

EFFECTS OF FLOW ON FILAMENTOUS ALGAE AND NUTRIENT LIMITATION IN  
LOTIC SYSTEMS

By

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To my parents Alan and Lauren and my wife Shannon

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This research examined the dual role of flow as a control on filamentous algae in lotic systems and its effect on nutrient limitation, with application to Florida spring-fed rivers. The primary goal was to understand how flow influences both algal growth by regulating nutrient supply and algal abundance by dictating drag forces. Four different approaches were used to address the research objectives: statistical analysis of North American stream datasets, a field survey and in situ experiment, laboratory stream channel experiments, and an ecological simulation model. The statistical analysis of North American streams utilized the LINX (Lotic Intersite Nitrogen eXperiment) datasets, and resulted in the development of a new metric, the autotrophic uptake length. The autotrophic uptake length accounts for nutrient concentration, discharge rate, and autotrophic metabolism and was a better predictor of nitrogen limitation than nitrogen concentration alone. A field survey and experiment at the Gum Slough spring system determined that increased filamentous algal abundance was related to declining discharge, and identified a flow velocity threshold of 35 cm/s above which algal abundance was minimal. The laboratory stream channel experiments tested the effect of flow velocity and nutrient concentration on the metabolism of the filamentous alga

*Lyngbya wollei*, whose response was measured by diel changes in pH. At low nitrate concentration, metabolism was stimulated by an increase in flow velocity from 1 to 5 cm/s, whereas at high nitrate concentration metabolic rates were similar at these two velocities, suggesting that the increase in velocity decreased nutrient limitation at low concentrations. Algal metabolism was lower at higher velocity (10 cm/s) regardless of nitrate concentration. Finally, a simulation model was created to help explain the results of the laboratory experiment. The model indicated that filamentous algae and flow velocity display a subsidy-stress relationship when nutrients are limiting, but velocity is only a stress if nutrients are readily available. In combination, these approaches demonstrate that flow has significant effects on algal growth, abundance, and autotrophic nutrient limitation in lotic systems.

## CHAPTER 1 OVERVIEW

### **Statement of the Problem**

Aquatic ecosystems throughout the world are experiencing dramatic changes as the result of anthropogenic activities. An increasingly common example is the proliferation of algae and subsequent shifts in ecosystem structure and function, which is often attributed to addition of nutrients that previously limited algal growth rates (Smith 2003). Florida springs and spring-fed rivers provide a recent example of algal proliferation and ecosystem change, yet the cause is still unclear (Brown et al. 2008; Heffernan et al. 2010b). Elevated nitrate concentrations have been measured in many springs (Scott et al. 2004), and therefore alleviation of nitrogen limitation has been suggested as the main driver of increased algal abundance. However, nutrient limitation was not detected in springs historically when nitrate concentrations were lower than at present (Odum 1957a; Duarte and Canfield 1990), and more recent studies of filamentous algae in springs also provide little evidence for the N-enrichment hypothesis (Stevenson et al. 2004, 2007).

In lotic systems (e.g. rivers and streams), nutrient availability is positively related to flow velocity (Stevenson 1996); therefore nutrient limitation may only occur where both velocity and nutrient concentration are low enough to significantly reduce nutrient availability. Discharge rates and flow velocities have been declining in most Florida springs over recent decades due to a combination of less rainfall and increased groundwater pumping (Copeland et al. 2009), which may increase the likelihood of nutrient limitation in low nutrient systems. However, flow velocity also dictates the drag force on algae and is the main determinant of both colonization and detachment (i.e.

export) rates (Stevenson 1996). In Florida springs, flow velocity is likely a major control on filamentous algal abundance, and declining flow velocities could reduce algal export and lead to algal proliferation. This research focuses on the dual role of flow on filamentous algae, as it acts both as a subsidy to algal growth by increasing nutrient availability and a stress on algal abundance by increasing drag force.

### **Research Objectives**

This research examines the dual role of flow as a control on filamentous algae in lotic systems and how this relates to nutrient limitation, with application to Florida spring-fed rivers. The primary goal was to understand how flow influences both algal growth by regulating nutrient supply and algal abundance by dictating drag forces. Much of this research focused on the filamentous alga *Lyngbya wollei*, since it is one of the main algal species proliferating in Florida spring-fed rivers. The research objectives were to:

- Determine how flow influences autotrophic nutrient limitation in lotic systems broadly
- Identify flow velocity thresholds which inhibit algal proliferation in Florida spring-fed rivers
- Quantify the interactive effects of flow velocity and nutrient concentration on *Lyngbya wollei*

Four different, yet complementary, approaches were used to address these objectives: statistical analysis of North American stream datasets, a field survey and in situ experiment, laboratory experiments, and a simulation model. The analysis of North American streams (Chapter 3) used the LINX (Lotic Intersite Nitrogen eXperiment) datasets to assess metrics for predicting nutrient limitation. Nutrient concentration was compared to autotrophic uptake length ( $S_{w,a}$ ) which is a new metric that takes into

account nutrient concentration, discharge rate, and autotrophic nutrient demand to better characterize nutrient limitation in lotic systems. A field survey and experiment (Chapter 4) were conducted at the Gum Slough spring system in order to determine the effect of flow velocity on filamentous algae in situ. The field survey consisted of detailed flow velocity and algal cover measurements at three transects every two months. The field experiment utilized plastic baffles to create high and low flow channels in which algal biomass was harvested on a periodic basis. Experimental stream channels (Chapter 5) were created in a greenhouse laboratory to test the effects of flow velocity and nutrient concentration on the metabolism and biomass of the filamentous alga *Lyngbya wollei*. Three velocities were tested and the algal response was measured by diel changes in pH, which were converted into metabolic rates. Finally, a simulation model (Chapter 6) was setup to explore the specific mechanisms that could explain the results of the laboratory experiment. In combination, these approaches generated multiple lines of evidence to fulfill the research objectives.

## CHAPTER 2 LITERATURE REVIEW

### **Nutrient Limitation in Aquatic Systems**

Eutrophication of water bodies is a global issue originating from increased nutrient loading, mostly as a result of intensive agricultural and urban development (Carpenter et al. 1998). Eutrophication is a process which occurs when the addition of limiting nutrients causes increased autotrophic productivity due to stimulation of algae and other aquatic plant growth. Since there are often substantial negative effects of eutrophication on ecosystem structure and function, recreation, aesthetics, and even human health (Lembi 2003), this phenomenon has received much attention in the realm of environmental management (USEPA 1998; USEPA 2001). In response to eutrophication, managers often focus on reducing nutrient loading to the impacted systems by targeting the sources of nutrients, although there are a variety of other techniques such as directly removing algae and nutrients from the systems themselves through harvesting of biomass or sediment removal (Cooke et al. 1993; Lembi 2003).

Nitrogen (N) and phosphorus (P) are the main nutrients of concern due to their low availability relative to their biotic demand; although other nutrients may also limit autotrophic productivity such as has been documented with diatoms and silica (Hecky and Kilham 1988). The particular form of nutrient is also important as dissolved inorganic forms of nitrogen (DIN) and phosphorus (DIP) are most readily utilized by autotrophs. The N:P ratio is often used as indicator of nutrient limitation status; ratios of 16:1 based on molar concentrations (the Redfield ratio for marine phytoplankton) are considered balanced. The optimal N:P ratio for algae varies by species, but generally ratios > 20:1 suggest P limitation and ratios < 10:1 suggest N limitation with

intermediate ratios possibly indicating co-limitation by N and P (Schanz and Juon 1983). The N:P ratio is not always indicative of nutrient limitation, as both nutrients may be available in sufficient quantities to saturate autotrophic nutrient demand (Snyder et al. 2002). Co-limitation by N and P is a common phenomenon in which the addition of one nutrient subsequently causes limitation by the other, and thus addition of both can have substantial effects.

Regional factors such as geologic setting can exert strong influence on N and P availability, and in some areas aquatic systems are naturally eutrophic (i.e. high nutrient levels and productivity). Based on early studies conducted in mostly temperate climates it was concluded that typically freshwater systems are P limited whereas estuarine systems are N limited (Schindler 1977; Howarth 1988). However, recent research shows that eutrophication is a complex process which warrants the consideration of both N and P control for all types of aquatic systems (Conley 2000; Howarth and Marino 2006; Elser et al. 2007; Lewis and Wurtsbaugh 2008).

### **Nutrient Limitation in Lotic Systems**

In lotic (i.e. flowing aquatic) systems, the effects of nutrient enrichment may generally be less dramatic in comparison to lentic systems. This is partially due to the influence of other factors that constrain autotrophs, such as light reduction by the riparian tree canopy and velocity induced export, but more importantly with regard to nutrient limitation is the role of flow. Stream discharge is inversely proportional to the hydraulic residence time of water, which determines the rate of nutrient replenishment in the water column and the type of algae that can persist. Regarding the later, there is a distinct difference between lotic systems with residence times long enough to allow free-floating algae (i.e. phytoplankton) to persist and those with shorter residence times in

which only algae attached to a substrate (i.e. periphyton) are viable (Hilton et al. 2006). In slow-flowing lotic systems dominated by phytoplankton, nutrient limitation may be stronger and the effects of nutrient enrichment may be more similar to lentic systems.

In lotic systems dominated by attached algae, nutrient limitation may only occur at relatively low nutrient concentrations, since flow generates a continuous flux of nutrients which can saturate nutrient demand (sensu Odum 1957a). Francoeur (2001) conducted a meta-analysis of 237 nutrient enrichment experiments in streams and determined that 43% did not show a significant response to either N or P additions. Six of ten streams assayed in Oklahoma (Ludwig et al. 2008) and 32% of stream assays in Idaho (Sanderson et al. 2009) did not indicate nutrient limitation. Studies of streams across North America spanning a wide range of biomes found that in most cases algal production and biomass did not exhibit nutrient limitation (Tank and Dodds 2003; Johnson et al. 2009). A study of six streams in Washington did not find a relationship between DIP and algal biomass despite  $N:P > 30$  (Welch et al. 1988).

There are many lotic systems which have displayed algal nutrient limitation; however, in each case nutrient levels were extremely low ( $DIN < 55 \mu\text{g/L}$ : Triska et al. 1983; Grimm and Fisher 1986; Hill and Knight 1988; Rosemond et al. 1993;  $DIP < 6 \mu\text{g/L}$ : Elwood et al. 1981; Peterson et al. 1983; Bothwell 1985; Pringle 1987; Bothwell 1988; Rosemond et al. 1993). Streams which did not indicate algal nutrient limitation had higher nutrient levels ( $DIN > 138 \mu\text{g/L}$  and  $DIP > 12 \mu\text{g/L}$ : Pringle et al. 1986; von Schiller et al. 2007). There are other nutrients which may limit algal growth rates, particularly when N and P are sufficiently available, such as certain micronutrients (e.g. Fe, Mn, Co) (Pringle et al. 1986). Light levels may also limit algal growth; therefore,

algal nutrient limitation is more prevalent in open canopy systems where light limitation is minimal (Grimm and Fisher 1986; Hill and Knight 1988; Rosemond et al. 2000; Tank and Dodds 2003; Johnson et al. 2009). In summary, nutrient saturation is common in lotic systems and nutrient limitation is more likely in open canopy environments.

The amount of algae may affect the nutrient concentrations required for nutrient limitation to occur. As algal biomass increases, nutrient transfer into the algal layer becomes increasingly diffusion limited and thus either higher nutrient concentrations or flow velocities may be necessary to inhibit nutrient limitation (Bothwell 1989). In lotic systems with substantial algal biomass, the threshold for limitation of peak biomass occurred at higher nutrient levels due to diffusion limitation (DIP < 25  $\mu\text{g/L}$ : Horner et al. 1983; Bothwell 1989; DIP < 38  $\mu\text{g/L}$  and DIN < 308  $\mu\text{g/L}$ : Rier and Stevenson 2006). Bothwell (1989) proposed that as nutrient levels increase there are three phases to algal nutrient limitation: phase 1 involves cellular uptake/growth rate saturation and displays Monod kinetics; phase 2 occurs as diffusion limits nutrient transfer into the algal layer which constrains total algal biomass and is a linear process; and phase 3 displays nutrient saturation in which there is no further positive effect of nutrient increases on algal growth or biomass. This conceptual model explains why Horner et al. (1983) found rapid increases in biomass up to 25  $\mu\text{g/L}$  DIP (phase 1) above which the biomass increased at a much slower rate (phase 2). Therefore, while algal growth rates may be nutrient saturated at low concentrations, the total amount of algal biomass may continue to respond to nutrient enrichment if nutrient transfer into the algal layer is limited by diffusion rates.

A general issue regarding nutrient limitation is whether the focus is on the entire system, a community, or a species. Methods for detecting nutrient limitation usually only focus on one of these aspects. For example, short-term nutrient enrichment of a stream reach measures the combined response of all organisms residing therein, whereas nutrient diffusing substrata assay the algal community that prefers the substrate type. Although these types of nutrient limitation assays may indicate that N or P is the primary limiting nutrient, results do not preclude the possibility that an individual species is limited by an alternative nutrient (Franceour 2001). Changes in both the total amount and relative abundance of nutrients often lead to altered algal community structure indicating that some species have strong preferences for particular nutrients (Stelzer and Lamberti 2001). One common occurrence is the dominance by cyanobacteria when N is limiting and N:P ratios are low, due to the ability of many cyanobacteria species to fix atmospheric N (Peterson and Grimm 1992; Schindler et al. 2008).

Regardless of the degree of nutrient limitation, there may be other factors that exert strong control on algae, particularly in lotic systems. For example, local flow velocities and the intensity and frequency of flood events can limit the amount of algal biomass in a given area (Biggs and Close 1989; Biggs 1996; Biggs et al. 1998). The type and amount of algal grazers such as snails and other invertebrates can also reduce algal biomass (Mulholland et al. 1991; Rosemond et al. 1993; Feminella and Hawkins 1995; Rosemond et al. 2000), through both consumptive and non-consumptive losses (Scrimgeour et al. 1991). Biotic controls on algae may be complex, since alteration of the density of one species in an interconnected food web can lead to

significant alterations of the other species (Carpenter et al. 1985). In shallow lakes in the United Kingdom, aquatic plants were ultimately controlled by fish densities rather than nutrients, since fish consumed invertebrates which consumed epiphytic algae which grew on the plants (Jones and Sayer 2003).

### **Nutrient Spiraling**

The concept of nutrient spiraling is the basis of a fundamental theoretical framework for analyzing nutrient cycling in lotic systems (Newbold et al. 1981). Nutrient spiraling describes how nutrients are cycled between inorganic and organic forms as they are displaced some distance downstream by the advective flow of water. Novel measurement methods created to test this theory involve adding nutrients to a stream, sometimes as isotopic or radioactive tracers, and measuring the decline in the added material with distance downstream. This rate of downstream decline can then be used to calculate the uptake length,  $S_w$ , which is the average distance downstream a dissolved inorganic nutrient molecule travels before being removed from the water by biotic and abiotic processes. Since  $S_w$  is dependent on the size of the system, it is often converted to an uptake velocity,  $v_f$ , or areal uptake rate,  $U$  (SSW 1990). The uptake velocity is also known as the mass transfer coefficient and is considered a measure of the biotic and abiotic demand for a nutrient relative to its water column concentration (Hall and Tank 2003).

Nutrient spiraling theory describes nutrient limitation in lotic systems as both uptake length and uptake velocity are proportional to the degree of nutrient limitation. With decreasing nutrient limitation, nutrient molecules travel farther before being utilized and thus the uptake length increases and the uptake velocity decreases. This theory has been applied to a model including different functional groups of invertebrates which

suggests that as nutrient availability varies, components of lotic systems adjust their cycling (i.e. uptake and remineralization) rates to compensate (Newbold et al. 1982). This tendency of the biota to self-organize to alleviate nutrient limitation, combined with the continuous replenishment of nutrients from upstream, indicates that nutrient limitation in lotic systems is most likely when nutrient levels are extremely low.

Nutrient spiraling measurements have been used to characterize nutrient limitation and saturation levels in lotic systems. Two studies of individual streams used N additions to examine relationships between spiraling metrics and N concentration based on Michaelis-Menton kinetics (Earl et al. 2006; Covino et al. 2010a). Another study determined spiraling metrics for a suite of streams spanning a wide range of N concentrations and found an efficiency-loss response across systems (O'Brien et al. 2007). Since nutrient spiraling is currently the accepted framework for understanding nutrient cycling in lotic systems, nutrient spiraling metrics could be useful for setting nutrient criteria whose goal is to maintain ecosystem integrity.

### **Effects of Flow on Lotic Algae**

The flow of water is the distinguishing characteristic of lotic systems. Within lotic ecosystems, both the magnitude and variation of flow largely determine the distribution of species and the rates of ecological processes. There are many aspects of flow and methods for its quantification; however, the overall discharge rate and local flow velocities are the primary factors affecting lotic algae. Temporal changes in discharge alter water depths and velocities, and sharp increases may cause scouring events. With regard to nutrient dynamics, discharge is directly related to the downstream advective nutrient flux in the water column, which controls the rate of nutrient replenishment. At a smaller scale, local flow velocities are a dominant driver of algal

community structure as some species prefer a certain velocity range (Whitford 1956; McIntire 1968; Traaen and Lindstrom 1983; Ghosh and Gaur 1991). A compilation of evidence from studies of lotic systems suggests that generally a subsidy-stress relationship exists between flow velocity and attached algae (Horner and Welch 1981; Stevenson 1996; Biggs et al. 1998); where velocity can aid algal growth by increasing nutrient uptake, but also constrains algal abundance due to drag. These opposing influences make flow a major determinant of both algal growth rates and the ultimate amount of algal biomass that can persist in a given area of a lotic system.

### **Flow as a Subsidy to Algae**

The subsidy effects of flow on attached algae in lotic systems largely involve enhancement of nutrient availability. There are two general mechanisms by which flow influences nutrient supply to algae: flow velocity regulation of diffusive boundary layer (DBL) thickness and the contribution of overall discharge to the advective flux of nutrients through the water column. These two processes act as controls on the rate of nutrient transfer from the water column to algal cells and the rate of nutrient replenishment in the water column, respectively. The magnitude of the subsidy effect is dependent on the degree to which algae are nutrient limited, as higher flow would not aid algal growth when nutrients are readily available (although see Mass et al. 2010).

The DBL is the stagnant layer of water that surrounds all submerged surfaces in aquatic systems, through which nutrients and other dissolved ions and gases must diffuse to reach cell surfaces. The mass transfer rate of nutrients due to molecular diffusion is proportional to the thickness of the DBL (i.e. the diffusion distance) and the concentration gradient (i.e. the difference in nutrient concentration on either side of the DBL). Larger algal species may protrude through the substratum DBL but nutrient

transfer may remain diffusion limited due to the DBL surrounding the algal cells themselves (Larned et al. 2004). The thickness of a DBL is inversely related to the local flow velocity, and as a DBL becomes thinner with increasing flow velocity the transfer rate of nutrients from the water column to algal cells increases (Jorgensen and Revsbech 1985; Gundersen and Jorgensen 1990; Hurd 2000). For mat-forming filamentous algae, higher velocities also increase diffusion of nutrients into the algal matrix (Stevenson and Glover 1993). As flow velocity increases, the DBL eventually becomes thin enough to no longer limit nutrient transfer, and instead cellular uptake rates (i.e. kinetics) control transfer rates (Sanford and Crawford 2000; Nishihara and Ackerman 2009). When diffusive mass transfer does not limit nutrient uptake, the concentration of nutrients in the water column may not be a good predictor of nutrient availability to algal cells since molecular diffusion is no longer the driving process.

For lotic systems, the rate at which nutrients are replenished throughout the water column is largely dependent on the discharge rate (advection) and turbulent mixing (dispersion). The advective nutrient flux is equivalent to the mass of nutrients flowing past a point per time and is derived from the product of discharge and nutrient concentration. Without inputs from groundwater or tributaries, nutrient concentrations typically decrease with distance downstream due to either biotic assimilation or dissimilation (e.g. denitrification). This rate of decline is inversely proportional to the advective nutrient flux, assuming that biotic demand remains constant. Therefore nutrient flux is related to nutrient supply to benthic algae, particularly when water column nutrient depletion is significant. Under conditions in which the water column is well-mixed and diffusion through the DBL is not the limiting factor, the advective nutrient

flux may be a better representation of nutrient availability to algal cells than the water column nutrient concentration. No studies have explicitly focused on the role of flow in relation to the advective nutrient flux in the water column, although some authors have acknowledged the potential importance of this factor (Allan 1995; Borchardt 1996; Tank and Dodds 2003).

There is abundant evidence that flow velocity can subsidize algal nutrient uptake and biomass. A positive relationship was documented in laboratory streams for flow velocity and algal nutrient uptake (Whitford and Schumacher 1961, 1964; Lock and John 1979; Horner et al. 1990; Larned et al. 2004) and algal biomass (McIntire 1966; Horner et al. 1983; Biggs and Hickey 1994). Welch et al. (1988) found a positive relationship between algal biomass and velocity (9 - 22 cm/s) in Washington streams. While most studies of the subsidy effect of flow velocity on algal growth focus on nutrient uptake, an equally important mechanism may be velocity enhancement of waste efflux. There is evidence that velocity induced efflux of oxygen (a byproduct of photosynthesis) away from cells has a strong influence on autotrophic production rates (Mass et al. 2010).

Some studies have found that the subsidy effect of flow on algae only occurred when nutrient concentrations were above a certain threshold (Horner and Welch 1981; Horner et al. 1983). This seems counterintuitive since the subsidy effect of flow should be greater when nutrient availability is less; however it is hypothesized that in some situations algae require sufficient nutrients to allow high enough growth rates to counteract the drag force induced by higher velocities (Horner and Welch 1981). Therefore a sufficient nutrient supply may be a prerequisite for some algae to become

established in a high flow environment. Also, if internal recycling within the DBL is a significant nutrient source (at low nutrient levels) then this recycling may be impeded with higher flow velocities resulting in lower algal growth rates.

Indirectly flow velocity can subsidize algae by increasing the drag on algal grazers and thus reducing their consumption rates (DeNicola and McIntire 1991; Poff and Ward 1992; Poff and Ward 1995; Opsahl et al. 2003). This relationship may be complex since each grazer species may have an optimal velocity range for feeding or may prefer certain algal species that have their own optimal velocity range. Also in some cases grazers can actually aid algal growth by excreting nutrients and removing overlying or senescent cells (McCormick and Stevenson 1991). When increasing velocity causes algal grazing rates to decrease then this is another mechanism that can lead to increased algal biomass, and may confound studies examining the direct relationship between flow velocity and algal biomass (e.g. Opsahl et al. 2003).

### **Flow as a Stress to Algae**

The influence of flow as a stressor to attached algae is more straightforward since only one mechanism is typically involved. Essentially with increasing flow velocity there is also an increase in drag force on algal cells, which both inhibits initial colonization and creates a sloughing effect whereby algae are detached from their substrate and exported downstream (Stevenson 1996). Drag force increases at a rate proportional to the square of the flow velocity, so that small changes in velocity can potentially lead to significant changes in algal export rates and community structure. Drag force has two components: viscous drag due to the shear force of water moving over a surface and form drag due to pressure variation over a surface (Larned 2010).

The sloughing effect is highly dependent on the physiognomic form of the algae, as the various forms are suited to particular hydraulic conditions (Biggs and Thomsen 1995; Biggs et al. 1998). Smaller algal species that adhere closely to a substrate are less likely to slough and may be sheltered within the boundary layer of the substrate. Some of these small species have strong attachment mechanisms and are therefore suited to high flow velocity environments. At the other end of the spectrum are some larger filamentous algal species which typically do not have strong attachment mechanisms and often form thick mats that consist of layers of intertwined filaments. These larger filamentous species usually penetrate the substrate boundary layer where they are exposed to higher velocities and drag. These features of mat-forming filamentous algae generally make them more suitable for lower velocity environments.

Some studies have shown that algal biomass (Poff et al. 1990; Biggs and Gerbeaux 1993) and algal export rates (McIntire 1966; Biggs and Thomsen 1995) are inversely related to flow velocity. High velocities may also reduce algal immigration and subsequent colonization rates (Stevenson 1983; Stevenson and Peterson 1989). One study did not detect increased export over a range of velocities (Horner et al. 1983), which may indicate that sudden increases in velocity are more important than the magnitude itself (Horner et al. 1990). At the ecosystem level, the amount of autotrophic production and biomass in a stream reach is strongly dependent on the intensity of and time since the last flood event for streams which have high flow variability (Tett et al. 1978; Fisher et al. 1982; Biggs and Close 1989; Young and Huryn 1996; Biggs 2000), highlighting the temporal aspect of flow velocity control on algae. Flow also affects the

type of substrate present, and since most algae prefer certain substrates this could have significant effects on algal community structure (Tett et al. 1978).

Flow may also act as a stress unrelated to drag induced export in some situations, although the mechanisms involved have not been determined. Humphrey and Stevenson (1992) showed negative effects of flow velocity on algal growth rates in low nutrient conditions, but no effect when nutrients were high. Likewise, Borchardt et al. (1994) found flow velocity was negatively related to P uptake and algal production rates at high current velocities, unrelated to sloughing. Various hypotheses have been posited to explain this stress effect of flow, such as compression of algal filaments, alteration of the microenvironment surrounding cells, stretching of cellular ion channels, and increased efflux of nutrients from cells (Borchardt et al. 1994).

### **The Subsidy-Stress Relationship between Flow and Algae**

While the subsidy and stress mechanisms involving flow effects on algae are well documented, there are few studies that have specified the velocity range for both the subsidy and stress responses, and few optimal intermediate velocities have been reported. A subsidy-stress relationship has been found between flow velocity and algal biomass in both natural and laboratory streams with a peak biomass in the range of 50-70 cm/s (Horner and Welch 1981; Horner et al. 1990; Biggs and Stokseth 1996). Biggs et al. (1998) found all three response types (subsidy, stress, and subsidy-stress) when examining algal species with different growth forms in relation to flow regime. In one study a stress effect of flow velocity was found for certain species, but velocity was determined to be a subsidy effect for the total amount of algal biomass (McIntire 1968). Overall, the effects of flow on algae are difficult to predict due to the variety of

mechanisms involved and the different responses of individual algal species, as well as interactions with other factors such as nutrients, light, and grazers.

### **Florida Springs**

Although the theoretical principles examined in this research regarding effects of flow on filamentous algae and autotrophic nutrient limitation are applicable to lotic systems in general, the goal was to apply this knowledge to artesian springs and spring-fed rivers in Florida. Florida springs as a whole constitute ecosystems at risk, as there have been significant changes in system structure and function over recent decades, particularly proliferation of filamentous algae (e.g. Munch et al. 2006). Algal proliferation has largely been attributed to increasing nitrate concentrations as consistent with the eutrophication paradigm; however, other factors have been suggested such as loss of algal consumers, declining discharge rates, and increased human disturbance. The lack of clear evidence that nitrate enrichment is the primary cause of algal proliferation indicates that changes in other factors are likely contributing to the degradation of these systems (Heffernan et al. 2010b).

### **Springs Ecology and Nutrient Limitation**

Florida's large artesian springs and spring-fed rivers have a long history of human use; however, their ecology did not receive much attention until they became the focus of research in the 1950s. Florida springs were ideal settings to test innovative methods for measuring whole ecosystem structure and metabolism (Odum 1957a, 1957b).

These studies provided a wealth of knowledge, including the determination that these springs are among the most productive ecosystems in the world due to their extremely clear water and relatively stable temperature, chemistry, and hydrology. Odum (1957a) also demonstrated that these spring ecosystems were highly tuned to light levels, with

no evidence of nutrient limitation. Odum (1957a) explained, “That the nutrients which limit production under transient conditions are not important in steady state seems reasonable since the large and continuous flows are continually renewing the aquatic medium so that no real deficit of nutrients can develop.” At this same time the algal community was studied in detail and it was found that flow velocity was a primary control on algal distribution patterns (Whitford 1956).

Decades later, Odum’s Silver Springs study was replicated by one of his students (Knight 1980), and no ecological changes were detected except for a decline in fish biomass which was attributed to construction of the Rodman Dam downstream. By the 1990s iconic springs such as Ichetucknee, Wakulla, Wekiwa, and Silver were showing signs of degradation, mainly in the form of increasing filamentous algae. These issues led to the formation of the Florida Springs Task Force, a multi-agency group charged with providing “recommended strategies for the protection and restoration of Florida’s springs”. In their first report (FSTF 2000), increasing nitrate levels and declining discharge rates were highlighted as potential causes of ecological degradation.

At the turn of the century two major ecological studies were conducted: a third follow-up to the Silver Springs studies (Munch et al. 2006) and a comprehensive study of the relationship between nutrients and algal proliferation (Stevenson et al. 2004, 2007). The Silver Springs study found a further decline in fish biomass and substantially more epiphytic algae and benthic algal mat biomass than in the 1950s. Ecosystem production and respiration rates had also declined as had discharge rates, and nitrate concentrations exceeded 1 mg N/L. The comprehensive study of algae and nutrients involved many parts including a survey of algal cover and water quality metrics

in 29 springs and various laboratory experiments testing the response of common filamentous algal species to different nutrient concentrations. The survey determined that *Vaucheria* was positively related to both N and P concentrations, but the other common algal species, *Lyngbya*, did not correlate with either nutrient. Among the springs studied there was no indication of the expected positive relationship between total algal cover and nitrate concentration, as there were multiple springs with high nitrate and low algae levels and vice versa. The experimental results were highly variable, as some found a relationship between filamentous algae and nutrient concentration and others did not.

Multiple other studies have focused on drivers of the autotrophic community, particularly filamentous algae; however much uncertainty remains regarding the role of nutrients. A survey of 31 spring-fed rivers did not find a relationship between either autotrophic biomass or productivity and nutrient concentration, but did find an inverse relationship with canopy cover suggesting that these systems were limited by light rather than nutrients (Duarte and Canfield 1990). An experiment involving nutrient additions to in situ tubes in the spring-fed Chassahowitzka River found that algal growth on glass slides was stimulated by P but not N, suggesting P limitation (Notestein et al. 2003). A laboratory experiment tested various nitrate concentrations on the growth of *Lyngbya wollei* and found a substantial increase in growth rates from 0.3 to 0.6 mg N/L (Cowell and Dawes 2004), contrary to other studies (e.g. Stevenson et al. 2004, 2007).

One explanation for the lack of correspondence between the results of these studies is the difference in the flow environment. The only experiments with flow representative of lotic systems were those which used semi-recirculating channels with

a moderate flow velocity of 25 cm/s (Stevenson et al. 2004, 2007). For these particular experiments, most did not detect nutrient limitation for either *Vaucheria* or *Lyngbya*; however, one found that *Lyngbya* growth rates were saturated at a nitrate concentration of 0.11 mg N/L. In comparison to studies which found higher thresholds for N saturation, these results may indicate that higher flow velocities can reduce the nutrient concentrations required to achieve saturation. High rates of water exchange utilized in other experiments likely did not compensate for effects of flow velocity on nutrient transfer through boundary layers (and potentially other effects), and therefore higher nutrient concentrations were required to alleviate nutrient limitation.

### **Autotrophic Community Shifts**

The autotrophic (i.e. photosynthetic) community in Florida springs was characterized by dense beds of submerged aquatic vegetation (SAV), primarily the grasses *Sagittaria kurziana* and *Vallisneria americana*, and a diverse assemblage of epiphytic and benthic algae (Whitford 1956). More recently, large filamentous algal mats have proliferated in many springs, and are likely competing with the epiphytic algae and SAV that defined the historic autotrophic community. Severe algal proliferation may cause shifts from SAV to algal dominance due to competition for light and nutrients (Duarte 1995; Hilton et al. 2006), which may explain the observed decline in SAV in some springs. Alternatively, SAV loss may be mostly due to increased uprooting resulting, in part, from either nutrient enrichment which can cause rooted SAV to develop longer leaves and less below ground material (Nixon et al. 2001) or shifts in sediment composition to finer particles with less cohesion (personal observation). Human activities such as wading and boating are also a direct cause of SAV loss and could exacerbate uprooting.

A recent survey of Florida springs identified 23 species of macroalgae, of which *Lyngbya wollei* and *Vaucheria* spp. were the two most common (Stevenson et al. 2007). These species were present in the past in smaller amounts, but now cover 50% of the benthic surface on average (Stevenson et al. 2007) and have surpassed SAV biomass in some systems (e.g. Munch et al. 2006). Although the classification of these species has varied over time, it appears that they are native, rather than invasive exotics, since they were found in Silver Springs in 1950s (Whitford 1956); therefore a significant alteration in their environmental controls must have occurred to allow their proliferation.

*Lyngbya* spp. is a particularly problematic alga which has received considerable attention globally over recent decades due to its ability to form expansive, resilient mats and its potential to produce toxins (Carmichael et al. 1997; Ahern et al. 2006). *Lyngbya wollei* is a filamentous cyanobacterium with multiple characteristics that give it the potential to proliferate in aquatic systems: adaptation to low light levels (Speziale et al. 1991), ability to fix atmospheric nitrogen (Phlips et al. 1992), and a thick sheath that serves to inhibit grazing (Camacho and Thacker 2006). *Lyngbya wollei* has been the focus of management efforts throughout the southeastern U.S., particularly in shallow lakes and reservoirs (Speziale et al. 1991; Phlips et al. 1992; Doyle and Smart 1998). *Lyngbya wollei* within Florida springs may actually consist of two or more species based on molecular and morphological data, and each species may have different water chemistry preferences (Joyner et al. 2008).

Despite the potential for some algal species to proliferate, algae are an important component of Florida springs ecosystems. Even problematic filamentous algae such as *Lyngbya* provide habitat for a large amount and diversity of invertebrates, especially

amphipods and gastropods (personal observation), and are likely significant contributors to chemical cycling through alteration of oxygen, carbon, and nutrient levels. Large-scale eradication techniques such as intensive herbicide application and use of mechanical harvesters should be given careful consideration as they may lead to further ecological degradation.

### **Potential Causes of Filamentous Algal Proliferation**

Florida springs are changing in a variety of ways, which makes it difficult to determine the specific causes and effects among drivers and components of the ecosystem. Overall, there is little historical ecological data available with which to examine changes over time, with Silver Springs having the best long-term record. In fact, much of the evidence for algal proliferation is based on anecdotes and comparisons with historical photographs; however, the current amount of filamentous algae in many springs appears to be unprecedented as are the changes in other ecological attributes (e.g. declines in SAV and fish biomass). The available long-term data consists mostly of discharge rates and water chemistry parameters, which generally show that discharge rates are declining and nitrate concentrations are increasing. Since the available data do not indicate a single definitive cause of filamentous algal proliferation in Florida spring systems, all potential factors should be given consideration including increased nitrate concentrations, loss of algal consumers, decreased discharge, increased human recreation, and other direct disturbances such as aquatic plant management (Heffernan et al. 2010b).

In many aquatic systems, nutrient enrichment is the primary cause of excessive aquatic plant and algal growth. Nutrients (primarily N and P) often limit algal growth because of their relatively low availability in relation to that required by algae; however,

once nutrients become readily available then algal growth is nutrient saturated and further increases in nutrients will have no additional effect on algal growth. For the main filamentous algal species proliferating in Florida springs, the saturating N concentrations were estimated to be 0.59 mg/L and 0.25 mg/L for *Vaucheria* and *Lyngbya*, respectively (Stevenson et al. 2007). It would take reductions in N below the saturating concentrations to begin constraining the growth of these algal species, and possibly much more to achieve a significant reduction in algal biomass. Historically, N concentrations in springs were likely 0.05 – 0.1 mg/L and today the average concentration is > 1 mg/L (Heffernan et al. 2010b); therefore, it is feasible that N enrichment has increased algal growth rates. However, there are multiple studies which have not found a strong relationship between nutrient concentrations and autotrophic biomass and productivity (including filamentous algae) within Florida spring systems (Odum 1957a; Duarte and Canfield 1990; Cowell and Botts 1994; Terrell and Canfield 1996) and the overall narrative that N enrichment is the primary cause of algal proliferation has been questioned (Heffernan et al. 2010b). Saturation concentrations for P have also been estimated (26 µg/L and 33 µg/L for *Vaucheria* and *Lyngbya*, respectively (Stevenson et al. 2007)); however, P enrichment in springs is uncommon since P is readily retained in the calcium carbonate matrix of the aquifer.

An alternative mechanism which could lead to filamentous algal proliferation is the loss of organisms that consume algae (i.e. algal grazers) such as certain snails and other invertebrates as well as some fish species. The magnitude of consumer loss and their effect on algae is currently under investigation; however, there is preliminary evidence that this could be a substantial cause of algal proliferation (Heffernan et al.

2010b). Consumer loss may be primarily due to declines in the dissolved oxygen (DO) content of spring water, which stresses the organisms and can lead to reduced algal consumption rates. Other potential drivers of reduced algal consumption could be loss of habitat such as SAV beds, and the presence of toxins such as pesticides and other man-made chemicals. At high enough concentrations, nitrate may also act as a toxin to certain aquatic organisms (> 2 mg N/L: Camargo et al. 2005), and these concentrations have been observed in some springs and may occur in many others in the near future.

Declining discharge is often mentioned as a cause of springs degradation; however, this is usually in reference to substantial declines in flow that lead to complete loss of a spring such as has occurred for Kensington, White, and Worthington Springs. The effect of smaller declines in discharge on algal control mechanisms has not received much attention. This may partially be due to the difficulty in determining whether the discharge for a given spring is actually declining, as there is inherent variation on a monthly to annual timescale. This variation is often correlated with recent rainfall in the spring recharge area; however, there are long-term declines in some springs which could be due to consumptive use of groundwater by humans (Weber and Perry 2006; Williams 2006). Alteration of the landscape in a way that lowers the permeability of the land surface would also lead to less recharge to the aquifer. The potential for groundwater pumping to affect aquifer levels regionally is only now becoming evident, as it has been shown that the groundwater divide in north-central Florida has shifted substantially over recent decades due to pumping in the Jacksonville area (Grubbs 2011).

Declining discharge rates could be involved in multiple mechanisms which result in filamentous algal proliferation including less algal export, decreased DO levels, and increased nutrient concentrations. In most spring systems, discharge rates are proportional to flow velocities throughout the channel. Since flow velocity is related to drag on filamentous algae, declining discharge rates could lead to less export and increased algal biomass. Lower DO levels observed in many springs could be related to lower discharge rates due to older, deeper groundwater making up a greater proportion of spring discharge (Katz et al. 1999), with subsequent reduction of algal consumption. In some springs, nitrate concentrations are inversely related to discharge (Katz et al. 1999). Thus, declining discharge may increase the potential for nutrient enrichment and stimulation of algal growth rates.

Some human activities directly disturb the ecosystem including increased recreational use, channel alterations, and aquatic plant management. Springs are enjoyed by many people for recreational purposes such as swimming, wading, tubing, snorkeling, SCUBA diving, paddling, boating, and fishing. While these activities likely have minimal effects on spring ecosystems when few people are involved, a large number of users can lead to significant impacts. Recreational impacts have received little attention by scientists; however, one study of the Ichetucknee River found that SAV damage and export increased with the amount of people tubing and SCUBA diving (DuToit 1979). As a result of this study the amount of tubers and divers on the Ichetucknee River are now regulated to minimize these impacts, but degradation related to recreation is still a problem for this spring system and many others.

Other impacts related to human activities are the result of channel alterations such as damming and dredging and large-scale aquatic plant management. Where these alterations lower flow velocities, conditions may favor filamentous algal proliferation. In some cases migration of manatees and fish such as mullet and catfish are also impeded, and the loss of their top-down control on spring food webs could have significant ramifications. Aquatic plant management is potentially a major disturbance to spring ecosystems where intensive methods such as widespread herbicide application and large mechanical harvesters are used. Exotic invasive plants such as hydrilla and water hyacinth became problematic in some spring systems, notably Kings Bay and Wakulla Springs, resulting in management plans for their eradication. In most cases this involved substantial application of various herbicides, which were largely unsuccessful at eliminating the target plant species and may have further contributed to ecosystem degradation. In Kings Bay, many of the nuisance plants eventually declined (mostly unrelated to management efforts) and were replaced by filamentous algae, primarily *Lyngbya* (Evans et al. 2007). When chemical application failed to control the algae, then mechanical harvesters were used to directly remove algae from the bottom of the bay, a practice which continues today. While control of invasive exotic plants is a laudable goal, the consequences of these efforts may actually promote filamentous algal proliferation by increasing algal dispersal and removing competitors for light and nutrients.

### **Flow Velocity Control of Filamentous Algae**

There is some evidence that the drag created by flow velocity is a significant control on filamentous algal abundance in Florida spring systems. There are observations that filamentous algal biomass is higher in low velocity areas such as

topographic depressions (Munch et al. 2006; Quinlan et al. 2008), as well as along channel margins and in the vicinity of spring boils where velocities are typically lower (personal observation). SAV and other stable substrates may be necessary to support substantial filamentous algal biomass in higher velocity areas because the algae can wrap around plant stems in order to stay in place and SAV beds may provide lower velocity areas where benthic algal mats may form. In Florida springs, the effect of flow velocity on filamentous algae has not received much attention; however, a study of three coastal systems found that algal biomass was minimal where velocities were  $> 25$  cm/s (Hoyer et al. 2004).

The available evidence suggests a negative (i.e. stress) relationship between flow velocity and filamentous algae in springs. Overall, this relationship may explain the spatial heterogeneity of algal biomass within a given spring more than it explains widespread algal proliferation. However, since flow velocity can have a large effect on algal export, a decline in velocity over time could be a mechanism for increased algal biomass in lotic systems. Although declining spring discharge is not necessarily related to declining flow velocity in a given area, substantially lower discharge would have less kinetic energy and therefore velocities would be lower overall throughout the system. The influence of SAV beds on local flow velocities may be important due to their role in constraining the channel area, which causes faster velocities above the beds and lower velocities within them. Additionally, since flow velocity is positively related to nutrient availability, lower velocities could increase the likelihood of nutrient limitation where nutrient concentrations are low enough.

## Synthesis

This research was inspired by the need to delve deeper into the causes of filamentous algal proliferation in Florida springs. There were apparent contradictions between available evidence and the narrative that nitrate enrichment and the alleviation of algal N-limitation (i.e. eutrophication) was the predominant cause. Lotic systems are fundamentally different than other aquatic systems and paradigms from these other systems should be applied with caution in the absence of sufficient evidence demonstrating their effectiveness. The role of flow as a primary control on nutrient availability should be considered when determining whether algae and other autotrophs are nutrient limited. Nutrient concentration may not be the best descriptor of nutrient availability in lotic systems, and instead nutrient flux should be assessed as a metric of nutrient limitation. In addition, local flow velocities also dictate whether algae can even begin to colonize an area due to drag forces, and are a strong determinant of the ultimate amount of algal biomass that can persist in an area. The dual role of flow as a control on algal abundance in lotic systems is an example of a subsidy-stress relationship, since some amount of flow positively affects the algae until a threshold is reached past which higher flow is increasingly a negative influence. There are many other factors that are also important when considering what controls algal abundance in a specific area: such as the amount of light, nutrients, algal consumers, the type of substrate, and the specific response of each algal species to all of these factors. However the dominant role of flow must be accounted for in order to determine whether nutrients are a limiting factor and whether drag forces are a significant constraint on algal abundance in a lotic system.

Lotic systems are expected to respond differently to nutrient enrichment than lentic systems, since in the latter flow is not a dominant factor and algal communities often consist primarily of free-floating phytoplankton rather than attached algae. In lentic systems the major controls on algal biomass are nutrients, consumers, and light availability. Algal consumers may exert significant control over algal biomass and autotrophic community shifts (Carpenter et al. 1985; Jones and Sayer 2003), but their influence may become less significant with increasing nutrient levels. Therefore, when nutrients are readily available algal biomass may accumulate until light penetration into the water column becomes the limiting factor for further growth. However, in lotic systems with sufficient flow it is unlikely that nutrients will be severely limiting to attached algae, so there must be other controls that prevent algae from covering the entire surface area. Local flow velocities and algal consumers constrain the amount of attached algae that can persist in an area, and therefore factors which reduce the strength of these mechanisms could lead to algal proliferation.

The influence of nutrients on the ultimate (i.e. steady state) amount of algal biomass in lotic systems remains uncertain. During algal succession, there is often an accrual phase that leads to peak biomass, which may be followed by loss of biomass due to sloughing that eventually levels off at an ultimate biomass (e.g. Rier and Stevenson 2006). Grimm and Fisher (1986) hypothesized that nutrients should not control the ultimate amount of algal biomass in streams, since eventually any necessary nutrients will flow by and become available. Therefore under conditions of nutrient limitation it may take longer for algae to reach the ultimate biomass due to lower growth rates, but over time the ultimate biomass would be equivalent regardless of nutrient

concentration. There is some support for this conceptual model as the ultimate biomass was similar for different nutrient concentrations in stream mesocosms, although peak biomass was correlated with nutrient concentration (Rier and Stevenson 2006). This model, however, omits the direct effects of flow velocity which can have both positive and negative effects on the ultimate algal biomass. Higher velocity may result in greater algal biomass (McIntire 1966) or it can prevent algal biomass from achieving the same level as in a lower velocity (Horner and Welch 1981; Poff et al. 1990; Peterson and Stevenson 1990). There are also complex temporal dynamics as peak biomass may increase with velocity, but subsequent drag may reduce the ultimate biomass to a lower level than would occur in a lower velocity environment (Biggs and Stokseth 1996). While differences in nutrient concentrations may not substantially affect the ultimate amount of algal biomass in lotic systems, differences in flow velocity can lead to a variety of possible outcomes resulting from the opposing effects of nutrient availability and drag during algal colonization and succession.

A more advanced conceptual model separates the subsidy and stress roles of flow. The subsidy effect on nutrient supply increases algal growth rates and peak biomass, but the stress effect of drag determines the ultimate amount of algal biomass in conjunction with nutrient concentration. This model is similar to the habitat suitability framework proposed by Biggs (1996) which focuses on the roles of “resources” and “disturbances” as controls on autotrophic growth and loss rates, respectively. If a portion of an algal population is continuously exported downstream due to drag forces, then a species with a higher growth rate should be able to achieve higher ultimate biomass through faster replacement of exported material. Algal growth rates may be

stimulated by increased nutrient availability due to either increased nutrient concentration or velocity, which could shift the balance between growth rates and export rates. This model highlights the importance of factors that affect both growth and export rates and therefore suggests a mass-balance approach would be beneficial for studying algal dynamics in lotic systems (e.g. Hecky and Kilham 1989).

In order to apply a mass-balance approach to filamentous algal proliferation in Florida springs, the influence of flow velocity on algal growth and export rates must be quantified. In all but the lowest nutrient springs, nutrient availability is likely high enough for algae to grow at maximum rates (i.e. nutrient saturation); however declining discharge may lead to lower velocities and less algal export which could be a significant cause of algal proliferation. Lower velocity areas within spring systems, such as near spring boils, may contain favorable conditions for algal proliferation and are where algae are most likely to respond to nutrient enrichment. Determining the response of filamentous algae, such as *Lyngbya wollei*, to a range of nutrient concentrations and flow velocities will improve understanding of the causes of algal proliferation and nutrient limitation in Florida springs and other lotic systems.

## CHAPTER 3 NUTRIENT FLUX, DEMAND, AND AUTOTROPHIC LIMITATION IN LOTIC SYSTEMS

### **Introduction**

Nutrient enrichment is often identified as the primary cause of changes in aquatic ecosystem productivity and community composition (Carpenter et al. 1998; Elser et al. 2007). Prior to enrichment the availability of a nutrient limits autotrophic production, and alleviation of nutrient limitation permits additional production, with a suite of potentially undesirable consequences. Typically, nutrient availability is inferred from water column concentration, with limitation assessed based on the concentration alone or from the ratio with other potentially limiting nutrients. This approach has been useful for diagnosing nutrient limitation in lakes, estuaries and reservoirs, but has been less successful for lotic systems (i.e. rivers and streams), where nutrient concentrations alone fail to reliably predict autotrophic biomass (Dodds et al. 2002b).

Several plausible reasons might account for the differential utility of concentration as a predictor of nutrient limitation in lentic and lotic systems. First, compared to lakes, lotic systems are more likely to be limited by factors other than nutrients such as flood disturbances and riparian shading (Biggs 2000; Rosemond et al. 2000). Perhaps more important, however, are the differences between lakes and streams with regard to hydraulic residence time. The initial development of models for predicting nutrient limitation in lakes explicitly considered system hydraulics (i.e. flushing rates; Vollenweider 1982). Despite substantially shorter residence times in streams than in lakes, hydraulics are generally not considered in models of stream and river eutrophication (Hilton et al. 2006). This study argues that the hydraulic properties of lotic systems limit the utility of concentration as a predictor of autotrophic nutrient

limitation, specifically because concentration alone does not take into account continuous nutrient resupply from advection and (re)mineralization.

Despite the centrality of nutrient spiraling theory (Newbold et al. 1982) to stream ecology and its explicit consideration of system hydraulics; metrics derived from that body of theory have yet to be incorporated broadly into predictions of lotic nutrient limitation. This study explores a new metric of lotic ecosystem nutrient limitation called the autotrophic uptake length ( $S_{w,a}$ ).  $S_{w,a}$  is based, in part, on nutrient flux, and thus takes residence time into account to better represent nutrient availability to the biota. The metric further considers whether the nutrient flux is sufficient to saturate autotrophic nutrient demand (which may be limited by light or post-flood successional status); since where fluxes are in excess of demand, nutrient enrichment may have little effect, even if concentrations are relatively low.

### **Nutrient Limitation in Lotic Systems**

Evidence abounds that nutrient concentrations predict autotrophic productivity and biomass more strongly for lentic than lotic systems. Dodds et al. (2002b) showed that total nitrogen (N) and total phosphorus (P) combined explained less than 40% of the variation in chlorophyll a for benthic algae in streams, whereas these variables explain more than 60% of the variation in planktonic algae in lakes. Likewise, ammonium uptake was only weakly linked ( $R^2 = 0.41$ ) to concentration in a study of headwater streams across biomes (Dodds et al. 2002a). A study of 30 streams also in multiple biomes (Lamberti and Steinman 1997) concluded that annual gross primary production (GPP) was only weakly explained by soluble reactive phosphorus (SRP;  $R^2 = 0.38$ ) and that total P,  $NH_4$ , and  $NO_3$  were not significant predictors. Several cross-system studies found that light rather than nutrients was the dominant control on autotrophic

production (North American streams - Mulholland et al. 2001; Florida spring-fed rivers - Odum 1957a; a New Zealand river continuum - Young and Huryn 1996). Finally, in a meta-analysis of nutrient diffusing substrata in streams, nearly half the experiments (42.6%) did not show a significant response to nutrient addition (Franceour 2001). Regional characteristics may influence this association, as a few studies conducted within a single region yielded higher correlations between algal biomass and nutrients ( $0.63 > R^2 > 0.46$ ) (Biggs and Close 1989; Lohman et al. 1992; Chetelat et al. 1999; Ludwig et al. 2008), but not in all cases ( $R^2 < 0.13$ ; Sanderson et al. 2009). The much higher predictive power ( $0.95 > R^2 > 0.49$ ) (Dillon and Rigler 1974; Jones and Bachmann 1976; Smith 1982; Vollenweider 1982; Brown et al. 2000) of lentic eutrophication models based on nutrient concentrations alone suggests some crucial knowledge gaps regarding how nutrients affect autotrophs in lotic systems.

Conceptual models of nutrient limitation in aquatic systems typically rely on water column nutrient concentrations, and omit the defining influence of flow in lotic systems. Flow may influence nutrient limitation through effects on diffusive boundary layers (DBLs) and mass transfer, the residence time of water, ecosystem-scale nutrient fluxes, and the identity and abundance of resident organisms. Nutrient transfer is diffusion limited when biotic uptake rates exceed rates of diffusion from the water column through a DBL, leading to a nutrient-depleted layer surrounding the biota (Borchardt 1996). Water column nutrient concentrations are theoretically most important when diffusion limitation occurs, but the prevalence of diffusion limitation in aquatic systems is currently uncertain. Diffusion limitation is more likely under relatively quiescent conditions in lentic systems, whereas more turbulent conditions in lotic systems minimize diffusion

limitation by reducing the size of DBLs. In one laboratory study, nutrient uptake was limited by diffusion rates through a DBL over a wide range of velocities (1 – 50 cm/s) (Larned et al. 2004). In contrast, however, Nishihara and Ackerman (2009) reported that concentration gradients were large enough to generate diffusion rates in excess of uptake rates even at low velocities (< 0.5 cm/s). When diffusion exceeds uptake then the water column nutrient concentration may cease to be a good predictor of nutrient availability, which may partially explain why lotic studies show highly variable correlations between nutrient concentrations and autotrophic metrics (i.e. productivity and biomass). Advective nutrient flux based on water residence times may be a better predictor of autotrophic metrics, particularly when diffusion limitation is minimal (i.e. concentration gradients are of minor importance). Moreover, when autotrophic uptake is large enough to reduce water column concentrations, nutrient flux controls the replenishment rate of nutrients in the water column and therefore the potential for nutrient limitation. Few studies have explicitly focused on the role of flow in relation to the advective nutrient flux in the water column, although several authors have acknowledged the potential importance of this factor (Allan 1995; Borchardt 1996; Tank and Dodds 2003).

When considering the influence of advective nutrient flux on autotrophic demand in lotic systems it is important to distinguish between systems which are dominated by autotrophs attached to the benthos versus those dominated by free-floating phytoplankton. The determining factor is whether the residence time of the water is long enough to allow phytoplankton to reproduce at rates that maintain a viable population (Hilton et al. 2006). For lotic systems with shorter residence times, only

autotrophs which remain attached to a substrate can persist. Attached benthic autotrophs are Eulerian (Doyle and Ensign 2009) in the sense that they remain in place while the nutrient molecules move past them due to advection, as quantified by the advective nutrient flux. In contrast, free-floating phytoplankton move with the water in a Lagrangian sense, occupying a single parcel of water where diffusive processes are likely to control nutrient availability.

In addition to system hydraulics, the influence of autotrophic demand on nutrient availability and limitation is also unaccounted for by water column nutrient concentrations alone. As autotrophic production increases so does the demand for nutrients, which may lead to a significant transfer of nutrients from the water column to autotrophic biomass (Mulholland et al. 2006; Roberts and Mulholland 2007; Hall et al. 2009; Heffernan and Cohen 2010). Nutrient spiraling theory supports this concept; since biotic uptake converts nutrients from dissolved inorganic forms to particulate organic forms, the ratio of inorganic to organic nutrients is indicative of nutrient limitation (Newbold et al. 1982). Autotrophic nutrient demand eventually becomes saturated as nutrient availability increases (Earl et al. 2006; Covino et al. 2010a), and past this saturation threshold nutrients do not limit autotrophic production even if concentrations are low.

A single metric that relates nutrient flux to nutrient demand would include multiple reach-scale factors (nutrients, discharge, and metabolism) and could be useful in characterizing the nutrient status of lotic systems. For lotic systems dominated by attached autotrophs, the ratio of advective flux to autotrophic demand for any nutrient is expected to correlate with the degree of nutrient limitation. Enumerating thresholds at

which nutrient limitation typically occurs would provide an a priori screening tool that could be used to prioritize more detailed studies and help set protective regulatory criteria.

### **Autotrophic Uptake Length**

This study adapts the concept of the nutrient uptake length ( $S_w$ ) from nutrient spiraling theory (SSW 1990; Dodds et al. 2002a) to create a new metric that quantifies the relative magnitudes of nutrient flux and autotrophic demand. The proposed metric, the autotrophic uptake length ( $S_{w,a}$ ), is obtained from a calculation that proceeds opposite the typical direction where  $S_w$  is measured and the uptake rate ( $U$ ) is inferred. Specifically,  $S_{w,a}$  is computed as follows:

$$S_{w,a} = \frac{QC}{wU_a}$$

where  $Q$  is the stream discharge ( $L^3 T^{-1}$ ),  $C$  is the nutrient concentration ( $M L^{-3}$ ),  $w$  is the stream width ( $L$ ), and  $U_a$  is the areal uptake rate (e.g. nutrient demand;  $M L^{-2} T^{-1}$ ) due to autotrophic production only. In this study, the autotrophic uptake rate,  $U_a$ , is estimated based on stream metabolism and autotroph stoichiometry.  $S_{w,a}$  is equivalent to the more typical uptake length,  $S_w$ , but excludes removal via other biotic and abiotic pathways.  $S_{w,a,N}$  and  $S_{w,a,P}$  refer to the autotrophic uptake length for N and P, respectively.

Ultimately, the purpose of this analysis was to advance understanding of conditions under which nutrient limitation occurs and to compare metrics for assessing nutrient limitation across a range of lotic systems. The specific objective was to test the hypothesis that flow mediates nutrient concentration effects on autotrophic nutrient limitation. It is expected that autotrophic uptake length,  $S_{w,a}$ , would be a better predictor

of autotrophic N and P limitation in lotic systems than water column nutrient concentrations. This study also sought to identify critical values of  $S_{w,a}$  below which nutrient limitation of autotrophic production is likely, and extrapolate that value to a global distribution of  $S_{w,a,N}$  and  $S_{w,a,P}$  for lotic systems obtained from the literature.

## Methods

### Data Sources

The two primary datasets for this analysis are the Lotic Intersite Nitrogen eXperiment (LINX) I and II studies, which combined include 82 stream assessments from across North America (Tank and Dodds 2003; Johnson et al. 2009). These studies utilized both inorganic (glass) and organic (cellulose) nutrient diffusing substrata (NDS) to deliver nutrients for nutrient limitation assays (NLAs). For each substrate type there were three nutrient treatments (N, P, and N+P) and a control. The response variable was attached biofilm biomass (as chlorophyll a) after 17-22 days.

Tank and Dodds (2003) was the source of the majority of the LINX I data, including NLA data (extracted from graphs using computerized analysis). This study assessed 10 streams resulting in 10 inorganic and 9 organic substrate NLAs total. This dataset was supplemented with stream width data (Dodds et al. 2002a; Mulholland et al. 2001). The LINX II data primarily came from Johnson et al. (2009), and the NLA data was obtained directly from the authors. Of the 72 streams assayed, 69 inorganic and 47 organic substrate assays were successfully completed. This dataset was supplemented with stream width (Mulholland et al. 2008) and gross primary production (GPP) data (Bernot et al. 2010). Mulholland et al. (2008) also contained  $\text{NO}_3$  uptake lengths ( $S_w$ ) using the traditional spiraling methodology with a  $^{15}\text{NO}_3$  tracer which is compared in this study to the calculated  $S_{w,a,N}$  values for the same LINX II streams.

Nutrient concentration data was based on dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP), since these inorganic forms are generally used by autotrophs. Combined, these sources provided the requisite data (DIN and SRP concentrations, NLA results, discharge, stream width, GPP) for 80 stream segments, including 79 inorganic and 56 organic substrate NLAs. The consistent methodology and mostly contemporaneous measurement of the variables in each stream make this dataset particularly appropriate for this analysis. A literature search did not reveal any additional studies which contained the necessary data measured contemporaneously.

A larger dataset was compiled from the literature to determine the broader distribution of  $S_{w,a,N}$  and  $S_{w,a,P}$  across diverse streams and rivers (Table 3-1). The required data were the same as above, except for the omission of NLA results. There were many datasets ( $n = 15$ ) that fit these criteria, with the limiting factor usually being contemporaneous GPP and nutrient concentration measurements. In some cases, inclusion of studies in this broader dataset required assumptions about some variables. For example, Ortiz-Zayas et al. (2005) included discharge at only 1 of the 3 stream reaches, so ratios of contributing areas were used to estimate discharge for the other 2 sites. In another case, total N and P data were used in lieu of inorganic N and P data (Duarte and Canfield 1990), although organic N and P are typically very low in these systems. Most studies used a 2-station method to measure GPP; however, some used a 1-station method or empirically derived relationships based on stream characteristics such as autotrophic biomass, nutrient levels, and PAR (Table 3-1). This analysis assumes these methods produce comparable results.

Florida's large artesian spring-fed rivers are prominent in the broader  $S_{w,a}$  dataset, as they are fairly unusual lotic systems due to their discharge and chemical stability and high autotrophic productivity, and their potential for nutrient limitation is uncertain. Previous work on these systems (Odum 1957a) concluded that they were light rather than nutrient limited; however subsequent N enrichment has been proposed as the cause for recent ecological changes (i.e. proliferation of benthic filamentous algae) via alleviation of N limitation. While this hypothesis for algal proliferation has been challenged (Heffernan et al. 2010b), understanding nutrient limitation in these systems is critical to their management.

### **Uptake Length Calculations**

Calculations of  $S_{w,a}$  for each stream reach, required contemporaneous measurements of  $Q$ ,  $C$ ,  $w$ , and  $U_a$ . Autotrophic nutrient demand ( $U_a$ ) was determined by converting GPP data to NPP assuming 50% of GPP was consumed by autotrophic respiration (e.g. Odum 1957a; Hall and Tank 2003). NPP was converted from  $O_2$  to C using a photosynthetic quotient of 1.00, then converted from C to either N or P using molecular ratios of C:N = 12 and C:P = 200 (approximate median values from Stelzer and Lamberti 2001). These stoichiometric assumptions for autotrophic production and biomass allowed the use of GPP data to approximate daily autotrophic N and P demand on an area basis. Nutrient flux alone ( $S_{w,a}$  excluding autotrophic demand,  $U_a$ ) was also calculated for each site as a predictor of nutrient limitation. In order to put the nutrient flux on an area basis, flux was divided by an arbitrary reach length of 100 m.

### **Statistical Analysis**

In all cases, the regressions fit a power function to the data where the NLA response ratio was the dependent variable, as this is a measure of the degree of

nutrient limitation. NLA response ratios were calculated by dividing the average chlorophyll a of each nutrient treatment by that of the control ( $RR_N$  and  $RR_P$  for N and P diffusing substrata respectively). A site was considered nutrient limited if RR was greater than 1. For each regression model, the  $S_{w,a}$  threshold indicative of nutrient limitation was determined by calculating  $S_{w,a}$  when the fitted line crossed  $RR = 1$ . For significant models (i.e.  $p < 0.05$ ) the 90% confidence interval of this  $S_{w,a}$  threshold was also calculated by determining where the confidence interval lines crossed  $RR = 1$ . Inorganic and organic substrate response ratios were analyzed separately for each stream (if applicable) due to substantial differences in their responses. Prior to statistical analysis, all values of nutrient concentrations,  $S_{w,a}$ , and RR were log transformed to meet the model assumptions of normality and homogeneity of variance.

The LINX II dataset included eight regions across North America in which three land use types (reference (REF), agricultural (AGR), and urban (URB)) were evenly sampled, allowing analysis by region or land use type. For the regional average analyses the values of nutrient concentration,  $S_{w,a}$ , and RR were averaged for the sites within each region and then followed the same regression procedure described above. The regional average analyses consisted of one which included all sites and another which only included reference sites. All statistical analyses were conducted using the software package R version 2.11.1 (R Foundation for Statistical Computing).

## **Results**

### **LINX I Analysis**

For the LINX I analysis,  $S_{w,a,N}$  was a much better predictor of  $RR_N$  than DIN concentration (Figure 3-1). The relationship between  $S_{w,a,N}$  and  $RR_N$  was significant for both NDS substrate types (Figure 3-1A-Organic:  $R^2 = 0.85$ ,  $p < 0.001$ ; Figure 3-1A-

Inorganic:  $R^2 = 0.46$ ,  $p = 0.03$ ), but not for DIN concentration and  $RR_N$  (Figure 3-1B-Organic:  $R^2 = 0.19$ ,  $p = 0.25$ ; Figure 3-1B-Inorganic:  $R^2 = 0.09$ ,  $p = 0.35$ ). The N limitation threshold for  $S_{w,a,N}$  on organic NDS was 2410 m (90% CI: 1210 – 5250 m). DIN flux alone was also a significant predictor of  $RR_N$  (Organic:  $R^2 = 0.54$ ,  $p = 0.02$ ; Inorganic:  $R^2 = 0.52$ ,  $p = 0.02$ ). Neither  $S_{w,a,P}$  nor SRP concentration were a significant predictor of  $RR_P$  for any substrate.

## LINX II Analysis

For the LINX II analysis,  $S_{w,a,N}$  and DIN concentration were both significant predictors of  $RR_N$ ; however, despite the increased statistical power (47 organic NDS and 69 inorganic NDS), neither explained as much variation as  $S_{w,a,N}$  did for the LINX I data (Figure 3-2A-Organic:  $R^2 = 0.11$ ,  $p = 0.02$ ; Figure 3-2A-Inorganic:  $R^2 = 0.07$ ,  $p = 0.03$ ; Figure 3-2B-Organic:  $R^2 = 0.15$ ,  $p = 0.007$ ; Figure 3-2B-Inorganic:  $R^2 = 0.07$ ,  $p = 0.03$ ). The N limitation threshold was beyond a  $S_{w,a,N}$  of 18,700 m (90% CI: 2510 – 2,930,000 m) on organic NDS. Compared to the LINX I  $S_{w,a,N}$  regressions, the slopes were much less steep, the intercepts were smaller, and both variables spanned a much larger range. As with LINX I, neither  $S_{w,a,P}$  nor SRP concentration explained significant variation in  $RR_P$ . A comparison of  $S_{w,a,N}$  values to  $S_w$  values for  $NO_3$  addition measured as part of the LINX II study found a weak but significant correlation ( $R^2 = 0.24$ ;  $p < 0.001$ ); however, these isotope-tracer-derived  $S_w$  values were no more effective at predicting  $RR_N$  than was  $S_{w,a,N}$  ( $R^2 < 0.1$  for both substrates).

To explore the dramatic differences in explanatory power of  $S_{w,a,N}$  between the LINX I and LINX II datasets land use effects were examined, since LINX I streams were all characterized as reference sites and LINX II included three sites of each land use type (reference, agricultural, and urban) within each region. When separated by land

use (Figure 3-3),  $S_{w,a,N}$  and DIN concentration were fairly poor predictors for all three land use types, although DIN concentration was significant in two instances. While significant scatter occurred below a  $S_{w,a,N}$  of approximately 1000 m for reference sites and 10,000 m for agricultural and urban sites, above these thresholds the points were tightly clustered together around  $RR_N = 1$  indicating a lack of nutrient limitation. The models for LINX II reference sites had similar intercepts and slopes as LINX I inorganic NDS, but the LINX I organic NDS had much higher values. For the LINX II reference sites, three streams had extremely low DIN flux and unexpectedly low  $RR_N$ ; when omitted, regressions improved markedly and more closely resembled LINX I. Upon further investigation these streams had high  $NH_4:NO_3$  ratios, which may explain why they did not respond to the  $NO_3$  diffusing NDS.

Overall, regressions of  $S_{w,a,N}$  and  $RR_N$  within the eight individual regions were inconsistent and not statistically significant. However, analysis of LINX II data on a regional average basis substantially improved the explanatory power of both  $S_{w,a,N}$  and DIN concentration (Figure 3-4), though not  $S_{w,a,P}$  and SRP concentration. As with LINX I,  $S_{w,a,N}$  was a significantly better predictor of  $RR_N$  than DIN concentration (Figure 3-4A-Organic:  $R^2 = 0.80$ ,  $p = 0.003$ ; Figure 3-4A-Inorganic:  $R^2 = 0.38$ ,  $p = 0.11$ ; Figure 3-4B-Organic:  $R^2 = 0.37$ ,  $p = 0.11$ ; Figure 3-4B-Inorganic:  $R^2 = 0.04$ ,  $p = 0.62$ ). In this regional analysis, the N limitation threshold for  $S_{w,a,N}$  on organic NDS was 24,600 m (90% CI: 6180 – 766,000 m). Compared to the LINX I  $S_{w,a,N}$  regressions the slopes were similar, but the intercepts were larger and overall the  $S_{w,a,N}$  values were higher. Because LINX II included agricultural and urban sites, regional average analyses conducted for reference sites only are also reported (Figure 3-4), for which  $S_{w,a,N}$  and

DIN concentration were both highly significant and each explained over 60% of the variation in  $RR_N$  on both organic and inorganic NDS.

Overall 10 different regression models were created with  $S_{w,a,N}$  as the explanatory variable for  $RR_N$  (Table 3-2). The LINX I and LINX II regionally averaged models for organic NDS were the best models for predicting  $RR_N$  ( $R^2 > 0.80$ ). In general  $S_{w,a,N}$  was a better predictor of  $RR_N$  on organic than inorganic NDS. While some LINX II analyses using individual sites (rather than averaged values) were significant, none explained much variation in  $RR_N$ , even for reference sites only. The threshold  $S_{w,a,N}$  values that may delimit nutrient limitation varied substantially across models. Most suggested the limitation threshold was at  $S_{w,a,N}$  values ca. 10,000 – 60,000 m, although the best model (LINX I – Organic NDS) had the lowest value of 2410 m. The 90% confidence intervals for these values also span a wide range, with some of the upper limits on the order of  $1 \times 10^7$  m; however this is likely due to small slopes of these models and high uncertainty in this data range.

### **Global Distribution of $S_{w,a}$**

The global analysis for both  $S_{w,a,N}$  and  $S_{w,a,P}$  yielded median values of 12,500 m and 10,100 m, respectively (Figure 3-5). This  $S_{w,a,N}$  value is above the estimated threshold of 2410 m from the LINX I organic NDS analysis but within the range of thresholds from the LINX II analyses. Median  $S_{w,a,N}$  and  $S_{w,a,P}$  values for the LINX streams (5260 m and 1760 m, respectively) were lower than the median of all streams combined. Florida springs had higher median values ( $S_{w,a,N} = 72,300$  m,  $S_{w,a,P} = 39,900$  m), although three systems had  $S_{w,a,N}$  values less than the LINX I threshold indicating potential N limitation. When Florida springs were analyzed assuming historic DIN concentrations of 0.05 mg N/L (keeping GPP, discharge, and width the same as in the

previous analysis), the median  $S_{w,a,N}$  value was 5480 m and nine additional systems were below the 2410 m  $S_{w,a,N}$  value indicative of N limitation from the LINX I dataset. For the entire global analysis, only 89 out of 276 (32%) stream reaches were below this threshold, although many of these systems may be nutrient enriched and likely had lower  $S_{w,a,N}$  values in the past.

## Discussion

### Nutrient Limitation and Spiraling Metrics

While the core conceptual model of nutrient limitation in lotic systems continues to require refinement, this analysis provides evidence that metrics of lotic nutrient limitation derived from the conceptual framework of nutrient spiraling may outperform use of nutrient concentration alone. The results generally support the hypothesis that the autotrophic uptake length ( $S_{w,a}$ ) indicates the severity of N limitation in lotic systems; however, P limitation was both rare and poorly predicted by all metrics. The two clearest examples of superior predictive power using  $S_{w,a,N}$  as compared to DIN concentration are for the LINX I and regionally averaged LINX II analyses (Figures 3-1 and 3-4). In both cases  $S_{w,a,N}$  was highly significant and explained approximately 80% of the response ratio variation for organic NDS, whereas DIN concentration was a non-significant predictor.

It appears that no other studies have examined nutrient spiraling metrics in relation to nutrient limitation as measured by NLAs; however, there are several studies which directly used spiraling metrics to assess nutrient limitation. Two studies used nutrient additions to characterize nutrient saturation (i.e. Michaelis-Menton uptake kinetics) for individual streams based on relationships between nutrient concentration and spiraling metrics (Earl et al. 2006; Covino et al. 2010a). Another study determined spiraling

metrics for a suite of streams spanning a wide range of  $\text{NO}_3$  concentrations and found a response characteristic of an efficiency-loss model across systems (O'Brien et al. 2007). In general, short  $S_w$  and high  $v_f$  are likely to be indicative of lotic nutrient limitation.

Considering the utility of nutrient spiraling theory with regard to nutrient transport in lotic systems, a conceptual model of lotic nutrient limitation should be based on nutrient spiraling metrics rather than nutrient concentrations and ratios. Three aspects of the autotrophic uptake length,  $S_{w,a}$ , make it a theoretically compelling metric: 1) it has a basis in spiraling theory, 2) it integrates information about stream GPP, which can vary for reasons other than nutrient availability, and 3) it is scale invariant, and therefore applicable across drainage networks.

$S_{w,a}$  explicitly assesses autotrophic nutrient limitation within the nutrient spiraling framework, and could potentially improve our ability to predict the effects of nutrient enrichment on autotrophic communities.  $S_w$  is a measure of advective nutrient flux per the rate of nutrient removal from the water column, which is useful but does not differentiate between autotrophic uptake and other removal processes (e.g. denitrification for N and sorption for P).  $S_{w,a}$  represents only the autotrophic portion of  $S_w$  while retaining other reach characteristics (i.e. discharge, width, GPP, and nutrient concentration) which affect nutrient availability and the degree of autotrophic limitation.

A measure of autotrophic nutrient limitation is important since concern for nutrient dynamics in aquatic systems is often related to overgrowth of algae and macrophytes (i.e. eutrophication). A metric which integrates autotrophic production (i.e. GPP), indirectly accounts for other factors such as light levels, scouring, and grazing which

may constrain production and nutrient demand. For example, in shaded lotic systems lower nutrient concentrations are required to saturate autotrophic demand since the low light levels limit the amount of autotrophic biomass (Hill and Knight 1988; Rosemond et al. 2000).

Another useful feature of  $S_{w,a}$  is that theoretically it is scale invariant, meaning that the ability of  $S_{w,a}$  to predict autotrophic nutrient limitation should apply to lotic systems of all sizes, as long as benthic production is dominant. This relationship may be different for lotic systems dominated by phytoplankton (e.g. lower portions of the St. John's River and the Mississippi River), since these organisms are suspended in the water column and nutrient transfer is more dependent on diffusive processes. Light intensity is a strong predictor of autotrophic nutrient limitation (Tank and Dodds 2003) across small streams, largely due to heavy canopy cover in many of these systems. For larger systems canopy cover does not substantially limit light availability, and therefore incident light intensity would not be able to explain differences in nutrient limitation between large river systems whereas  $S_{w,a}$  should retain predictive power. Since it is difficult to assess autotrophic nutrient limitation and spiraling metrics in larger lotic systems due to methodological constraints, a scale invariant metric such as  $S_{w,a}$  could be useful in under-studied (Ensign and Doyle 2006) large systems.

### **Assessing Autotrophic Nutrient Demand and Limitation**

Our ability to assess autotrophic nutrient demand at the scale of whole ecosystems is currently limited by available measurement techniques. In this analysis it was assumed that there were consistent C:N, C:P, GPP:NPP, and photosynthetic quotients among all systems in order to convert GPP values (based on oxygen changes) into autotrophic nutrient demand ( $U_a$ ). Calculations of  $U_a$  do not presently

account for variation in these values, and the effects of this omission on precision and bias of  $S_{w,a}$  are not known. The measurement of diel variations in N and P using improved in situ sensors may obviate these stoichiometric and physiological assumptions by providing a direct measure of nutrient demand (Heffernan and Cohen 2010).

Nutrient diffusing substrata (NDS) of various types have been the primary method for characterizing the degree of nutrient limitation in lotic systems to date, and their utility is apparent based on extensive use in many settings. However NDS are limited in their ability to assess reach-scale autotrophic nutrient limitation, since they generally target biofilms which may not be representative of the autotrophic community within that reach. Larger autotrophs (particularly rooted macrophytes) would not be captured by most NDS, whereas their production could be a significant component of the total amount of autotrophic production in a reach. Rooted macrophytes may also experience different nutrient regimes than autotrophs in benthic biofilms due to their ability to acquire resources directly from sediment porewaters.

Results of NDS assays are also influenced by temporal attributes of a system such as algal immigration rates and storm events. In this analysis, some of the streams with the lowest DIN flux had a minimal response to the N-enriched NDS despite moderate light and GPP levels, which could reflect such limitations of NDS. There were also different relationships for the two substrate types used in LINX (e.g. organic and inorganic), and  $S_{w,a,N}$  was a better predictor of organic NDS response ratios overall. Recently developed techniques which measure the degree of nutrient limitation for pre-existing autotrophic communities in situ, such as the tracer additions for spiraling curve

characterization (TASCC) methodology recently proposed by Covino et al. (2010b), may provide better metrics of the severity of nutrient limitation.

Evidence is accumulating that NDS are particularly poor assays of autotrophic P limitation. In several cases P enriched NDS have shown less algal accrual than controls indicating that P enrichment may have inhibited algal growth (Hill and Knight 1988; Tank and Dodds 2003; Sanderson et al. 2009). Although the mechanism behind this phenomenon and its significance are unknown, it is likely related to the extremely low P concentrations required for growth rate saturation ( $< 4 \mu\text{g/L}$ : Bothwell 1985) and that P cycling in the benthos may be sufficient to alleviate any substantial P limitation. In this analysis, both SRP concentration and  $S_{w,a,P}$  were unable to explain any variation in the P response ratios, and the majority of these response ratios were less than 1 (i.e. less algae grew on the P enriched NDS than on the un-enriched controls).

A general issue with nutrient limitation assays is the distinction between instantaneous and chronic nutrient enrichment effects. Short-term assay techniques may not capture effects of long-term chronic nutrient enrichment which can cause substantial changes in autotrophic community composition with altered nutrient cycling and potentially higher nutrient demand. O'Brien et al. (2007) did not find evidence of saturation in biotic demand among streams spanning a range N concentrations due to chronic N enrichment, in contrast to the findings of two studies which assessed nutrient limitation using instantaneous N additions (Earl et al. 2006; Covino et al. 2010a). One mechanism explaining this distinction is the formation of large algal mats under chronic nutrient enrichment, and the difference between nutrient limitation of peak algal biomass and cellular growth rate. Peak algal biomass requires time to develop and nutrient

saturation occurs at higher levels than for cellular growth rate (Bothwell 1989). NDS would mostly capture growth rate nutrient limitation (since they usually collect a thin layer of algae), and are therefore unlikely to characterize nutrient limitation for systems with substantial algal mats. This could explain why some streams with lower  $S_{w,a}$  values did not exhibit nutrient limitation, as GPP may have been high due to thick algal layers throughout the reach but nutrient limitation was not evident due to thin algal growth on NDS. Overall, the extent to which these results reflect the idiosyncrasies of NDS (as opposed to limitations of the  $S_{w,a}$  metric) is not known.

### **Effects of Land Use and Region**

For the LINX II dataset, it was expected that  $S_{w,a}$  would be a good predictor for each land use type since it accounts for any major differences related to autotrophic nutrient limitation. However both  $S_{w,a,N}$  and DIN concentration were fairly poor predictors of  $RR_N$  when separated by land use, which may indicate that the differences between each land use type were minimal. Although DIN concentration was a significant predictor of  $RR_N$  in two instances, the results overall are inconsistent and there are no clear trends related to land use effects.

It was also expected that  $S_{w,a}$  would be a good predictor of nutrient limitation both among and within regions. Dodds et al. (2002b) suggest that relationships between nutrient concentrations and algal biomass within a region should be stronger than among regions, as found in some studies (Biggs and Close 1989; Lohman et al. 1992; Chetelat et al. 1999; Ludwig et al. 2008). Dodds and Welch (2000) describe how region-specific data have led to more accurate predictions of algal biomass. While they caution that it is possible to have high variation both temporally and spatially at the stream reach scale, they conclude that if conditions are similar (i.e. light, grazers, etc.)

then the nutrient concentration relationship with algal biomass should be valid among regions. While nutrient concentration may not be the best predictor of autotrophic biomass, there is nothing inherent in a region or individual stream that should affect the relationships discussed here. Differences in factors such as light intensity, grazer abundance, sediment or dissolved organic particle effects on light availability, and toxins in runoff should all be indirectly accounted for in the autotrophic demand component of  $S_{w,a}$ , since this reflects the current level of primary production.

In contrast to other studies of lotic systems which indicated that stronger relationships between nutrient levels and autotrophic biomass occur within a single region than among regions,  $S_{w,a}$  was not a very good predictor within any of the eight regions covered by the LINX II dataset. One partial explanation is that there was not a large range of both  $S_{w,a}$  and response ratio values within most of the regions. Overall, it is unclear why regression models for  $S_{w,a}$  on organic NDS are significant when using regional averages (i.e. among regions) and not otherwise, but it may point to some unaccounted for regional differences. The fact that the LINX I dataset was compiled from individual streams in similar regions as for LINX II and shows fair model fits indicates that the  $S_{w,a}$  metric is a good predictor of nutrient limitation among regions.

### **Nutrients and Alternative Controls**

Nutrients are one of many factors which can strongly affect autotrophic production and biomass accrual in aquatic systems. In lotic systems, top-down controls and velocity induced sloughing may limit algal biomass as much as bottom-up controls (e.g. nutrients and light). Recent research indicates that a preponderance of lotic systems do not display nutrient limitation (Francoeur 2001; Tank and Dodds 2003; Johnson et al. 2009), suggesting that these other factors dominate. The global analysis of N limitation

further supports this finding, as 68% of the systems were above the provisional  $S_{w,a,N}$  threshold of 2410 m indicative of N limitation. This finding does not diminish the need for nutrient reduction in anthropogenically enriched systems, but does indicate that in many lotic systems nutrients are not the primary driver of autotrophic production. Due to the difficulty of decreasing elevated nutrient levels combined with the potential for hysteresis in degraded ecosystems, management actions that go beyond nutrient control and reduction appear warranted. Further research that focuses on the full suite of controls on autotrophic production and biomass would complement efforts to reduce the potential effects of nutrient enrichment by providing additional techniques for effective ecosystem management.

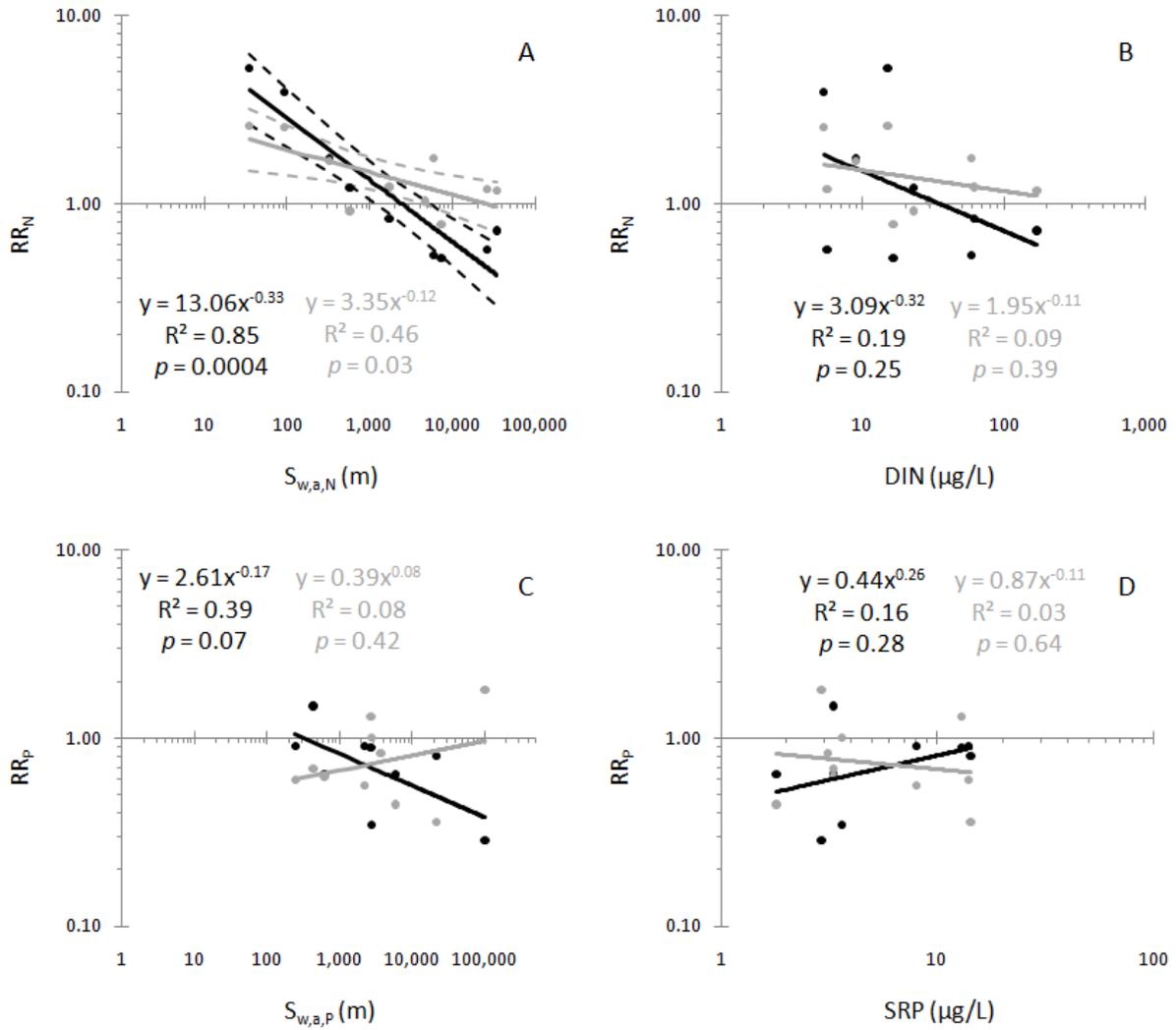


Figure 3-1. Comparison of autotrophic uptake lengths ( $S_{w,a}$ ) and nutrient concentrations in relation to the NLA response ratios ( $RR_x$ ) from the LINX I dataset. Black indicates organic NDS and gray indicates inorganic NDS.

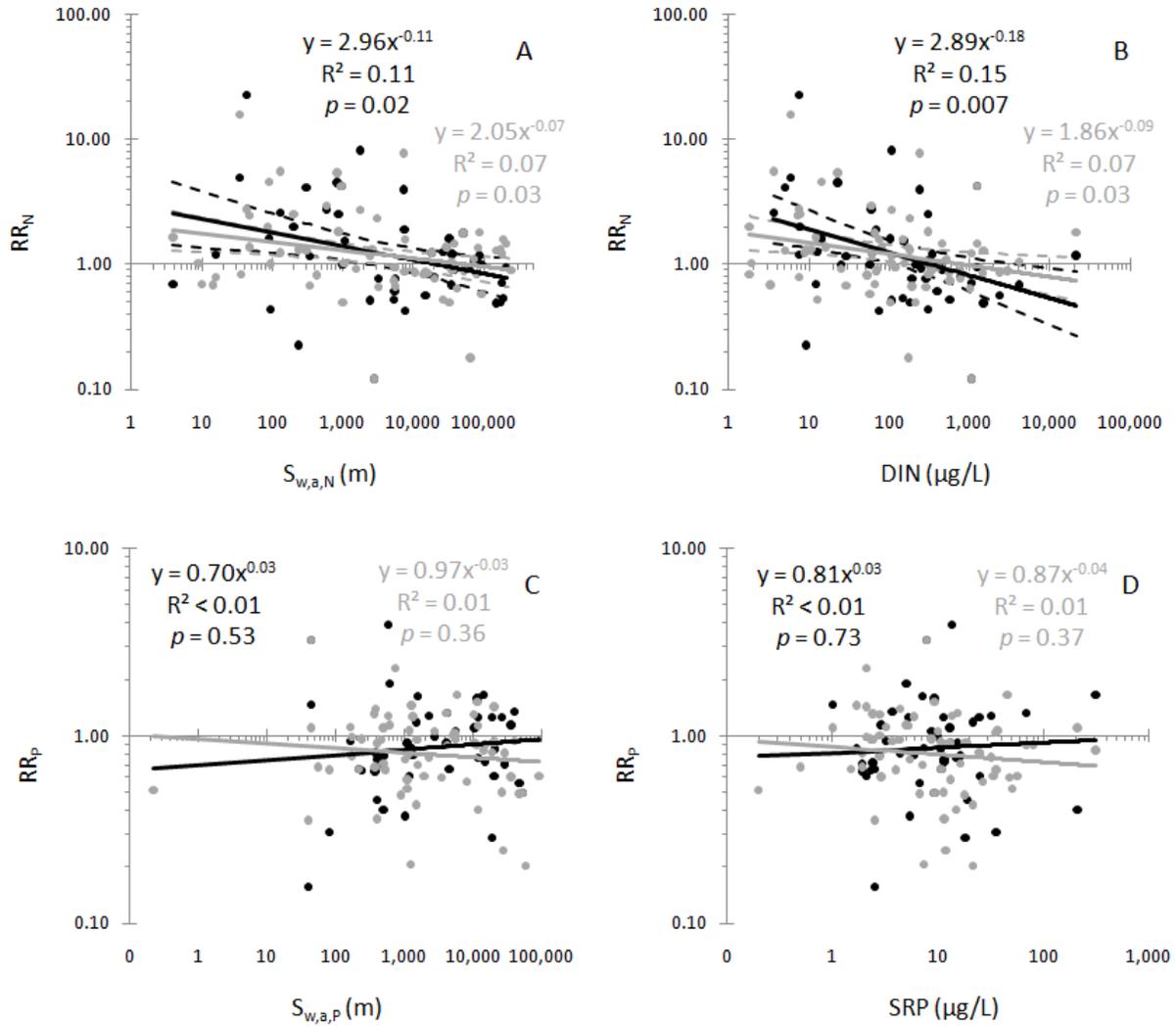


Figure 3-2. Comparison of autotrophic uptake lengths ( $S_{w,a}$ ) and nutrient concentrations in relation to the NLA response ratios (RR<sub>x</sub>) from the LINX II dataset. Black indicates organic NDS and gray indicates inorganic NDS.

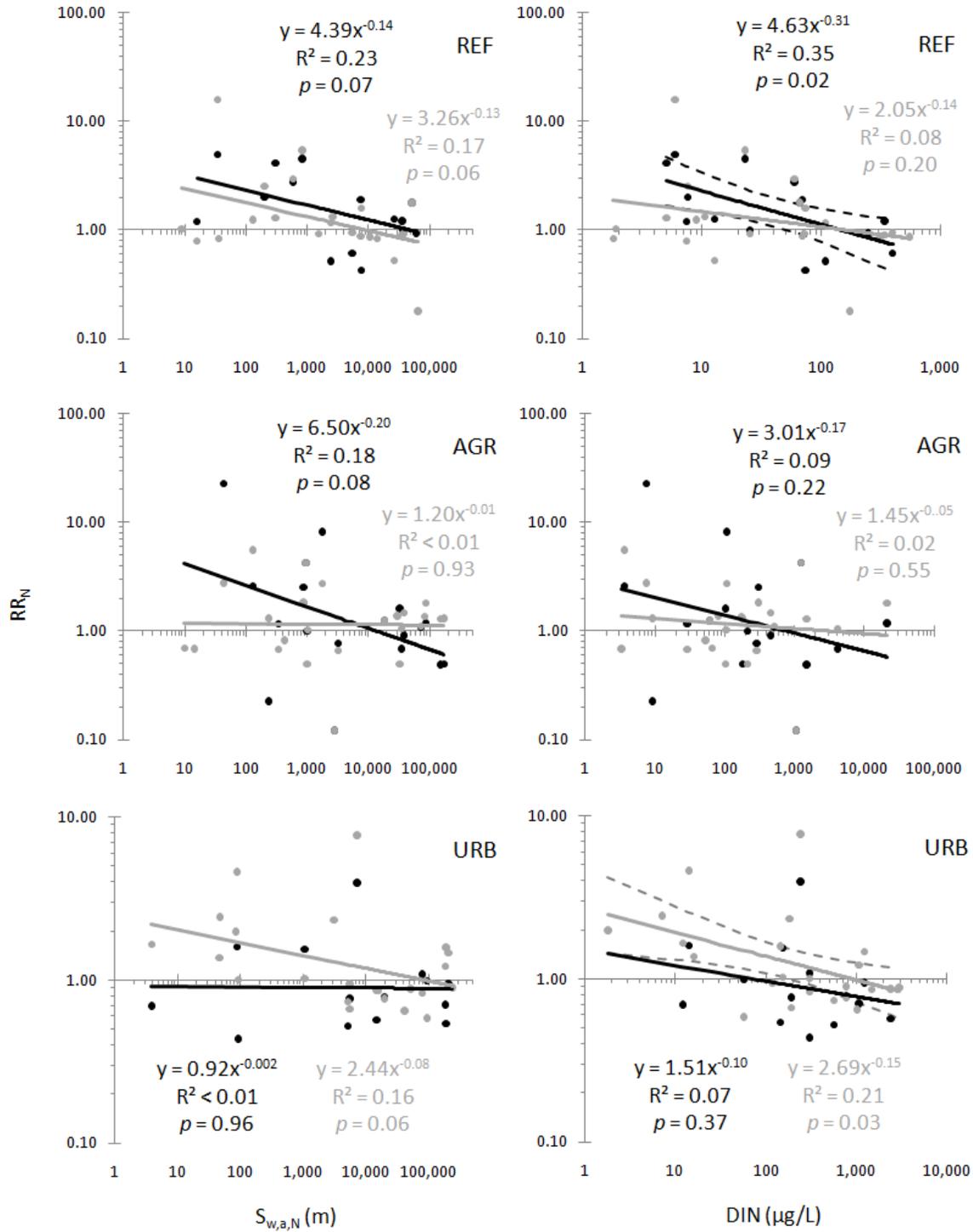


Figure 3-3. Comparison of autotrophic N uptake length ( $S_{w,a,N}$ ) and DIN concentration for the three land use types in relation to  $RR_N$  for individual streams from the LINX II dataset. REF – reference, AGR – agricultural, URB – urban. Black indicates organic NDS and gray indicates inorganic NDS.

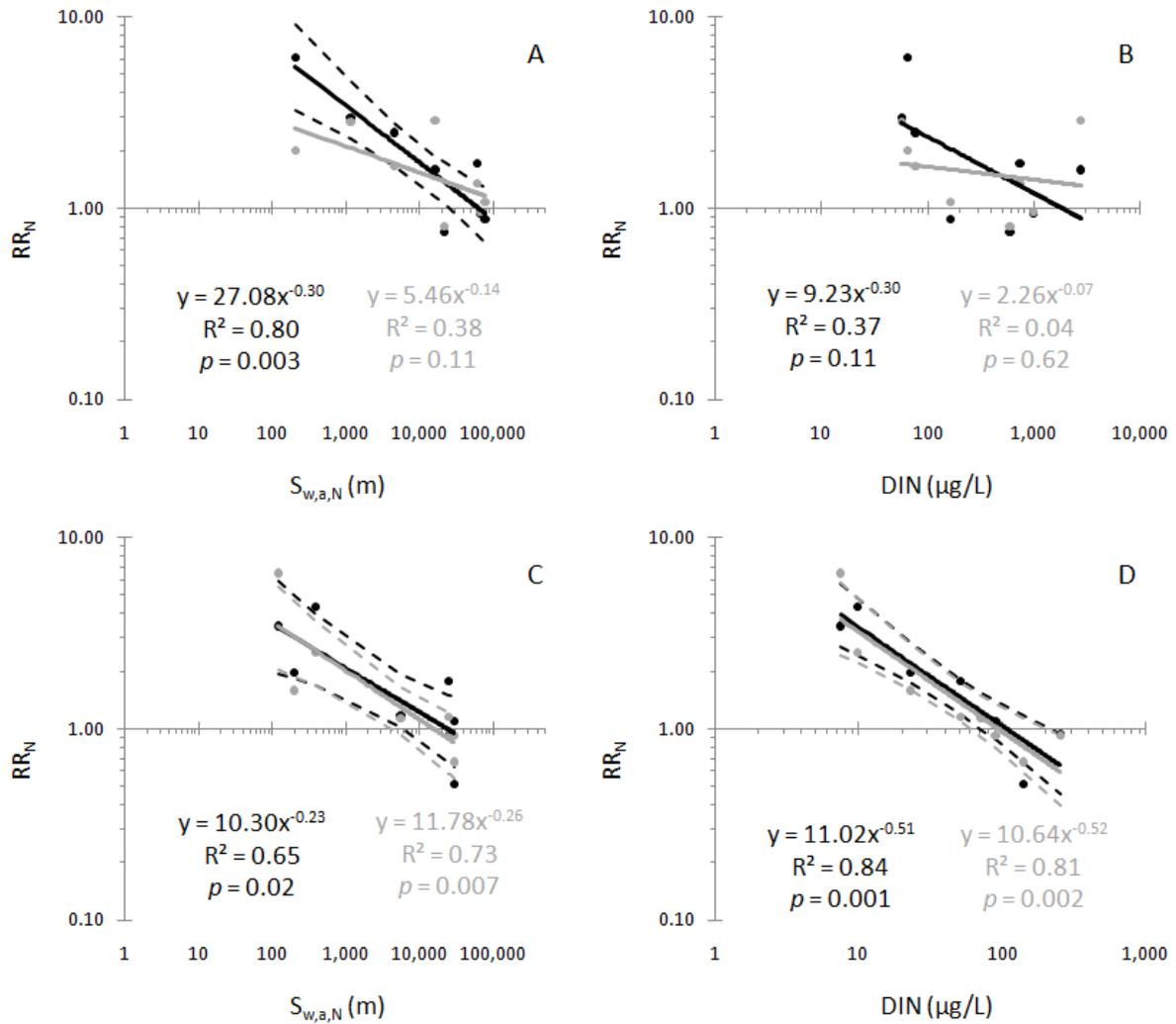


Figure 3-4. Comparison of autotrophic N uptake length ( $S_{w,a,N}$ ) and DIN concentration in relation to the  $RR_N$  from the regionally averaged LINX II dataset (A and B) and for the regionally averaged reference sites only (C and D). Black indicates organic NDS and gray indicates inorganic NDS.

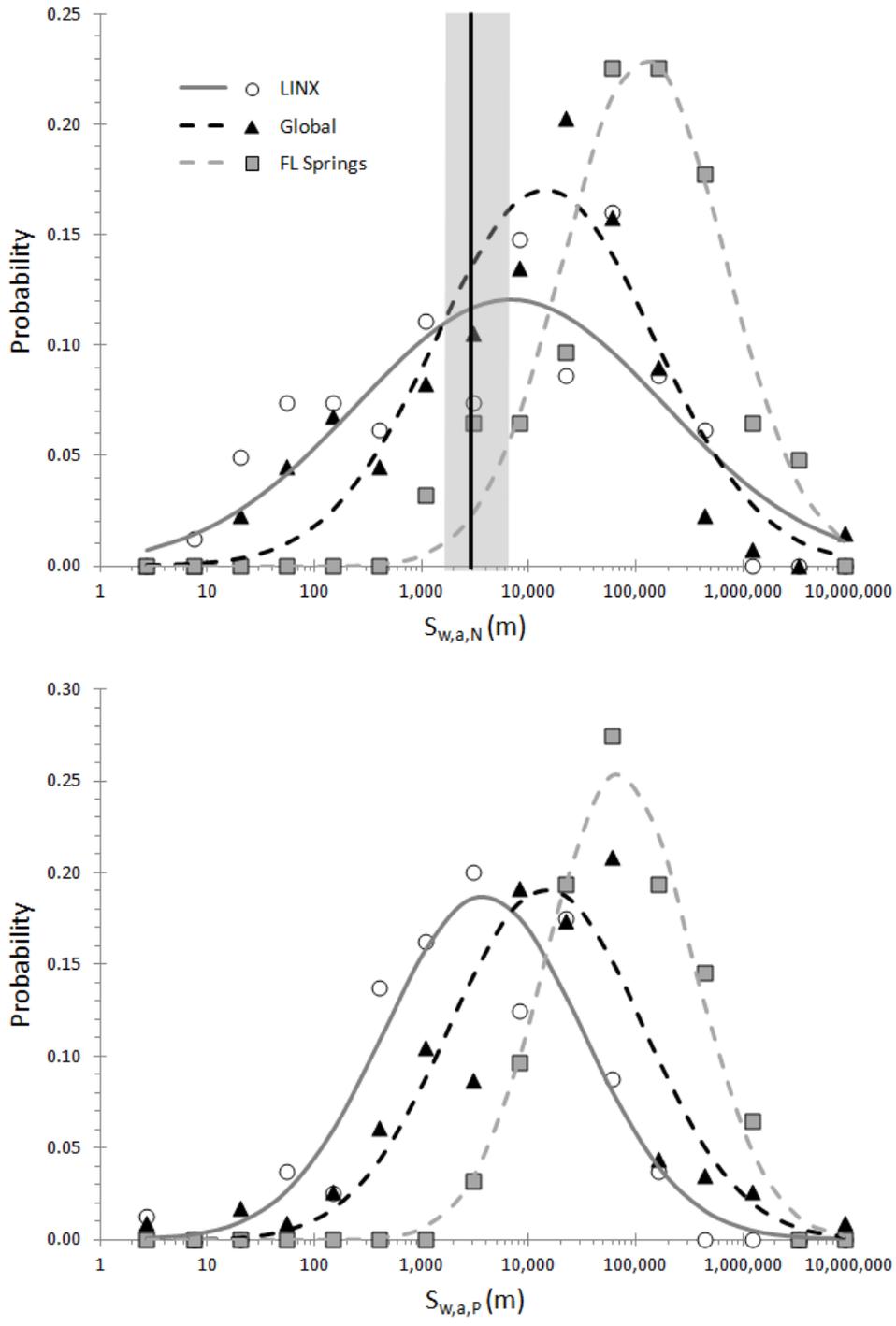


Figure 3-5. Normal probability distributions for  $S_{w,a,N}$  and  $S_{w,a,P}$  of the three groups of datasets fit to their relative frequency of occurrence. Florida spring-fed rivers were included separately to demonstrate where they are located in terms of expected nutrient limitation. The  $S_{w,a,N}$  value at which  $RR_N = 1$  is shown (vertical black line) with the 90% confidence interval (gray bar) based on the LINX I organic NDS analysis.

Table 3-1. Global analysis datasets. In some studies a stream reach was sampled on multiple occasions and these values were included as separate points. Florida springs systems are listed separately at the bottom of the table.

Reference	Location	Reaches sampled	Total data points	GPP methodology
Elosequi and Pozo 1998 <sup>a</sup>	Spain	2	8	diel DO change
Fellows et al. 2006	New Mexico, USA	2	2	2-station DO change
	Tennessee, USA	1	1	2-station DO change
	North Carolina, USA	1	1	2-station DO change
Fisher et al. 1982	Arizona, USA	1	7	empirical equations
Grimm 1987	Arizona, USA	1	5	in situ chambers
	Arizona, USA	1	1	diel DO change
Hall and Tank 2003	Wyoming, USA	11	11	2-station DO change
Hall et al. 2003	Wyoming, USA	1	3	2-station DO change
Meyer et al. 2005	Georgia, USA	6	6	diel DO change
Mulholland et al. 1997	Tennessee, USA	1	1	2-station DO change
	North Carolina, USA	1	1	2-station DO change
O'Brien et al. 2007	Kansas, USA	9	9	diel DO change
Ortiz-Zayas et al. 2005 <sup>b</sup>	Puerto Rico	3	28	diel DO change
Wright and Mills 1967	Wyoming, USA	5	5	2-station pH change
Young and Huryn 1999	New Zealand	5	5	2-station DO change
Duarte and Canfield 1990 <sup>c</sup>	Florida, USA	32	32	empirical equations
WSI 2008	Florida, USA	6	24	2-station DO change
WSI 2010 <sup>d</sup>	Florida, USA	12	12	2-station DO change

<sup>a</sup> nutrients and discharge values are annual averages

<sup>b</sup> discharge estimated for two of the streams, based on the discharge of the main stream and the basin area ratio

<sup>c</sup> widths were estimated by dividing discharge by velocity and depth; TN and TP data used instead of DIN and SRP

<sup>d</sup> maximum widths rather than average

Table 3-2. Summary of the  $S_{w,a,N}$  regression results. The  $S_{w,a,N}$  indicative of N limitation (i.e.  $S_{w,a,N}$  when  $RR_N = 1$ ) is also included along with the 90% confidence interval for significant models.

Model	Parameter	Value	SE	$p$	Model $R^2$	Model $p$	Limiting $S_{w,a,N}$ (m)	90% Confidence Interval	
								Lower	Upper
1) LINX I - Organic NDS	Intercept	1.12	0.17	<0.001	0.85	<0.001	2.41E+03	1.21E+03	5.25E+03
	$S_{w,a,N}$	-0.33	0.05	<0.001					
2) LINX I - Inorganic NDS	Intercept	0.53	0.15	0.009	0.46	0.031	2.34E+04	4.25E+03	3.03E+07
	$S_{w,a,N}$	-0.12	0.05	0.031					
3) LINX II - Organic NDS all sites	Intercept	0.47	0.18	0.011	0.11	0.023	1.87E+04	2.51E+03	2.94E+06
	$S_{w,a,N}$	-0.11	0.05	0.023					
4) LINX II - Inorganic NDS all sites	Intercept	0.31	0.11	0.006	0.07	0.030	2.68E+04	3.24E+03	2.33E+07
	$S_{w,a,N}$	-0.07	0.03	0.030					
5) LINX II - Organic NDS reference sites	Intercept	0.64	0.25	0.023	0.23	0.070	3.73E+04	N/A	N/A
	$S_{w,a,N}$	-0.14	0.07	0.070					
6) LINX II - Inorganic NDS reference sites	Intercept	0.51	0.22	0.029	0.17	0.055	8.38E+03	N/A	N/A
	$S_{w,a,N}$	-0.13	0.06	0.055					
7) LINX II - Organic NDS regional averages	Intercept	1.43	0.25	0.001	0.80	0.003	5.84E+04	2.49E+04	2.56E+05
	$S_{w,a,N}$	-0.30	0.06	0.003					
8) LINX II - Inorganic NDS regional averages	Intercept	0.74	0.30	0.048	0.38	0.106	1.93E+05	N/A	N/A
	$S_{w,a,N}$	-0.14	0.07	0.106					
9) LINX II - Organic NDS regional avg - reference	Intercept	1.01	0.26	0.008	0.65	0.016	2.46E+04	6.18E+03	7.66E+05
	$S_{w,a,N}$	-0.23	0.07	0.016					
10) LINX II - Inorganic NDS regional avg - reference	Intercept	1.07	0.23	0.004	0.73	0.007	1.30E+04	4.44E+03	7.98E+04
	$S_{w,a,N}$	-0.26	0.06	0.007					

\*all variables log10-transformed

## CHAPTER 4 FLOW VELOCITY CONTROL OF FILAMENTOUS ALGAE IN A SUB-TROPICAL SPRING-FED RIVER

### **Introduction**

Algae are an integral component of most aquatic systems due to their influence on oxygen and nutrient dynamics and their role as a food source for grazing organisms that, in turn, support the production of higher trophic levels. However, when algal abundance increases rapidly (i.e. proliferates) there are often a broad suite of negative consequences such as degraded ecosystem structure and function, impaired recreational use and reduction in aesthetic quality, and even human health impacts (Lembi 2003; Smith 2003). Algal proliferation is typically attributed to nutrient enrichment (i.e. eutrophication) which may stimulate the growth rate of nutrient-limited algae, and for systems initially dominated by submerged vascular plants this can cause a shift to attached algae and then potentially to free-floating planktonic algae (Duarte 1995; Valiela et al. 1997). While nutrient enrichment is often the primary cause of algal proliferation in aquatic systems in general, there are other possible explanations such as loss of algal consumers (Jones and Sayer 2003) or changing flow conditions (Wade et al. 2002).

In lotic systems (i.e. rivers and streams), flow is known to have a significant influence on algal metabolism, abundance, and community structure (Biggs 1996; Stevenson 1996), but when algal proliferation occurs flow conditions are rarely considered as a regulating factor. Most of the focus on flow has been in relation to periodic flood events which scour channels and dislodge attached algae (Biggs 2000). However, in lotic systems where scouring events are infrequent, attached algae may accumulate over inter-annual time periods, and algal abundance may ultimately be

controlled by longer- term hydrologic fluctuations. This study examined a sub-tropical spring-fed river with minimal scouring events to assess whether long-term declines in flow might account for observed filamentous algal proliferation.

### **Flow Velocity and Algae**

The flow of water is involved in multiple mechanisms which regulate algal abundance and distribution in lotic systems (Biggs 1996; Stevenson 1996). Flow velocity has been shown to have a positive effect on algal growth due to increased transfer of nutrients to cells (Whitford and Schumacher 1961, 1964; McIntire 1966; Lock and John 1979; Horner et al. 1990) and waste products away from cells (Mass et al. 2010). However, with increasing flow velocity, the negative effects of drag force will eventually override the positive effects on chemical transfer, creating a “subsidy-stress” relationship (Odum et al. 1979) between flow and biomass accumulation. This has been demonstrated over a wide velocity range (Horner and Welch 1981; Horner et al. 1990), but a synthesis indicates that the strength and direction of the flow velocity-algae relationship varies widely (see Table 1 in Stevenson 1996), making it difficult to predict with certainty how algae will respond to changing flow conditions. While some of the variation in the flow velocity-algae relationship can be attributed to interactions with other key environmental variables such as nutrients, light intensity, and algal grazers; much is due to differential response among algal species that exhibit a wide range of morphological characteristics and attachment capabilities. Particularly important is the growth form (i.e. physiognomy) of the algal species as small, closely adhering species are more likely to show a positive relationship with velocity due to increased chemical transfer whereas larger filamentous forms of algae are likely to show a negative relationship due to increased drag force (Biggs et al. 1998).

In lotic systems, the drag force due to flow velocity can have two main effects on algae: 1) inhibiting establishment and 2) dictating sloughing (i.e. export) rates. High velocities can inhibit colonization for some species of algae (Stevenson 1983; Stevenson and Peterson 1989). Once algal colonization has occurred, velocity controls the sloughing rate by which algal cells are detached from their substrate and exported downstream, thus contributing to the overall loss rate of algal biomass (McIntire 1966; Biggs and Thomsen 1995). Drag force due to friction increases at a rate proportional to the square of the flow velocity, so that small changes in velocity can potentially lead to significant changes in algal export rates. Algal cells may be sheltered from drag force by residing within the boundary layers that surround submerged surfaces. Local flow velocity controls the thickness of the boundary layer, and therefore can alter the hydraulics of the near surface environment in which attached algae reside. While most field studies of flow velocity effects focus on the role of flood (i.e. scouring) events (Tett et al. 1978; Biggs and Close 1989; Peterson and Stevenson 1992), the inhibitory effect of velocity has also been recorded in lotic systems with relatively stable hydrology (e.g., low gradient systems fed by groundwater) (Poff et al. 1990; Ghosh and Gaur 1991). This study is one of the first to examine the effect of declining flow velocity on algal abundance over an inter-annual time period in a system with low hydrologic variation.

### **Factors Affecting Flow Velocity**

The magnitude and distribution of flow velocity within a lotic system are largely controlled by the overall rate of discharge and the geomorphology of the channel, although woody debris and submerged aquatic vegetation (SAV) may have significant influence on velocity at smaller spatial scales. Due to friction, velocities are typically highest in the center of the channel near the water surface and lower at the channel

margins and near the sediment/water interface. Submerged aquatic vegetation, particularly dense monotypic patches (i.e. beds), obstruct the flow of water and alter the distribution of flow velocities such that velocity is lower within the beds, but higher throughout the rest of the water column (Sand-Jensen and Mebus 1996). Algal mats may significantly alter the flow velocity distribution in a similar manner (Dodds 1991), though algae are sloughed downstream more easily than rooted vascular plants due to the difference in substrate attachment capability. It is hypothesized that the biomass of filamentous algae and SAV are positively related, since SAV may decrease velocities locally and provide stable substrate for algal colonization; however over longer time periods algae may lead to the decline of SAV due to factors such as light competition.

### **Spring-fed Rivers in Florida**

Florida contains among the highest density of first magnitude ( $> 2.8 \text{ m}^3/\text{s}$ ; Copeland 2003) artesian springs in the world as a result of unique karst geology and a highly productive aquifer system (Scott et al. 2004). Many of these springs form spring-fed rivers, which are characterized by high water clarity, stable hydrology, chemistry, and temperature, and dense SAV beds (Odum 1957b; Canfield and Hoyer 1988). These spring-fed rivers were the focus of early ecological studies which determined that flow velocity and light availability were the primary drivers of algal community structure and autotrophic production rates, rather than nutrients (Whitford 1956; Odum 1957a). Over recent decades, filamentous algae such as the cyanophyte *Lyngbya wollei* and the xanthophyte *Vaucheria* spp. have proliferated in many of Florida's spring systems (Stevenson et al. 2004; Stevenson et al. 2007). Anthropogenic nitrogen enrichment is widely considered to be the cause of algal proliferation, but a recent synthesis of the evidence for this contention suggests that nutrient enrichment is not likely the primary

causal factor and that alternative hypotheses should be evaluated (Heffernan et al. 2010b).

The influence of flow conditions and hydraulics on filamentous algal abundance in Florida spring-fed rivers has not been adequately investigated. These rivers typically respond to rainfall over a period of months (e.g., Florea and Vacher 2006; Heffernan et al. 2010a) and do not exhibit scouring events which limit algal abundance in many lotic systems. Many spring-fed rivers have experienced declining discharge over the last few decades (Munch et al. 2006; Weber and Perry 2006; Copeland et al. 2009; Grubbs 2011), which would generally lead to lower velocities and less drag on algae. It is hypothesized that lower flow velocities allow greater amounts of filamentous algae to accumulate in Florida spring systems, and there is a velocity threshold above which algal abundance is minimal.

## **Methods**

### **Site Description**

The Gum Slough spring system is a second magnitude springs complex located near the central west coast of peninsular Florida in Sumter County (Figure 4-1). Six larger and numerous smaller springs combine to form an approximately 8 km river that discharges into the Withlacoochee River. The larger springs are all within the upper 2 km of the river where the water is extremely clear and tree canopy cover is moderate, thus providing sufficient light for high rates of autotrophic production. Further downstream, the river becomes more forested and braided (i.e. a slough) with less autotrophic biomass. Most of the adjacent land is undeveloped and the river receives sparse human traffic due to its remote location and relative inaccessibility.

Discharge downstream of the main springs complex averaged 2.62 m<sup>3</sup>/s from October 2003 to December 2011 (the period of record at USGS gage #02312764), fitting the classification as a large second magnitude spring system (Figure 4-2). The period of record for discharge at Gum Slough is not sufficient to assess long-term flow conditions; however, it is likely that over the past decade discharge was lower than for previous decades due to substantially less rainfall as observed for other spring systems (Copeland et al. 2009). Over the period of record, Gum Slough was subject to the effects of multiple tropical storms followed by low flow periods in which sections of the upper river ceased flowing due to minimal discharge from the upstream springs. During this study, discharge decreased from moderate to extremely low levels, which provided an opportunity to examine the effects of declining discharge on flow velocity and filamentous algal cover.

Gum Slough water chemistry is typical of a hard freshwater spring (e.g., Whitford 1956) with higher sulfate concentrations than many similar inland springs (Champion and Starks 2001) (Table 4-1). Nitrate concentrations have increased from 0.5 mg N/L in 1972 (Scott et al. 2004) to a high of 1.53 mg N/L in 2011, and were likely less than 0.1 mg N/L prior to human settlement (Heffernan et al. 2010b). The increased nitrate concentrations in many Florida springs are due to a combination of agricultural and residential fertilizer application, intensive animal operations, land application of wastewater effluent and residuals, and septic tank discharge within the recharge area (Katz 2004; Phelps 2004).

The autotrophic community is dominated by the rooted macrophytes *Sagittaria kurziana* and *Vallisneria americana*, which shift in dominance from the former to the

latter with distance downstream from spring inputs. A variety of epiphytic algal species contribute significantly to overall primary production in spring systems (Whitford 1956; Odum 1957b). Over the past decade, filamentous algae, primarily the cyanophyte *Lyngbya wollei*, has increased in abundance and formed persistent mats which occasionally proliferate to extreme nuisance levels, especially near the upper most spring vents. Despite the shift towards filamentous algal dominance, there remains a large and diverse fish population dominated by large-mouth bass (*Micropterus salmoides*) and various sunfish (*Lepomis* spp.) (WSI 2011). There is also a large snail population comprised primarily of *Elimia* spp. but also including apple snails (*Pomacea* spp.) and other species (personal observation). These snails likely have a significant influence on algal dynamics in their role as algal grazers (Heffernan et al. 2010b).

### **Field Surveys**

Field data were collected at Gum Slough at three transects perpendicular to the river channel, which were sampled approximately every two months ( $n = 10$ ) (Figure 4-1). The three sites were chosen based on river morphology and relative flow velocities; T1 is wide and deep with low velocity (width = 30 m; depth = 1.2 m), T2 is narrow and shallow with high velocity (width = 13 m; depth = 0.6 m), and T3 is deep with moderately high velocity (width = 18 m; depth = 1.2 m). Sampling began on 6/21/10 and ended on 12/14/11, and over this time period discharge (and water levels) decreased substantially below the longer-term average. All discharge rates and stage levels refer to measurements at the USGS gage located just downstream of all spring inputs (Figure 4-1), since discharge rates calculated from the velocity data for each transect were all linearly related to the USGS gage discharge rate ( $R^2 > 0.98$ ;  $p < 0.001$ ).

Flow velocity with depth and filamentous algal cover were measured at 2 m intervals along each transect. Flow velocity was measured with a portable velocity meter (Model 2000; Marsh-McBirney Inc., Frederick, Maryland), using a 5-second average. At each sampling point vertical variation in flow velocity was measured at 10 cm increments starting just below the water surface and ending at the sediment surface. T1 was sampled in less vertical detail (20 cm depth increments) due to its width and low variation in velocity. At each transect sampling point the areal percent cover of filamentous algae was visually estimated with a 0.25 m<sup>2</sup> quadrat. When a thick algal mat or SAV bed was present, the depth at the top of the mat or bed was recorded.

Transect data were analyzed in two ways: for transects as a whole and for individual sampling points. The analysis of transects as a whole used the average (v-avg) and maximum (v-max) of all velocities measured with depth across each transect, and averaged the filamentous algal cover recorded at each sampling point. The analysis of individual sampling points along a given transect also used average and maximum velocities, but in these cases the average and maximum velocities refer to the measurements with depth for each sampling point.

### **Experimental Manipulation of Flow Velocity**

Plastic baffles were used to construct two side-by-side channels with different flow conditions to experimentally test the effect of flow velocity on both filamentous algae and SAV abundance as indicated by biomass (Figure 4-3). Three clear plastic sheets (0.65 m wide and 3 m long) were used to create the baffles. Each sheet was trimmed to 2.5 m to make the two channels. The 0.5 m trimmed sections were used as inlet control structures with one side open (i.e. \ /) and the other semi-closed (i.e. / \), thus forming high and low flow channels, respectively. Each section of plastic sheet was attached to

wooden stakes which were inserted into the sediment so that the bottom of the sheet was flush with the sediment surface. The baffles were placed in three different locations in the upper section of the river where SAV density was homogeneous and water depths were < 0.65 m. The first two locations were only harvested once while the third location was harvested three times, resulting in five sampling events. At this third location, there were two harvests when samples were taken in previously un-harvested areas, then the entire baffle area was cleared of algae and SAV to test the effect of SAV absence. Additional sampling was precluded by declining discharge which eliminated appropriate installation locations.

The baffles were installed for approximately 7 weeks (46 to 52 days) to allow for filamentous algal colonization prior to harvesting (except for the one instance when the baffles were harvested a second time, which occurred 28 days after the first harvest). After the colonization period, a metal cylinder (0.05 m<sup>2</sup>) was used to harvest all autotrophic biomass from three equidistant points within the center of each channel. This material was transported on ice back to the laboratory where it was separated into filamentous algae and SAV components, and rinsed of other materials (e.g., sediment, leaves, invertebrates). The separated sample material was then weighed and dried in an oven at 70 °C until constant weight to determine the dry weight, after which the dried samples were ashed in a muffle furnace at 440 °C for four hours to determine the ash-free dry mass (AFDM). Just prior to biomass harvest, flow velocity measurements were taken at three equidistant points in the center of each channel, at depth increments of 10 cm throughout the water column.

## Statistical Analysis

Linear regression was used to assess the relationships between discharge and flow velocity, and between flow velocity and filamentous algal cover. Temporal variation in filamentous algal cover was investigated by numbering each day of a year (1 – 365) starting on 10/15, since this date was approximately the end of the growing season. The effects of both flow velocity and temporal variation (i.e. day of the year) on filamentous algal cover were assessed with additive multiple linear regression. Linear regression and a t-test were used to assess the effects of experimental manipulation of flow velocity on filamentous algae and SAV biomass (omitting the last harvest event when SAV was absent). All statistical analyses were conducted using the software package R version 2.11.1 (R Foundation for Statistical Computing).

## Results

### Field Surveys

During the course of this study discharge decreased from a high of 2.98 m<sup>3</sup>/s on 6/21/10 to a low of 0.94 m<sup>3</sup>/s on 6/29/11 (Figure 4-2). Subsequently, discharge increased, but never exceeded 1.70 m<sup>3</sup>/s. Discharge for each sampling event was highly correlated with both the average velocity ( $R^2 > 0.89$ ;  $p < 0.001$ ) and maximum velocity ( $R^2 > 0.85$ ;  $p < 0.001$ ) measured for each transect. Overall the sampling dates fell into three distinct groups based on discharge; high flow:  $> 2.75$  m<sup>3</sup>/s (6/21/10 – 10/8/10); medium flow: 1.13 to 1.64 m<sup>3</sup>/s (1/20/11 – 5/9/11 and 8/16/11 – 12/14/11); and low flow:  $< 1.0$  m<sup>3</sup>/s (6/29/11).

For each transect, the distribution of flow velocity was generally highest near the top of the water column in the center of the channel and decreased to zero near the sediment surface. Where SAV beds were present (for T2 and T3) there was a sharp

change in velocity with depth, and velocities within SAV beds were typically < 10 cm/s. Large fluctuations in velocity ( $\pm 5$  cm/s) occurred just above SAV beds due to turbulence, particularly at high discharge. SAV beds were absent at T1 throughout the study, and while velocity decreased near the sediment surface the change with depth was less pronounced.

The low velocity transect, T1, contained  $\geq 60\%$  filamentous algal cover during the entire study period (Figure 4-4). When flow velocity was the lowest (on 6/29/11) algal cover was greatest; although on 1/20/11 the algal cover was also high despite slightly greater flow velocity. Large floating algal mats were present at the water surface towards the transect edges throughout the study period; flow velocity at these edges was always < 3 cm/s. On the lowest flow date (6/29/11), when velocity was barely detectable, floating algal mats were present across the entire transect.

The other two transects (T2 and T3) had much higher flow velocities throughout the study period and filamentous algal cover was generally low except at low velocities, suggestive of a threshold value. For T2, algal cover was  $\leq 26\%$  until 3/9/11 after which it increased to 85% during the summer months. This increase in algal cover coincided with a decline in average flow velocity from > 20 cm/s to a low of 5 cm/s on 6/29/11. For T3, algal cover was < 6% until 3/9/11 and also reached 80% by the end of the summer with similar changes in flow velocity. Overall, the highest values of filamentous algal cover occurred below 15 cm/s average velocity (22 cm/s maximum velocity), which corresponded to a discharge rate of approximately  $1.5 \text{ m}^3/\text{s}$  at the downstream USGS gage.

A seasonal pattern of filamentous algal cover was apparent, as winter values were low both years but summer values were only low when flow velocity was high. During two winter sampling events (1/20/11 and 12/14/11), algal cover was < 15% despite low flow velocity for the two higher velocity transects (T2 and T3). However, algal cover remained > 50% for the low velocity transect (T1). Filamentous algal cover substantially increased to > 80% from 3/9/11 to 8/16/11 for T2 and T3; however measurements from the previous summer were < 20% when flow velocities were much higher. This suggests that there is potential for filamentous algal proliferation in the spring and summer months, but if the average velocity is above the 15 cm/s threshold then algal accrual is inhibited.

The linear relationship between flow velocity (both average and maximum) and filamentous algal cover was weak and insignificant for transects T1 and T2 ( $R^2 < 0.30$ ,  $p > 0.1$ ); however for T3 maximum velocity was a significant predictor ( $R^2 = 0.41$ ,  $p = 0.046$ ) and average velocity was borderline ( $R^2 = 0.37$ ,  $p = 0.059$ ). When a seasonal effect was included in these regression models by accounting for the day of the year, then the models for T2 and T3 improved substantially and were significantly related to maximum velocity (Table 4-2). Inclusion of both the seasonal effect and maximum flow velocity did not generate a significant model for T1, likely because the velocities were always below a threshold value that might otherwise inhibit algal accrual.

Analysis of individual sampling points along transects yielded an average velocity threshold of 25 cm/s and a maximum velocity threshold of 35 cm/s beyond which algal cover was < 5% (Figure 4-5). Just below these velocities algal cover increased to  $\geq$  50% at multiple locations, suggesting that velocity induced drag is a significant

constraint on filamentous algal accrual. These apparent threshold values are larger than those derived from average transect values, since there were individual sampling stations with moderate algal cover despite higher velocities. Overall, many low flow velocity stations contained low filamentous algal cover, which indicates that factors other than velocity also limit algal cover. The primary role of local flow velocities was to create an upper-bound that constrained the maximum potential algal cover, unless velocities were  $< 5$  cm/s at which point 100% algal cover was frequent.

### **Experimental Manipulation of Flow Velocity**

The baffle experiment results generally supported the survey results, as filamentous algal biomass was negatively correlated with both average and maximum flow velocity (Figure 4-6). The average algal biomass (AFDM) was  $216.3$  g/m<sup>2</sup> and  $137.7$  g/m<sup>2</sup> in the low and high flow channels, respectively, although values varied widely depending on baffle location and associated flow velocity differences. The t-test did not find a significant difference in algal biomass between the low and high flow channels, likely due to a combination of relatively small differences in flow velocity and low statistical power (4 samples total). The flow velocity threshold that inhibited substantial algal biomass was approximately 10 cm/s. There was a positive correlation between filamentous algal biomass and SAV biomass (Figure 4-6), which suggests that filamentous algae may increase with SAV density. However, when the baffles were left at the third location for three harvest events, the resulting algal biomass for each channel was similar despite the absence of SAV for the final harvest ( $208$ - $230$  g/m<sup>2</sup> in the low flow channel;  $128$ - $153$  g/m<sup>2</sup> in the high flow channel).

## Discussion

### Flow Velocity and Filamentous Algae

This study involved both in situ field surveys and an experimental manipulation, and the combined results suggest that flow velocity constrains filamentous algal abundance in the Gum Slough spring system. The early part of the study period was ideal for testing the effect of declining discharge and velocity on filamentous algal cover, and also allowed comparison of two growing seasons with different flow conditions. Over this period, filamentous algal cover increased substantially at the two transects with initial velocities above the implied threshold of 25 cm/s depth-averaged velocity (35 cm/s maximum velocity), whereas the low velocity transect was below the velocity threshold for the entire study period and always had > 50% algal cover. This flow velocity threshold is consistent with findings by Hoyer et al. (2004) who found that above 25 cm/s filamentous algal biomass was minimal in three coastal Florida spring-fed rivers, which suggests that this velocity threshold applies to lotic systems with similar characteristics. While a few other studies of Florida springs have anecdotally mentioned that less algae occurs where velocity is greater (Munch et al. 2006; Quinlan et al. 2008), this study and that by Hoyer et al. (2004) are the only ones which have quantified the relationship between flow velocity and filamentous algae in sufficient detail to specify a velocity threshold that likely inhibits algal proliferation.

Few studies have focused on how declining discharge rates in lotic systems may contribute to attached algal proliferation. Since flow velocity is directly related to the drag force exerted on attached algae, declines in flow velocity should allow more algae to establish. This process, however, may be non-linear due to the presence of velocity thresholds which substantially constrain algal abundance. Lower velocities could also

exacerbate the effects of nutrient enrichment by reducing the export rate of attached algae. Wade et al. (2002) created a simulation model to examine effects of nutrient enrichment and flow declines in an English river, and found that lower flow reduced the loss of epiphytic algae which, in turn, reduced SAV biomass. The concept that declining discharge is as important a contributor to algal proliferation as nutrient enrichment should be more fully explored since flow is expected to change in many lotic systems globally due to climate change effects on rainfall and increasing water withdrawal by humans.

### **The Relationship between SAV and Filamentous Algae**

In many Florida spring-fed rivers, filamentous algal abundance is increasing as SAV abundance is decreasing (e.g., Quinlan et al. 2008), but it is currently unknown if these two trends are directly related. The results of the baffle experiments suggest that filamentous algae may utilize SAV as a substrate, but SAV presence is not necessary if flow velocities are sufficiently low. This study also found evidence that SAV beds decrease flow velocities near the sediment surface and increase turbulence just above SAV beds. Overall, it is unclear whether SAV beds promote filamentous algal proliferation by lowering velocities within the beds and providing substrate for attachment, or inhibit proliferation by creating higher turbulence above the beds, reducing light intensity within the beds, and supplying habitat for algal consumers. Conversely, it is likely that filamentous algae have contributed to the loss of SAV, since it has been shown that vascular macrophytes are often replaced by filamentous algae due to competition for light (Duarte 1995; Valiela et al. 1997). Loss of SAV could ultimately lead to less filamentous algal abundance (Frazer et al. 2006), since this algae appears to use SAV as a substrate, particularly in higher velocities.

## Management Implications

Maintenance of flow velocities above identified thresholds may be necessary to minimize filamentous algal proliferation and associated impacts in many lotic systems. Typically, variation in flow velocities over time is driven by fluctuations in overall discharge, which are largely related to rainfall patterns. However, where consumptive use or other factors have lowered discharge, there are opportunities to ameliorate these effects by reducing water use and restoring landscapes to promote pre-development hydrologic conditions. In addition, there may be more direct methods to prevent decreased flow velocities, such as minimizing dredging and damming activities which result in lower velocities. Intensive aquatic plant management may also be problematic as removal of substantial amounts of vegetation may alter the distribution of flow velocities in the water column, which in addition to the effects of disturbance and increased light availability, could create conditions that favor a shift to algal dominance. Finally, while managers do not usually have direct control over river discharge; in regulated rivers, controlled flow releases can be used to suppress or reduce filamentous algal blooms (Mitrovic et al. 2011).

In Florida, many spring-fed rivers have exhibited reduced discharge over recent decades due to a combination of less rainfall and increased groundwater pumping which, in turn, has lowered aquifer levels and associated springs discharge (Munch et al. 2006; Weber and Perry 2006; Copeland et al. 2009; Grubbs 2011). Until the late 1990s, water policy in Florida focused more on water quality than quantity; however, dwindling water supplies have increased the attention to water quantity and spurred the implementation of minimum flows and levels (MFLs) for Florida water bodies (Munson et al. 2005). To date, MFLs have been based on factors such as fish passage, aquatic

habitat preservation, and floodplain inundation, but MFLs could also account for filamentous algal proliferation by setting a minimum flow that maintains velocities above the thresholds identified in this study.

Reducing nitrate loading to groundwater has been the primary focus for springs management to date, but while nutrient enrichment may increase algal growth rates, the flow conditions in these systems have been shown here to play an important role in controlling algal abundance. Nitrate concentrations and loading rates may have to be reduced substantially to inhibit algal proliferation, which is expected to require long time periods and may not reduce already established algal mats. While decreased flow velocities are likely to contribute to algal proliferation in Florida spring-fed rivers, the importance of this factor in comparison to other changes such as nutrient enrichment and loss of algal consumers is currently unknown.

The negative relationship between flow velocity and filamentous algae may, in part, explain why algal proliferation frequently occurs near spring vents (i.e. locations of discharge from the aquifer). Spring vent areas tend to resemble pools more than rivers, with lower flow velocities except near the vent itself. Downstream of vent areas, spring systems usually become more channelized with higher velocities, which might reduce the potential for algal accumulation. Nutrient enrichment may also have a greater effect where velocities are lower, since nutrient availability becomes more limited with increasing water residence time. Other factors that promote filamentous algae such as human recreation disturbance and loss of algal consumers may also have more influence in the vicinity of spring vents (DuToit 1979; Heffernan et al. 2010b).

When examining the causes of algal proliferation in lotic systems, factors affecting both the growth of algae (e.g., nutrients, light) and the loss of algae (e.g., drag-induced export, consumption) should be considered. A multi-faceted approach would draw attention to the full suite of factors potentially controlling algal abundance in a given system. For Florida spring-fed rivers, filamentous algal proliferation is likely a multi-causal response to the combined effects of lower flow velocities, less algal consumption, and increased nutrient concentrations, as well as increased disturbance in general. A management strategy that acknowledges and incorporates a broad suite of potential drivers of algal proliferation will provide additional options and techniques for maintaining the ecological integrity of Florida springs.

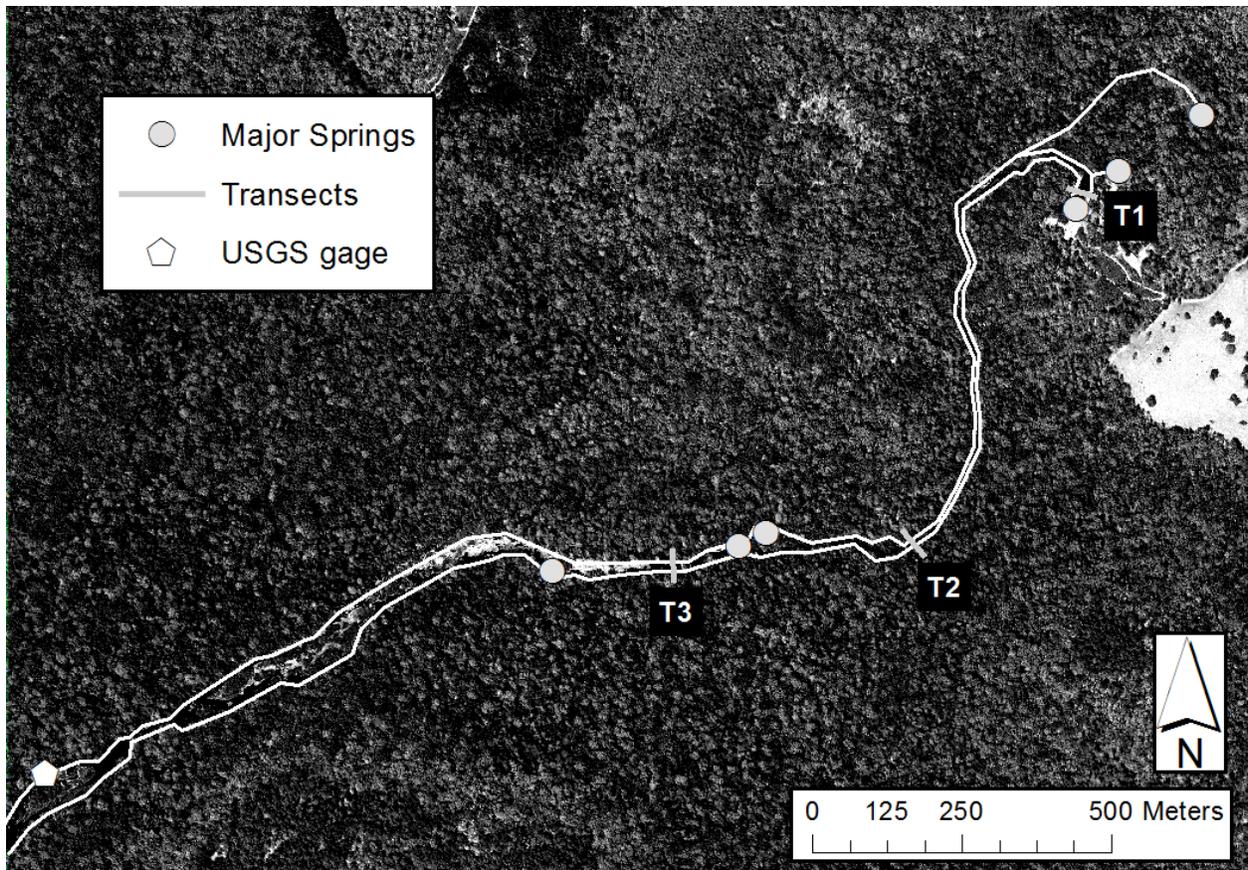


Figure 4-1. Aerial view of Gum Slough. The spring-fed river begins in the northeast and flows towards the southwest. The majority of the water comes from six large springs (circles), whose combined discharge rate is recorded at a USGS gage downstream (pentagon in the lower-left). The three transects sampled every two months (solid lines) are shown.

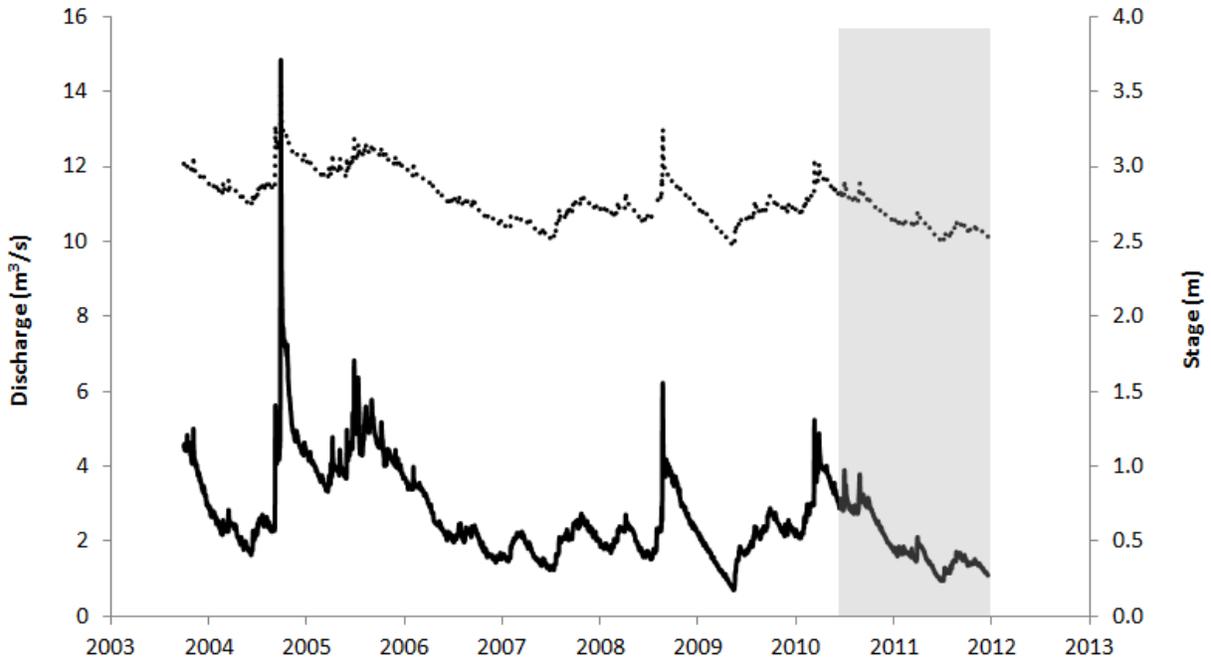


Figure 4-2. Discharge (—) and stage (---) for the period of record (October 2003 to December 2011) from the USGS gage downstream of the spring inputs. The study period is highlighted in gray.

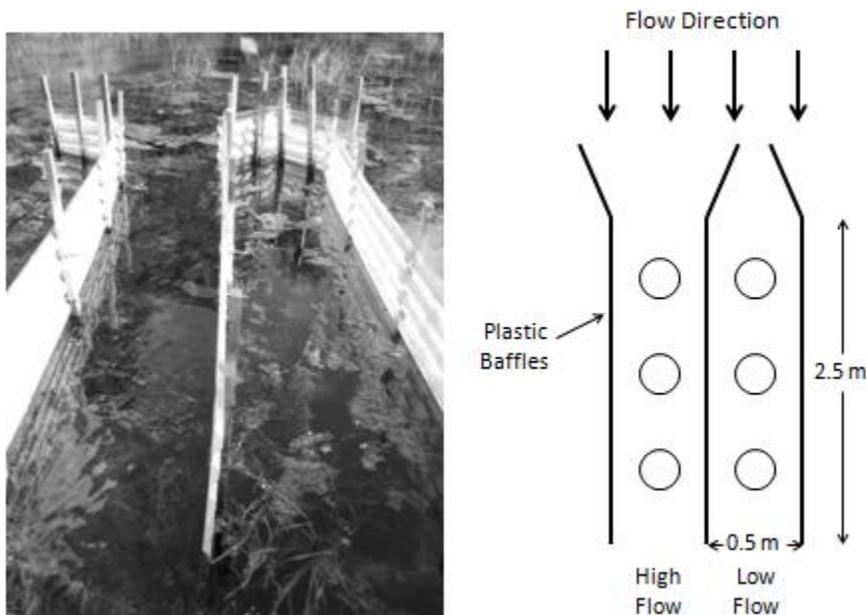


Figure 4-3. Picture and diagram of the experimental baffles. Circles indicate the locations of flow velocity measurements and algae/SAV sampling.

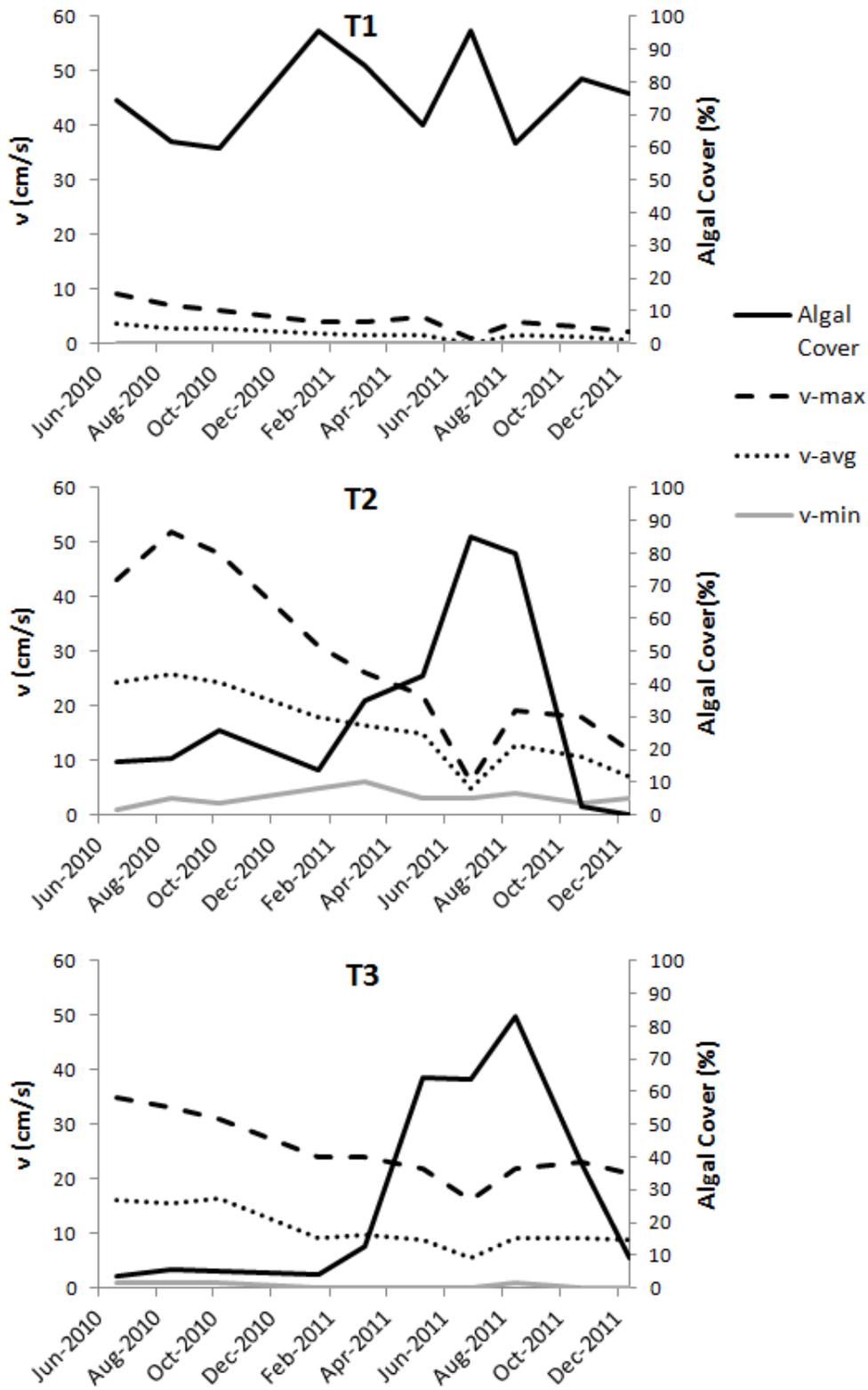


Figure 4-4. Changes in flow velocity and filamentous algal cover over time at three transects with different morphology.

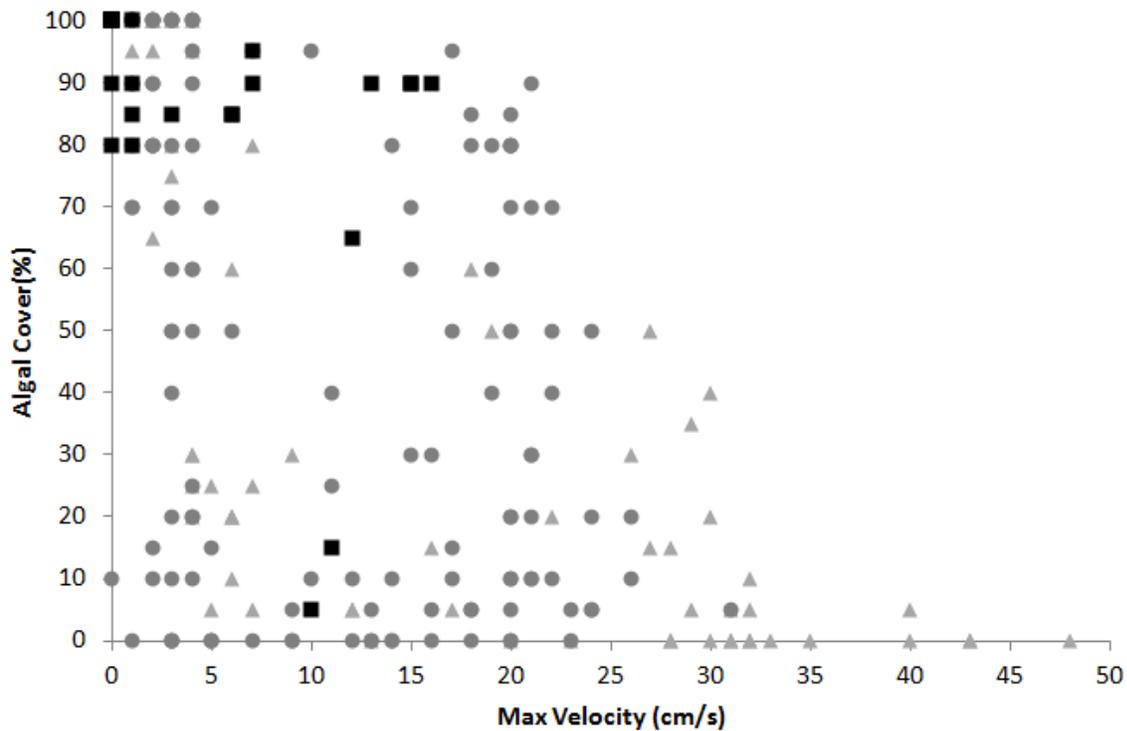
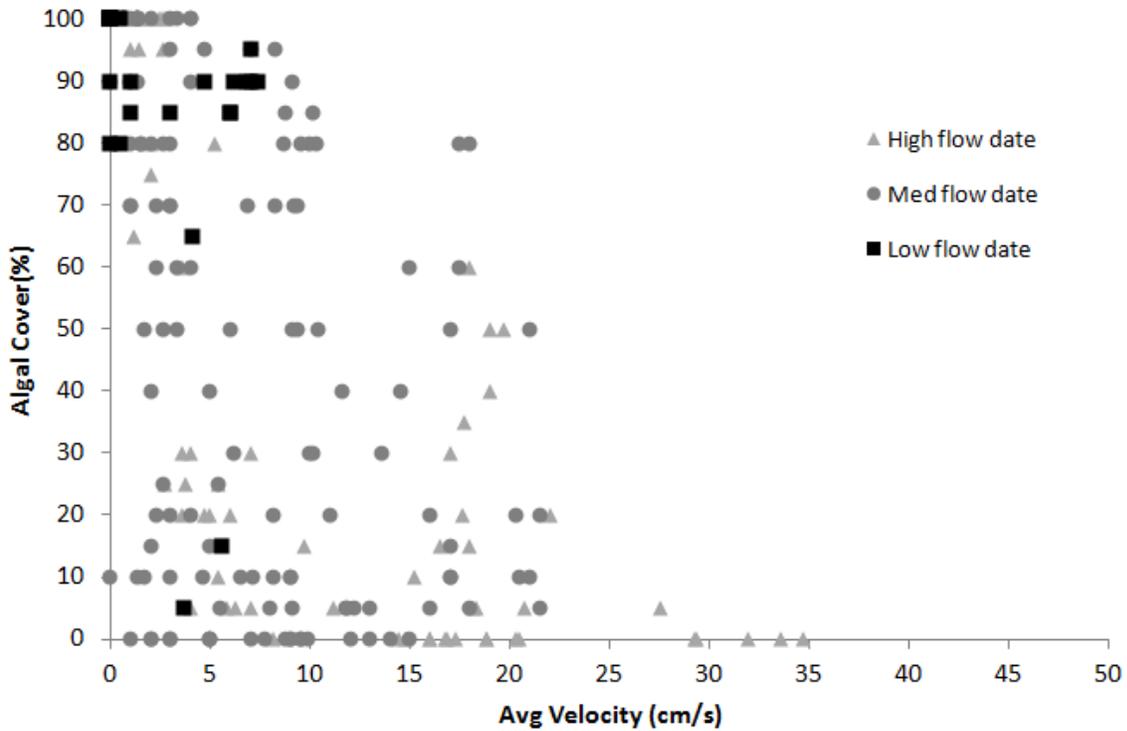


Figure 4-5. Filamentous algal cover in relation to average and maximum flow velocity for individual sampling points across all transects. High Flow: n = 3; Med Flow: n = 6; Low Flow: n = 1; where n is the number of sampling dates in that flow category.

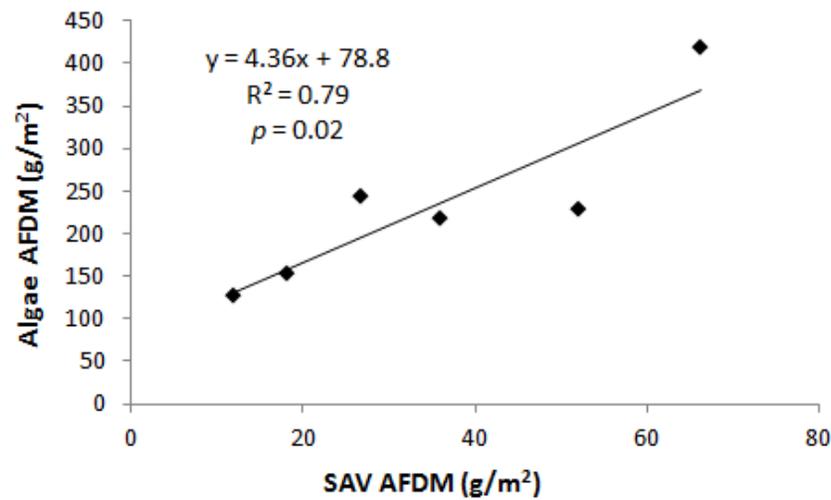
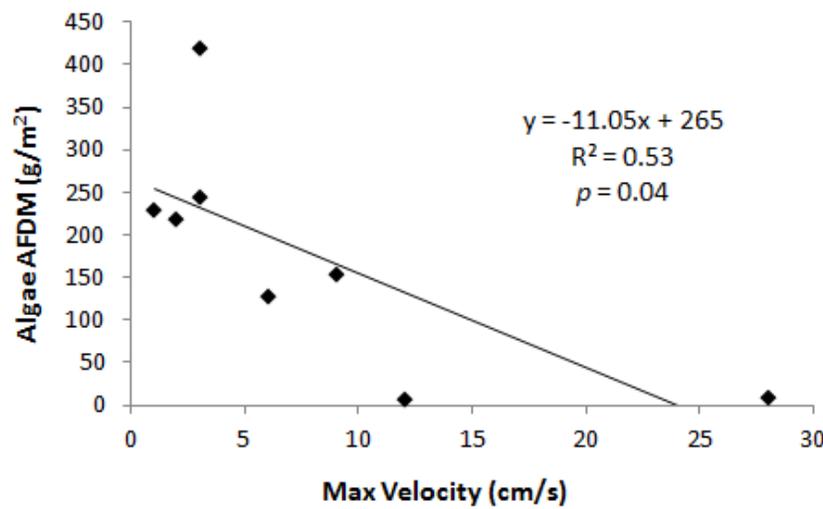
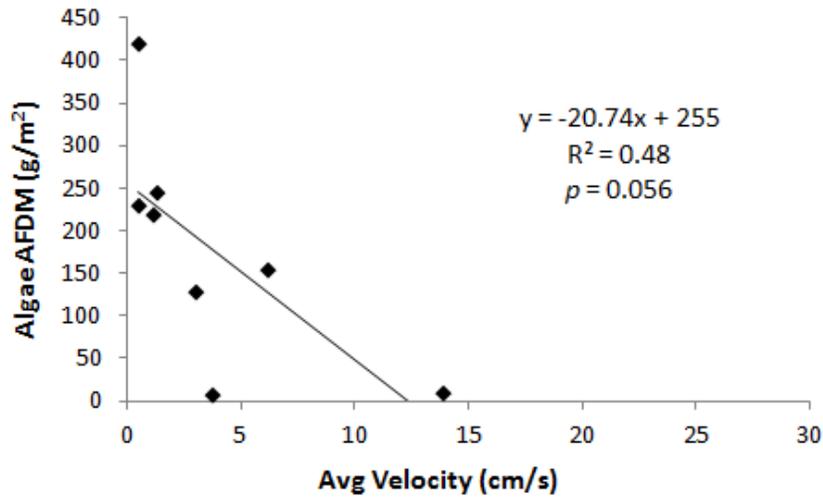


Figure 4-6. Baffle experiment results. Filamentous algal biomass (as ash-free dry mass (AFDM)) in relation to average and maximum flow velocity, and SAV biomass.

Table 4-1. Average water chemistry for the main spring at Gum Slough during the study period (data from quarterly monitoring by the Southwest Florida Water Management District).

Temp (°C)	Sp. Cond (µS/cm)	Alkalinity (mg/L CaCO <sub>3</sub> )	pH	DO (mg/L)	NO <sub>3</sub> +NO <sub>2</sub> (mg N/L)	Ortho-P (mg P/L)	Ca (mg/L)	Cl (mg/L)	Fl (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)	SO <sub>4</sub> (mg/L)
23.04	332	125.1	7.73	3.96	1.45	0.028	54.7	7.5	0.13	0.47	7.03	4.17	27.6

Table 4-2. Results of multiple linear regression models for each transect, using maximum flow velocity (v-max) and the day of the year (day) to predict filamentous algal cover.

Transect	Parameter	Value	SE	p	Model	
					R <sup>2</sup>	p
T1	Intercept	93.5	8.9	<0.001	0.41	0.156
	v-max	-1.81	1.85	0.360		
	day	-0.05	0.04	0.240		
T2	Intercept	29.0	9.4	0.018	0.86	0.001
	v-max	-1.68	0.31	0.001		
	day	0.25	0.04	<0.001		
T3	Intercept	111.4	30.9	0.008	0.62	0.034
	v-max	-4.33	1.31	0.013		
	day	0.13	0.07	0.093		

CHAPTER 5  
EFFECTS OF FLOW VELOCITY AND NUTRIENTS ON THE FILAMENTOUS  
CYANOPHYTE *LYNGBYA WOLLEI* IN LABORATORY STREAM CHANNELS

**Introduction**

In lotic systems, hydraulic conditions are known to be a major determinant of autotrophic structure and function (Biggs 1996; Stevenson 1996), but are usually not explicitly considered when assessing the conditions under which autotrophs might be nutrient limited. Nutrient availability (primarily N and P) often limits autotrophic production, particularly under conditions of low nutrient concentration and high light availability (Grimm and Fisher 1986; Hill and Knight 1988; Rosemond et al. 2000). However, there are many lotic systems which do not display nutrient limitation (Franceour 2001; Tank and Dodds 2003; Johnson et al. 2009) and nutrient concentrations do not correlate with algal biomass to the same degree as for lentic systems (Dodds et al. 2002b). Part of the difficulty in assessing nutrient limitation in lotic systems is due to the influence of hydraulic conditions on benthic autotrophs. Many studies have found that flow velocity substantially affects algal nutrient uptake, production, and biomass (see Stevenson 1996 for a review); although the specific response varies greatly due to factors such as algal grazing (Poff and Ward 1995; Opsahl et al. 2003) and algal growth form (Biggs et al. 1998). This study examined the response of the filamentous cyanophyte *Lyngbya wollei* to nutrients under different flow velocities, to better understand how flow mediates nutrient availability and limitation for this species.

**Positive Effects of Flow Velocity on Algae**

Assessments of hydraulic conditions in lotic systems often focus on flow velocity (i.e. current), which may affect nutrient availability to benthic autotrophs by regulating

the thickness of diffusive boundary layers (DBLs). A DBL is a stagnant layer of water that surrounds submerged surfaces in aquatic environments, and may limit the mass transfer of nutrients and other dissolved ions and gases to cell surfaces. A DBL becomes thinner with increasing flow velocity, thus increasing the diffusive mass transfer rate of nutrients from the water column to cellular uptake sites (Jorgensen and Revsbech 1985; Gundersen and Jorgensen 1990; Hurd 2000). As flow velocity increases, DBLs may become thin enough to no longer limit nutrient transfer, and instead cellular uptake rates become the limiting factor (Sanford and Crawford 2000; Nishihara and Ackerman 2009). In addition, flow velocity has recently been found to enhance waste efflux (such as oxygen) away from autotroph cells with a corresponding increase in production rates (Mass et al. 2010). This latter mechanism could lead to a positive relationship between flow velocity and algae independent of nutrient availability. The effects of flow velocity on DBLs and waste efflux could have significant implications for assessing nutrient limitation in lotic systems.

### **Filamentous Algae**

The proliferation of filamentous algal mats is a growing problem globally, and often results in degraded ecosystem structure and function as well as diminished aesthetics and recreational opportunities (Lembi 2003; Smith 2003). Flow velocity can regulate nutrient transfer to algal mats as previously described, and additionally stimulates nutrient flux into the mats (Stevenson and Glover 1993). Internal nutrient cycling within filamentous algal mats can be substantial, although its significance as a nutrient source is dependent on external nutrient concentration (Mulholland et al. 1994; Sickman et al. 2009). In this study, portions of filamentous algal mats (i.e. clumps) were used, rather

than single filaments or a thin biofilm, since the goal was to determine the response of algal mats to flow velocity and nutrients.

The test organism in this study was the filamentous cyanophyte *Lyngbya wollei* which has received much attention globally due to its tendency to form prolific floating mats of high biomass and its potential to generate neurotoxins (Carmichael et al. 1997). *Lyngbya* often forms dense mats on the benthic surface of freshwater systems which may detach from the benthos and become floating mats due to buoyancy from gas bubbles. *Lyngbya* is shade adapted with a low light compensation point of 32  $\mu\text{mol}/\text{m}^2/\text{s}$  (Speziale et al. 1991) and has a thick sheath which may make it unpalatable to most algal grazers (Camacho and Thacker 2006). Although this study is focused on a single filamentous algal species, *Lyngbya wollei*, the general principles involved are presumed to apply to other mat-forming species.

*Lyngbya wollei* is one of the primary algal species currently proliferating in Florida springs (Stevenson et al. 2004) and may actually consist of two or more species based on molecular and morphological data (Joyner et al. 2008). Attempts to find a relationship between *Lyngbya* and nutrient (N and P) concentrations within Florida springs have been largely inconclusive (Cowell and Botts 1994; Stevenson et al. 2004) and most laboratory experiments which found a relationship were conducted in relatively static hydraulic conditions not representative of lotic systems (Cowell and Dawes 2004; Stevenson et al. 2007). One laboratory study using recirculating channels found some evidence that nitrate concentration was related to *Lyngbya* growth rates, although the threshold which would saturate *Lyngbya* growth was relatively low (< 110  $\mu\text{g N/L}$ ) in comparison to the levels currently found in most springs (Stevenson et al.

2007). The uncertainty associated with the nitrate relationship may be partially due to *Lyngbya* being a nonheterocystous cyanobacterium which has the ability to fix atmospheric nitrogen (N<sub>2</sub>) under certain conditions (Phlips et al. 1992). An alternative hypothesis tested in this study is that flow velocity is a significant determinant of *Lyngbya* metabolism and biomass.

This study tested the effects of flow velocity on clumps of the filamentous alga *Lyngbya wollei* at different nutrient concentrations. Algal response was assessed based on metabolic rates derived from diel changes in pH and DO, and changes in algal biomass over time. The first experiment examined algal response to flow velocity at high (1.00 mg N/L) and low (< 0.05 mg N/L) nitrate concentrations. It was hypothesized that algal metabolism and biomass would increase with flow velocity at low nitrate concentration; but would be relatively similar among velocities at the high nitrate concentration due to nutrient saturation. A second experiment involved a series of nutrient additions, beginning with amended deionized (DI) water, followed by P and micronutrient addition, and finally N addition. For this experiment it was expected that algal metabolism and biomass would increase with each nutrient addition. The combined results of both experiments were evaluated to determine how flow velocity influences the metabolism of filamentous algal clumps in a variety of nutrient conditions ranging from severe nutrient limitation to nutrient saturation.

## **Methods**

### **Stream Channel Design**

Three laboratory stream channels were constructed out of a 3 m section of 15 cm diameter PVC pipe with the top quarter cut out, creating a channel open to light and gas exchange (Figure 5-1). A 1.9 cm diameter PVC pipe was inserted perpendicularly

within the channel near the inlet and outlet in order to brace the pipe open, as the pipe would slightly constrict with the top cut out. PVC caps were fitted to the ends of each channel with a 3.2 cm hole cut near the top of the upstream cap for the inlet pipe and a 5 cm hole cut near the bottom of the downstream cap for the outlet pipe. Each channel was connected to separate 190 L plastic tanks from which the water was recirculated to the channel inlet using a pump.

Each pump moved water to the channel inlet through a short section of flexible plastic tubing attached to 3.2 cm diameter PVC pipe configured to enter through the hole in the upstream end cap. Near the inlet, 1.9 cm diameter PVC was fitted into the channel perpendicular to the flow direction in order to diffuse the inflowing water, thus generating more uniform flow through the channel. A 5 cm diameter PVC outlet pipe was fitted into the hole in the channel outlet cap and ran directly into the plastic water tank. The outflow rate and channel water level were controlled with a gate valve on the outlet pipe. This configuration resulted in three identical artificial stream channels with adjustable flow rates (as dictated by pump choice). The three pumps were rated at 6.3, 31.5, and 75.7 L/min and generated average flow velocities of 1, 5, and 10 cm/s, respectively, with a water depth of 10 cm.

Each channel contained a rack to hold the filamentous algal clumps in place, which was made using 2 m of the cut out top section of the PVC channel into which 1 cm diameter wooden dowels (made from poplar) were inserted perpendicular to the water flow. This created a substrate for the algae similar to macrophyte stems on which they were observed to grow in the spring-fed river where collected. Fine mesh was placed over the channel outlets in order to capture export of filamentous algae. Coarse

mesh (2 cm x 2 cm spacing) was placed just below the water surface to prevent the algae from floating to the surface, which ensured that gas exchange resulting from algal metabolism occurred in the water and not directly with the atmosphere.

### **Experimental Conditions**

The experiments were conducted within an air-conditioned greenhouse laboratory at the University of Florida in Gainesville. Photosynthetically active radiation (PAR) was maintained at  $< 700 \mu\text{mol}/\text{m}^2/\text{s}$  (with shade cloth if necessary), since *Lyngbya wollei* is light saturated at  $< 664 \mu\text{mol}/\text{m}^2/\text{s}$  (Speziale et al. 1991) and an unidentified small green alga occasionally became abundant when light levels were higher. PAR was measured using a LI-COR meter (LI-1400 model) with a PAR sensor and averaged  $7.42 \text{ mol}/\text{m}^2/\text{d}$  (SD:  $2.84 \text{ mol}/\text{m}^2/\text{d}$ ). Water temperature was recorded every 15 min with a YSI 556 MPS, and submersible heaters were used to help maintain the desired water temperature of  $24 \text{ }^\circ\text{C}$  ( $\pm 6 \text{ }^\circ\text{C}$ ). Temperature varied slightly among the channels ( $< 1 \text{ }^\circ\text{C}$ ) due to differential heat from the pumps. Flow velocity was measured in the middle of the water column within each channel downstream of the algae using a Marsh-McBirney, Inc., Flo-Mate (Model 2000) portable flow meter. The three flow rates were randomly assigned to each channel prior to each trial, and the velocity in the center of the channel averaged either 1 cm/s (low v), 5 cm/s (med v), or 10 cm/s (high v).

Two experiments were conducted which differed in their duration, water source, and nutrient conditions. Both experiments utilized the three laboratory stream channels which were identical except for the flow velocity (1, 5, and 10 cm/s). The first experiment tested two nitrate concentrations (1.0 and  $< 0.05 \text{ mg}/\text{L}$ ) using amended tap water, and consisted of five short (5-6 day) trial runs at each nitrate concentration. The second experiment tested the effect of sequential nutrient additions over a 19 day

period, beginning with amended deionized water without N and P, addition of P and micronutrients after six days, and addition of N after twelve days.

Prior to each trial, all channels and tanks were scrubbed clean, filled with tap water, dosed with 400 mL of bleach, and circulated for 48 hrs. For the first experiment, the water was then replaced with fresh tap water and circulated for 48 hrs in order for residual chlorine to diffuse out of the water and for gas equilibration with the atmosphere, as initially  $\text{CO}_2$  and  $\text{O}_2$  were undersaturated. Tap water was analyzed for cations and anions by ion chromatography (Dionex ICS-3000) (Table 5-1). Specific conductivity ranged from 100 to 400  $\mu\text{S}/\text{cm}$ . An in situ submersible ultraviolet nitrate analyzer (SUNA; Satlantic) was placed in the tank of the high flow channel for multiple trials and recorded nitrate concentration every 15 min. Tap water initially contained some nitrate (0.02-0.05 mg N/L) and  $\text{NaNO}_3$  was added to generate the higher  $\text{NO}_3$  concentration tested (1.0 mg N/L). In each trial, phosphorus was elevated to 1.0 mg P/L using  $\text{KH}_2\text{PO}_4$ , and this concentration was assumed to saturate biotic P demand. These were the initial N and P target concentrations and there was no effort to maintain concentrations due to the short duration of each trial.

The second experiment used deionized water instead of tap water and consisted of a series of nutrient additions. Initially, each tank was filled with 150 L of DI water and amended with 10 g  $\text{CaCl}_2$ , 1 g  $\text{MgSO}_4$ , and 8 g of  $\text{NaHCO}_3$  in order to raise conductivity, pH, and alkalinity to levels resembling the tap water. After six days, 10 mL of a micronutrient solution (Brightwell Aquatics FlorinMulti) was added to each tank and phosphorus was elevated to 1.0 mg P/L using  $\text{KH}_2\text{PO}_4$ . After six more days, nitrogen

was added for the first time using  $\text{NaNO}_3$  to generate 1.0 mg N/L. Prior to each nutrient addition, the tanks were refilled to 150 L with DI water to replenish evaporative loss.

### **Algal Collection and Processing**

Samples of fresh filamentous algal mats dominated by the cyanophyte *Lyngbya wollei* were collected in the vicinity of the main spring vent at the Gum Slough spring system in Sumter County, Florida, USA. The algae samples were placed in plastic containers and transported on ice back to the greenhouse. The algae were washed with deionized water and all macroscopic objects such as leaves and invertebrates were removed. The algae samples were then acclimated for at least two days in a tank which contained water with the same nutrient concentrations and chemistry as the intended trial. To initiate a trial, algae samples were spun dry and separated into 10 g wet weight ( $\pm 1$  g) clumps, and seven clumps were added to each channel (six clumps in the DI water experiment). At the end of each trial the algae was collected, spun dry, and weighed to determine the change in wet weight over the course of the trial.

### **Metabolism Measurements**

To measure the filamentous algal response to each combination of flow velocity and nutrients, diel pH changes were recorded and converted into  $\text{CO}_2$  changes using the equilibrium equation for total  $\text{CO}_2$  and alkalinity measurements. Downstream of the algae in each channel, pH was measured every minute using a pH meter (Artisan PH2000), equipped with a double junction pH probe (Oakton) and an automatic temperature compensation (ATC) probe. These pH meters were calibrated by three-point calibration prior to each trial, and when checked for drift at the end of each trial were always within 0.08 pH units. Carbonate alkalinity (CA; meq/L) was measured every 1-2 days in each channel by titrating a 100 mL sample with 0.2 N  $\text{H}_2\text{SO}_4$  until the

pH was 4.5. Initial carbonate alkalinity varied from 0.59 to 0.80 meq/L in tap water and was 0.5 meq/L in the amended DI water. Alkalinity increased linearly over the course of each trial, so a linear relationship was developed for each channel to account for changing alkalinity over time.

Hourly averages of the pH were converted to total CO<sub>2</sub> and [CO<sub>2</sub>] (i.e. free CO<sub>2</sub>) using the following equilibrium equations modified to include carbonate alkalinity:

$$Total\ CO_2 = CA \left[ \frac{\{H^+\}^2 + \{H^+\}K_1 + K_1K_2}{[\{H^+\} + 2K_2] * K_1} \right]$$

$$[CO_2] = CA \left[ \frac{\{H^+\}^2}{[\{H^+\} + 2K_2] * K_1} \right]$$

where  $\{H^+\}$  is the activity of the hydrogen ion derived from the pH, and  $K_1$  and  $K_2$  are equilibrium coefficients (from Stumm and Morgan 1996:  $pK_1 = 6.35$  at 25°C and  $pK_2 = 10.33$  at 25°C; note that these K values are given as pK).

The contribution of CO<sub>2</sub> reaeration (i.e. atmospheric gas exchange) to changes in total CO<sub>2</sub> was determined by setting the clean channels at the target velocities (1, 5, or 10 cm/s) without algae and measuring the change in pH in fresh tap water (initially undersaturated with CO<sub>2</sub>) for five trials. An additional trial was conducted for the amended deionized water prior to adding algae. The results of these trials were used to calculate an average CO<sub>2</sub> reaeration coefficient ( $k_{CO_2}$ ; m/hr) for each of the three target velocities. It was assumed that the change in total CO<sub>2</sub> was due only to CO<sub>2</sub> diffusion into and out of the water in each channel; therefore CO<sub>2</sub> reaeration rates ( $D_{CO_2}$ ; mmol/L/hr) were estimated by adjusting  $k_{CO_2}$  to best match the measured total CO<sub>2</sub> change.  $D_{CO_2}$  was estimated using the following model:

$$D_{CO_2} = \frac{A}{V} k_{CO_2} (K_H * p_{CO_2} - [CO_2])$$

where  $A$  is the channel surface area ( $m^2$ ),  $V$  is the total system volume ( $m^3$ ),  $K_H$  is the Henry's constant for  $CO_2$  (mmol/L/atm), and  $p_{CO_2}$  is the partial pressure of  $CO_2$  in the atmosphere ( $3.91E-4$  atm from NOAA/ESRL ([www.esrl.noaa.gov/gmd/ccgg/trends](http://www.esrl.noaa.gov/gmd/ccgg/trends))).

The effect of temperature variation on  $K_H$  was accounted for by using the van't Hoff equation:

$$K_H = K_{H,T\theta} * \exp\left(C\left(\frac{1}{T} - \frac{1}{T\theta}\right)\right)$$

where  $K_{H,T\theta}$  is the Henry's constant at standard conditions (34 mmol/L/atm),  $C$  is an enthalpy coefficient (2400 K),  $T$  is the water temperature (K), and  $T\theta$  is standard temperature (298 K). In order to match the observed reaeration rates, it was necessary to multiply the temperature adjusted  $K_H$  values by a constant amount ( $C_2$ ) depending on the channel velocity. It is currently unknown what caused the deviation in  $K_H$  from typical values, but this effect was consistent and may be related to conditions in the greenhouse or water chemistry.

For the trials with algae, the  $CO_2$  reaeration rate was calculated on an hourly basis and subtracted from the total  $CO_2$  change. This diffusion corrected total  $CO_2$  change was then used to calculate the rate of net ecosystem production per algal biomass (NEP; mg C/g biomass/hr). Algal biomass was assumed to change linearly over the course of each trial based on the initial and final weights. Daily NEP (mg C/g biomass/d) was determined by summing the hourly NEP for each day. Daily community respiration (CR) was estimated by averaging the hourly NEP values over the previous night, and assuming that this average hourly value applied throughout the day. Daily CR was then added to NEP to determine gross primary production (GPP) on a daily basis due to the filamentous algae.

Diel changes in dissolved oxygen (DO) were also used to estimate filamentous algal metabolism using a similar methodology to the pH changes just described. Data sondes equipped with optical DO probes (YSI 6600) were placed in the tank of each channel where they recorded DO concentration, DO saturation, specific conductivity, and temperature every 15 min. Oxygen reaeration was estimated for each velocity based on changes in DO saturation in the clean channels without algae. Metabolism results were calculated from diel changes in DO concentration corrected for reaeration, as described for pH.

### **Statistical Analysis**

Daily values of NEP, CR, and GPP were averaged for each trial. Analysis of variance (ANOVA) was used to test for the separate effects of nitrate concentration and flow velocity on the overall change in algal biomass and daily average NEP, CR, and GPP. For significant ANOVA models, post-hoc Tukey's honest significant difference (HSD) was used to examine the significance of differences between means of each level (e.g. velocity). All statistical analyses were conducted using the software package R version 2.11.1 (R Foundation for Statistical Computing).

## **Results**

### **CO<sub>2</sub> and DO Reaeration**

For the five reaeration trials in tap water without algae, initially the fresh water was undersaturated with CO<sub>2</sub> and therefore had an elevated pH that declined with time at a rate dependent on the channel velocity. The time required to reach CO<sub>2</sub> equilibrium was approximately 1 day at 10 cm/s, 2 days at 5 cm/s, and 4 days at 1 cm/s (Figure 5-2). After reaching CO<sub>2</sub> equilibrium, total CO<sub>2</sub> varied slightly due to temperature variation, and the entire trial period was used to calculate the  $k_{CO_2}$  and  $C_2$  coefficients for each

velocity for each of the five trials in tap water (Figure 5-3; Table 5-2). For the experiment in deionized water, the same  $k_{CO_2}$  values were used, but  $C_2$  was adjusted to 1.80 for all three velocities. Dissolved oxygen was also undersaturated initially in the tap water, but equilibrated much faster than  $CO_2$  due to reaeration rates which were approximately two orders of magnitude greater. Due to the large influence of reaeration on diel DO changes, DO was deemed unsuitable for algal metabolism calculations using these laboratory stream channels.

### **Algal Biomass and Metabolism**

For the experiments with filamentous algae, diel pH changes were dictated by a combination of  $CO_2$  reaeration and biotic metabolism associated with the filamentous algal clumps (Figure 5-4). In the trials of the first experiment pH ranged from 7.6 to 9.0 (usually not exceeding 8.6), but reached 9.4 towards the end of the longer experiment. In the low velocity channel, pH routinely spiked which appeared to be related to algal metabolism rather than poor mixing or measurement error. Generally, the amplitude of the diel pH changes was inversely related to flow velocity, which illustrated that flow velocity increased  $CO_2$  reaeration and kept the higher velocity channel closer to  $CO_2$  equilibrium with the atmosphere. Water in the high velocity channel routinely reached  $CO_2$  equilibrium with the atmosphere at night whereas the two channels with lower velocity did not completely equilibrate during each trial. Despite the apparent influence of  $CO_2$  reaeration on pH changes, changes in total  $CO_2$  were mostly due to biotic production and respiration rather than reaeration (Figure 5-5).

In the first experiment, biomass of the filamentous algal clumps declined in almost all of the trials and was not significantly affected by either nitrate concentration or flow velocity; although the largest average decrease was at high velocity (Table 5-3). Algal

export was found to be minimal over the course of a trial (< 2 g wet weight), but slightly increased with flow velocity. Net ecosystem production was negative for most trials and was significantly lower with high nitrate concentration ( $p = 0.0008$ ), but not strongly affected by flow velocity. Both flow velocity and nitrate concentration affected CR and GPP of the filamentous algal clumps (Figure 5-6). At low nitrate concentration, CR and GPP were much higher at a flow velocity of 5 cm/s than 1 cm/s, whereas at 10 cm/s both were intermediate and highly variable. At high nitrate concentration, CR and GPP were similar at 1 and 5 cm/s, but much lower at 10 cm/s. The only significant flow velocity relationship was for GPP at high nitrate concentration ( $p = 0.028$ ), which confirmed that at 10 cm/s GPP was lower than at 1 and 5 cm/s. Nitrate concentration declined substantially over the duration of the high nitrate trials and leveled at 0.5 mg/L; in contrast nitrate concentration was relatively stable in the low nitrate trials (Figure 5-7).

In the second experiment, the filamentous algal clumps gained substantial biomass over the 19 day duration although the timing of growth was highly variable between the three flow velocities (Table 5-4). Algal biomass in the low velocity channel increased from 60 to 80 g wet weight over the first six days without any N, P, or micronutrient addition, but then declined slightly once these nutrients were added. Algal biomass in the medium velocity channel increased by 11.7 g over the first six days, and then remained steady until growing another 11.1 g once N was added. Algal biomass in the high velocity channel was steady except for an increase of 11.8 g during the six day period after P was added.

Overall, metabolism of the filamentous algal clumps increased with time and nutrient additions as shown by the amplitude of the diel pH and NEP changes over the

19 day experiment (Figures 5-8 and 5-9). Net ecosystem production was uniformly negative over the first six days in the amended DI water, despite the steady or increasing amounts of algal biomass over the same time period. Once nutrients were added then NEP increased in each channel, and was positive after N addition. In all channels, CR and GPP increased with nutrient additions over the course of the experiment, except for CR in the medium velocity channel which oscillated around an average value of 1.72 mg C/g biomass/d. CR and GPP were highest in the medium velocity channel initially, but by the end of the experiment the low and high velocity channels were greater. On day 16 of this experiment, pH and NEP increased substantially which coincided with the end of a storm system that lowered the light levels for the first few days after N addition. Nitrate addition also stimulated the metabolism of organisms not associated with the filamentous algal clumps as the water in all channels became visibly green and cloudy, and a biofilm was apparent on most surfaces exposed to light.

## **Discussion**

### **Algal Metabolism Response to Flow Velocity and Nutrients**

The two experiments in this study indicate that flow velocity and nutrient concentration influence the metabolism of filamentous algal mats dominated by *Lyngbya wollei*. In the first experiment, higher flow velocity appeared to stimulate algal metabolism in the low nitrate concentration trials, whereas at high nitrate concentration the higher velocity had an inhibitory effect. The positive effect of flow velocity on algal metabolism at low nitrate concentration only, suggests that velocity increased N availability and therefore decreased N limitation. This effect has been observed in other similar studies (Whitford and Schumacher 1961, 1964; Lock and John 1979; Horner et

al. 1990) and indicates that flow velocity can be an important driver of algal metabolism, particularly when nutrient availability is limited. Additionally, the lack of a positive effect of flow velocity at high nitrate concentration suggests that the main effect of flow velocity was to increase nutrient availability rather than waste efflux (e.g. Mass et al. 2010).

The decrease in filamentous algal metabolism at high flow velocity in the high nitrate concentration trials is more difficult to explain, particularly since a similar effect was not observed in the deionized water experiment. With regard to nutrient availability, N saturation was expected due to the high initial nitrate concentration (1 mg N/L). If N is saturating then the stimulatory effect of flow velocity on nutrient availability would not be observed, and algal metabolism should remain relatively constant across this range of flow velocities. Nitrogen saturation was likely based on the similar magnitude of CR and GPP at low and medium flow velocity at high nitrate concentration, which were also comparable to CR and GPP at high and medium flow velocity at low nitrate concentration. One possibility is that the high velocity (10 cm/s) negatively affected algal metabolism as occurred in a similar study (Borchardt et al. 1994); although the specific mechanism is unknown. This unknown mechanism may also explain why at low nitrate concentration algal metabolism at high flow velocity was highly variable and slightly lower than at medium velocity.

An alternative explanation for the decrease in algal metabolism at high flow velocity and high nitrate concentration is that another organism interfered with the filamentous algae in these conditions. Two observations suggest that a species of bacteria with high N demand thrived in the high nitrate concentration trials. First, nitrate concentrations declined asymptotically to 0.5 mg N/L and this could not be accounted

for by algal uptake alone since there was no net accrual of algal biomass and nitrate declined regardless of light availability (i.e. at night). Second, CR was higher and NEP was much more negative in the high nitrate trials, even though GPP was relatively similar for both nitrate concentrations. Denitrification is suspected to be the mechanism of nitrate loss, which may seem unlikely in these well-oxygenated stream channels but has been shown to occur within filamentous algal mats if nitrate concentration is sufficiently high (Triska and Oremland 1981; Duff et al. 1984). It is possible that denitrifying bacteria negatively affected filamentous algal metabolism in the higher flow velocity when nitrate concentration was elevated above 0.5 mg N/L.

### **Algal Biomass Response to Flow Velocity and Nutrients**

In this study, the effect of flow velocity and nutrients on filamentous algal biomass is unclear. In the first experiment algal biomass declined in almost all trials, largely independent of flow velocity or nitrate concentration. However, in the second experiment algal biomass increased in all velocities, and the influence of nutrient additions was highly unpredictable. The largest increase in algal biomass (20 g wet weight) occurred over the first six days at low flow velocity in the amended deionized water without any addition of N, P, or the micronutrient solution. Overall, there was no clear trend in biomass changes related to nutrient additions, even though the additions did seem to consistently stimulate algal metabolism.

The decrease in algal biomass in the first experiment has many possible explanations. Algal export from the channel could only account for a small fraction of this decline, which indicates that most of this loss was due to mortality or consumption. Small invertebrates were observed in the algal clumps, and consisted mostly of amphipods which are able to consume *Lyngbya* though they do not prefer it (Camacho

and Thacker 2006). The influence of these invertebrates on algal consumption is unknown but expected to be minimal as most were removed prior to each trial. Both export and consumption are likely minor sources of biomass loss, since these would have occurred in the deionized water experiment as well.

A likely explanation for the loss of algal biomass in the first experiment is that the chemistry of the tap water was unsuitable, which caused the algae to slowly decay. There are two possible sources of negative water chemistry effects: the presence of a toxin such as chlorine or copper, or the chemistry was simply not appropriate for *Lyngbya*. Tap water is typically chlorinated, but no chlorine was detected in the fresh tap water and the water was aerated for 48 hours prior to adding algae. Overall, the presence of a toxin seems unlikely since algal production was measurable, and the algae persisted in other tanks with tap water for over a year.

The chemistry of the tap water was comparable to that of most Florida springs and originated from the same source (the Floridan aquifer), but still may not have been ideal for *Lyngbya*. Since the algae did accrue biomass in the deionized water experiment, there was apparently a key difference in water chemistry. Although the deionized water was amended to resemble the tap water, the calcium concentration was lower (16 mg/L versus 30 mg/L) and bicarbonate was added directly. Calcium can affect *Lyngbya* growth over this range of concentrations, but the effect may depend on poorly understood interactions with N and P (Cowell and Botts 1994). *Lyngbya* is known to prefer bicarbonate as a carbon source (Beer et al. 1992); however it was expected that bicarbonate was the dominant form of carbonate in the tap water as well since bicarbonate dominates in the pH range of 6.3 to 10.3. There are likely other relevant

differences in water chemistry such as iron (Ahern et al. 2006), but without further experimentation it is difficult to specify the reason why algal biomass increased in deionized water and not tap water.

### **Determining Nutrient Response of Attached Algae**

A common goal of many laboratory experiments with algae is to determine algal response over a range of nutrient concentrations, which can help identify nutrient criteria that inhibit algal proliferation. The results of this study do not allow a specification of the range of N concentrations that stimulate *Lyngbya wollei*. However, there is indication that N demand is saturated at relatively low concentrations as shown by the similar production rates in the low and high nitrate concentrations tested. Additionally, while there was less algal production in low velocity and low nitrate concentration, a modest velocity increase to 5 cm/s appeared to generate the maximum production rate. Other studies which have examined the relationship between *Lyngbya* and nitrate concentration did not explicitly account for the influence of flow velocity, which may explain why their results differed. One *Lyngbya* experiment used small flasks in which the water was continuously replenished but with little flow velocity, and found that growth rates were stimulated when nitrate increased from 0.3 to 0.6 mg N/L (Cowell and Dawes 2004). In contrast, a study that used semi-recirculating channels with a velocity of 25 cm/s found that *Lyngbya* growth rates were N saturated at a much lower nitrate concentration of 0.11 mg N/L (Stevenson et al. 2007). A likely explanation for the different saturating nitrate concentrations is that the higher velocity increased nutrient availability, and therefore lower nitrate concentrations were required to saturate N demand and inhibit N limitation. The substantial effect of flow velocity on algae in general (Biggs 1996; Stevenson 1996) implies that experiments meant to assess the

range of nutrient concentrations that stimulate a particular algal species or community in a lotic environment must consider hydraulic conditions.

In conclusion, flow velocity and nutrient concentration interact to determine nutrient availability to the filamentous cyanophyte *Lyngbya wollei*, but flow velocity may also negatively affect this species in other ways. Since flow velocity can increase nutrient availability and decrease nutrient limitation for filamentous algae, flow velocity should be considered in conjunction with the nutrient concentration when assessing the potential for algal nutrient limitation. Laboratory stream channel experiments may offer a controlled approach to determining nutrient limitation thresholds for lotic algae by examining the species-specific relationship with flow velocity in conjunction with nutrient concentrations, as different species have been shown to have wide variation in responses. The degree to which flow affects nutrient availability deserves further attention, since flow may confound the use of nutrient concentration alone as a predictor of nutrient limitation in lotic systems.

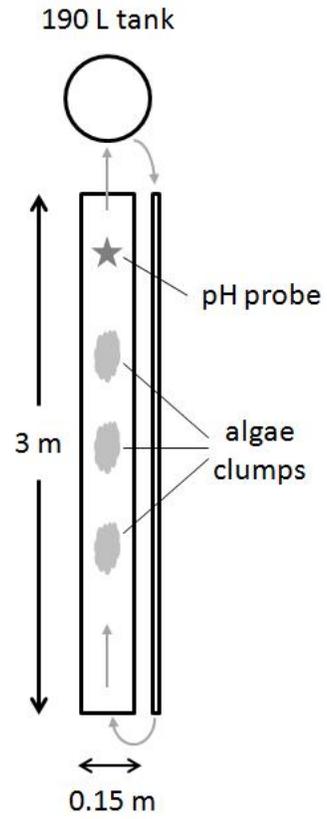
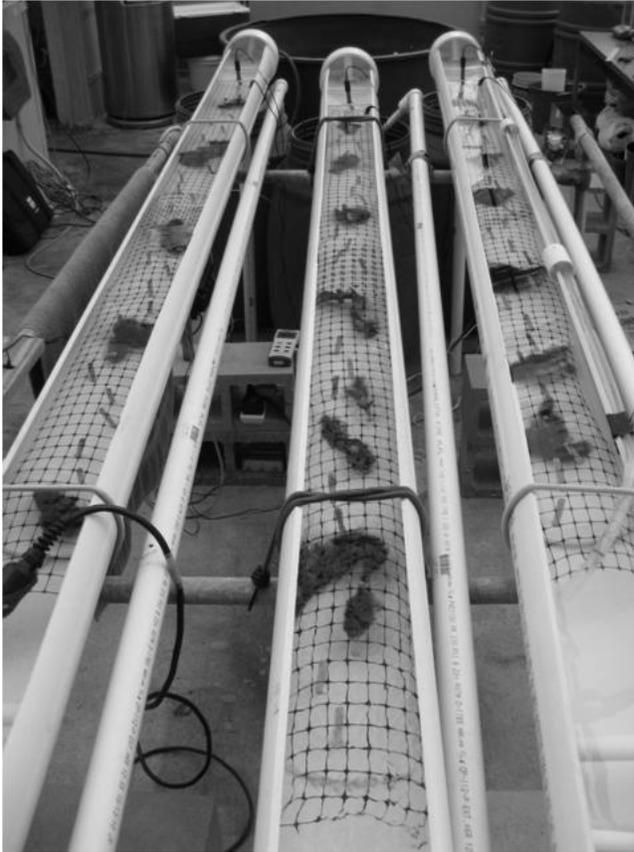


Figure 5-1. Diagram of the experimental laboratory stream channels.

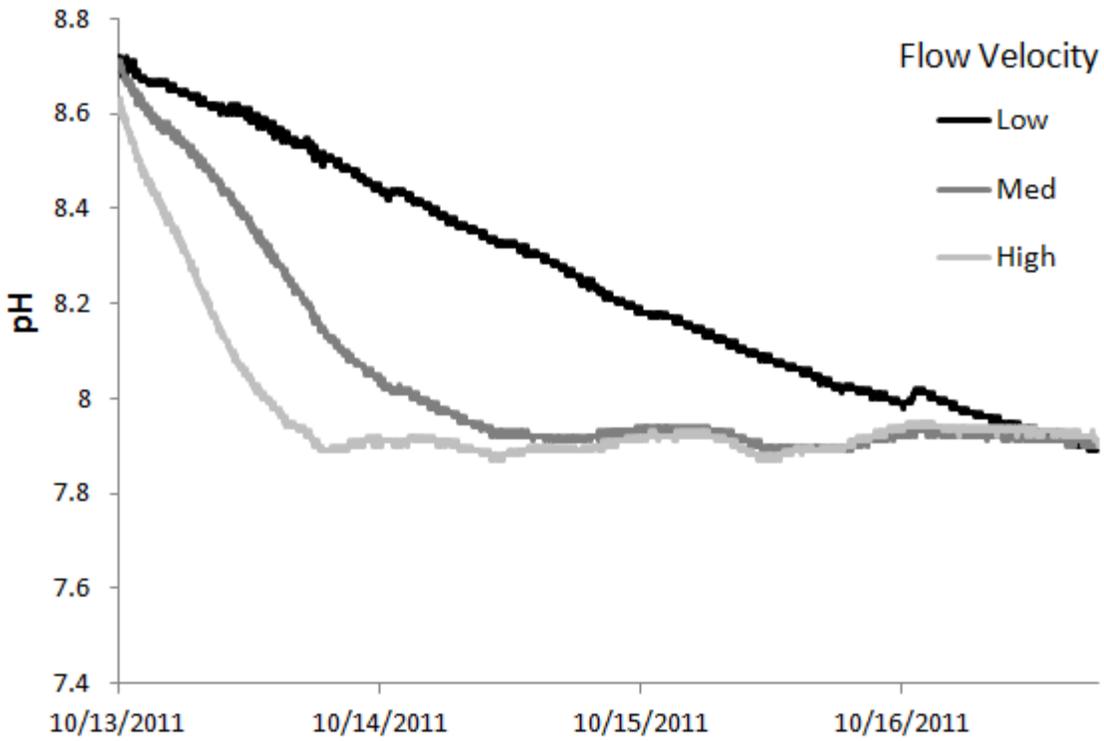


Figure 5-2. Initial pH change in fresh tap water at the three flow velocities due to CO<sub>2</sub> reaeration.

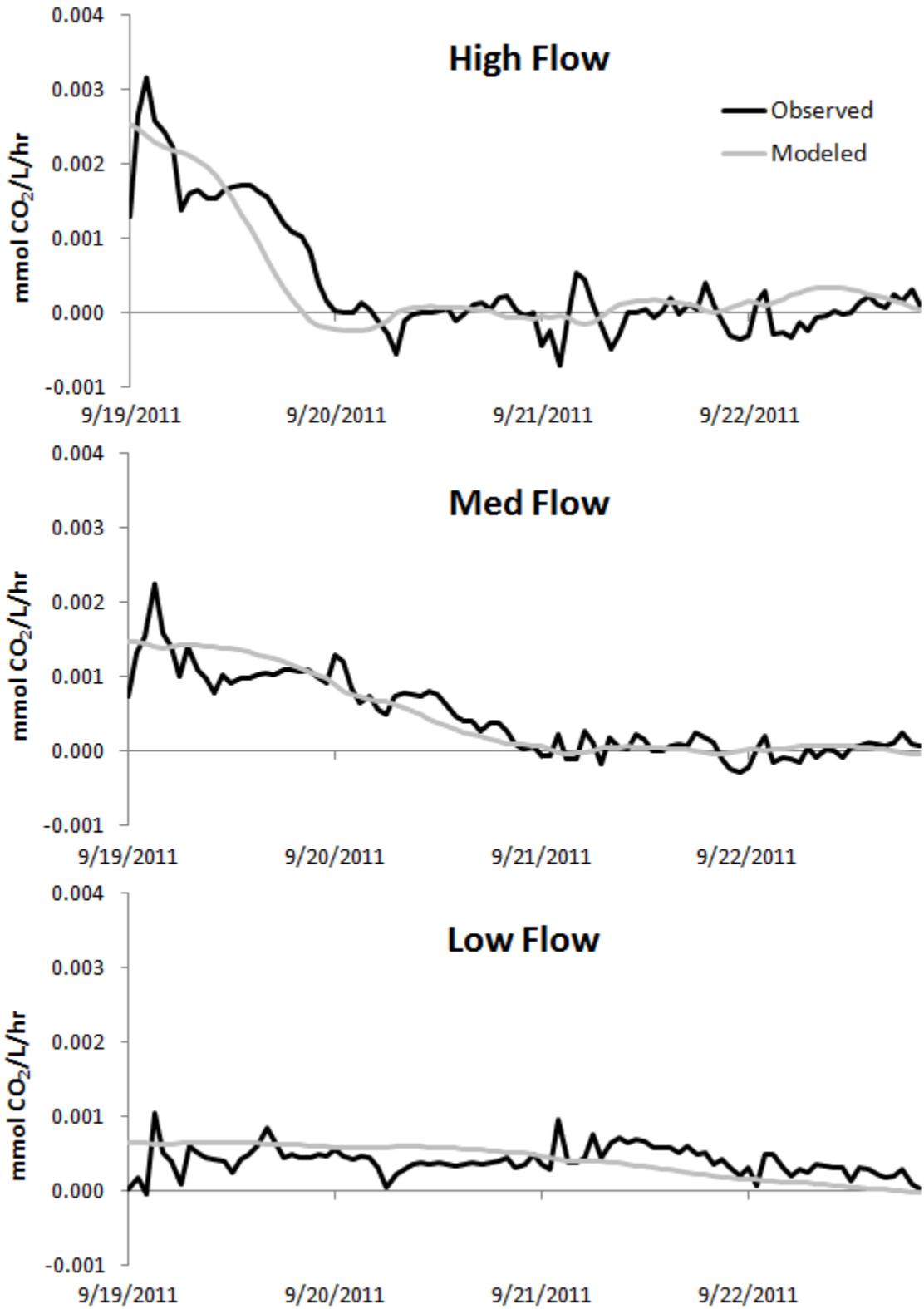


Figure 5-3. CO<sub>2</sub> reaeration rate estimation based on total CO<sub>2</sub> change in fresh tap water from 9/19/11 to 9/22/11.

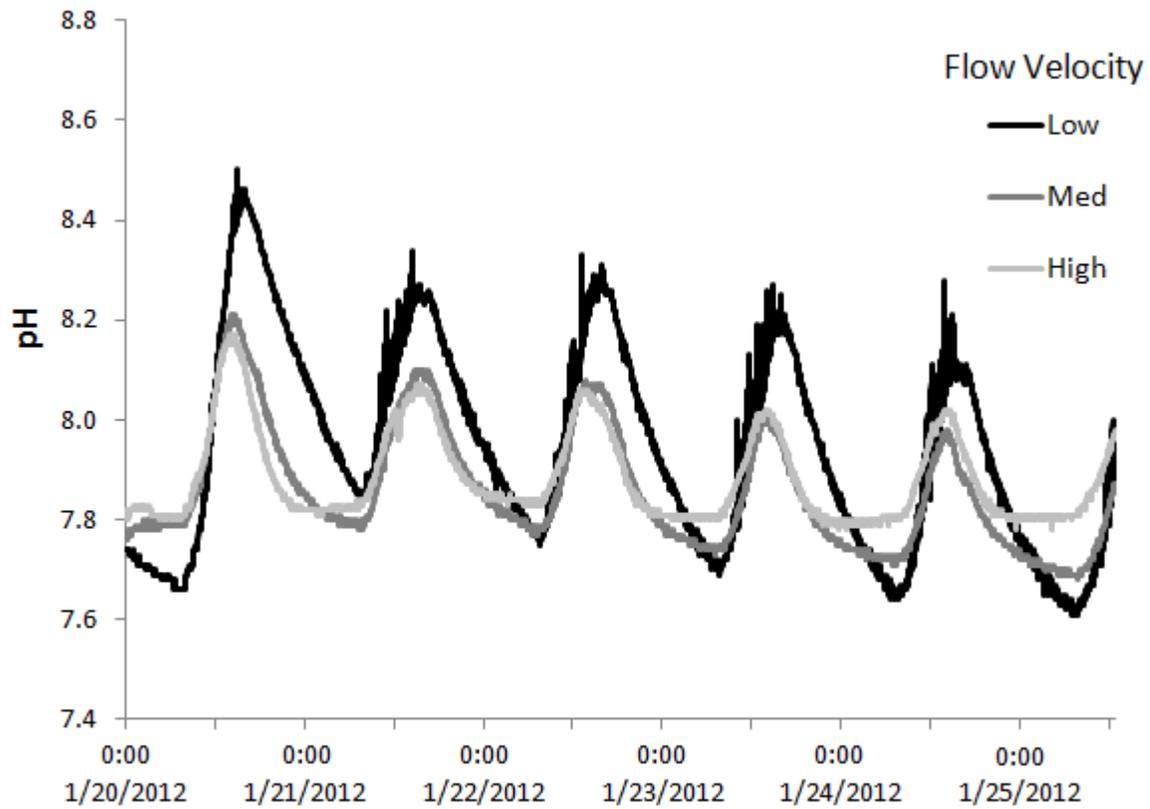


Figure 5-4. Example of diel pH changes for each velocity from the trial beginning on 1/19/12.

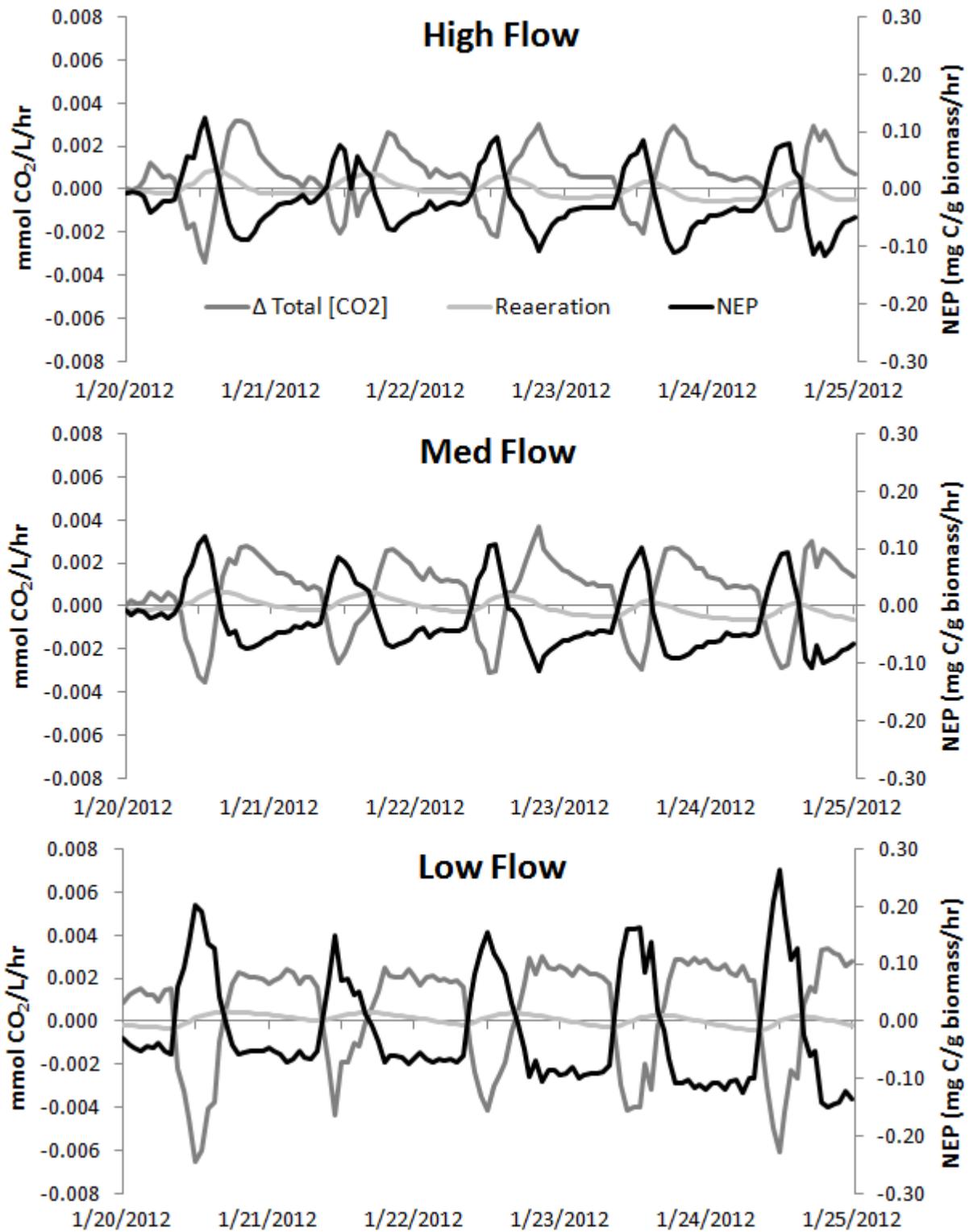


Figure 5-5. Example of  $\Delta \text{Total [CO}_2\text{]}$ , reaeration rate, and NEP for each velocity from a high nitrate trial.

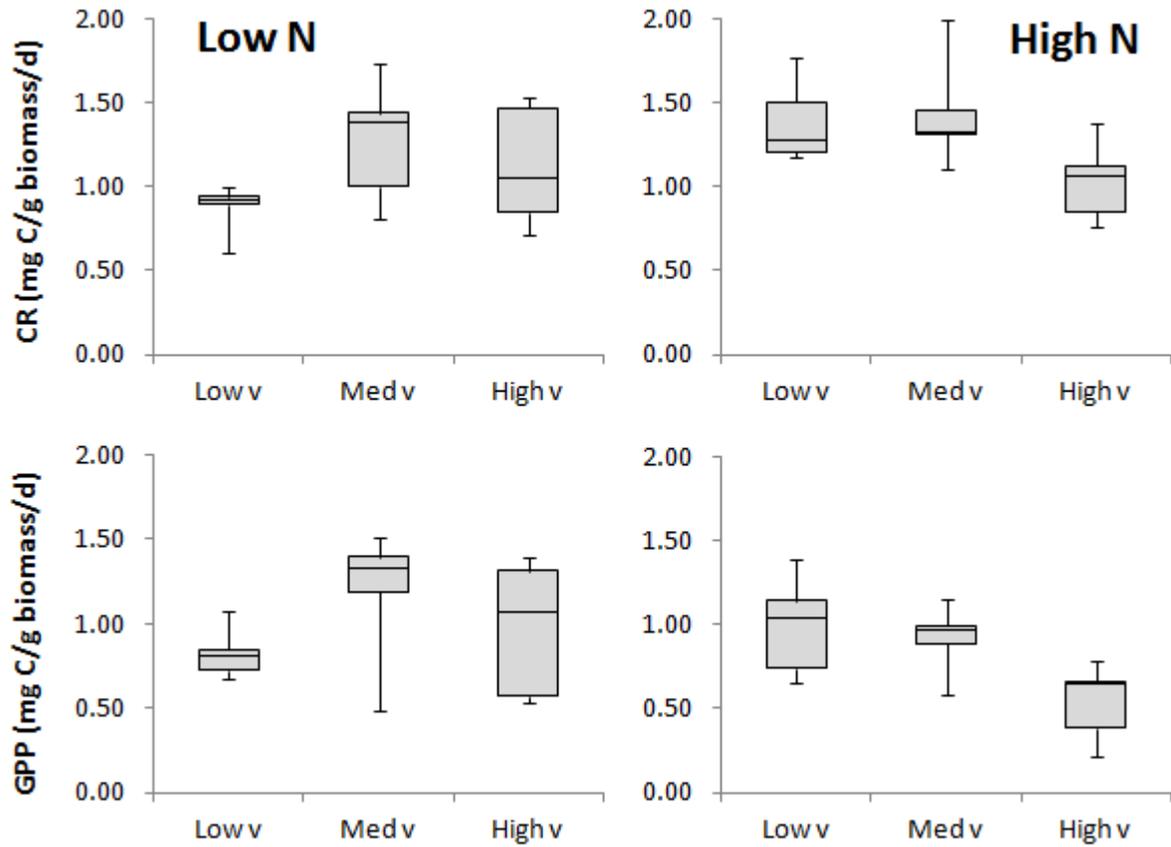


Figure 5-6. Boxplot comparison of CR and GPP results from the experiment testing flow velocity effects at low and high nitrate concentration.

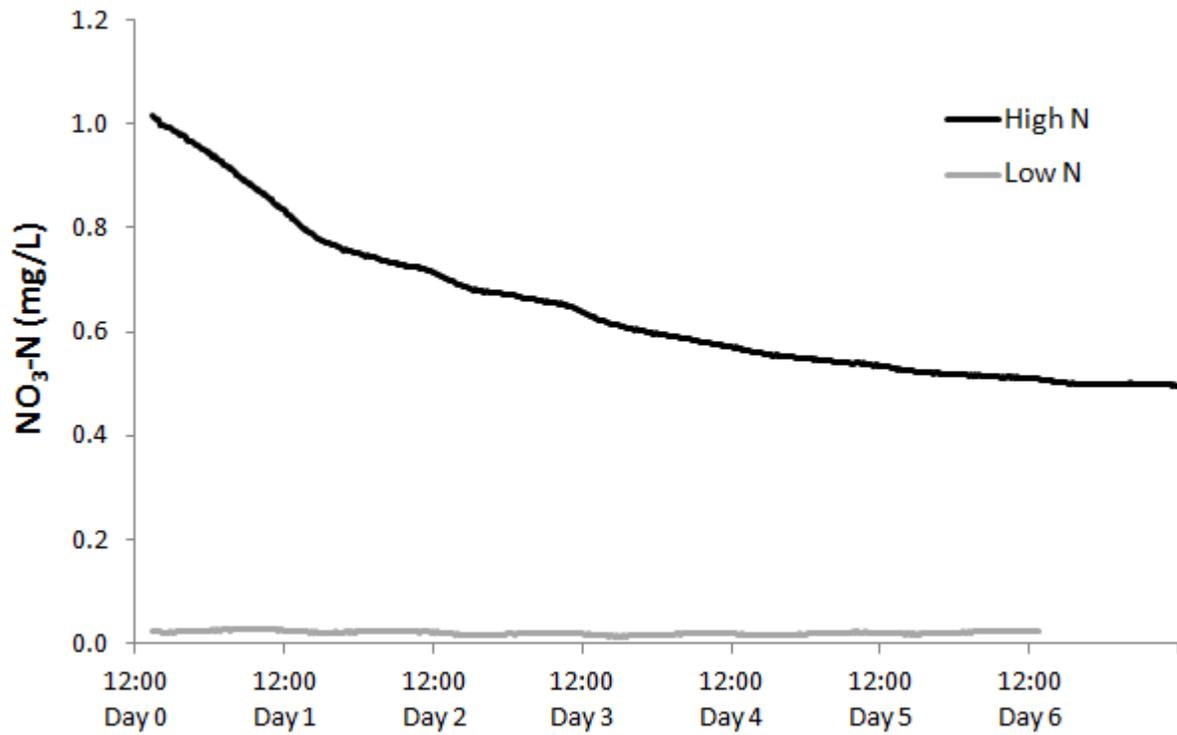


Figure 5-7. Nitrate concentration change over time at high flow velocity for both low and high initial nitrate concentration.

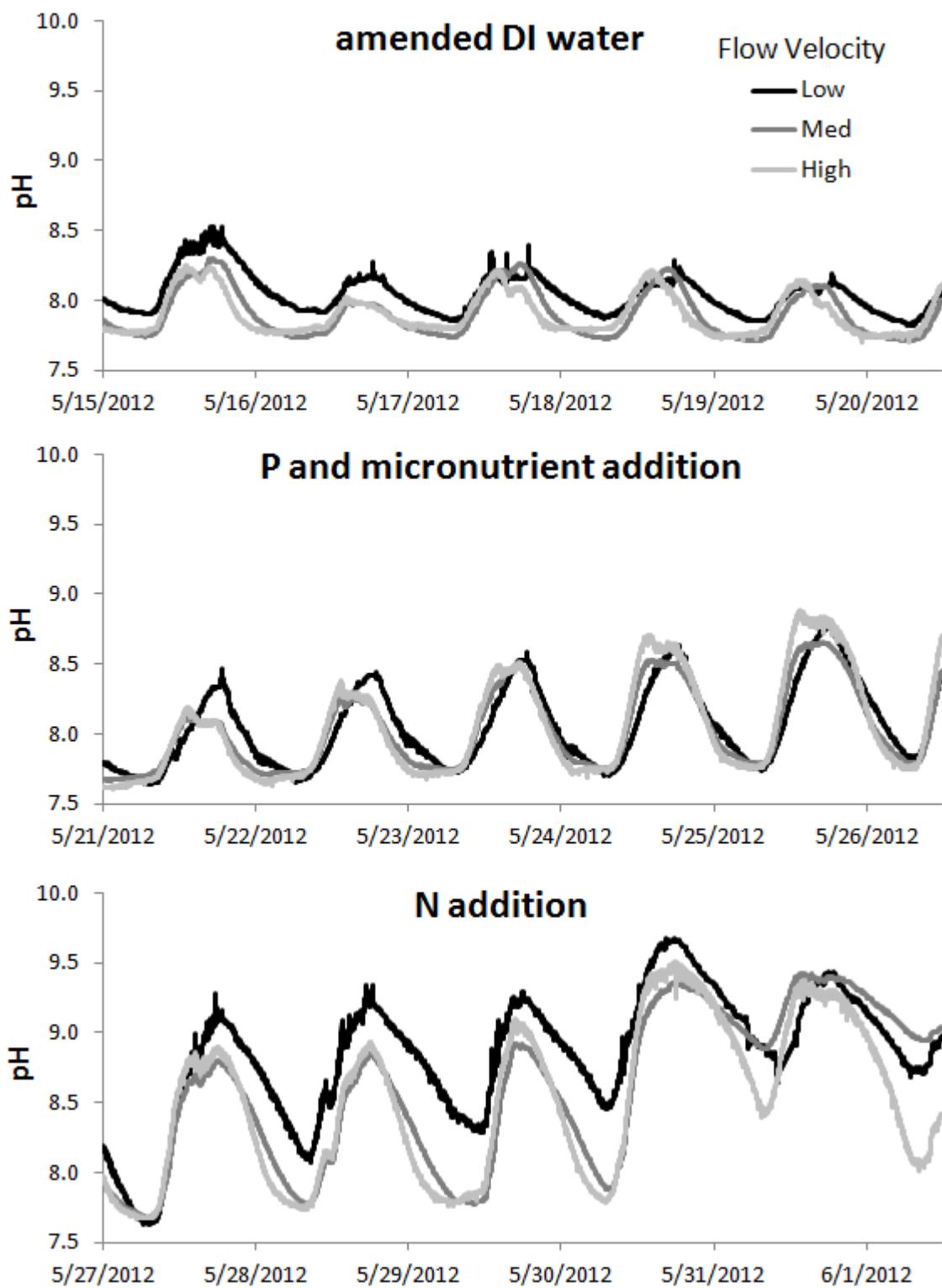


Figure 5-8. Diel pH changes in the 19 day experiment with sequential nutrient additions.

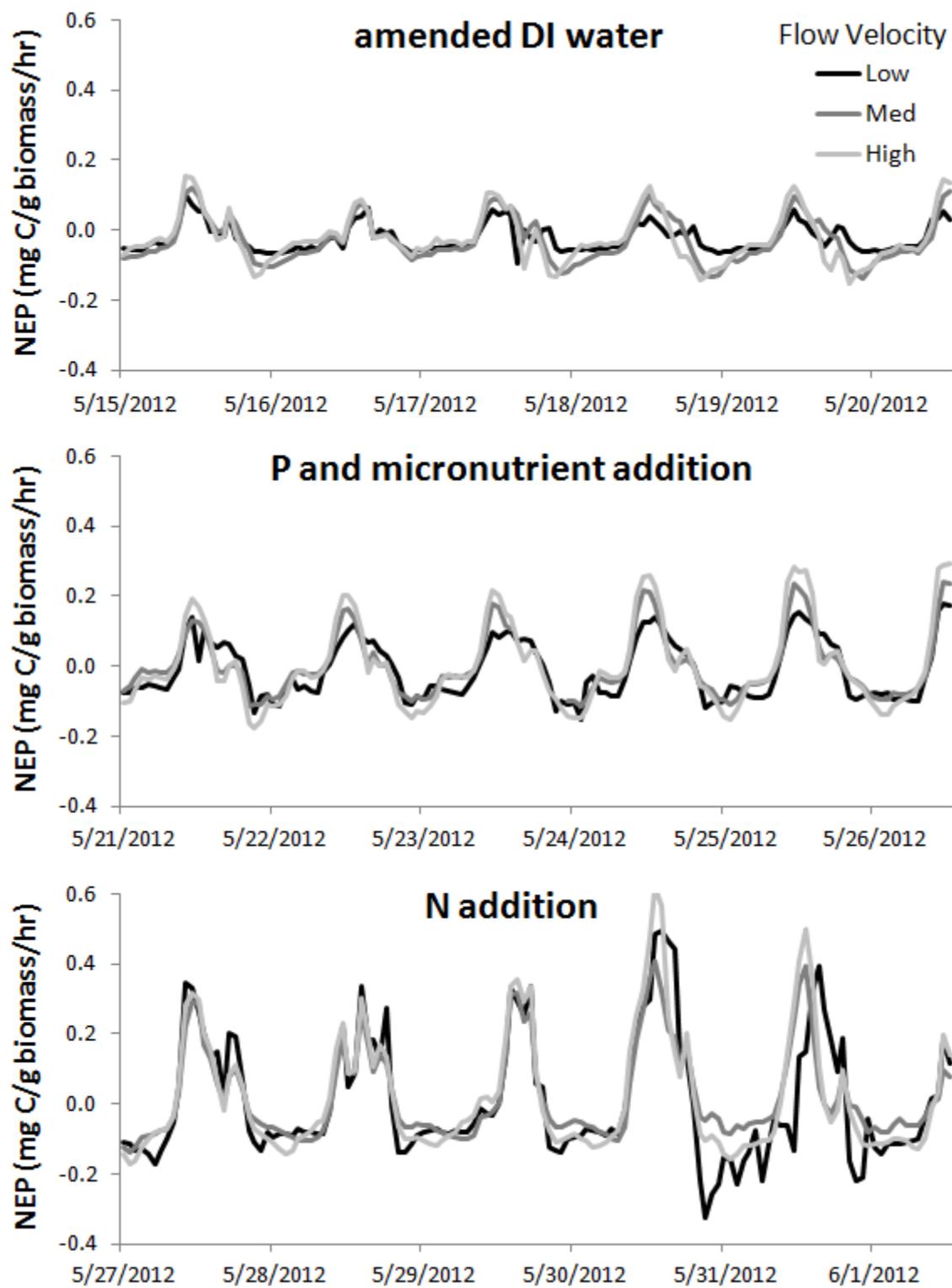


Figure 5-9. Diel NEP changes in the 19 day experiment with sequential nutrient additions.

Table 5-1. Chemistry of fresh tap water prior to nutrient addition. BDL – below detection limit.

Cations	mg/L	Anions	mg/L
Ca	30.37	Br	BDL
K	1.29	Cl	23.3
Li	BDL	I	BDL
Mg	18.28	SO <sub>4</sub>	62.6
Na	10.79		
NH <sub>4</sub>	BDL		

Table 5-2. CO<sub>2</sub> reaeration coefficients estimated from the five trials without algae.

	<i>k</i> <sub>CO<sub>2</sub></sub> (m/hr)			C <sub>2</sub>		
	Low v	Med v	High v	Low v	Med v	High v
Average	1.15E-02	2.53E-02	3.76E-02	1.55	1.56	1.50
Max	1.40E-02	4.20E-02	4.71E-02	1.77	1.85	1.67
Min	6.72E-03	1.52E-02	2.81E-02	1.30	1.35	1.29

Table 5-3. Algal biomass and metabolism results from the experimental trials comparing the effect of flow velocity at high and low nitrate concentration.

Trial	Δ Biomass (g - wet wt.)			NEP avg (mg C/g biomass/d)			CR avg (mg C/g biomass/d)			GPP avg (mg C/g biomass/d)		
	Low v	Med v	High v	Low v	Med v	High v	Low v	Med v	High v	Low v	Med v	High v
High N1	-0.2	-18.1	-14.3	-0.14	-0.16	-0.12	1.18	1.31	0.76	1.05	1.16	0.65
High N2	-5.5	6.0	-11.1	-0.54	-1.03	-0.90	1.28	2.00	1.06	0.74	0.97	0.22
High N3	-4.7	0.0	-4.0	-0.56	-0.44	-0.59	1.21	1.33	1.38	0.65	0.89	0.78
High N4	-28.9	-12.4	-21.9	-0.38	-0.52	-0.47	1.77	1.11	0.86	1.39	0.59	0.38
High N5	-16.9	-18.5	-19.0	-0.36	-0.46	-0.48	1.51	1.46	1.13	1.15	1.00	0.65
Average	-11.2	-8.6	-14.1	-0.40	-0.52	-0.51	1.39	1.44	1.04	1.00	0.92	0.54
SD	11.6	11.0	7.0	0.17	0.32	0.28	0.25	0.34	0.24	0.30	0.21	0.23
Low N1	-5.7	-14.9	-9.0	-0.19	-0.33	-0.52	0.92	0.81	1.05	0.72	0.48	0.53
Low N2	-10.2	-17.7	-22.4	-0.19	-0.25	-0.28	0.99	1.44	0.85	0.81	1.19	0.57
Low N3	-15.4	-12.0	-14.0	-0.05	-0.23	0.68	0.89	1.74	0.71	0.85	1.51	1.39
Low N4	-22.9	-19.7	-20.6	0.13	0.33	-0.23	0.94	1.00	1.54	1.07	1.33	1.31
Low N5	-8.3	-5.7	-9.9	0.08	0.02	-0.39	0.60	1.38	1.47	0.68	1.40	1.07
Average	-12.5	-14.0	-15.2	-0.04	-0.09	-0.15	0.87	1.27	1.12	0.83	1.18	0.97
SD	6.8	5.5	6.1	0.15	0.27	0.48	0.15	0.37	0.37	0.15	0.41	0.41

Table 5-4. Algal biomass and metabolism results from the 19 day experiment with a series of nutrient additions.

Days	Nutrients	Δ Biomass (g - wet wt.)			NEP avg (mg C/g biomass/d)			CR avg (mg C/g biomass/d)			GPP avg (mg C/g biomass/d)		
		Low v	Med v	High v	Low v	Med v	High v	Low v	Med v	High v	Low v	Med v	High v
0-6	----	20.0	11.7	0.6	-0.57	-0.73	-0.60	1.31	1.92	1.46	0.74	1.19	0.86
6-12	P, micro	-5.9	-0.3	11.8	-0.21	0.06	0.08	1.97	1.46	1.94	1.75	1.52	2.01
12-19	N, P, micro	-3.8	11.1	-0.6	0.33	0.64	0.69	2.85	1.77	2.49	3.18	2.41	3.19

CHAPTER 6  
A SIMULATION MODEL FOR FLOW AND NUTRIENT EFFECTS ON FILAMENTOUS  
ALGAE AND BACTERIA IN LABORATORY STREAM CHANNELS

**Introduction**

In lotic systems, two of the primary factors which control attached benthic algae are nutrients and flow velocity (Borchardt 1996; Stevenson 1996). Algal production (i.e. growth) may be stimulated by increased nutrient concentrations or flow velocity due to greater nutrient availability, until nutrient demand is saturated and further increases no longer have a stimulatory effect. Flow velocity can also negatively affect algal colonization and biomass accrual due to drag, and therefore an intermediate flow velocity is usually optimal which varies by algal species (Biggs 1996).

When managing aquatic systems, a common goal is to minimize nutrient enrichment so that algae are not overly stimulated and the formation of large colonies (i.e. blooms or mats) is inhibited. This approach often relies on a target nutrient concentration that if not exceeded will prevent the overstimulation of algae and maintain the integrity of the aquatic ecosystem. This approach has proven useful when managing algae in lakes and slow moving rivers in which free-floating phytoplankton is the dominant form of algae; however, it has been more difficult to specify a relationship between nutrient concentration and attached benthic algae found in most streams and rivers (Dodds et al. 2002b). This is partially due to the effect of flow velocity on both nutrient availability and drag, and therefore it is necessary to consider nutrient-algae relationships in the context of flow conditions.

The simulation model described here specifies the effects of nitrate concentration and flow velocity on the filamentous cyanophyte *Lyngbya wollei*. The relationships simulated were derived from an experiment using laboratory stream channels in which

nitrate concentration and flow velocity were manipulated to test the response of algal metabolism and biomass (see Chapter 5). The simulation model was used to help explain the experimental results by examining multiple hypotheses regarding the mechanistic relationships between the variables measured.

### **Methods**

The simulation model was designed to determine the potential mechanisms that could lead to the observed changes in algal biomass and metabolism in a laboratory stream channel experiment. Metabolism was measured as gross primary production (GPP), community respiration (CR), and their difference, net ecosystem production (NEP). The experiment consisted of six scenarios: two nitrate concentrations (0.05 and 1.0 mg N/L) at three flow velocities (1, 5, and 10 cm/s). The overall performance of the model was judged on its ability to recreate five observed results for each scenario: overall change in algal biomass and nitrate concentration, and average daily GPP, CR, and NEP.

After multiple rounds of testing different models, only one model was able to adequately approximate the experimental results (Figure 6-1). The model explicitly involves two organisms, filamentous algae and bacteria, whose nitrate uptake rates follow a Michaelis-Menton saturation function. During the simulation, a portion of the algae biomass senesces and goes to a detritus storage from which remineralized nitrate flows back into the nitrate storage, completing a closed cycle. At high nitrate concentrations ( $> 0.5$  mg N/L), the bacteria becomes active and converts nitrate into nitrogen gas through the process of denitrification, which causes removal of nitrate from the system. A unique aspect of this model is that nitrate availability to the algae

increases with flow velocity, but flow velocity also negatively affects algal production, and therefore peak algal production occurs at an intermediate velocity.

The simulation model was created in the energy circuit language (Odum 1994), which can be used for any systems containing storages of material (or energy) and the flows between them. The model consists of sets of differential equations that represent changes in the quantity of stored material over time (Tables 6-1 and 6-2). The model was run with a one minute time step for five days, and was calibrated by adjusting coefficients in the model to match the results of a laboratory stream channel experiment (Table 6-2). The estimated turnover time of each storage was used to constrain the coefficients and aid calibration, by reducing the number of unknown variables. Light (based on photosynthetically active radiation (PAR)) was modeled with a sin function that initiated at 7am, peaked at  $270 \mu\text{mol}/\text{m}^2/\text{s}$  at 1pm, and ended at 7pm. Metabolism values were summed up for each day of the simulation model and divided by the average algal biomass for that day. This resulted in five daily average metabolism values on a biomass basis ( $\text{mg C}/\text{g biomass}/\text{d}$ ), which were then averaged to yield average daily metabolism for each scenario, comparable to the observed experimental values.

Initially, the model was run at steady state to ensure that it was parameterized correctly (i.e. steady state was achieved). The steady state simulation was setup for the base conditions of low flow velocity (1 cm/s) and low nitrate concentration (0.05 mg N/L). Other initial conditions were set based on measured experimental conditions and estimated turnover times of each storage (Table 6-1). To use the steady state approach when a storage had multiple inflows or outflows, the flows were partitioned. This was

necessary for the two outflows from nitrate to algae and bacteria. At low nitrate concentration, only the algae was involved in nitrate uptake, whereas at high nitrate concentration (1.0 mg N/L) uptake was split evenly between the algae and bacteria. After checking for steady state, it was necessary to increase the mortality rate of the algae to match the observed results since the actual system was not in steady state initially.

## **Results**

The simulation model was able to approximate the results observed in the laboratory stream channel experiment for the six combinations of nitrate concentration and flow velocity tested (Table 6-3). Only for a small range of nitrate uptake and velocity coefficients was the model able to reproduce the peak in algal production observed at low nitrate concentration and 5 cm/s flow velocity (Figure 6-2). For the modeled experimental conditions, the highest algal production occurred at 3 cm/s flow velocity when nitrate concentration was 0.05 mg/L, but when nitrate was readily available at 1.00 mg/L velocity only had a negative effect. Similarly, algae responded to increased nitrate concentration at low velocity, but at higher velocity nitrate uptake was constant with a lower saturation concentration due to the inhibitory effect of velocity (Figure 6-3). Bacteria required higher nitrate concentration for saturation than the algae, and was not directly affected by flow velocity.

The main goal of the model was to reproduce the observed algal biomass and metabolism results. In each scenario, algal biomass declined by 9 to 13 g wet weight, similar to experimental results (Figures 6-4 to 6-7). At low initial nitrate concentration, the peak in GPP at medium flow velocity was captured, although GPP was not quite as large as measured. CR also peaked at medium flow velocity, and was higher than GPP

for all three velocities tested, resulting in slightly negative NEP as observed. At high initial nitrate concentration, GPP decreased with increasing flow velocity, but all values were higher than observed, especially at 10 cm/s. CR increased due to the stimulation of the bacteria, which caused lower NEP. CR was also much higher than observed at 10 cm/s.

A secondary goal of the model was to simulate the observed fluctuations in nitrate concentration. The modeled nitrate dynamics at both low and high initial nitrate concentration did correspond with the experimental measurements. At low initial concentration, nitrate oscillated slightly around the initial value due to uptake at day, followed by remineralization at night. At high initial concentration, there was an asymptotic decline in nitrate due to the denitrifying bacteria, which leveled off at about 0.55 mg N/L.

### **Discussion**

A simulation model of filamentous algae under different nitrate concentrations and flow velocities that included bacteria was the only model proposed which was able to approximate the observed results of the laboratory stream channel experiment. Within a specific range of model coefficients, the subsidy-stress relationship (Odum et al. 1979) between algae and flow velocity was reproduced. When nitrogen availability was limited, higher flow velocity subsidized algal production by increasing nitrate availability. With increasing velocity, eventually the negative effect of velocity became greater than the subsidy effect, resulting in peak algal production at an intermediate velocity. Typically, the negative effect of flow velocity on attached algae is associated with physical drag; however, drag would affect algal biomass rather than production rates. A mechanism by which flow velocity negatively affects algal production has not been

determined, but this effect has been observed in another study and may be related to compression of algal filaments or disruption of cellular uptake processes (Borchardt et al. 1994).

One observed result which was not captured by the model, was the substantial decline in GPP and CR at 1.0 mg N/L and 10 cm/s velocity. It is possible that the negative effect of velocity was stronger than modeled, but if this velocity effect is increased then metabolism also declines substantially at low nitrate concentration, which was not observed. There appears to be an unknown mechanism involved, which caused lower overall metabolism only when both nitrate concentration and flow velocity were elevated. The decline of CR in these conditions could partially be attributed to the decline in algal production and associated respiration; however lower algal respiration cannot account for this entire decline. This indicates that bacterial respiration was also lower in these conditions, and may be dependent on algal production to provide the labile carbon necessary for denitrification.

Bacteria was not directly quantified in the laboratory experiment; however, evidence of increased heterotrophy and declining nitrate concentration in the high nitrate trials as compared to the low nitrate trials suggests that a denitrifying bacteria was present (see Chapter 5). Inclusion of bacteria in the model was necessary to simulate CR and NEP accurately which is further evidence of the influence of bacteria in the experiment. In order to reproduce the observed asymptotic decline in nitrate at high initial concentration, the Michaelis-Menton uptake function was altered so that uptake did not occur unless nitrate concentration was greater than 0.5 mg N/L. While this is

atypical, it is feasible that a threshold concentration was required in order for the bacteria to become sufficiently active for substantial denitrification.

One purpose of this experiment and model was to determine the nitrate concentration that limits *Lyngbya* growth, and how this threshold concentration may change due to flow velocity. The half saturation constant ( $k_{A-Half}$ ) of 0.2 mg N/L used in the Michaelis-Menton function indicates that nitrate uptake by *Lyngbya* would be saturated at 0.4 mg N/L. This saturation concentration lies between the values found in similar *Lyngbya* studies (0.60 mg/L: Cowell and Dawes 2004; 0.11 mg/L: Stevenson et al. 2007). Since flow velocity is known to increase nutrient availability, it seems appropriate that the saturation concentration found in this study would be intermediate between these other two studies because the velocities tested were also intermediate (1 to 10 cm/s: this study; approximately 0 cm/s: Cowell and Dawes 2004; 25 cm/s: Stevenson et al. 2007).

However, the inclusion of flow velocity effects on nitrate uptake in the model substantially alters the saturation concentration. The simulated response of the algae to nitrate concentration at a velocity of 1 cm/s suggests that saturation would not occur until the concentration was > 1.0 mg N/L. Furthermore, the saturation concentration becomes irrelevant at flow velocity > 5 cm/s, since nitrate uptake is no longer related to concentration and is instead controlled by flow velocity alone. Nitrate concentration did appear to affect *Lyngbya* growth in a similar study at a flow velocity of 25 cm/s (Stevenson et al. 2007), so the model may require further adjustment to accurately represent the effects of both nitrate concentration and flow velocity on *Lyngbya* growth and nitrate uptake. While models such as this can be useful for mechanistic description

of experimental outcomes, the specific model equations and parameters should be regarded with caution as the model itself is a hypothesis and does not include all of the components and processes occurring in the actual system.

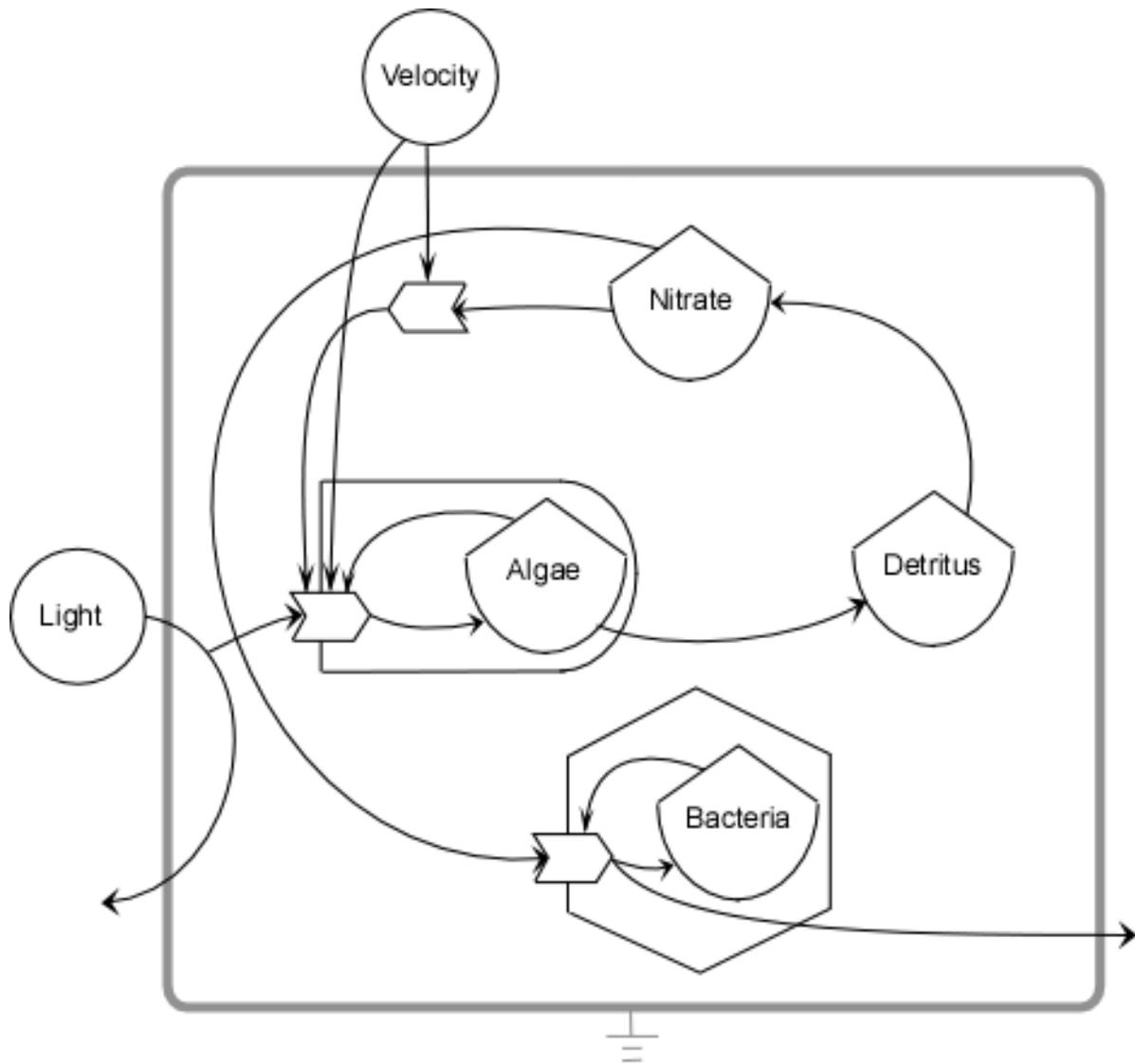


Figure 6-1. Systems diagram of the simulation model for nitrate and flow velocity effects on algae and bacteria.

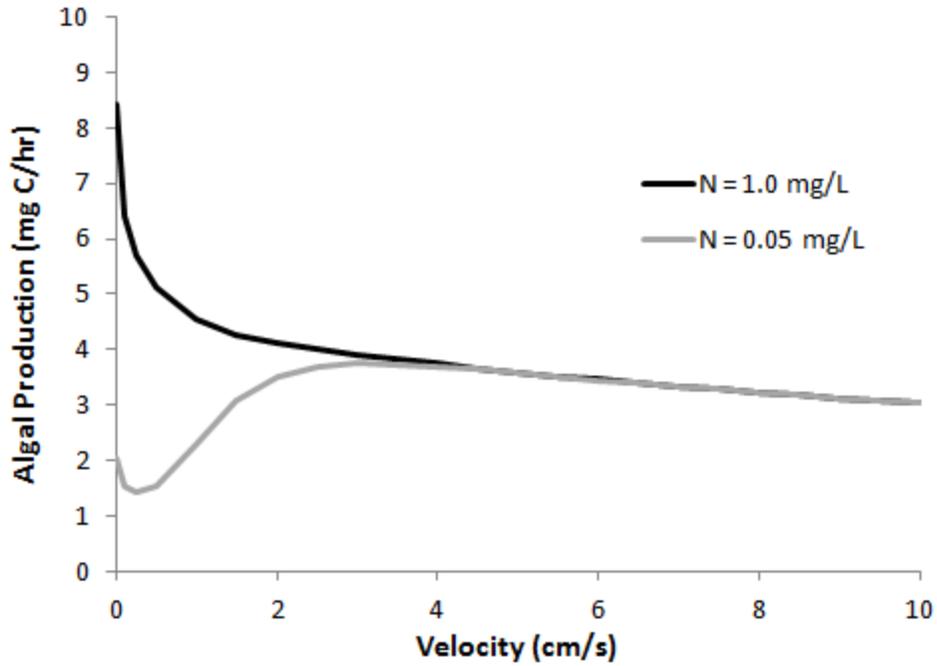


Figure 6-2. Response of algal production to flow velocity at two nitrate concentrations (1.0 and 0.05 mg N/L).

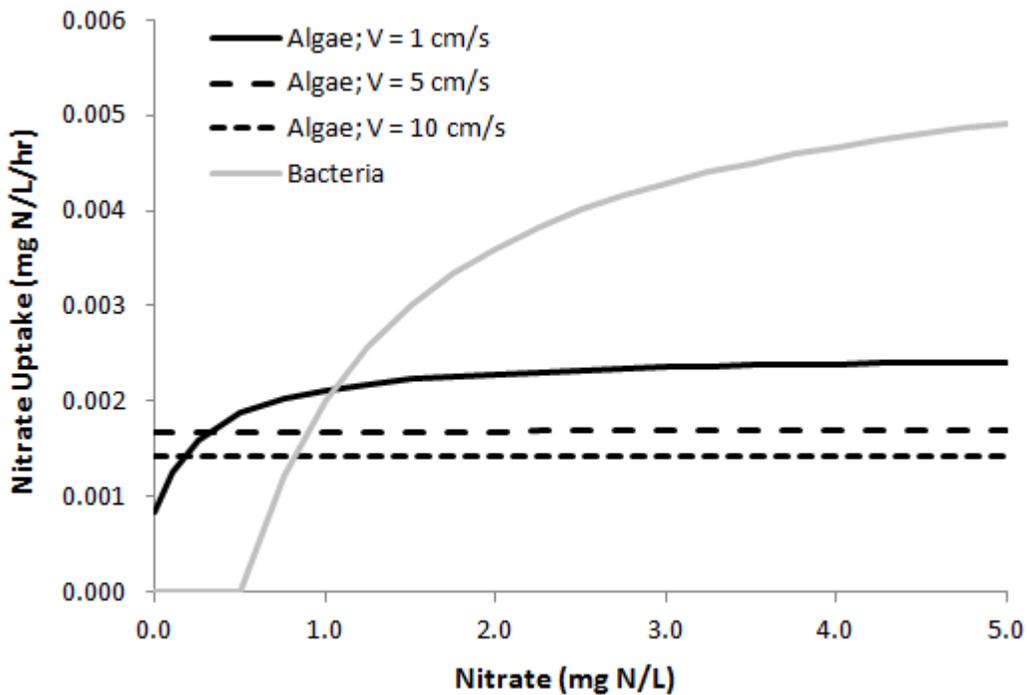


Figure 6-3. Relationship between nitrate uptake and nitrate concentration for bacteria and algae at different flow velocities.

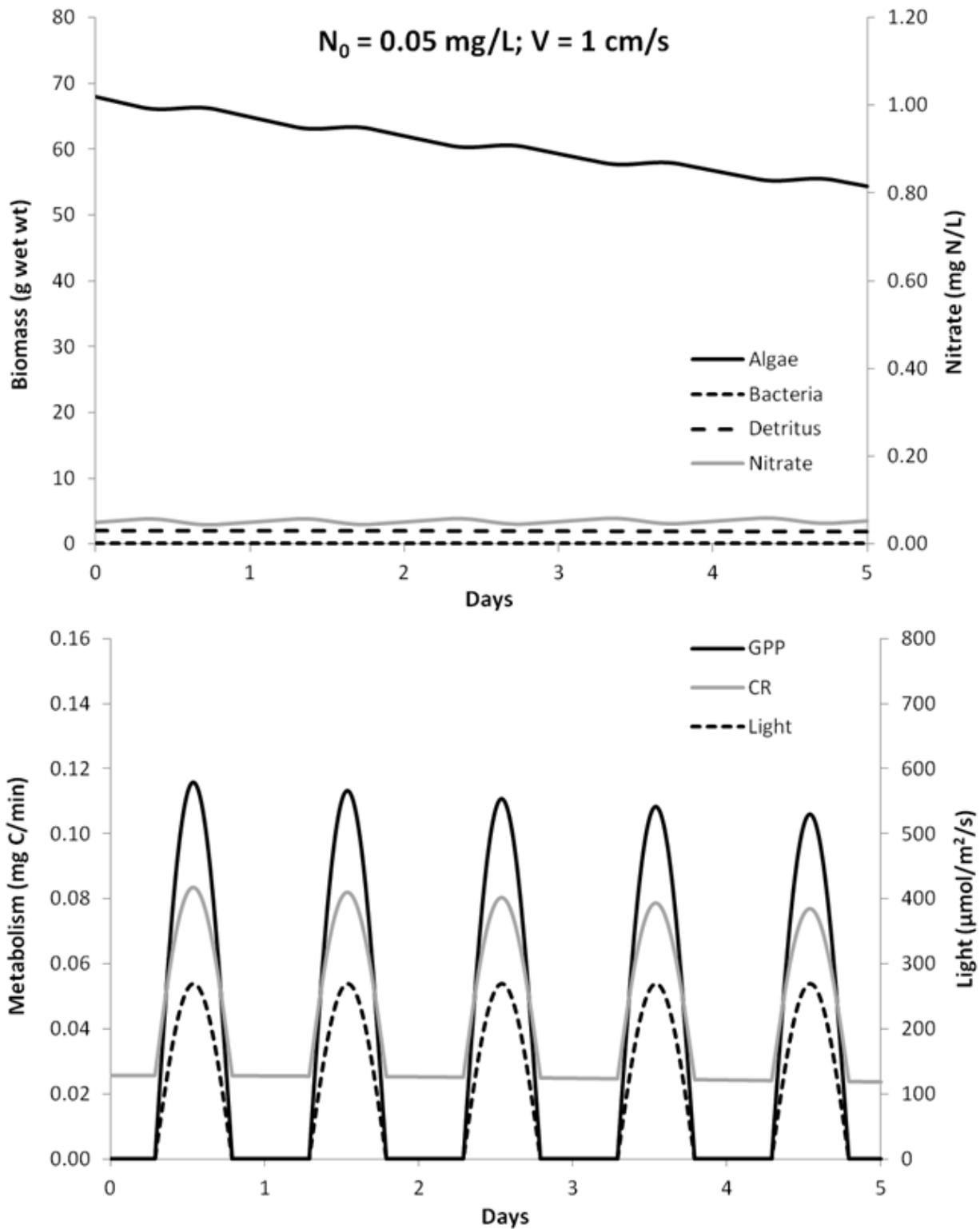


Figure 6-4. Simulation model results of storages and metabolism at low initial nitrate concentration (0.05 mg N/L) and 1 cm/s flow velocity.

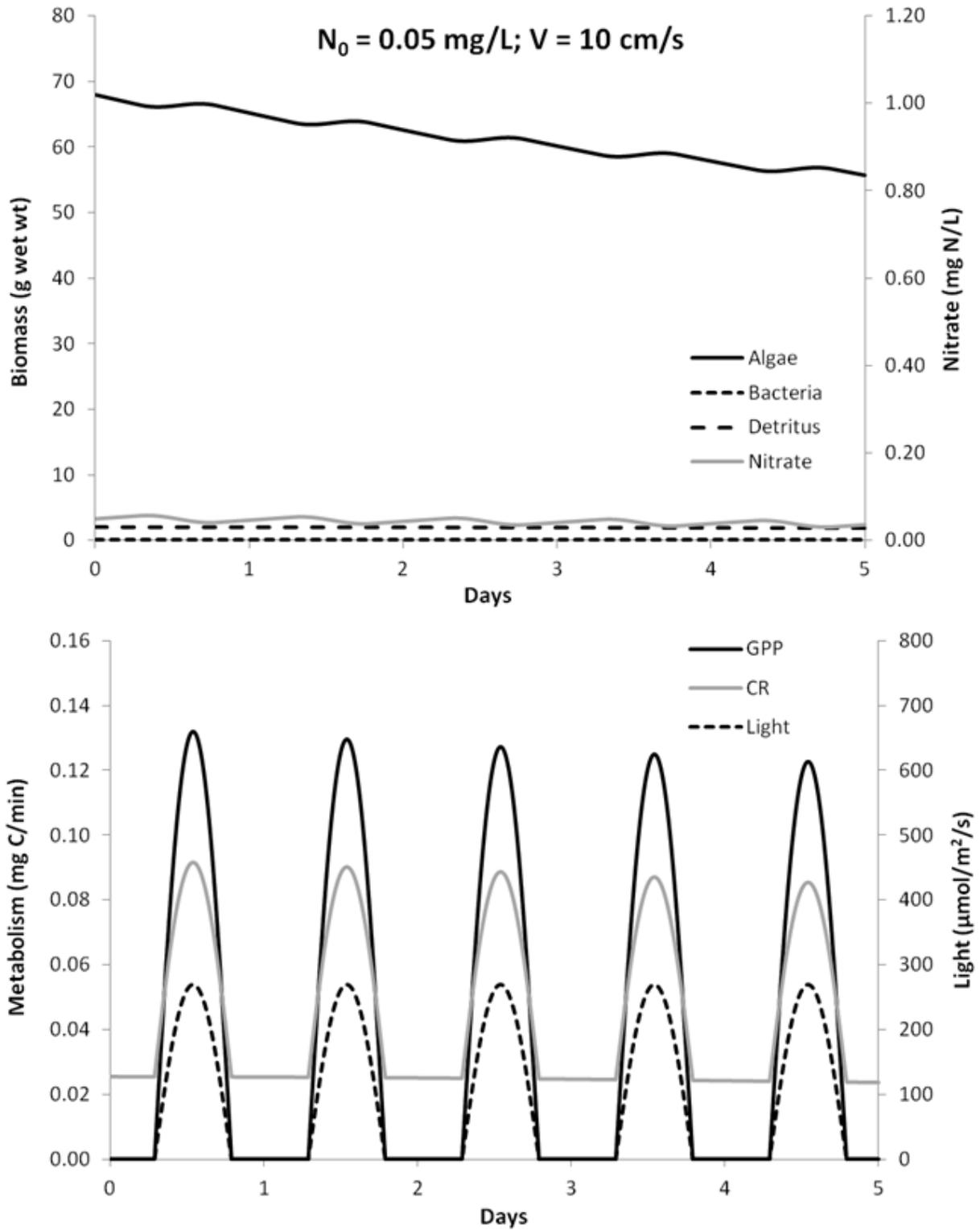


Figure 6-5. Simulation model results of storages and metabolism at low initial nitrate concentration (0.05 mg N/L) and 10 cm/s flow velocity.

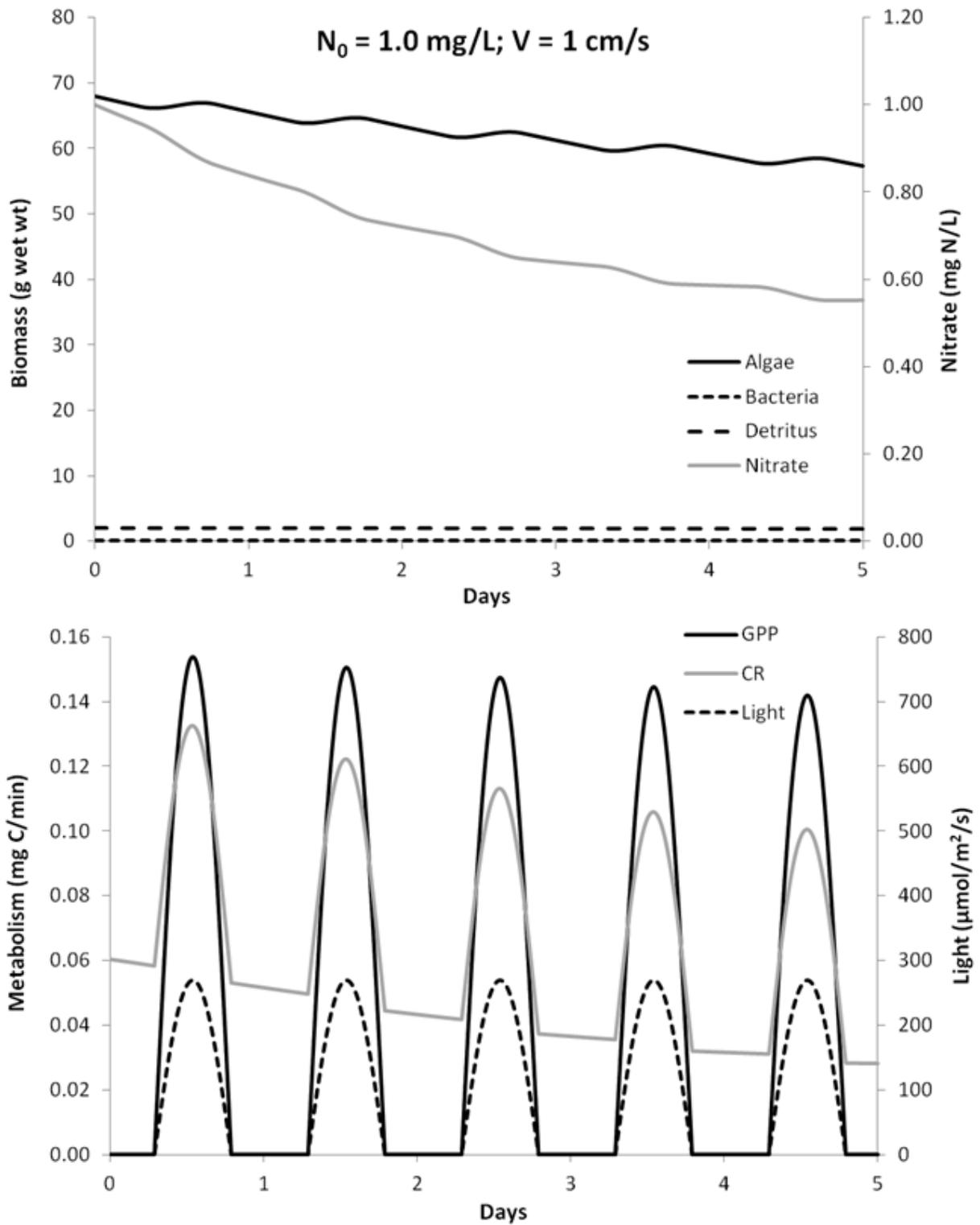


Figure 6-6. Simulation model results of storages and metabolism at high initial nitrate concentration (1.0 mg N/L) and 1 cm/s flow velocity.

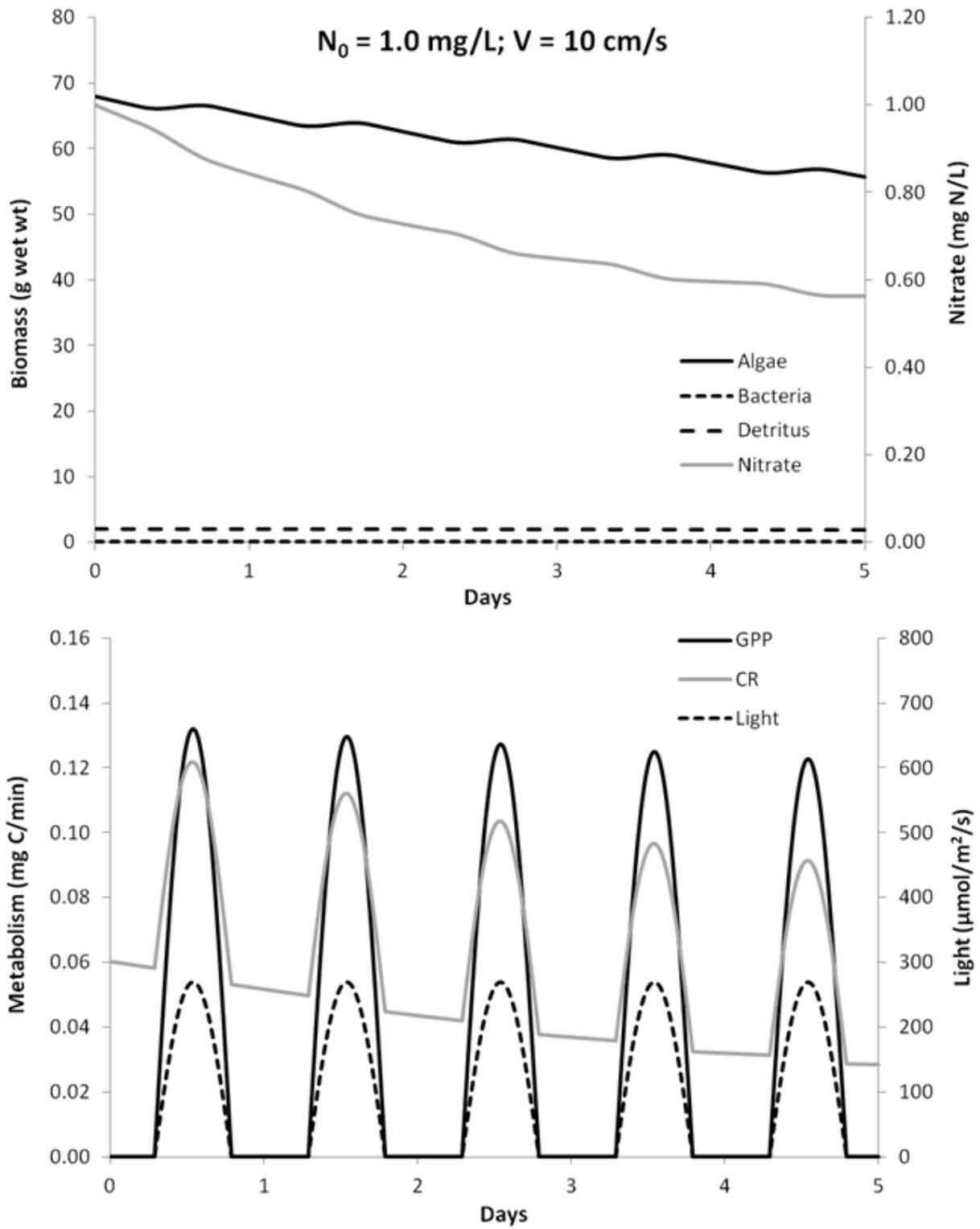


Figure 6-7. Simulation model results of storages and metabolism at high initial nitrate concentration (1.0 mg N/L) and 10 cm/s flow velocity.

Table 6-1. Sources and storages used as variables in the simulation model. All storage turnover times were estimated.

Sources/Storages	Variable	Initial Value	Units	Source
Light	L	86	$\mu\text{mol}/\text{m}^2/\text{s}$	Measured
Available Light	$R_L$	43	$\mu\text{mol}/\text{m}^2/\text{s}$	50% of L – Estimated
Velocity	V	1	cm/s	Measured
Algae (30 days)	A	68	g wet weight	Measured
Bacteria (10 days)	B	0.1	g wet weight	Estimated
Detritus (5 days)	D	2	g wet weight	Estimated
Nitrogen (2 days)	N	0.05	mg/L	Measured

Table 6-2. Model equations and coefficients.

Name	Variable	Equation	Coefficients
Algae	A	$\frac{dA}{dt} = \frac{k_1 R_L \mu_A A}{1 + V^{k_{VA}}} - k_2 A$	$k_1 = 5.02E-2$ $k_2 = 5.56E-5$ $k_{VA} = 0.4$
Bacteria	B	$\frac{dB}{dt} = k_3 \mu_B B - k_4 B$	$k_3 = 2.08$ $k_4 = 6.94E-5$
Nitrate	N	$\frac{dN}{dt} = k_5 D - \frac{k_6 R_L \mu_A A}{1 + V^{k_{VA}}} - k_7 \mu_B B$	$k_5 = 8.68E-6$ $k_6 = 5.54E-4$ $k_7 = 36.46$
Detritus	D	$\frac{dD}{dt} = k_8 A - k_9 D$	$k_8 = 4.09E-6$ $k_9 = 1.39E-4$
Available Light	$R_L$	$R_L = L / \left(1 + \frac{k_{RL} \mu_A A}{1 + V^{k_{VA}}}\right)$	$k_{RL} = 1.37E3$
Algal N Uptake	$\mu_A$	$\mu_A = \left(\frac{k_{A-Max} * (N + k_{V1} V^{k_{V2}})}{k_{A-Half} + (N + k_{V1} V^{k_{V2}})}\right)$	$k_{A-Max} = 5.0E-5$ $k_{A-Half} = 0.2$ $k_{V1} = 0.1$ $k_{V2} = 2.5$
Bacterial N Uptake	$\mu_B$	$N > 0.5 \quad \mu_B = \left(\frac{k_{B-Max} * (N - 0.5)}{k_{B-Half} + (N - 0.5)}\right)$ $N \leq 0.5 \quad \mu_B = 0$	$k_{B-Max} = 1.0E-4$ $k_{B-Half} = 1.0$
Gross Primary Production	GPP	$GPP = \left(\frac{k_1 R_L \mu_A A}{1 + V^{k_{VA}}}\right) k_A$	$k_A = 23.7$
Community Respiration	CR	$CR = \left(\frac{k_1 R_L \mu_A A}{1 + V^{k_{VA}}}\right) k_A * 0.5 + (k_3 \mu_B B) k_B + (k_9 D) k_D$	$k_B = 5000$ $k_D = 92$
Net Ecosystem Production	NEP	$NEP = GPP - CR$	

Table 6-3. Comparison of observed experimental results and modeled results for metabolism of filamentous algae and bacteria in the six scenarios simulated.

Initial Conditions		Metabolism Results (mg C/g biomass/d)			
Nitrogen (mg/L)	Velocity (cm/s)		GPP	CR	NEP
0.05	1	Observed	0.83	0.87	-0.04
	5		1.18	1.27	-0.09
	10		0.97	1.12	-0.15
0.05	1	Modeled	0.84	1.01	-0.17
	5		1.02	1.09	-0.07
	10		0.95	1.06	-0.11
1.0	1	Observed	1.00	1.39	-0.39
	5		0.92	1.44	-0.52
	10		0.54	1.04	-0.50
1.0	1	Modeled	1.09	1.49	-0.40
	5		1.02	1.47	-0.45
	10		0.95	1.44	-0.49

## CHAPTER 7 CONCLUSIONS

The purpose of this research was to examine the effects of flow on filamentous algae and nutrient limitation in lotic systems, with application to algal proliferation in Florida springs and spring-fed rivers. The first task was to assess predictors of nutrient limitation in lotic systems broadly by comparing nutrient concentration to a new metric, the autotrophic uptake length, which also accounts for discharge rate and autotrophic metabolism. Autotrophic uptake length was a better predictor of autotrophic N limitation, suggesting that flow does affect nutrient limitation in lotic systems. An autotrophic uptake length threshold was quantified above which N limitation is unlikely, and the majority of Florida springs were above this threshold value indicating that N limitation is minimal in all but a few spring systems.

Based on a literature review, a subsidy-stress relationship between flow velocity and filamentous algae was hypothesized for Florida springs. The laboratory experiment found that at low nitrate concentration ( $< 0.05$  mg N/L), a flow velocity increase from 1 to 5 cm/s stimulated production rates of the filamentous cyanophyte *Lyngbya wollei*. At high nitrate concentration (1.0 mg N/L), no stimulatory effect of flow velocity was observed. This indicates that flow velocity can control nutrient availability to algae, and algal N demand may be saturated even at fairly low concentrations with moderate velocity (at least for *Lyngbya wollei*). An inhibitory effect of flow velocity on algal production was observed at 10 cm/s, which is relatively low in comparison to velocities in many spring-fed rivers and was apparently unrelated to export. The field survey and experiment at Gum Slough found that filamentous algal abundance, composed mostly of *Lyngbya wollei*, was negatively affected across a range of flow velocities from 5 – 35

cm/s, with very little filamentous algae above 35 cm/s and the potential for large algal mats below 5 cm/s.

Combined, these results indicate that filamentous algae in Florida springs are sensitive to flow velocity, and the presence of a subsidy effect is dependent on N concentration. The velocity threshold for a shift from a subsidy to a stress effect may occur at about 5 cm/s when N concentration is low ( $< 0.05$  mg/L), but when N concentration is higher then any subsidy effect would be minimal since N availability is no longer a limiting factor. Overall, this suggests that filamentous algae is likely to increase substantially as flow velocity decreases below 35 cm/s regardless of N concentration, until a threshold is reached around 5 cm/s below which N concentration may become important. Since declining discharge usually leads to lower velocities, this could be a primary cause of filamentous algal proliferation in some spring systems.

For Florida springs, the pertinent question is not whether one of the proposed sources of degradation (e.g. N enrichment, declining flow velocity, loss of consumers) is an issue but rather to what degree. Currently there is no indication that a single cause is responsible for observed ecological degradation but rather a plurality of causes (i.e. multiple stressors) which may have complex interactions. This situation is particularly difficult for managers as it requires a more holistic approach in order to maintain or restore the ecology of each system, and each spring or region may have its own particular suite of challenges and solutions. Unfortunately it is more difficult to communicate complex issues with multiple causes and high uncertainty to the public, whose participation is often necessary to resolve local and regional problems. The simplicity of the nutrient enrichment narrative is likely the reason it has been so widely

adopted; however, nutrient reduction alone is unlikely to inhibit filamentous algal proliferation in most Florida springs. Although maintenance (or restoration) of historic nutrient levels is a desirable goal, in many cases it would be appropriate to adopt a more holistic approach that considers alternative management options in addition to nutrient control.

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## BIOGRAPHICAL SKETCH

Sean Alan King was born in 1983 in Columbia, South Carolina where he lived for eighteen years. In 2001, he moved to Gainesville, Florida to begin college at the University of Florida where he met his wife, Shannon McMorrow. He received a Bachelor of Science degree in environmental engineering in 2005. During the final year of his undergraduate studies, he began graduate work at the University of Florida with his advisor Mark T. Brown, who introduced him to the concepts of systems ecology and ecological engineering. His master's research focused on hydrologic modeling for wetland restoration on phosphate mining land, and he received a Master of Engineering degree in 2007. That year he received a fellowship to begin his doctorate research at the University of Florida under the same advisor. He was also accepted as an associate member of a NSF Interdisciplinary Graduate Education and Research Traineeship (IGERT) program, which was focused on adaptive management of water resources and involved trips to the Florida Everglades and the Okavango Delta in Botswana, Africa. As a result of this program, he became interested in issues involving the ecology of Florida springs and rivers, and decided to focus on this topic for the next four years of his life. Sean now considers himself both an engineer and a scientist, and plans to use what he has learned to continue working at the interface of human and natural systems.