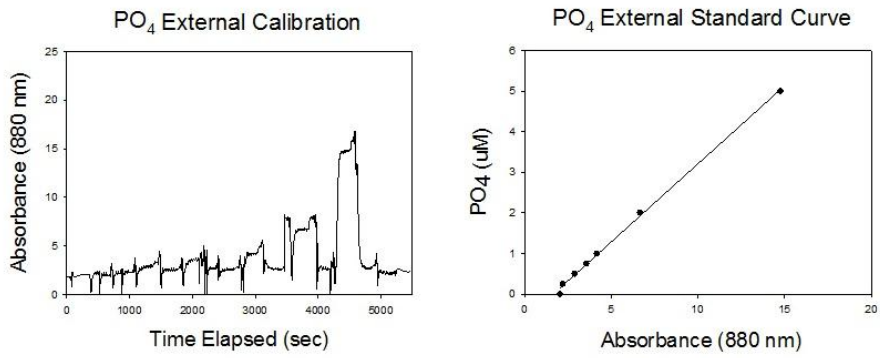


Figure 4.3: Example of an “external” standard curve trial using the APNA

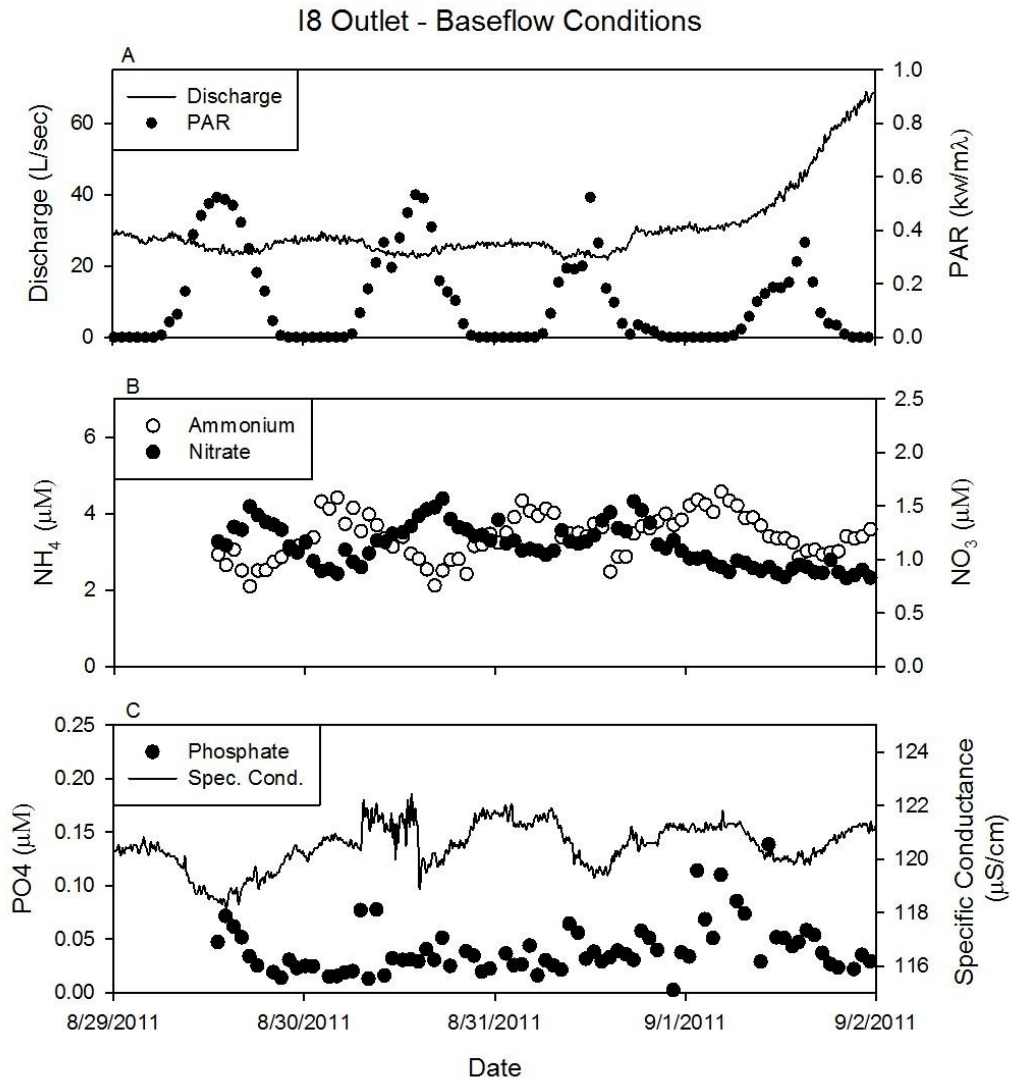


Figure 4.4: Trends in discharge and photosynthetically active radiation (PAR) (A), ammonium and nitrate (B), and phosphate and specific conductance (C) during baseflow conditions in I8 Outlet.

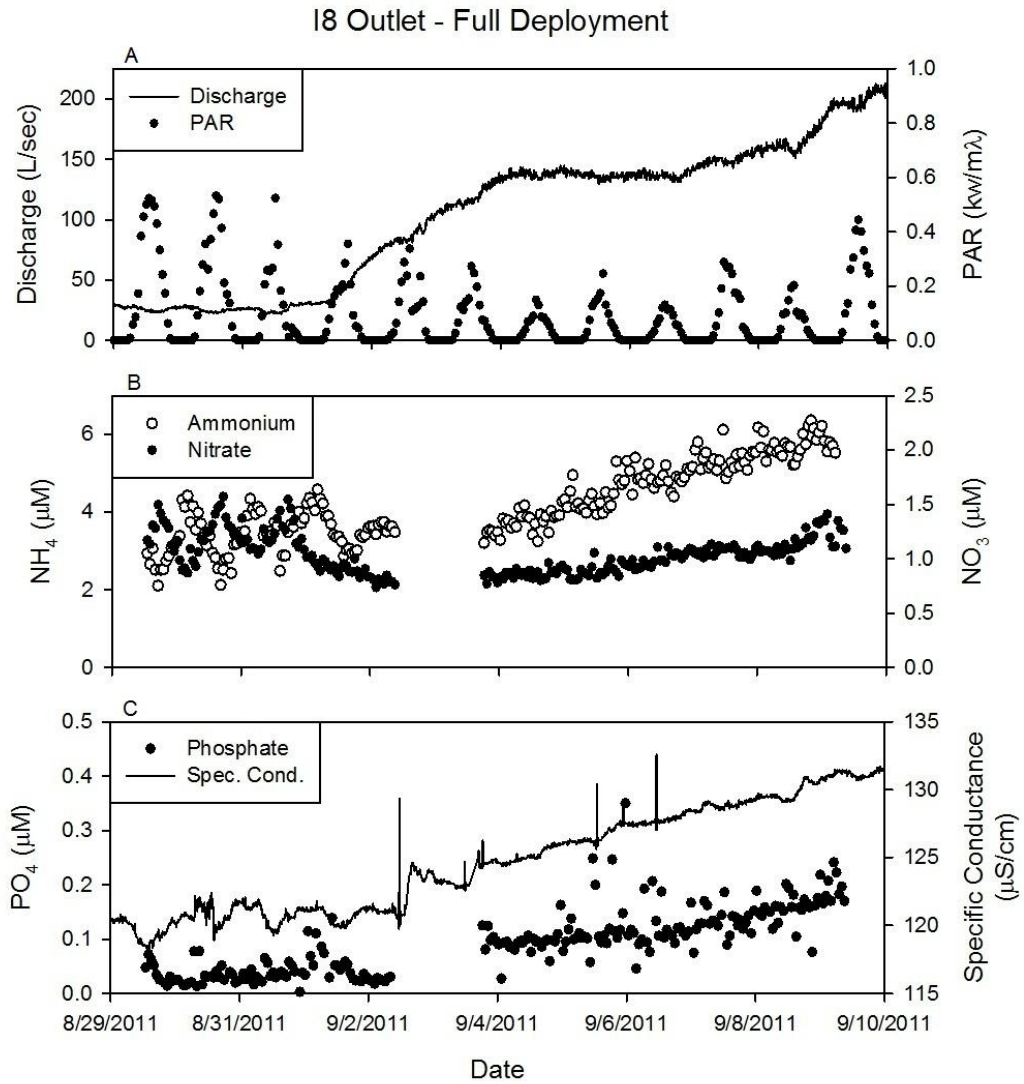


Figure 4.5: Trends in discharge and photosynthetically active radiation (PAR) (A), ammonium and nitrate (B), and phosphate and specific conductance (C) during throughout the APNA deployment in I8 Outlet.

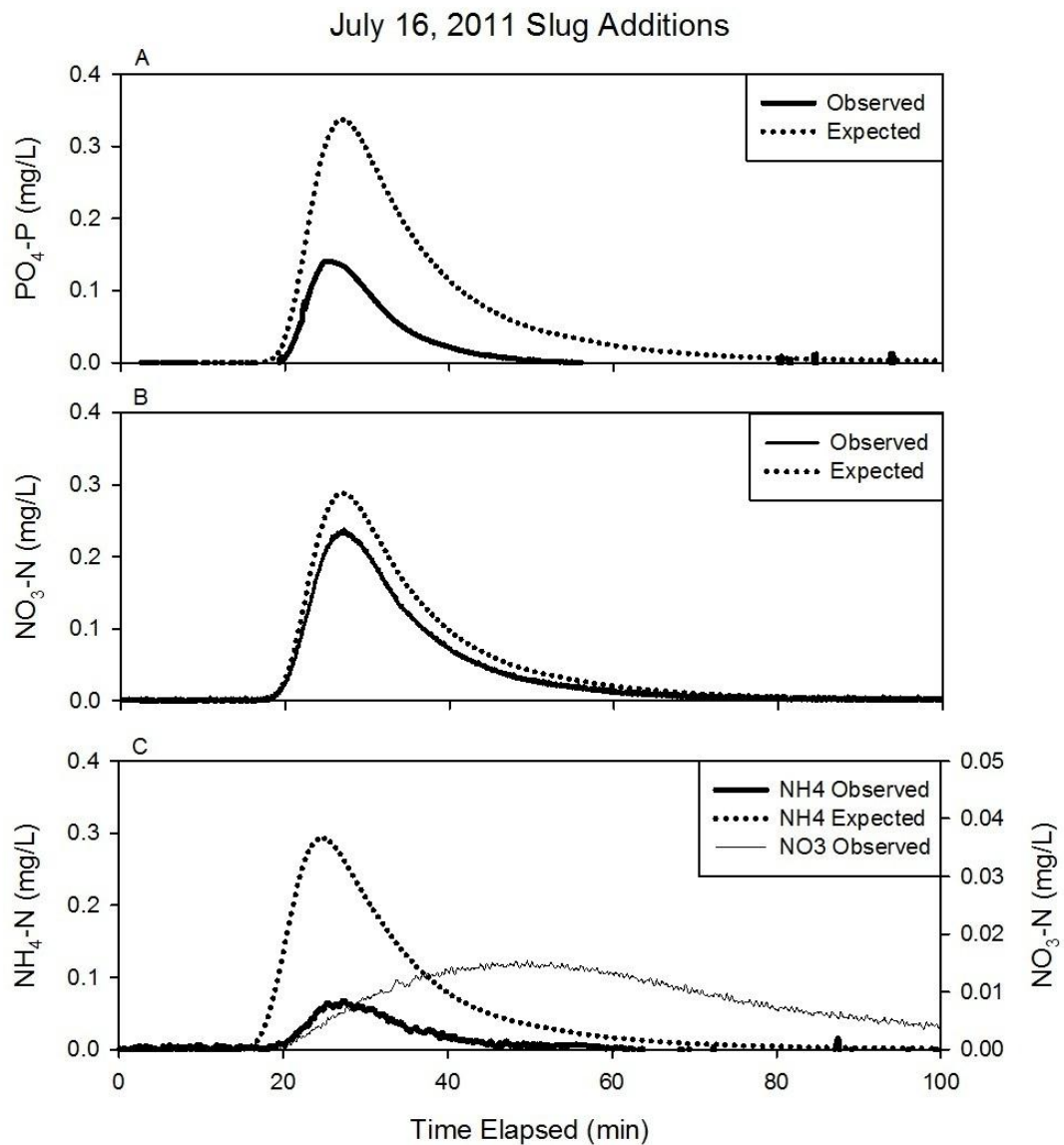


Figure 4.6: Slug nutrient additions of nitrate + phosphate with chloride as a conservative tracer (A and B) and ammonium with chloride as a conservative tracer (C) on July 16, 2011. “Observed” represents the concentration of nutrient over background as determined by the APNA and “Expected” means the anticipated nutrient concentration with zero uptake or transformation.

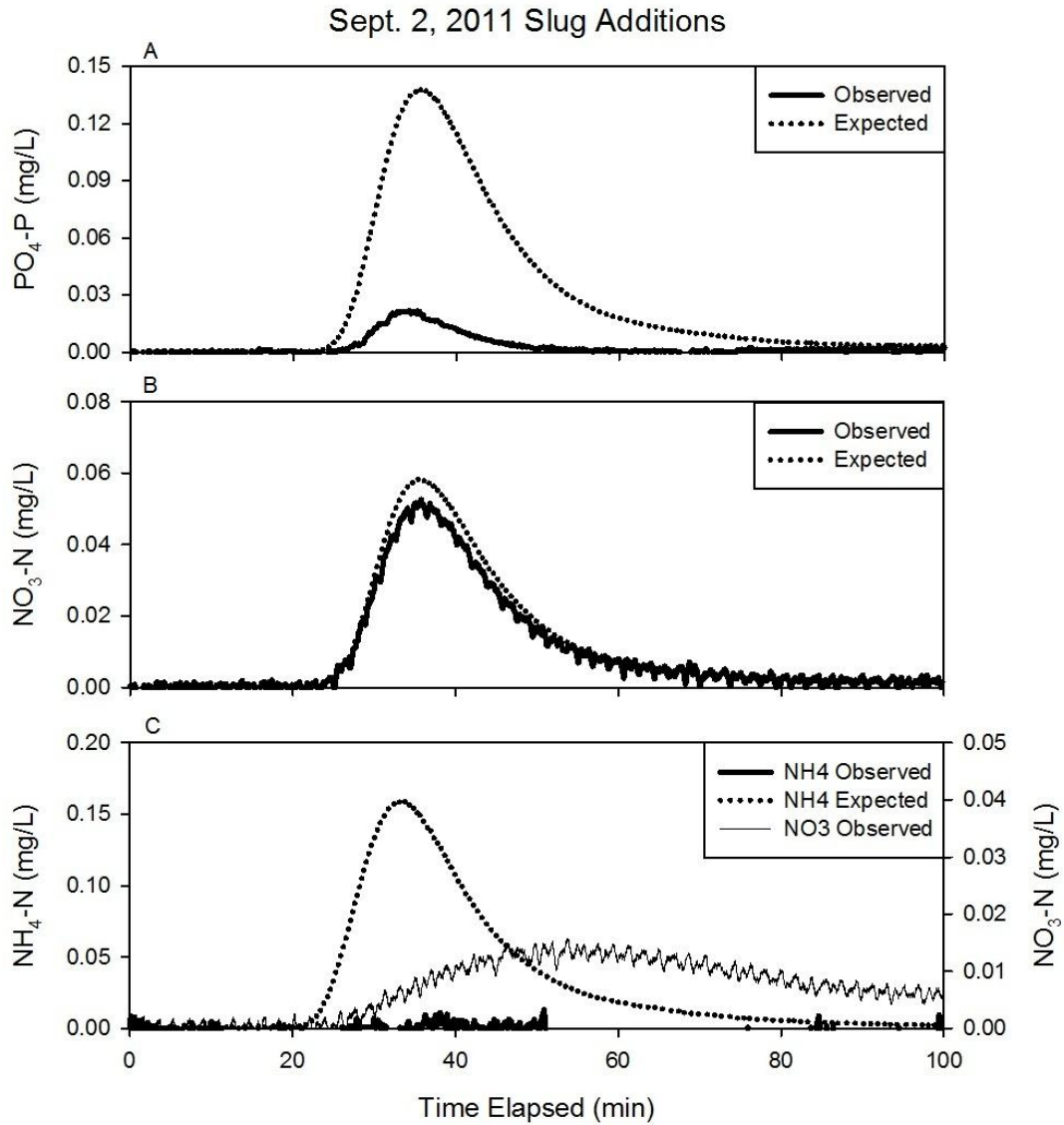


Figure 4.7: Slug nutrient additions of nitrate + phosphate with chloride as a conservative tracer (A and B) and ammonium with chloride as a conservative tracer (C) on September 2, 2011. “Observed” represents the concentration of nutrient over background as determined by the APNA and “Expected” means the anticipated nutrient concentration with zero uptake or transformation.

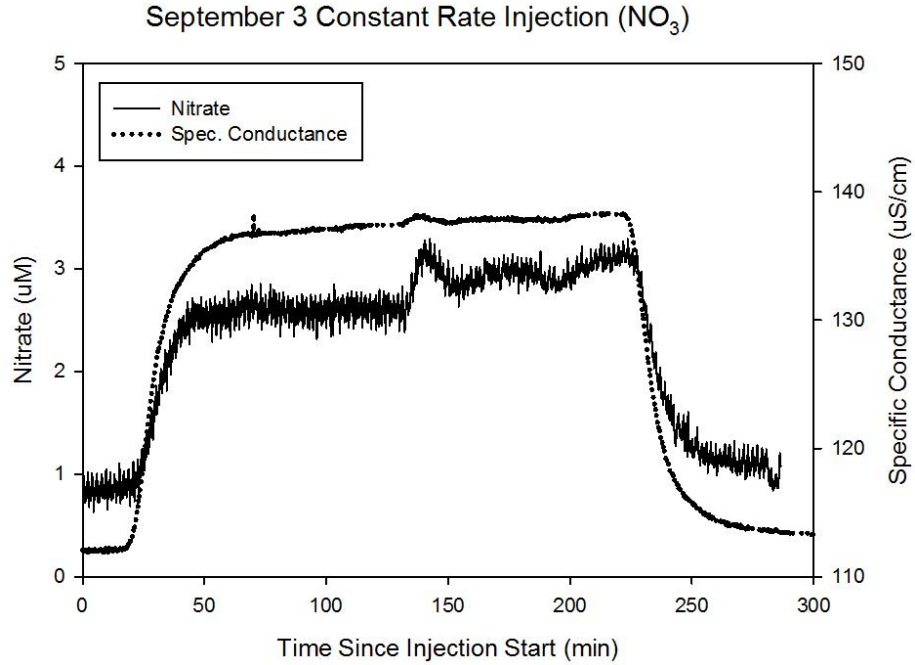


Figure 4.8: Nitrate concentration throughout a Constant Rate Addition (CRA) on September 3, 2011

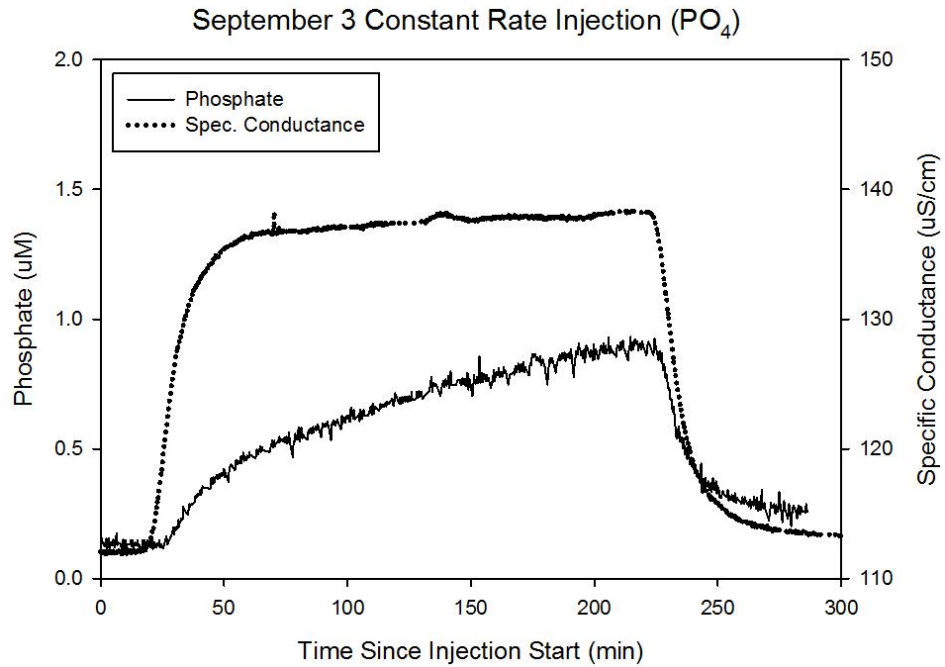


Figure 4.9: Phosphate concentration throughout a Constant Rate Addition (CRA) on September 3, 2011

July 16, 2011 Nutrient Injection Validation

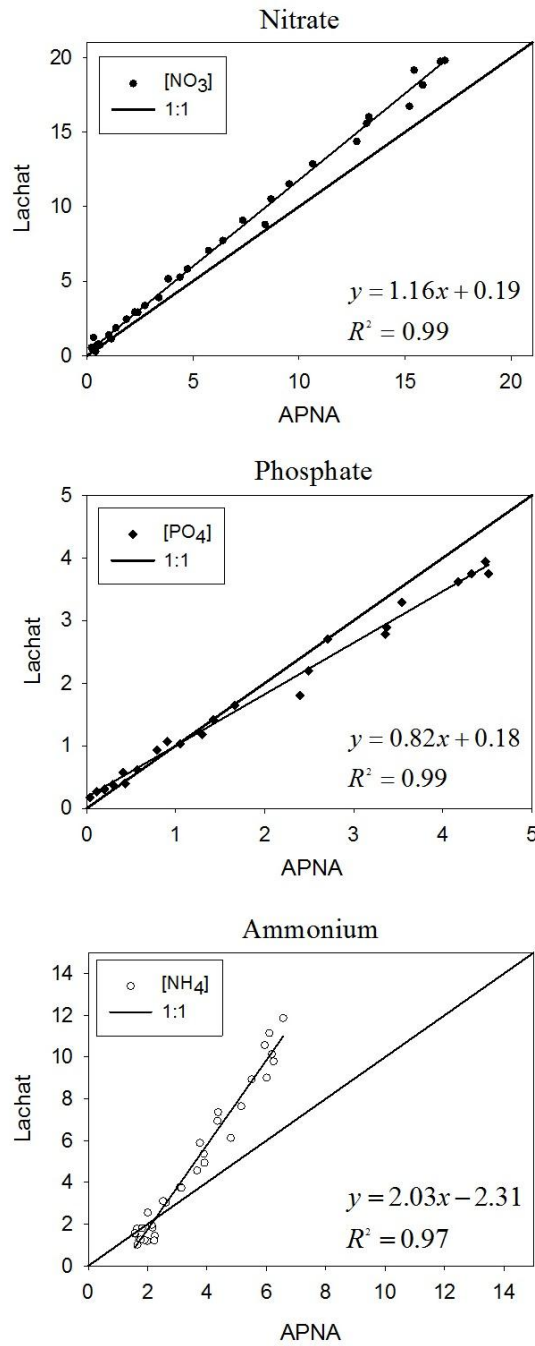


Figure 4.10: Nutrient concentration measurement comparison between APNA and grab samples analyzed in the laboratory from July 16, 2011 TASC injections. All units are reported in μM .

September 2, 2011 Nutrient Injection Validation

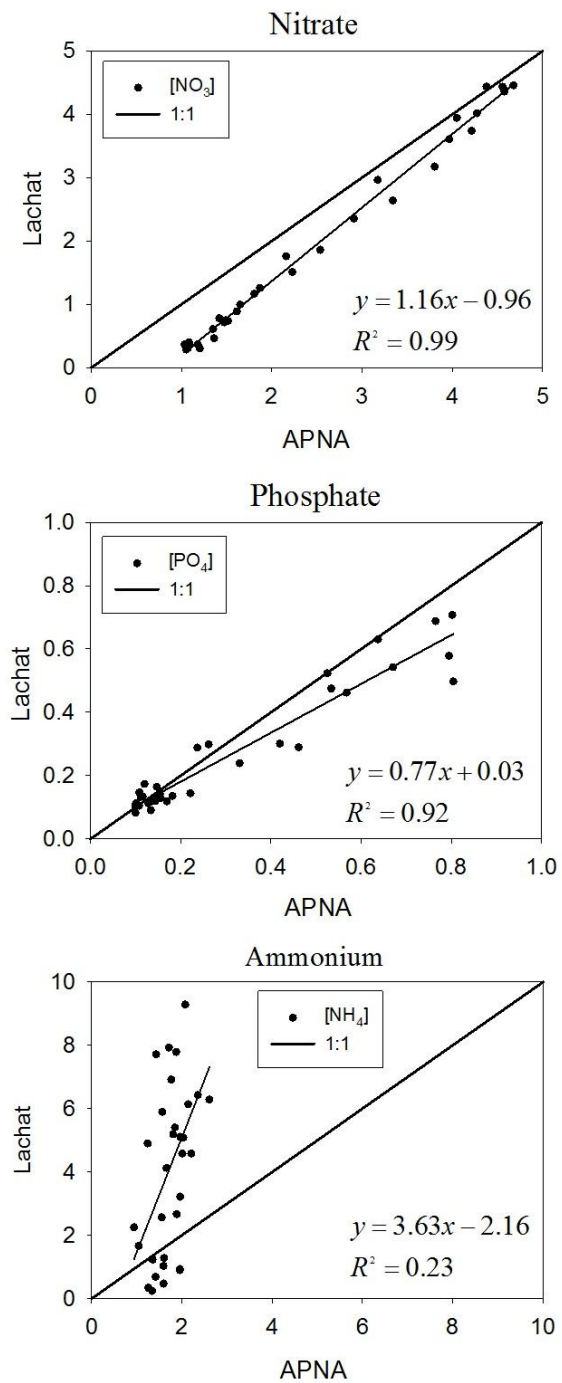


Figure 4.11: Nutrient measurement comparison between APNA and grab samples analyzed in the laboratory from September 2, 2011 TASC injections. All units are reported in μM .

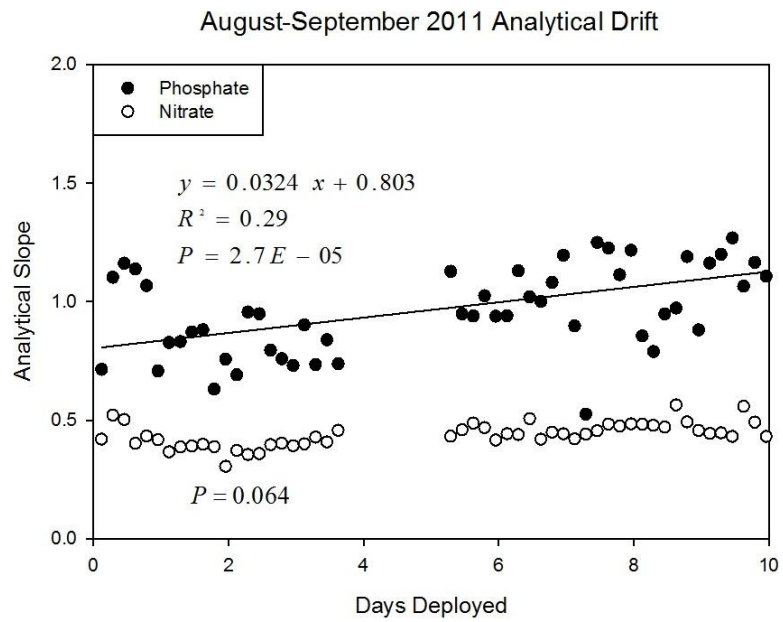


Figure 4.12: Drift in nitrate and phosphate analytical slope measured in situ throughout extended deployment

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