EFFECTS OF GROWING SEASON FLOW REGIME ON STREAM PERIPHYTON GROWTH IN A COASTAL PLAIN STREAM

by

DAVID LEONARDO DIAZ

(Under the Direction of Alan P. Covich)

ABSTRACT

Increasing water extractions for irrigation coupled with climate shifts in the lower Flint River Basin in southwestern Georgia can decrease water flows in local stream systems. We conducted controlled experiments to examine the relationships between low flow stream discharges, associated environmental factors and periphyton growth, biomass, and composition in Ichawaynochaway Creek (IC), a major Flint River tributary during the summer of 2014. We used an artificial stream facility to manipulate discharge, nutrients, and grazers in a series of experiments. Our study indicated that there is rapid periphyton accumulation potential during the summer growing season. Nutrient enrichment effects were greatest at higher discharge and grazers exerted limited control over periphyton growth. All flow treatments were dominated by diatoms but lower flow treatments had higher relative abundance of green algae. Flow alterations can potentially affect periphyton accumulation rates and shift algal composition with impacts on in-stream habitats and food web dynamics.

INDEX WORDS: Environmental Flows, Periphyton, Streams, Discharge, Nutrients, Grazers, Gastropods

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continue my journey of learning and hope to communicate to others the importance of rivers and their ecology.

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LITERATURE REVIEW AND SUMMARY OF OBJECTIVES

Literature Review

The lower Flint River Basin (LFRB), located in southwestern Georgia, is one of the most productive agricultural regions in the U.S (McKissick, 2004). Since the 1970s the regional economy has depended on row-crop agriculture supported by center-pivot irrigation, which increased water withdrawal from both surface and groundwater sources**.** Withdrawals often coincide with reductions in summertime flows and exacerbate normal low flow conditions caused by droughts (Torak et al. 1996, Rugel et al. 2012). Prolonged dry periods are projected to be more common as a result of global climate change in Georgia (Cook et al. 1999, Viger et al 2011, Sun et al. 2013,), and their impact on aquatic ecosystems is important to consider for future water management and decisions regarding the conservation of the high freshwater biodiversity in the southeastern USA (Hopkinson et al. 2013).

Variation in stream discharge can alter the colonization, production, and biomass of periphyton (Biggs & Close 1989), a key functional component and energy source in stream systems (Stevenson et al. 1996). Experimental studies indicate that greater algal biomass accumulation is often associated with lower discharge (Poff et. al 1990, Suren et al. 2003, Biggs et al. 2005). Increases in the density of periphyton in an aquatic system may negatively affect water clarity, limit growth of submerged aquatic vegetation, alter habitat quality and biodiversity, decrease aesthetic and recreational values (Biggs 2000, Suplee et al. 2009) and affect invertebrate grazing communities and food web dynamics (Suren et al. 2003). Stream grazers often limit algal biomass but may not be able to respond to a rapid increase in algal growth rates that exceeds herbivory rates (Lamberti et al. 1989).

The purpose of this study is to investigate how summer low-flow conditions affect algal growth and biomass and the ability of stream herbivores to respond to changes in algal abundance. This research will help identify environmental thresholds of stream discharge with respect to periphyton growth and the ability of a common stream herbivore (*Elimia albanyensis*) to respond to changes in periphyton growth rates. The following review provides a background on flow related factors that affect periphyton biomass, composition and interactions with grazers.

Background

Freshwater ecosystems are being altered in an unprecedented manner due to human use of water resources for household and industrial consumption, irrigation, energy production, and other uses (Dudgeon et al. 2006). The chemical, physical and ecological condition of streams worldwide are being altered to an extent that may negatively affect wildlife and humans who depend on the services that these ecosystems provide (Postel & Richter 2003). Some of these ecosystem services include water purification, habitat for aquatic biota, and recreational opportunities. Increased human water consumption coupled with finite freshwater supplies makes it difficult to balance human and ecological needs for water (Dewson et al. 2007). Therefore, a major challenge of the 21st century is to resolve issues concerning the management of freshwater ecosystems and to increase the sustainability of water resources to benefit both ecological and human systems (Palmer et al. 2004).

Aquatic ecologists recognize that the flow regime is a key driver of ecosystem function for rivers and riparian ecosystems because flow determines stream geomorphology and the types and condition of available physical habitat. Physical structure in turn affects the movement and persistence of aquatic biota (Bunn & Arthington 2002). Accordingly, flow regime modification is considered one of the most prominent threats to the ecological sustainability of fluvial ecosystems (Ward et al. 1999, Worrall et al. 2014). Scientists and resource managers are employing management strategies to develop appropriate environmental flow targets, which are needed to sustain freshwater and estuarine systems while at the same time supporting human needs. These targets include the quantity, timing and duration of specific flows needed to sustain healthy aquatic systems.

Flow Effects on Periphyton

Periphyton is a primary energy source in many lotic food webs (Lamberti & Moore 1984) and can influence chemical and physical habitat conditions by regulating nutrient availability, dissolved oxygen, pH, and substrate suitability for stream organisms. Major factors affecting algal growth and biomass at a local scale include temperature, light, nutrient availability, grazing pressure, and water velocity (Hill & Knight 1988). Loss of periphyton is affected by dislodgment due to high current velocity (Biggs & Thomsen 1995) and grazing (Hart 1985). The factors limiting algal growth can vary seasonally and spatially (Pringle et al. 1988), and in some cases two or more factors may be limiting simultaneously (Rosemond 1994, Dodds et al. 2002).

Increasing current velocity has two opposing effects on biomass accumulation: a positive effect by increasing nutrient supply (Horner et al. 1990) and a negative effect by increasing shear stress (Biggs & Thomsen 1995). Current velocity affects scouring rates and substrate makeup, which influences periphyton community composition based on individual algal species tolerance to different water velocities (McIntire 1966, Peterson & Stevenson 1990). Velocity gradients within a stream can produce periphyton patches of varying density and taxonomic composition.

Hart et al. (2013), for instance, found that the spatial arrangement of patches of filamentous green algae and cyanobacteria resulted from differing water velocity tolerances that affected their colonization and development.

Light is often a factor limiting periphyton productivity and biomass (Hill 1996). Stream discharge can influence light availability by affecting water depth and loads of suspended sediment and other light blocking materials. Major groups of algae respond differently to changes in light intensity. For instance, diatoms and cyanobacteria typically have lower light requirements than green algal species (Hill 1996). Additionally, increased solar radiation can raise stream temperature, which increases rates of metabolism and can alter algal assemblages based on species' temperature tolerances (Phinney & McIntire 1965).

Variations in flow can affect the rate of nutrient delivery to periphyton in lotic systems. Nitrogen (N) and phosphorus (P) are the most common nutrients limiting algal growth. There are a variety of periphyton growth responses that can take place due to the concentration and availability of nutrients in streams. For instance, benthic algal composition and periphyton biomass were not affected by enrichment of N and P in a forested stream in Georgia (Greenwood & Rosemond 2005). However, Tank and Dodds (2003) found that colimitation by N and P was common in periphyton because each algal matrix is composed of multiple species with different nutrient requirements. Other studies have found either N or P to be limiting (Stelzer & Lamberti 2001).

Periphyton-Grazer Interactions

Grazers have the ability to regulate periphyton biomass, growth rates, and taxonomic composition (e.g., Hintz & Wellnitz 2013). Algal responses (e.g., changes in biomass) to grazers are influenced by various factors including herbivore type, herbivore density and developmental state of the algal community (Feminella & Hawkins 1995). Many studies have concluded that algal biomass almost always declines in the presence of herbivores (Rosemond et al. 1993, Hillerbrand et al. 2005), although the outcome can depend on the type of algae and herbivore involved. There are rare cases in which algal biomass can be enhanced by grazing, which may be attributed to grazers removing older cells allowing for more vigorous algal cell growth or to nutrient regeneration taking place at the algal matrix scale (McCormick & Stevenson 1991). Algal taxonomic composition can be affected by herbivory since many of these consumers feed preferentially on certain algal species. Herbivores have specific adaptations for selectively feeding on particular algal community levels; for example, snails have rasping mouthparts and are suited to feed in zones where low-profile, tightly attached algae grow (Lamberti et al. 1987).

PROJECT OBJECTIVES

Extremely low stream and river flows in the LFRB during recent droughts have been associated with the formation of thick periphyton mats. We conducted a series of controlled experiments to examine (1) the relationship between stream discharge and periphyton growth and biomass, (2) low discharge and nutrient interactions on periphyton biomass and composition and (3) periphyton-grazer interactions under low discharge conditions. The following studies can provide insight into the linkages between reductions in stream discharge and periphyton growth and biomass. Exploring factors that promote rapid periphyton growth will provide a better understanding of the ecological impacts of human water consumption and low flows on lotic systems in this region.

References:

Biggs, B. J. (2000) Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society, 19, 17-31.

Biggs, B.J.F. & Close, M.E. (1989) Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients, Freshwater Biology, 22, 209–231.

Biggs, B. J., & Thomsen, H. A. (1995) Disturbance of stream periphyton by perturbations in shear stress: time to structural failure and differences in community resistance1. Journal of Phycology, 31, 233-241.

Biggs, B. J. F., Nikora, V. I. & Snelder, T. H. (2005) Linking scales of flow variability to lotic ecosystem structure and function. River Resource Applications, 21, 283– 298.

Bunn, S.E. & Arthington, A. H. (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management, 30, 492–507.

Cook E.R., Meko D.M., Stahle D.W. & Cleaveland M.K. (1999) Drought reconstructions for the continental United States. Journal of Climate, 12, 1145–1162.

Dewson Z.S., James, A.B.W. & Death R.G. (2007) Stream ecosystem functioning under reduced flow conditions. Journal of Applied Ecology, 17, 1797–1808.

Dodds, W. K., Smith, V. H., & Lohman, K. (2002) Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences, 59, 865-874.

Dudgeon D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D., et al. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Biological-Review, 81, 163–168.

Feminella, J. W., & Hawkins, C. P. (1995). Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. Journal of the North American Benthological Society, 14, 465-509.

Greenwood, J.L. & Rosemond, A.D. (2005) Periphyton response to long-term nutrient enrichment in a shaded headwater stream. Canadian Journal of Fisheries and Aquatic Sciences, 62, 2033-2045

Hart, D. D. (1985) Grazing insects mediate algal interactions in a stream benthic community. Oikos, 44, 40-46.

Hart, D. D., Biggs, B. J., Nikora, V. I., & Flinders, C. A. (2013) Flow effects on periphyton patches and their ecological consequences in a New Zealand river. Freshwater Biology, 58, 1588-1602.

Hill, W. R., & A.W. Knight. (1988) Nutrient and light limitation of algae in two northern California streams. Journal of Phycology, 24, 125-132.

Hill, W. Effects of light. pp 121-148. In: Stevenson, R. J., Bothwell, M. L., Lowe, R. L. (1996) Algal ecology: Freshwater benthic ecosystems. Academic Press, San Diego.

Hillebrand, H., Kahlert, M., Haglund, A. L., Berninger, U. G., Nagel, S., & Wickham, S. (2002) Control of microbenthic communities by grazing and nutrient supply. Ecology, 83, 2205-2219.

Hillebrand, H. (2005) Light regime and consumer control of autotrophic biomass. Journal of Ecology, 93, 758-769.

Hintz, W. D., & Wellnitz, T. (2013) Current velocity influences the facilitation and removal of algae by stream grazers. Aquatic Ecology, 47, 235-244.

Hopkinson, C.S., A.P. Covich, C.B. Craft, T.W. Doyle, N. Flanagan, M. Freeman, E.R. Herbert, A. Mehring, J.E. Mohan, C.M. Pringle, & C. Richardson. The effects of climate change on natural ecosystems of the southeastern United States. Pp.237-270. In: K.T. Ingram, K. Dow, L. Carter, and J. Anderson. (2013) Climate of the Southeast United States in the National Climate Assessment Regional Technical Input Series, Island Press, Washington, D.C.

Horner, R. R., Welch, E. B., Seeley, M. R., & Jacoby, J. M. (1990) Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. Freshwater Biology, 24, 215-232.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., Steinman, A. D., & McIntire, C. D. (1989) Productive capacity of periphyton as a determinant of plant-herbivore interactions in streams. Ecology, 70, 1840-1856.

McCormick, P. V., & Stevenson, R. J. (1991) Grazer control of nutrient availability in the periphyton. Oecologia, 86, 287-291

McIntire, C. D. (1966) Some effects of current velocity on periphyton communities in laboratory streams. Hydrobiologia, 27, 559-570

McKissick J.C. (2004) The Economic Importance of Irrigated Food and Fiber Production: A Spotlight on Georgia's Flint River Basin. The University of Georgia Center for Agribusiness and Economic Development, Athens, GA

Palmer M.A., Bernhardt E. & Chornesky E (2004) Ecology for a crowded planet. Science, 304, 1251–1252.

Peterson, C. G., & Stevenson, R. J. (1989). Substratum conditioning and diatom colonization in different current regimes. Journal of Phycology, 25**,** 790-793.

Peterson, C. G., & Stevenson, R. J. (1990) Post-spate development of epilithic algal communities in different current environments. Canadian Journal of Botany, 68, 2092-2102.

Phinney, H. K., & McIntire, C. D. (1965). Effect of temperature on metabolism of periphyton communities developed in laboratory streams1. Limnology and Oceanography, 10(3), 341-345.

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., & Stromberg, J. C. (1997). The natural flow regime. BioScience, 47, 769-784.

Poff N.L., Richter B.D., Arthington A.H. Bunn, S. E., Naiman, R. J., Kendy, E. & Warner, A.(2010) The ecological limits of hydrologic alteration (ELOHA): a new framework for developing regional environmental flow standards. Freshwater Biology, 55, 147-170

Postel S, & Richter B (2003) Rivers for life: Managing water for people and nature. Island Press, Washington, DC

Pringle, C. M., Naiman, R. J., Bretschko, G., Karr, J. R., Oswood, M. W., Webster, J. R., & Winterbourn, M. J. (1988) Patch dynamics in lotic systems: the stream as a mosaic. Journal of the North American Benthological Society, 7(4), 503-524.

Rosemond, A. D. (1994) Multiple factors limit seasonal variation in periphyton in a forest stream. Journal of the North American Benthological Society, 13, 333-344.

Rugel, K., C. R. Jackson, J. J. Romeis, S. W. Golladay, D. W. Hicks, & J. F. Dowd. (2012) Effects of irrigation withdrawals on streamflows in a karst environment: lower Flint River Basin, Georgia, USA. Hydrological Processes, 26, 523-534.

Shea, C.P., J.T. Peterson, M. J. Conroy, & J.M Wisniewski.(2013) Evaluating the influence of land use, drought, and reach isolation on the occurrence of freshwater mussel species in the lower Flint River Basin, Georgia (U.S.A.). Freshwater Biology, 58, 382–395.

Stelzer, R. S., & Lamberti, G. A. (2001). Effects of N: P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography, 46, 356-367.

Stevenson, R. J. (1983) Effects of current and conditions simulating autogenically changing microhabitats on benthic algal immigration. Ecology, 64, 1514-1524.

Sun, G., S. Arumugam, P.V. Caldwell, P.A. Conrads, A.P. Covich, J. Cruise, J. Feldt, A.P. Georgakakos, R.T. McNider, S.G. McNulty, D.A. Marion, V. Misra, T. C. Rasmussen, L. Romolo, & A.Terando. (2013) Impacts of climate change and variability on water resources in the southeast USA. Pages 210-236, In: Climate of the southeast United States - Variability, change, impacts and vulnerability. K.T. Ingram, K. Dow, L. Carter, and J. Anderson (eds.). Island Press, Washington, D.C.

Suplee, M. W., Watson, V., Teply, M., & McKee, H. (2009). How Green is Too Green? Public Opinion of What Constitutes Undesirable Algae Levels in Streams. Journal of the American Water Resources Association, 45, 123-140.

Suren, A. M., Biggs, B. J., Duncan, M. J., Bergey, L., & Lambert, P. (2003) Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment 2. Invertebrates. New Zealand Journal of Marine and Freshwater Research, 37, 71-83

Tank, J. L., & Dodds, W. K. (2003) Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology, 48, 1031-1049

Thompson, F.G. (2004) An identification manual for the freshwater snails of Florida. University of Florida Press, Gainesville.

Torak L.J., Davis G.S., Strain G.A. & Herndon J.G. (1996) Geohydrology and Evaluation of Stream- Aquifer Relations in the Apalachicola-Chattahoochee-Flint River Basin, Southeastern Alabama, Northeastern Florida, and Southwestern Georgia.U.S. Geological Survey, Water-Supply Paper 2460, Denver,CO.

Viger, R. J., Hay, L. E., Markstrom, S. L., Jones, J. W., & Buell, G. R. (2011) Hydrologic effects of urbanization and climate change on the Flint River basin, Georgia. Earth Interactions, 15, 1-25.

Ward, J. V., Tockner, K., & Schiemer, F. (1999) Biodiversity of floodplain river ecosystems: Ecotones and connectivity. Regulated Rivers: Research & Management, 15(1), 125-139

Worrall, T. P., Dunbar, M. J., Extence, C. A., Laizé, C. L. R., Monk, W. A., & Wood, P. J. (2014). The identification of hydrological indices for the characterization of macroinvertebrate community response to flow regime variability. Hydrological Sciences Journal, 59, 645-658

CHAPTER 2

INFLUENCE OF LOW FLOW AND NUTRIENT INTERACTIONS ON PERIPHYTON BIOMASS AND COMPOSITION[1]

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[1] Diaz, D.L., P.V. McCormick, A.P. Covich, to be submitted to *Freshwater Science*

Abstract

Increasing water demand coupled with climate change predictions will likely result in declining stream flows in southwest Georgia with potential effects on the growth of periphyton, which serves as an important basal energy resource in many streams. We used an experimental stream facility to conduct controlled experiments to examine the effects of low flows and flow-related nutrient availability on periphyton biomass accumulation and composition in Ichawaynochaway Creek (IC), a major Flint River tributary in southwest Georgia, in the summer of 2014. One of three nutrient-enrichment treatments was assigned to a replicate of each discharge treatment: (1) no enrichment (control); (2) phosphorus (P) enrichment (3) P and nitrogen (N) enrichment. Enrichment was achieved by placing small mesh bags of slow-release fertilizer at the upstream end of the channel. Accumulation of AFDM increased while chlorophyll a accumulation decreased with increasing discharge. Diatoms were the dominant algal group in all treatments, but the relative abundance of filamentous green algae increased with lower discharge. Nutrient enrichment treatments, especially with combined N+P, increased peak AFDM biomass at higher discharges. If water demand and drought conditions continue to increase in the future, then changes to low flow habitats will affect periphyton growth, biomass and composition in southwest Georgia streams.

Introduction

The flow regime is considered to be a key driver of ecosystem function and structure in aquatic systems (Bunn & Arthington 2002, Arthington 2012). Scientists and environmental managers increasingly face the challenge of meeting human needs for water while providing the flows necessary to sustain aquatic ecosystems (Postel & Richter 2003). This increasing competition for water coupled with increased temperatures and precipitation extremes (Hopkinson et al. 2013) has the potential to further reduce stream and river flows in many regions, including the southeastern USA (Seager et al. 2009). A better understanding of flow-ecological relationships is necessary for managers to establish appropriate environmental flow requirements to protect the ecological integrity of lotic ecosystems (Poff et al. 2010, Davies et al. 2014).

Reductions in stream discharge have the potential to influence a suite of environmental factors that control growth and biomass of attached algae (periphyton), an important basal energy source in aquatic food webs in most lotic systems (Jardine et al. 2013). Stream discharge affects both water velocity and nutrient supply (Biggs 2000), two factors that exert strong control over periphyton growth. Water discharge can alter the colonization, production, and biomass of periphyton in streams (Biggs & Close 1989). Shear stress on algae at higher velocities can decrease periphyton biomass due to higher rates of cell sloughing and reduced rates of algal cell colonization (Biggs & Thomsen 1995). Discharge can also influence the concentration and uptake potential of nutrients by benthic algae (Riseng et al. 2004). Higher water discharge has the potential to increase nutrient supply rates and decrease the diffusive boundary layers for higher nutrient uptake by algae. These changes in nutrient availability can positively affect algal growth rates (Biggs 2000).

Experimental studies indicate that reduced stream flow leads to greater algal biomass accumulation due to changes in factors that promote growth, including increases in temperatures and light (Suren et al. 2003, Biggs et al. 2005). Nuisance levels of periphyton due to flow alterations can affect water quality (e.g., dissolved oxygen), substrate quality, and food availability. Increases of algal biomass can also limit growth of submerged aquatic vegetation, affect invertebrate communities, and alter food web dynamics (Suren et al. 2003). A current priority for river science is to establish quantitative relationships between flow-related factors, algal biomass, and algal assemblage composition to better understand the ecological effects of reduced flows in aquatic systems (Poff et al. 2010).

Streams in the lower Flint River Basin in southwest Georgia USA exhibit broadly predictable seasonal discharge patterns that correspond with changes in underwater light availability and in-stream primary production. Discharge is typically highest during the winter and spring due to greater rainfall amounts that increase inflows of surface runoff and soil water. These water sources contribute dissolved organic matter content to the stream, resulting in high color values and low light penetration to the stream bottom. During the drier summer months, stream discharge is supported primarily by groundwater, resulting in low stream color and high light penetration. These periods of high light availability can coincide with rapid periphyton growth and periods of high biomass in these streams. Anecdotal observations indicate that the biomass and composition of periphyton during these low-discharge periods is spatially heterogeneous and possibly related to variation in local hydraulic environments.

This study was designed to quantify the relationship between summertime stream flow and periphyton growth and biomass in an agricultural watershed that experiences reduced stream flow in response to drought conditions and growing season water withdrawals for irrigation. We conducted controlled experiments to establish these relationships by investigating how discharge affects growth directly and indirectly via its influence on nutrient availability.

Methods

Site Description

Our study was conducted in the lower portion of Ichawaynochaway Creek, a 5th order tributary of the Lower Flint River (Fig. 2.1). This section of stream flows through a mantled karst landscape underlain by the Ocala limestone, the principal bedrock stratum containing the Floridan Aquifer. The region is characterized by high connectivity of groundwater and surface water (Rugel et al. 2012). Upstream surface-water withdrawals and groundwater pumping for irrigation during the growing season can result in abnormally low stream flows during dry years. These low flows are associated with changes in physical and chemical habitat conditions and biological responses, including extensive growths of periphyton that cover the entire stream bottom in some reaches of the stream.

Experimental Design

An artificial stream channel facility (Fig. 2.2) was constructed in an unshaded location adjacent to the creek to allow for controlled manipulations of discharge and nutrients. Channels were made from white plastic gutters (10-cm wide x 3.1 m long) that were secured in notches of a shallow flow-through water bath that served as a heat-sink. This design allowed channels with different discharge volumes to maintain water temperatures similar to that in the stream (Fig. 2.3). A solid cap was secured to the upstream end of each channel and a perforated cap was secured to the downstream end to allow for flow-through conditions and to maintain a channel water depth of approximately 6 cm. This channel system was supported in a wooden frame that included a clear plastic (Suntuf ®) roof to protect the channels from heavy rainfall that might cause scouring. Stream water was pumped through the facility using a pool pump, and discharge through individual channels was controlled by spigots. After flowing through the channels, water drained into a trough and returned to the stream through plastic drain pipe.

In our first experiment, we manipulated channel discharge to determine influences on periphyton growth rate, biomass, and composition. We hypothesized that lower discharge would result in slower initial periphyton accumulation rates due to nutrient supply constraints but allow for maintenance of higher biomass due to reduced shear stress and associated sloughing. Based on our in-stream observations, discharge was also predicted to influence the composition of the periphyton assemblage due to differences in flow tolerances for algal groups. In a second experiment, we assessed the influence of nutrient limitation on these same periphyton parameters under the same discharge range used in the first experiment. Because stream water is high in dissolved NO_3-N (nitrate) and extremely low in dissolved phosphorus (SRP), we hypothesized that phosphorus was the primary nutrient limiting periphyton growth and that nutrient limitation would intensify with decreasing discharge due to lower nutrient supply rates.

Experiment 1: Effects of Stream Discharge on Periphyton Growth

Stream water was pumped through 15 replicate channels lined with unglazed, ceramic tiles (2.5 cm x 2.5 cm) to achieve 5 discharge treatments that simulated a broad range of stream flows (3 replicates/flow treatment): .9L/min, 1.9L/min, 3.8 L/min, 9.5 L/min, 18.9 L/min. Because water depth and cross sectional area were the same for all treatments, current velocities differed by the same amount: .005ft/s, .011ft/s, .021ft/s, .055ft/s, .1ft/s. Water discharge for each channel was measured at least once a day during the experiment and adjustments were made to keep each channel within 10% of the target discharge. Temperature readings were also taken at the same time.

For the purpose of sampling, 4 sections of equal length were delineated from upstream to downstream in each channel, and one randomly selected tile was sampled from each section every 3-4 days for 28 days. Material accumulated on the tiles was transferred to a Whirlpak® bag by scraping with a razor blade, brushing with a stiff bristle toothbrush, and rinsing with deionized water. Scraped tiles were returned to the channel and turned over to avoid re-sampling. Sample bags were stored in the dark on ice and returned to the laboratory.

Initial processing of samples occurred within 2 hours of collection. Deionized water was added to each sample to achieve a known volume. Samples were then homogenized using a hand blender to disperse the material, and homogenized subsamples of know volume were removed to quantify chlorophyll a, ash-free dry mass (AFDM), and pigment composition. Chlorophyll subsamples were filtered onto a glass fiber filter (Zefon #AE2500**)**, which was then folded, wrapped in aluminum foil, and frozen for no more than 1 month. Chlorophyll a was extracted by grinding filters using a sonicator in 90% acetone using USEPA Method 445.0 (Arar and Collins, 1997) and quantified using a Aquaflour® handheld fluorometer (Turner Designs, Sunnyvale, CA). AFDM samples were poured onto pre-combusted and pre-weighed foil cups, dried at 105° C for 24 h, and weighed after cooling in a desiccator. Samples were then ashed at 500 °C for 1h. Ashed sample were rewetted (to account for water bound to clay particles), and dried and weighed as described above. Periphyton AFDM was determined as the difference between dried and ashed sample mass (APHA 2012). The relative dominance of major algal taxa (i.e., diatoms, green algae, and cyanobacteria) was determined on days 4, 7, 18 and 20 by High Performance Liquid Chromatography (HPLC) following the methods of Leavitt and Hodgson (2001). Pigment analysis samples were extracted with 90% acetone using USEPA Method 445.0 (Arar and Collins, 1997). Following extraction, samples were centrifuged and filtered through 0.22 µm nylon syringe filters (Millex) to remove particulate matter. Samples were placed in an autosampler tray where they were mixed with an ion-pairing agent prior to injection. 75 µL of sample extract and 25 µL of ion-pairing agent were injected into a Shimadzu HPLC system following the mobile phase and time sequence of Leavitt and Hodgson (2001). Pigments were separated using C18 column. Pigment identification was made using retention times of known standards (DHI Lab Products, Denmark) and pigment specific spectra recorded by a photodiode array detector for carotenoids and a fluorescence detector for chlorophylls. Pigment concentrations were calculated by comparing peak areas against standards of known concentration.

Experiment 2: Effects of nutrient enrichment on periphyton growth in different discharge regimes

Following completion of the first experiment, channels and tiles were thoroughly cleaned, were returned to the channels, and discharge treatments for each channel were reinstated. One of three nutrient-enrichment treatments was assigned to a replicate of each discharge treatment: (1) no enrichment (control); (2) phosphorus enrichment (3) phosphorus and nitrogen enrichment. Enrichment was achieved by placing small mesh bags of slow-release fertilizer (“Bonide triple superphosphate fertilizer ®” and “Monterey SR-N3 Slow Release Fertilizer ®”) at the upstream end of the channel. Water quality data were collected from each individual channel on sampling day 15 to confirm that nutrients, in the form of nitrate and phosphate, were being released. Periphyton sampling and processing methods were identical to those described for experiment 1, with the exception of HPLC sampling taking place only on the first and last day of the experiment.

Data analyses

For the first experiment, mean AFDM and chl a concentrations were calculated for each discharge treatment on each sampling date. Polynomial (linear and quadratic) equations were fitted to these data to estimate growth rates (linear coefficient) of algal biomass and chl a concentrations for each treatment and account for potential sloughing of biomass as the experiment progressed (quadratic coefficient). Linear coefficients were compared among treatments using 95% confidence intervals to test for significant differences. Log transformed maximum values of AFDM and chl a values and untransformed data for the day they were achieved in each channel were determined and compared among discharge treatments using 1-way ANOVA followed by multiple comparisons (Tukey's Honest Significant Difference test). Autotrophic index (AI) ratios (AFDM divided by chlorophyll a, Feio et al. 2010) were calculated and compared among treatments for each sampling date in the same manner as for maximum values.

Log transformed concentrations of total pigments and concentrations of pigments indicative of diatoms, green algae, and cyanobacteria were compared among treatments for each measurement date using 1-way ANOVA followed by Tukey Honestly Significant Difference test was used to test for treatment differences. Maximum pigment values and the day on which they were achieved were determined for each channel and compared among treatments as previously described.

For the second experiment, which examined the interaction of flow and nutrients, mean AFDM and chl a concentrations were calculated for each discharge and nutrient treatment combination on each sampling date. Polynomial (linear and quadratic) equations were fit to the data to estimate growth rates (linear coefficient) for algal biomass and chl a for each treatment as described for the first experiment. Comparison of linear coefficients using 95% confidence intervals was used to examine significant differences in accumulation rates among the flow and nutrient treatment combinations, and significant quadratic terms were used to indicate biomass sloughing. Maximum values of AFDM and chlorophyll a values and the day they were achieved in each channel were determined and compared among discharge treatments using two-way ANOVA's followed by Tukey Honestly Significant Difference test. Autotrophic index ratios were calculated and compared among treatments for each sampling date in the same manner as for maximum values.

Concentrations of total pigments and those indicative diatoms and green algae, and were compared across discharge and nutrient treatment combination for the first and last day of experimentations using two-way ANOVA's followed by Tukey Honestly Significant Difference test (R core team 2012).

Results

Experiment 1

Treatments with greater discharge generally had greater AFDM throughout the 28-day experiment. (Figure 2.4). Regression analysis demonstrated a significant linear relationship between AFDM and time for all discharge treatments (Figure 2.5). The accumulation rate increased with discharge and was significantly faster in the three highest discharge treatments as compared to lower discharges (Figure 2.6). Consequently, maximum production of AFDM during the experiment also increased with discharge (F=27.24, p<0.001) and was nearly 3-fold higher in the highest discharge treatment as compared to the lowest discharge (Figure 2.7a). Higher discharge treatments tended to accrue AFDM throughout the 28-day experiment whereas the two lowest treatments accumulated maximum AFDM after approximately 3 weeks (Figure 2.7b); however, these differences were not significant (F=1.751, p=0.215).

Accumulation of chl a followed a different pattern from that exhibited by AFDM as concentrations tended to be greater in lower discharge treatments as the experiment progressed (Figure 2.8). Regression analysis demonstrated a significant linear relationship between chl a and time for all the discharge treatments, with the exception of the highest (H) discharge treatment which demonstrated a quadratic relationship (Figure 2.9). Initial growth rates (linear coefficient) were similar between the two highest and lowest treatments (Figure 2.10), but the H treatment experienced a significant downward trend (quadratic coefficient) later in the experiment. Maximum chl *a* concentration varied significantly among discharge treatments (F=9.73, p=0.002) and was significantly greater in the medium-low discharge treatment as compared to higher discharges (Figure 2.11). Lower discharge treatments tended to reach maximum chl a concentrations later in the experiment, but day of maximum biomass did not differ significantly among treatments (F=1.45, p=0.288, Figure 2.11).

The autotrophic index increased with increasing discharge. The two lowest discharge treatments maintained an AI near 250:1 throughout the experiment whereas the AI in higher discharge treatments tended to increase during the experiment and reached a ratio near 2000:1 in the H discharge treatment by the end of the experiment (Figure 2.12). The AI was significantly different among treatments from day 21 onward ($F \geq 7.263$, $p \leq 0.005$). The AI in the two highest discharge treatments reached significantly higher levels than the low (L) and medium-low (ML) discharge treatments on days 21, 25, and 28 ($p<0.050$) and exceeded that of the medium discharge on days 25 and 28 ($p<0.050$).

Total pigment concentrations exhibited different accumulation patterns among discharge treatments (Figure 2.13). Concentration increases were initially faster in the higher discharge treatments as indicated by a significant difference among treatments on day 4 (F=3.696, p=0.043), but lower discharges achieved higher concentrations by day 28 (F=5.053, p=0.017). Maximum total pigment concentrations were marginally higher at lower discharges than at highest discharges (F=3.276, p=0.058). Day of maximum pigment accumulation was significantly earlier for the highest discharge compared to lower discharges (F=9.39, p=0.002) (Figure 2.14). Chl a pigment composed the greater proportion of the total pigment concentrations ranging from 40 to 50% of the total pigment concentration. The results of the HPLC analysis were positively correlated with the chl a measurements attained by fluorometry (Figure 2.15).

Concentrations of diatom pigments (Figure 2.16) composed 25 to 35% of the total pigment concentrations and exhibited accumulation patterns similar to those for total pigment concentrations, i.e., lower discharge treatments having lower concentrations than the two highest treatments on day 4 (F=3.93, p=0.036) and marginally higher concentrations on day 28 (F=2.073, p=0.052). Maximum pigment concentrations did not differ significantly among treatments, but the day of maximum concentration occurred earlier at the H discharge treatment compared to the rest of the treatments (p=0.002, Figure 2.17). These patterns were consistent with observations of rapid diatom mat development followed by noticeable sloughing at high discharge and more gradual mat development at low discharges.

Concentrations of green algae pigments remained low compared to diatom pigments (<0.4 nmol/cm^2) in the three highest discharge treatments throughout the experiment but increased steadily at lower discharges and accounted for as much as .1% of total pigment concentration (Figure 2.18). Concentrations differed significantly among discharge treatments on day 18 (F=6.719, p=0.007) with treatment differences between the L and H treatment (p=0.034), ML and MH (p=0.044) and ML and H (p=0.006) and on day 28 (F=12.25, p<0.001). L and ML treatments were significantly higher than H discharge treatments (p<=.041). Maximum concentrations were significantly higher in the L discharge treatment as compared to the three highest treatments (F=6.575, p=0.007, Figure 2.19) and occurred at the end of the experiment as opposed to near the beginning at the H discharge (F=6.853, p=0.006). These patterns were consistent with the development of dense masses of filamentous algae (order Zygnematales) at the two lowest discharges as the experiment progressed.

Cyanobacterial pigments were a minor component (<0.01%) of total pigments throughout the experiment. Peak concentrations occurred on day 18 in most treatments and there were no significant differences in the maximum concentration or the day when this maximum occurred.

Experiment 2

Many of the results were comparable to those found in experiment 1. Nutrient concentrations were determined on day 24 to see if nutrient dissolution was occurring within the channels (Figure 2.20). Many of the results were comparable to those found in experiment 1. Accumulation of AFDM increased with discharge, particularly in treatments with nutrient additions (Figure 2.21). Accumulation curves in unenriched channels were best described by a quadratic polynomial equation for all discharge treatments except for the L treatment, which was best described by a linear model (Figure 2.22). This response pattern was consistent with diminished or negative accumulation rates in higher discharge treatments later in the experiment due to density-dependent reductions in cell division and/or increased sloughing as compared to slower but persistent accumulation at the L discharge. Accumulation patterns in P-enriched channels were best described by quadratic relationships for the three highest discharge treatments and linear relationships for the two lowest treatments. Linear relationships best described accumulation in all discharge treatments in channels enriched with N+P. The accumulation rate for the L discharge treatment was significantly lower than the other discharge treatments under unenriched conditions (Figure 2.22). Lower discharges had significantly lower accumulation rates compared to M, MH, and H discharge treatments for P enriched treatments. For N+P enrichment treatments, AFDM accumulation rates were significantly lower at L and ML discharge as compared to MH and H discharge (Figure 2.23). Maximum AFDM achieved during the experiment increased with discharge (F=5.962, p=0.016) and was nearly 4-fold higher in the two highest discharge treatment as compared to the L discharge (Figure 2.24). Maximum biomass accumulation did not differ significantly among nutrient treatments. Neither discharge nor nutrient enrichment significantly affected the time required to achieve maximum biomass.

Chl a concentration tended to decrease with increasing discharge as the experiment progressed (Figure 2.25). Accumulation patterns were linear for all unenriched channels except for the H discharge channel, which was best described by a quadratic model (Figure 2.26). Channels with P and N+P additions were best described by linear models for all discharge treatments. Accumulation in unenriched channels was similar across all discharge treatments (Figure 2.27). For P additions, the L discharge treatment was significantly higher than the three highest discharge channels, and a similar but nonsignificant. There was no difference in growth rates from L to H discharge channels. There was no difference in maximum chl a concentrations across either discharge or nutrient treatments (Figure 2.28) nor were there differences in the day when maximum concentrations were achieved (Figure 2.29).

The autotrophic index tended to increase with increasing discharge, as with experiment 1. The two lowest discharge treatments maintained an AI below a 1000:1 ratio, while the higher discharges remained above this ratio and were highly variable. From day 18 onward, higher discharge exhibited higher AI ratios than the L and ML flow treatments (p>.05).

Total pigment concentrations and diatom and green algal pigments exhibited different accumulation patterns among discharge treatments (Figure 2.30). On day 4, there were significant differences in total pigment concentrations across discharge treatments (F=9.888, p=0.003), and the L discharge was significantly lower than both M (p=0.003) and MH (p=0.038). Discharge also positively influenced diatom pigment concentration at the start of the experiment (F=10.068, p=.003) with significant differences between M and L (p=0.002) and M and H (p=0.024) discharges. There were significant differences across discharge treatments on green algae pigment accumulation (F=6.123, p=0.015) with L and H both being significantly lower than M (p=0.024, p=0.029) on the first sampling day. Pigment concentrations did not vary significantly among discharge or nutrient treatments on the final day of sampling. L discharge channels tended to have the highest pigment concentrations, but this pattern was not significant (F=.273, p=0.887).

Discussion

Changes in stream discharge exert multiple influences on periphyton growth, including both stimulatory effects on cell metabolism and negative effects related to greater shear stress (Stevenson 1996, Flinders & Hart 2009). These contrasting influences complicate predictions of how periphyton accumulation and biomass will respond to changes in flow regimes. For our experiment, we explored periphyton responses to a range of lower flow conditions, simulating lower flows observed during the summer months in streams in this region. We focused on periphyton responses to a 20-fold range of discharge treatments and found complex responses.

In our experimental channels, we observed faster accumulation of AFDM but not chl a with increasing discharge. High discharge can stimulate algal accumulation by allowing for increased uptake of CO_2 and nutrients through the reduction of the diffusion boundary layer (Larned et al. 2004). Rapid initial growth in higher discharge treatments can, however, be countered by greater potential for sloughing as biomass accumulated and aged (Ahn et al. 2013). Ultimately, there can be a greater potential for standing crop at lower flows despite slower accumulation rates. This process was observed in our experiment. Initially, the highest accumulation rate of chl a occurred in the highest discharge treatment. However, as the experiment progressed, the low discharge accumulated more chl a than higher discharge treatments, apparently due to increased growth of green algae in lower discharge channels. AI ratios we observed also indicated more algal biomass at the end of sampling for lower flow treatments. The lack of increase in chl a concentrations could be attributed to large amounts of extracellular mucilage in higher discharge channels as compared to lower discharge treatments (Biggs, Goring, & Nikora 1998). Taxonomic differences across our discharge treatments, as indicated by pigment composition, were consistent with observed spatial variation in periphyton assemblages across different flow conditions within the adjacent stream during the experimental period (D.Diaz, personal observation). Other studies have also noted that velocity gradients within a stream can produce periphyton patches of varying density and taxonomic (e.g., Hart et al. 2013).

Growth rates and biomass of major algal groups can respond differently to changes in discharge (Stevenson et al. 2006), which, in turn, influences algal community structure (Peterson & Stevenson 1990). We observed that discharge affected taxonomic composition as higher discharge treatments maintained diatom dominance while lower discharge treatments tended towards green algal dominance. We attribute diatom dominance at higher discharges to increased algal metabolic rates in higher flow conditions where the diffusive boundary layer is thinner. This increased metabolic rate can promote greater nutrient uptake and disposal of cellular waste, allowing for greater growth potential (Musielak et al. 2009). Additionally, in-stream habitats with higher velocities tend to be dominated by mat-forming algal species, such as diatoms. Lower discharge favored dominance of filamentous green algae of the order Zygnematales. Other studies (e.g., Peterson & Stevenson 1992, Hart et al. 2013) also have shown that filamentous green algal dominance is a normal outcome of periphtyon succession at lower currents but not at high current since these taxa do not possess holdfast structures or other means of firmly attaching to the substrate. At the end of the experiment, total pigment concentrations were greatest in lower discharge treatments. Higher discharges had greater biomass accrual than lower discharges, but had lower chl a concentrations leading to higher autotrophic index values. This result may be attributed to decreased pigment levels per algal cell when cell density increases in the higher flows (Pinto et al. 2003).

Contrary to our prediction, nutrient enrichment had the greatest influence on periphyton accumulation at higher discharges. Response to nutrient enrichment across flow regimes suggests that current velocity rather than overlying (water-column) nutrient concentrations mediate nutrient supply rates to the periphyton mat (Royer et al. 2006). The highest AFDM peak biomass overall was reached in treatments of combined N+P additions in high flow treatments. Greater discharge, which generated more turbulent flow conditions that would facilitate nutrient transfer to algal cells through a decrease in the diffusive boundary layer, coupled with higher concentrations of nutrients at higher flows could provide an ideal scenario for algal growth (Larned et al. 2004).

This research provides greater insight into how alterations to summertime flow regimes affect periphyton ecological conditions within the LFRB. This research shows that flow alterations affect algal resources by changing periphyton abundance and composition. These changes are known to influence stream food webs and nutrient cycling (Finlay et al. 1999, Wellnitz & Poff 2012, Jardine et al. 2013). Our study highlights the significance of flow and nutrient effects on algal biomass, particularly in agricultural areas where present and future anthropogenic modifications can greatly alter lotic systems (Poff et al.1997, Smith and Schindler 2009).

References:

Ahn, C. H., Song, H. M., Lee, S., Oh, J. H., Ahn, H., Park, J. R. & Joo, J. C. (2013) Effects of water velocity and specific surface area on filamentous periphyton biomass in an artificial stream mesocosm. Water, 5, 1723-1740.

American Public Health Association, American Water Works Association, and the Water Environment Federation (2012) Standard methods for the examination of water and wastewater. American Public Health Association,Washington,D.C.

Arar, E. J., & Collins, G. B. (1997) Method 445.0: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence. United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory

Arthington A. (2012) Environmental flows. University of California Press, Berkeley, CA.

Leavitt, P. R., & Hodgson, D. A. (2001) Sedimentary pigments. In Tracking environmental change using lake sediments (pp. 295-325). Springer Netherlands

Biggs (2000) Eutrophication of streams and rivers: Dissolved nutrient-chlorophyll relationships for periphyton. J. North Am. Benthological Society. 19: 17–31

Biggs & Close (1989). Periphyton biomass dynamics in gravel bed rivers: The relative effects of flows and nutrients. Freshwater. Biology. 22: 209–231

Biggs, B. J., Goring, D. G., & Nikora, V. I. (1998). Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology, 34, 598-607.

Biggs, B. J. (2000) Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of the North American Benthological Society, 19, 17-31

Biggs, B. J. F., Nikora, V. I. & Snelder, T. H. (2005) Linking scales of flow variability to lotic ecosystem structure and function. River Resource Applications. 21: 283– 298

Davies, P. M., Naiman, R. J., Warfe, D. M., Pettit, N. E., Arthington, A. H., & Bunn, S. E. (2014). Flow–ecology relationships: closing the loop on effective environmental flows. Marine and Freshwater Research, 65, 133-141.

Dodds, W. K., & Welch, E. B. (2000) Establishing nutrient criteria in streams. Journal of the North American Benthological Society, 19, 186-196

Dodds, W. K., Smith, V. H., & Lohman, K. (2002) Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences, 59, 865-874

Feio, M. J., Alves, T., Boavida, M., Medeiros, A., & Graça, M. A. S. (2010). Functional indicators of stream health: a river-basin approach. Freshwater Biology, 55, 1050-1065.

Finlay, J. C., Power, M. E., & Cabana, G. (1999). Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. Limnology and Oceanography, 44, 1198-1203.

Flinders, C. A., & Hart, D. D. (2009) Effects of pulsed flows on nuisance periphyton growths in rivers: a mesocosm study. River Research and Applications, 25, 1320-1330

Hart, D. D., Biggs, B. J., Nikora, V. I., & Flinders, C. A. (2013) Flow effects on periphyton patches and their ecological consequences in a New Zealand river. Freshwater Biology, 58, 1588-1602

Hopkinson, C.S., A.P. Covich, C.B. Craft, T.W. Doyle, N. Flanagan, M. Freeman, E.R. Herbert, A. Mehring, J.E. Mohan, C.M. Pringle, C. Richardson. (2013) The effects of climate change on natural ecosystems of the southeastern United States. In: Climate of the Southeast United States in the National Climate Assessment Regional Technical Input Series (ed. by K.T. Ingram, K. Dow, L. Carter, and J. Anderson). Island Press,Washington, D.C.

Jardine, T. D., Hunt, R. J., Faggotter, S. J., Valdez, D., Burford, M. A., & Bunn, S. E. (2013) Carbon from periphyton supports fish biomass in waterholes of a wet–dry tropical river. River Research and Applications, 29, 560-573

Larned, S. T. (2010). A prospectus for periphyton: Recent and future ecological research. Journal of the North American Benthological Society 29:182-206.

Larned, S. T., Nikora, V. I., & Biggs, B. J. (2004) Mass-transfer-limited nitrogen and phosphorus uptake by stream periphyton: A conceptual model and experimental evidence. Limnology and Oceanography, 49, 1992-2000.

Lewis, J. R., William, M., McCutchan, J. R., & James, H. (2010) Ecological responses to nutrients in streams and rivers of the Colorado mountains and foothills. Freshwater Biology, 55, 1973-1983

McKissick J.C. (2004) The Economic Importance of Irrigated Food and Fiber Production: A Spotlight on Georgia’s Flint River Basin. The University of Georgia Center for Agribusiness and Economic Development, Athens, GA

Musielak, M. M., Karp-Boss, L., Jumars, P. A., & Fauci, L. J. (2009) Nutrient transport and acquisition by diatom chains in a moving fluid. Journal of Fluid Mechanics, 638, 401-421

Peterson, C. G., & Stevenson, R. J. (1990). Post-spate development of epilithic algal communities in different current environments. Canadian Journal of Botany, 68, 2092-2102.

Peterson, C. G., & Stevenson, R. J. (1992) Resistance and resilience of lotic algal communities: importance of disturbance timing and current. Ecology, 73, 1445-1461.

Pinto, E., Van Nieuwerburgh, L., de Barros, M. P., Pedersén, M., Colepicolo, P., & Snoeijs, P. (2003). Density-dependent patterns of thiamine and pigment production in the diatom Nitzschia microcephala. Phytochemistry, 63, 155-163

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., & Stromberg, J. C. (1997) The natural flow regime. BioScience, 47, 769-784

Poff, N. L., B. D. Richter, A. H. Arthington, S. E. Bunn, R. J. Naiman, E. Kendy, M. Acreman, C. Apse, B. P. Bledsoe, M. C. Freeman, J. Henriksen, R. B. Jacobson, J. G. Kennen, D. M. Merritt, J. H. OaKeeffe, J. D. Olden, K. Rogers, R. E. Tharme, and A. Warner. 2010. The ecological limits of hydrologic alteration (ELOHA): a new framework for developing regional environmental flow standards. Freshwater Biology 55, 147-170.

Riseng, C. M., Wiley, M. J., & Stevenson, R. J. (2004) Hydrologic disturbance and nutrient effects on benthic community structure in midwestern US streams: a covariance structure analysis. Journal of the North American Benthological Society, 23, 309-326

Royer, T. V., David, M. B., & Gentry, L. E. (2006) Timing of riverine export of nitrate and phosphorus from agricultural watersheds in Illinois: Implications for reducing nutrient loading to the Mississippi River. Environmental Science & Technology, 40, 4126-4131

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/

Rugel, K., C. R. Jackson, J. J. Romeis, S. W. Golladay, D. W. Hicks, and J. F. Dowd. (2012) Effects of irrigation withdrawals on streamflows in a karst environment: lower Flint River Basin, Georgia, USA. Hydrological Processes 26:523-534

Seager, R., A. Tzanova, & J. Nakamura. (2009) Drought in the southeastern United States: causes, variability over the last millennium, and the potential for future hydroclimate change. Journal of Climate, 22, 5021-5045

Smith, V. H., & Schindler, D. W. (2009) Eutrophication science: where do we go from here? Trends in Ecology & Evolution, 24, 201-207

Stevenson, R. J., Bothwell, M. L., Lowe, R. L., & Thorp, J. H. (1996) Algal ecology: Freshwater benthic ecosystem. Academic Press, San Diego.

Stevenson, R. J., Rier, S. T., Riseng, C. M., Schultz, R. E., & Wiley, M. J. (2006) Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia, 561, 149-165

Suren, A. M., Biggs, B. J., Duncan, M. J., Bergey, L., & Lambert, P. (2003) Benthic community dynamics during summer low flows in two rivers of contrasting enrichment 2. Invertebrates. New Zealand journal of marine and freshwater research, 37, 71-83

Torak L.J., Davis G.S., Strain G.A. and Herndon J.G. (1996) Geohydrology and Evaluation of Stream-Aquifer Relations in the Apalachicola-Chattahoochee-Flint River Basin, Southeastern Alabama, Northeastern Florida, and Southwestern Georgia.U.S. Geological Survey, Water-Supply Paper 2460, Denver, CO

Viger, R. J., Hay, L. E., Markstrom, S. L., Jones, J. W., & Buell, G. R. (2011) Hydrologic effects of urbanization and climate change on the Flint River basin, Georgia. Earth Interactions, 15, 1-25

Wellnitz, T., & Poff, N. L. (2012). Current-mediated periphytic structure modifies grazer interactions and algal removal. Aquatic Ecology, 46, 521-530.

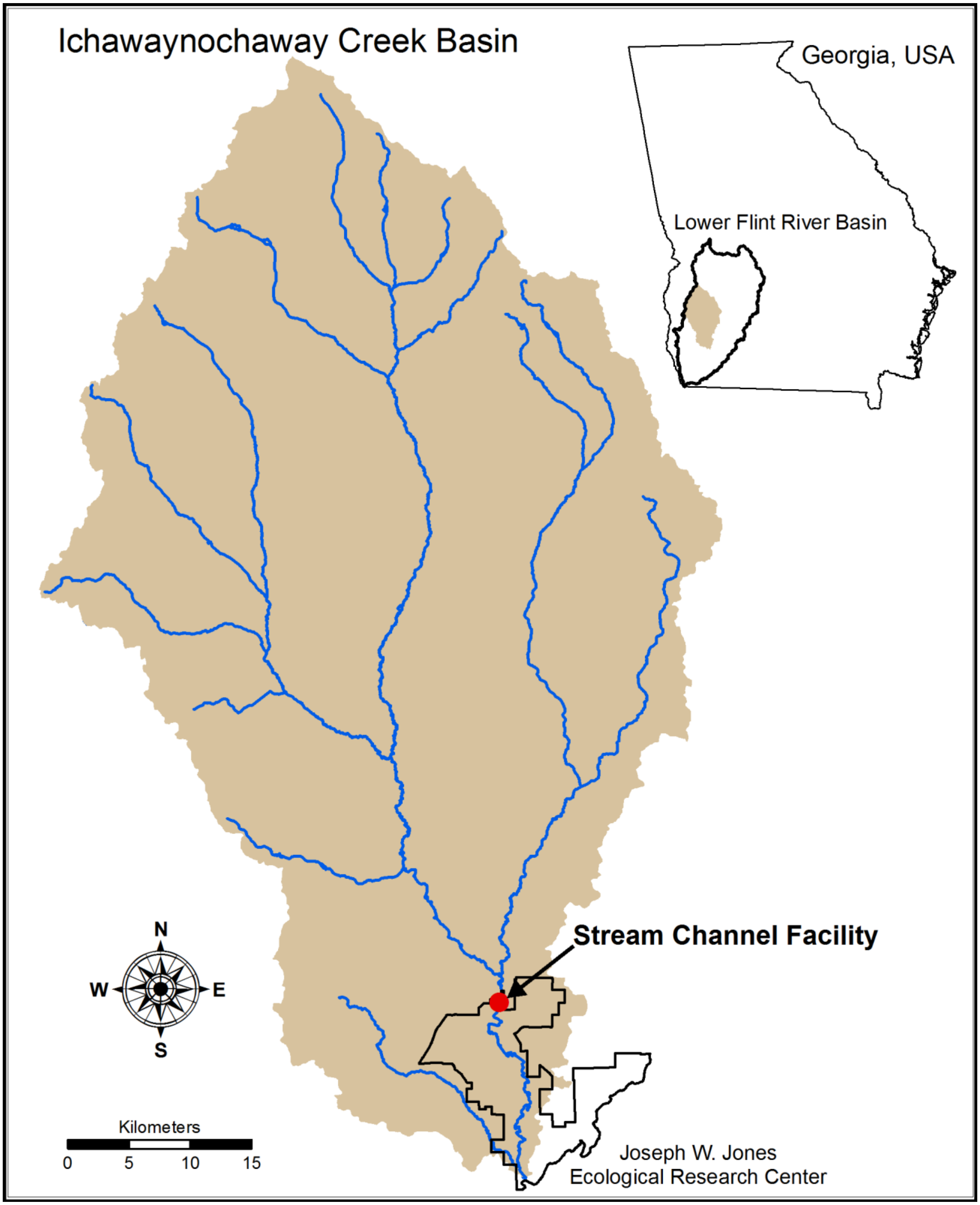


Figure 2.1: Map of study site: Lower Flint River Basin and Ichawaynochaway Creek basin in southwest Georgia, USA.

Figure 2.2: Artificial channel system with clear plastic roofing. Water was pumped from the Ichawaynochaway creek and flow treatments were controlled via spigots.

Figure 2.3: Artificial stream channels (width = 6 inches, length = 10 ft) on top of a flow-through water bath.

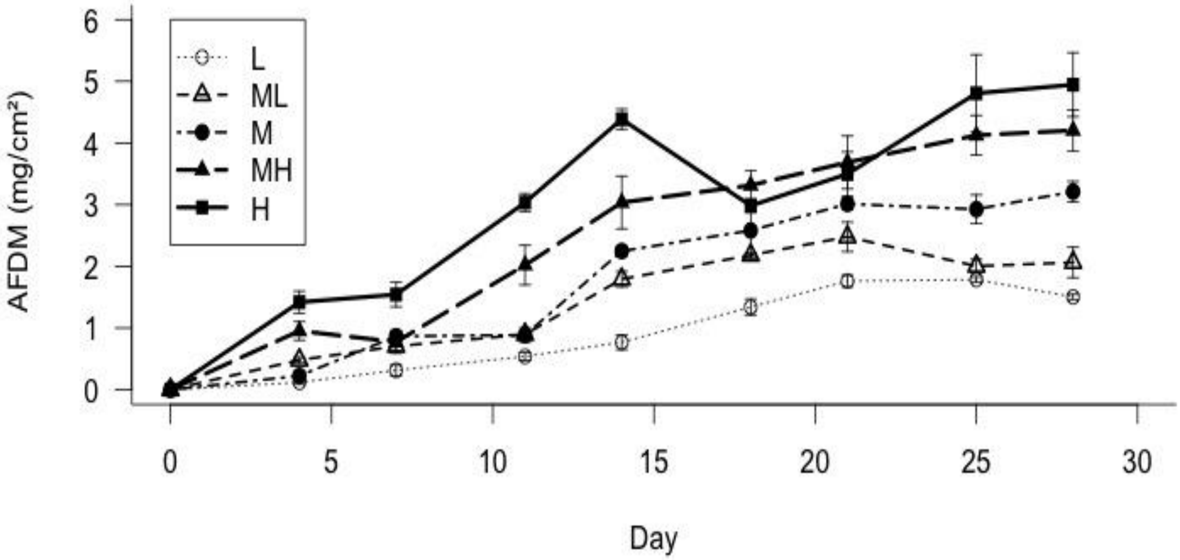


Figure 2.4: Accumulation of AFDM in different discharge treatments (see methods for treatment descriptions). Points are means of 3 replicate channels ± 1 SE.

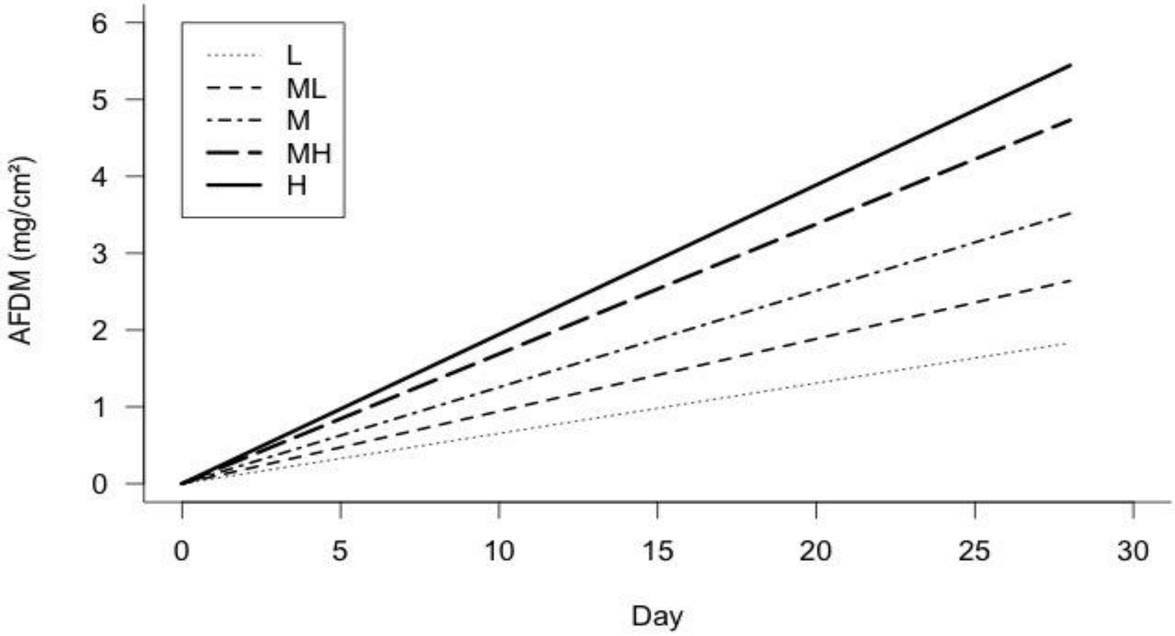


Figure 2.5: Regression models for AFDM accumulation for each discharge treatment

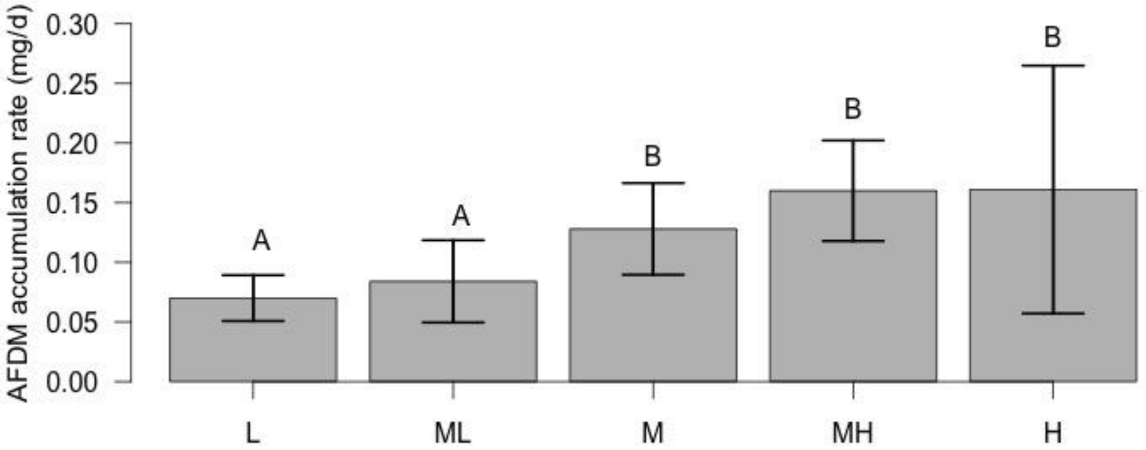


Figure 2.6: Mean AFDM accumulation rates in each discharge treatment. Bars are means of regression slopes for 3 replicate channels± 95% CI. Discharge treatments with the same letters are not statistically different.

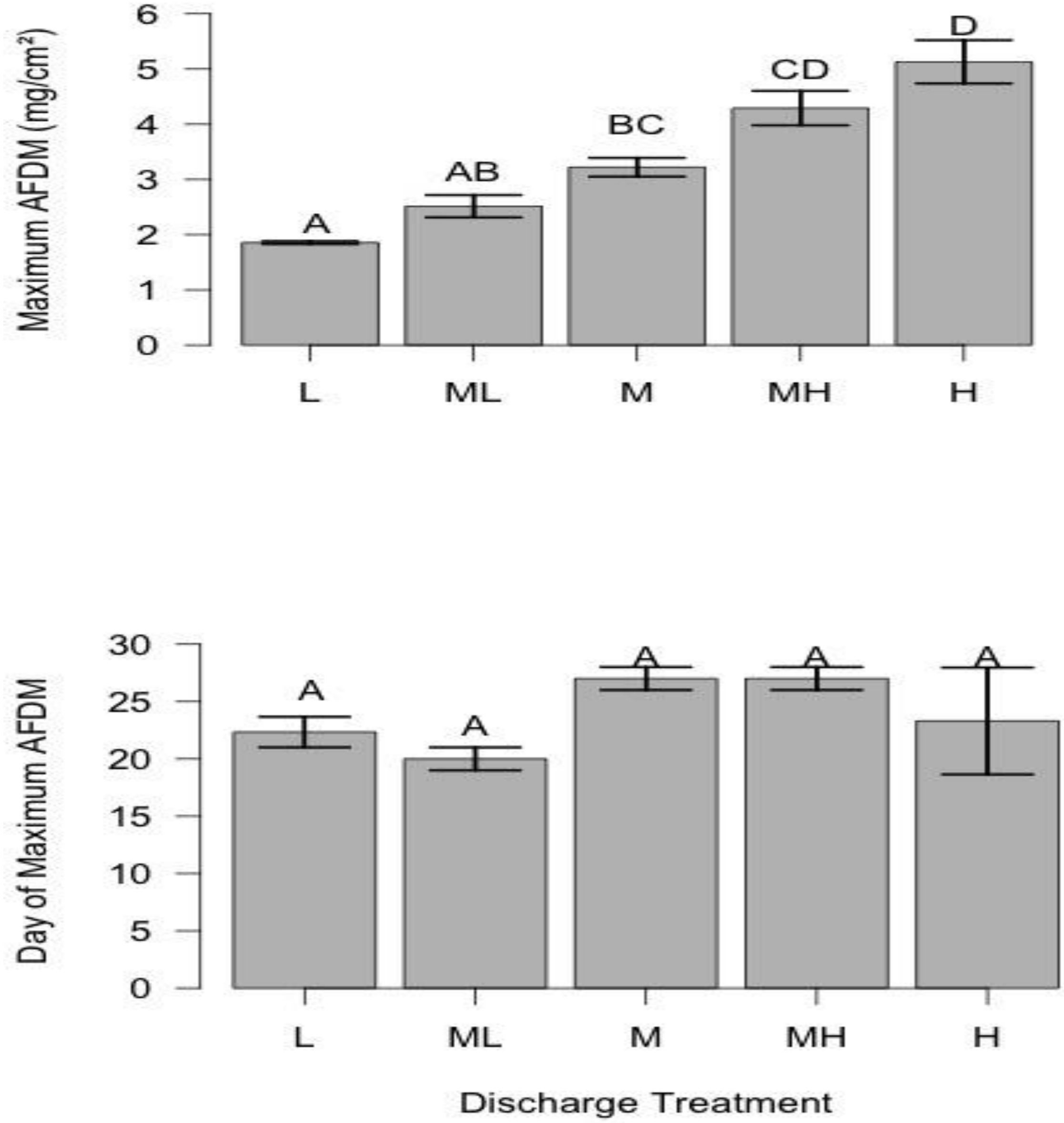


Figure 2.7: Maximum AFDM achieved in each discharge treatment and day this maximum was achieved. Bars are means for 3 replicate channels ± 1 SE. Discharge treatments with the same letters are not statistically different.

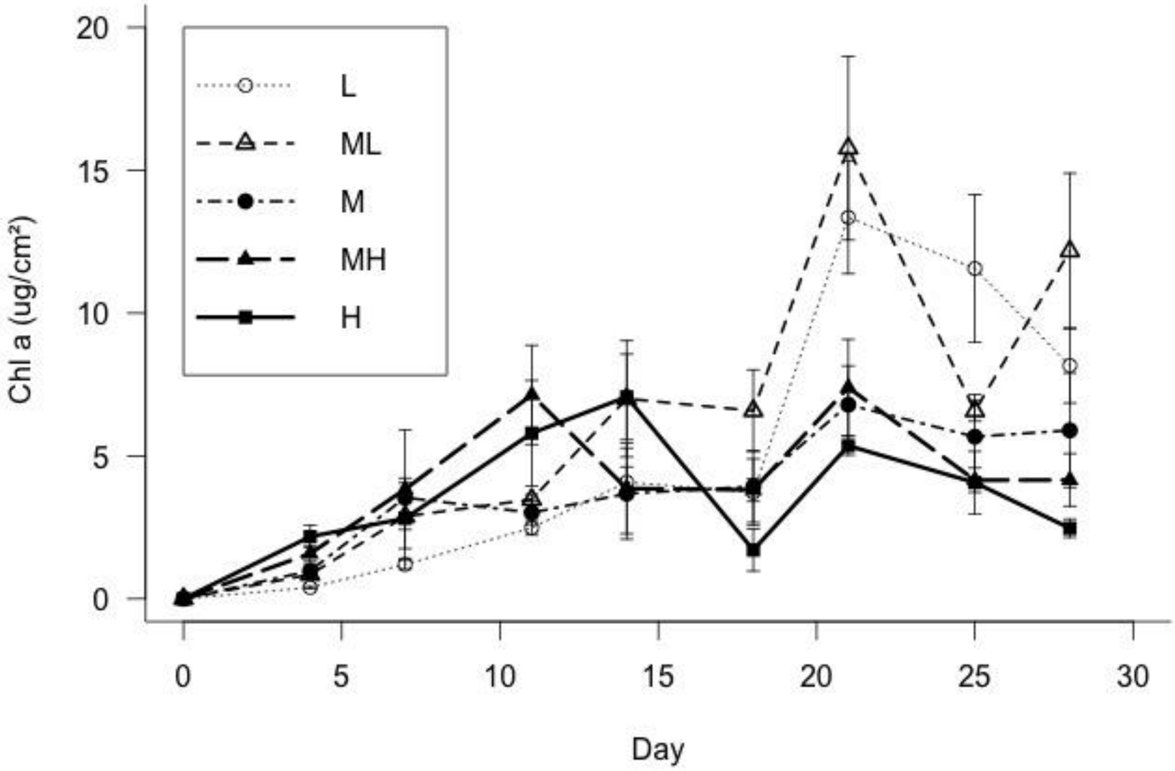


Figure 2.8: Mean chl a for different discharge treatments. Points are means of 3 replicate channels ± 1 SE.

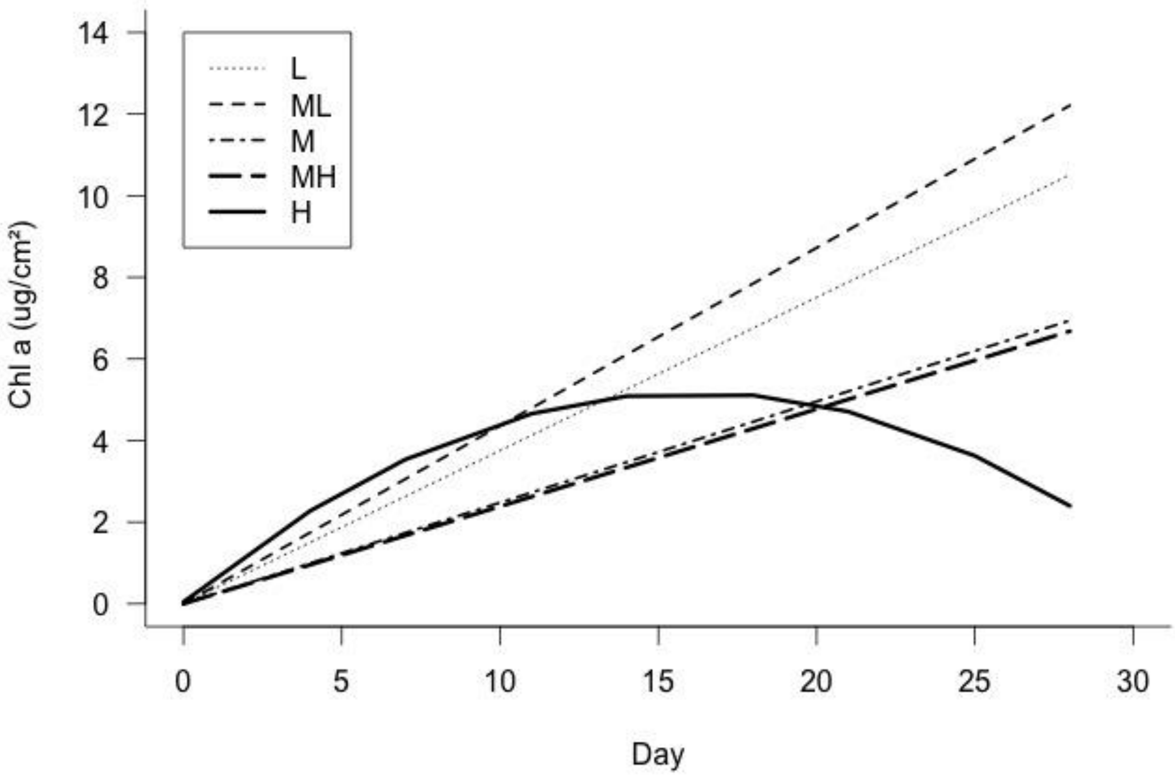


Figure 2.9: Regressions models for chl a accumulation in each discharge treatment.

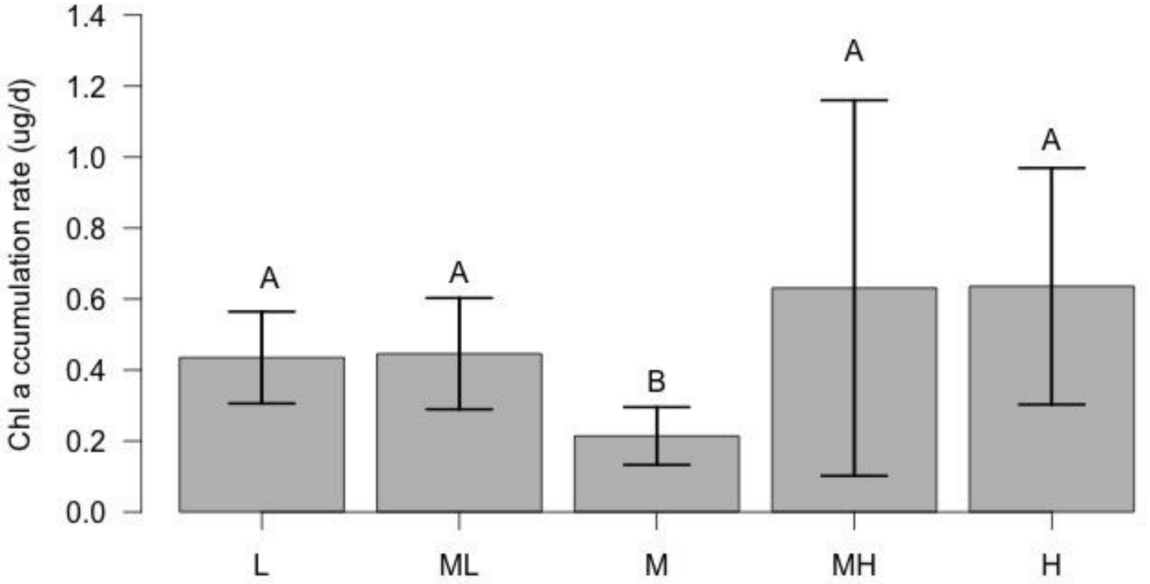


Figure 2.10: Mean chl a accumulation rates for discharge treatments. Bars are means of regression slopes for 3 replicate channels ± 95% CI.

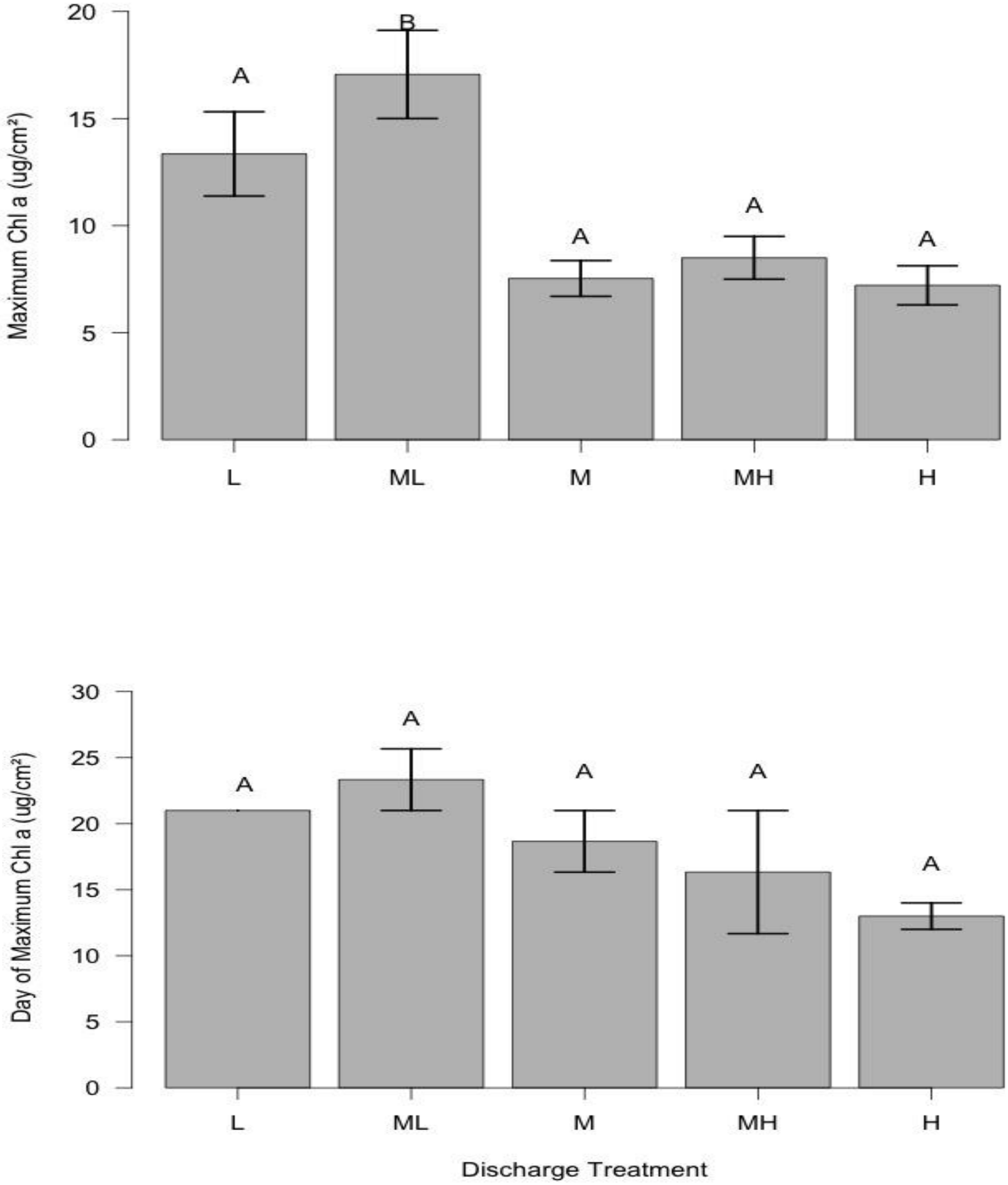


Figure 2.11: Maximum chl a concentration (ug/cm^2) and day of maximum accumulation. Bars are means for 3 replicate channels ± 1 SE. Discharge treatments with the same letter are not statistically different.

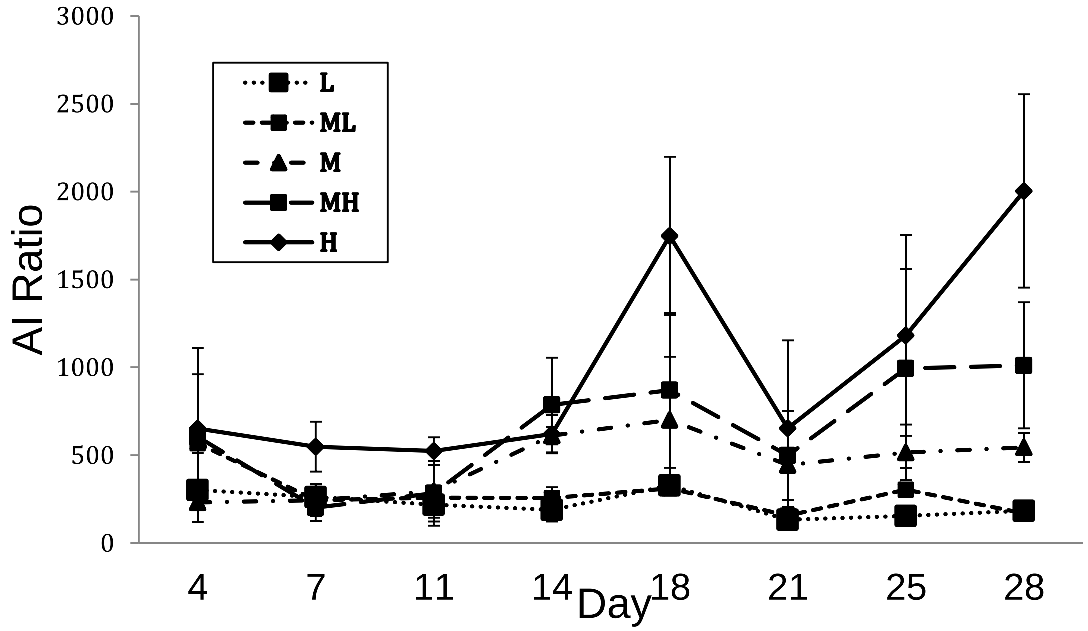


Figure 2.12: Mean AI ratios for different discharge treatments.

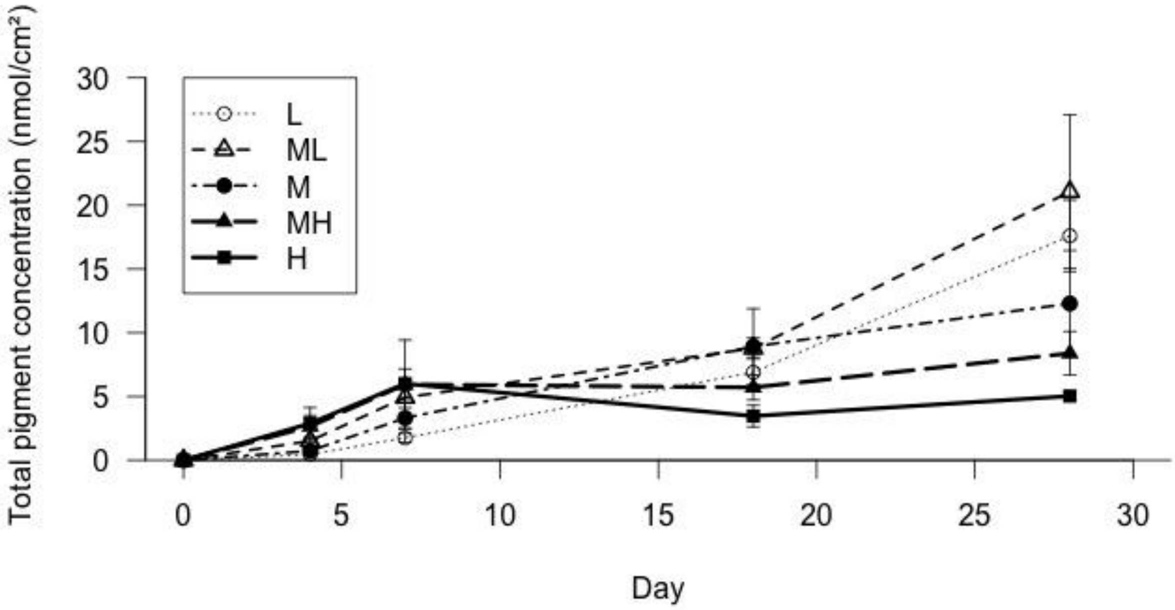


Figure 2.13: Mean total pigment concentrations. Points are means of 3 replicate channels ± 1 SE

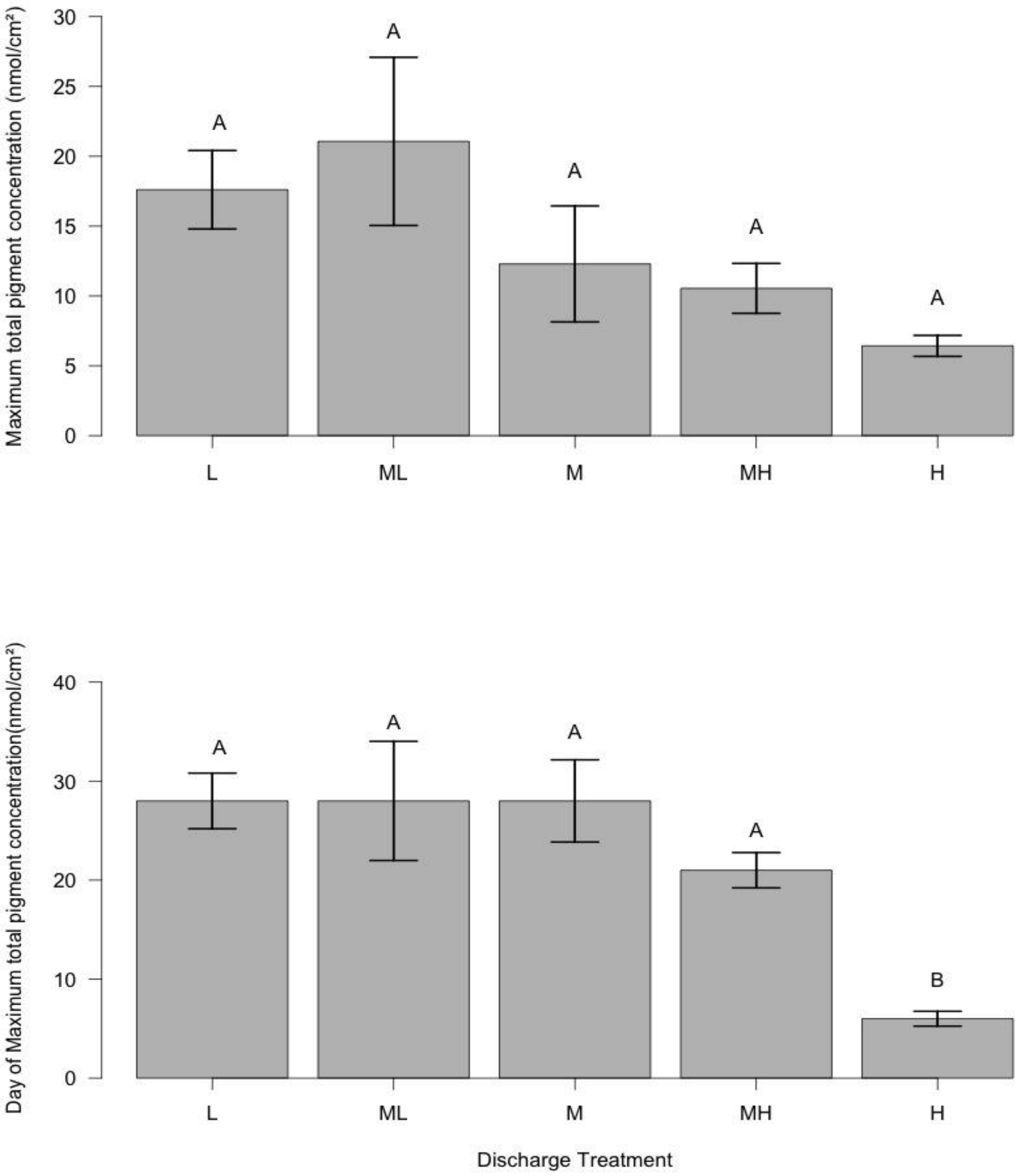


Figure 2.14: Maximum total pigment concentration (nmol/cm^2) and day of maximum accumulation. Bars are means for 3 replicate channels ± 1 SE. Discharge treatments with the same letter are not statistically different.

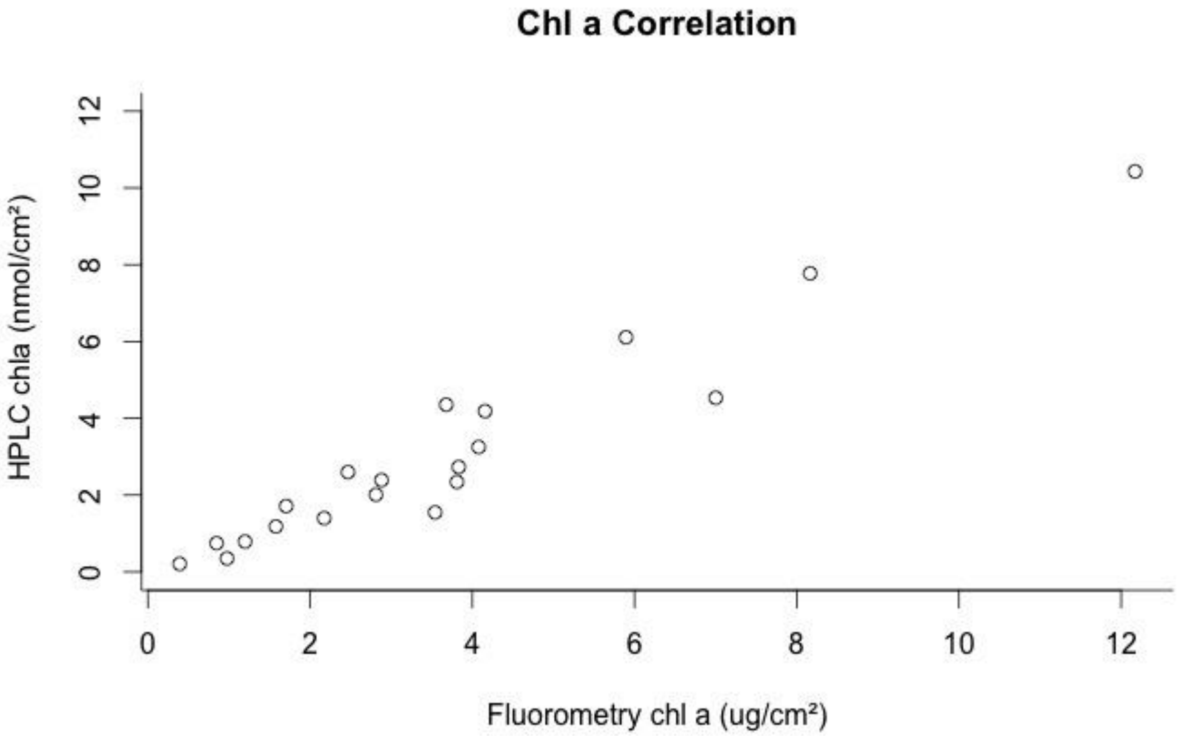


Figure 2.15: Correlation between chl a measured by fluorometry and by HPLC.

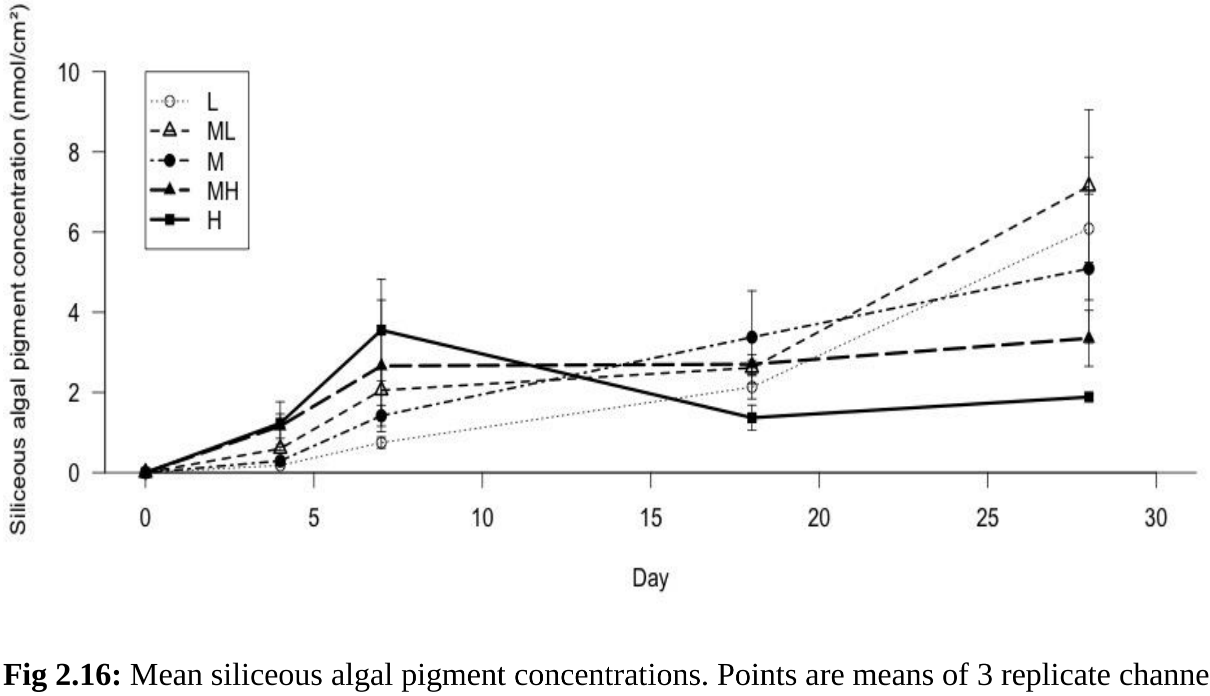


Fig 2.16: Mean siliceous algal pigment concentrations. Points are means of 3 replicate channels ± 1 SE

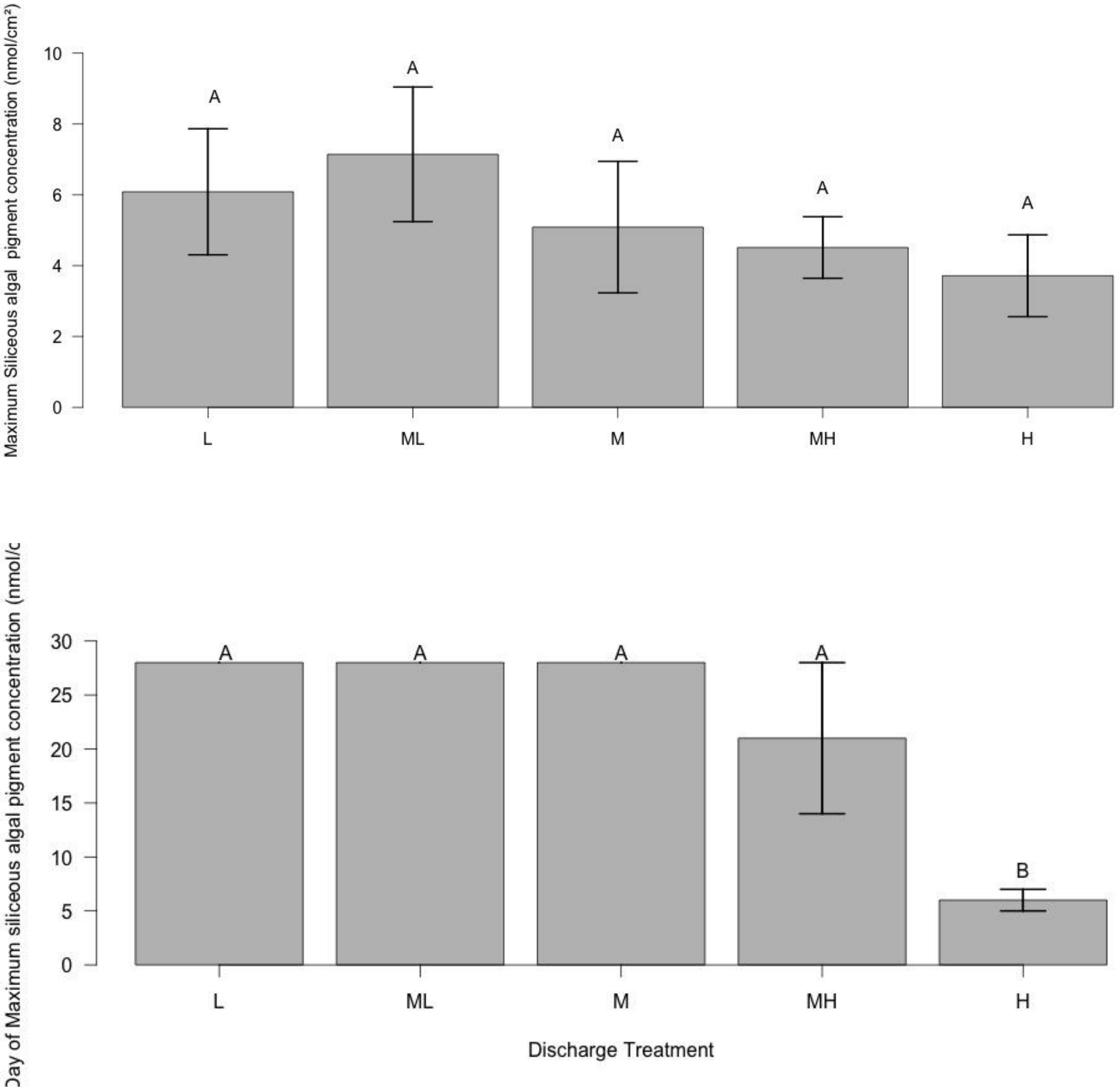


Figure 2.17: Maximum siliceous algal pigment concentrations (nmol/cm^2) and day of maximum accumulation. Bars are means for 3 replicate channels ± 1 SE. Discharge treatments with the same letters are not statistically different.

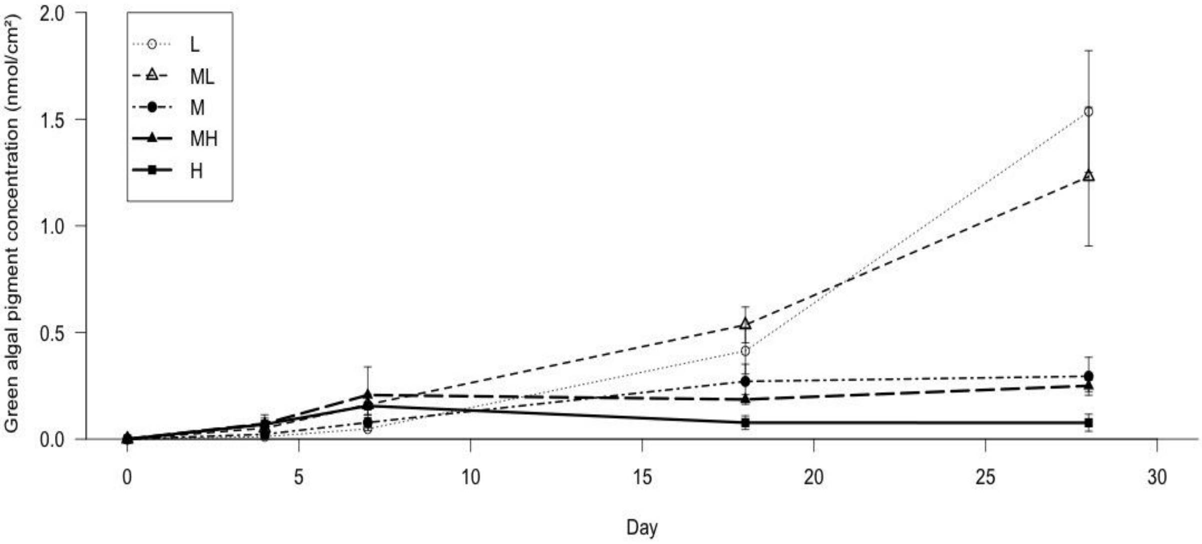


Fig 2.18: Mean green algal pigment concentrations over a 28-day experiment. Points are means of 3 replicate channels ± 1 SE

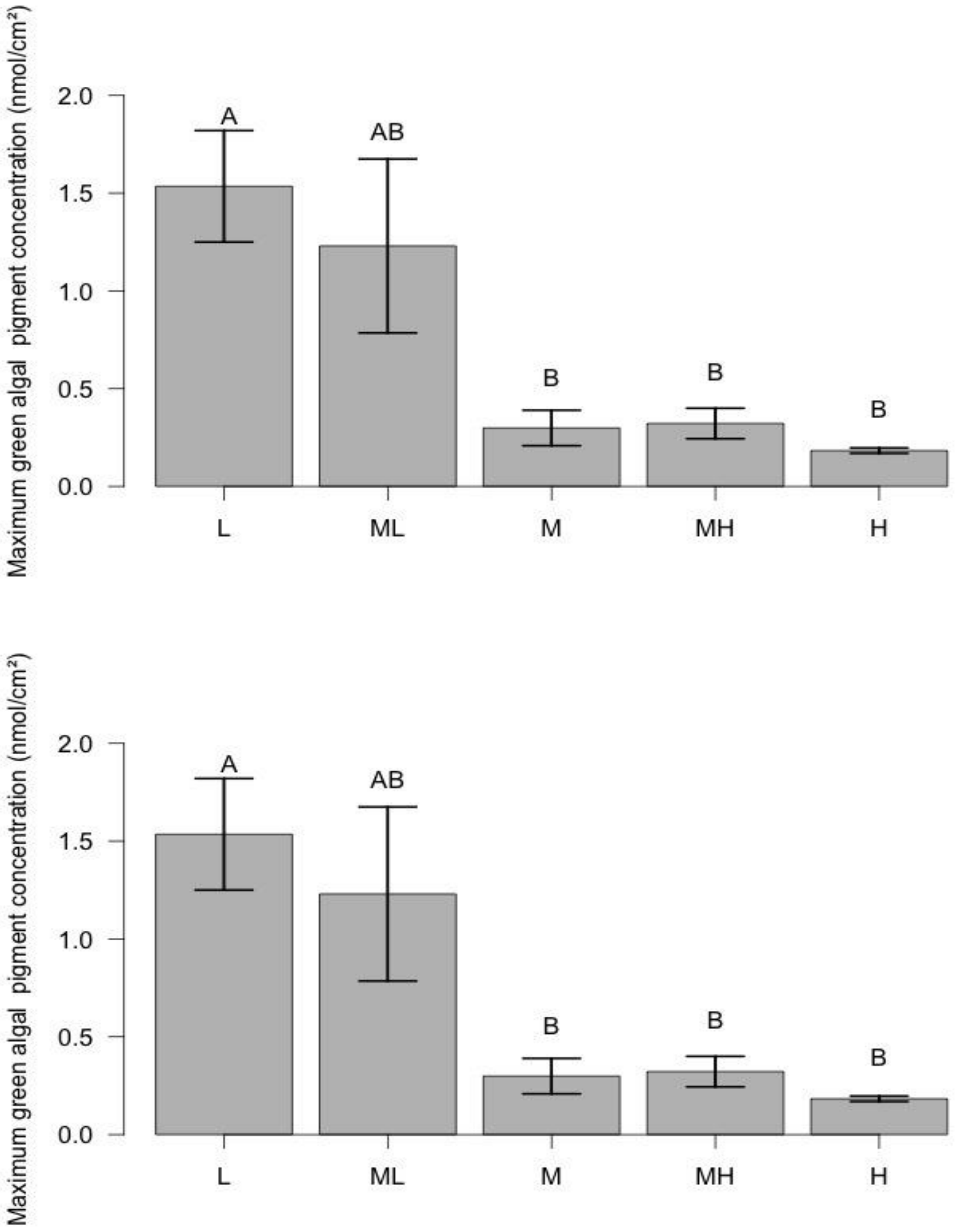


Figure 2.19: Maximum green algal pigment concentration (nmol/cm^2) and day of maximum accumulation. Bars are means for 3 replicate channels ± 1 SE. Discharge treatments with the same letters are not statistically different.

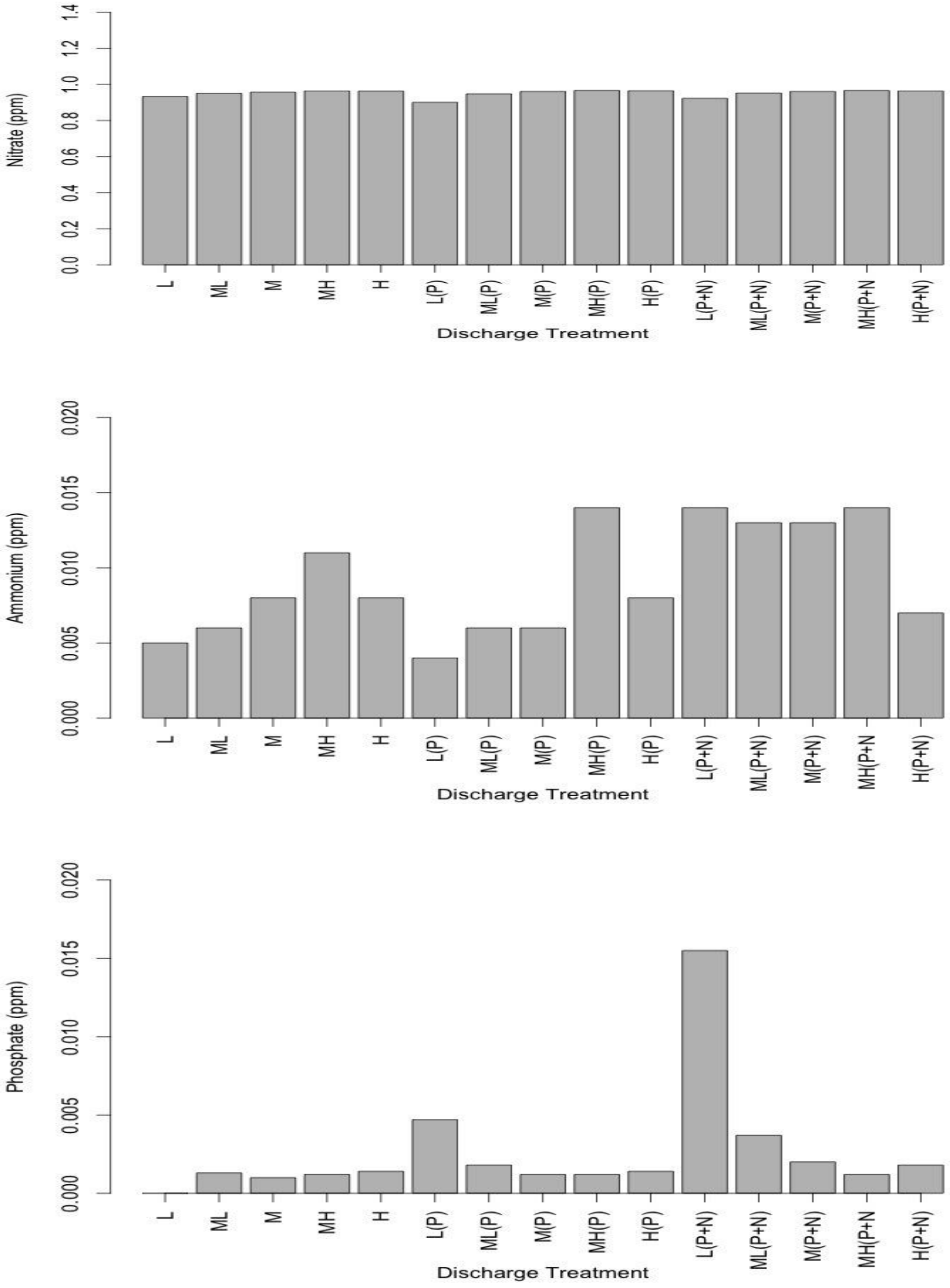
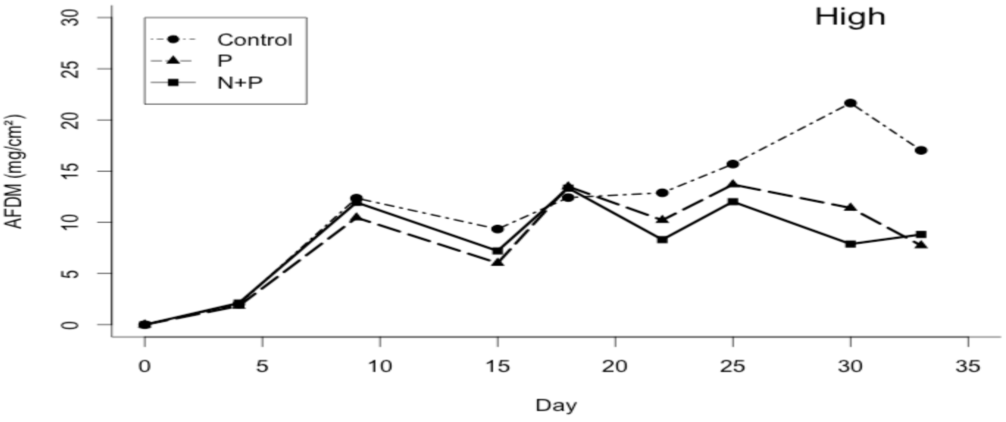
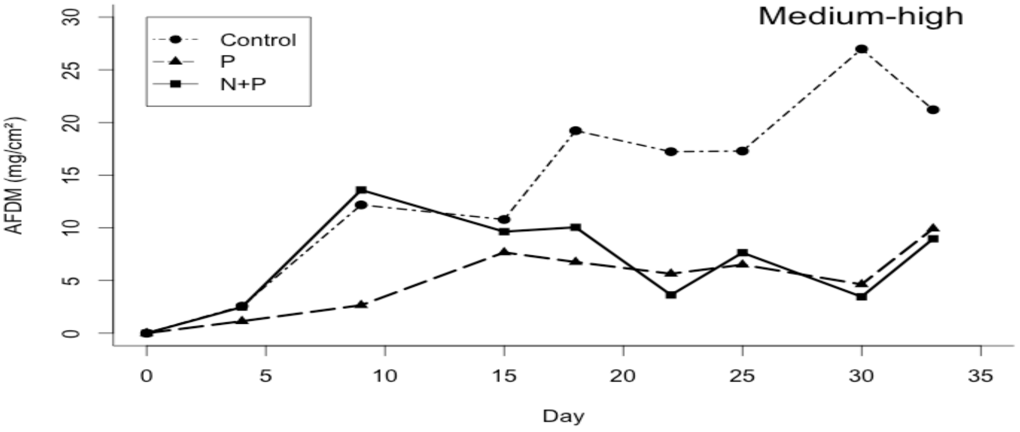


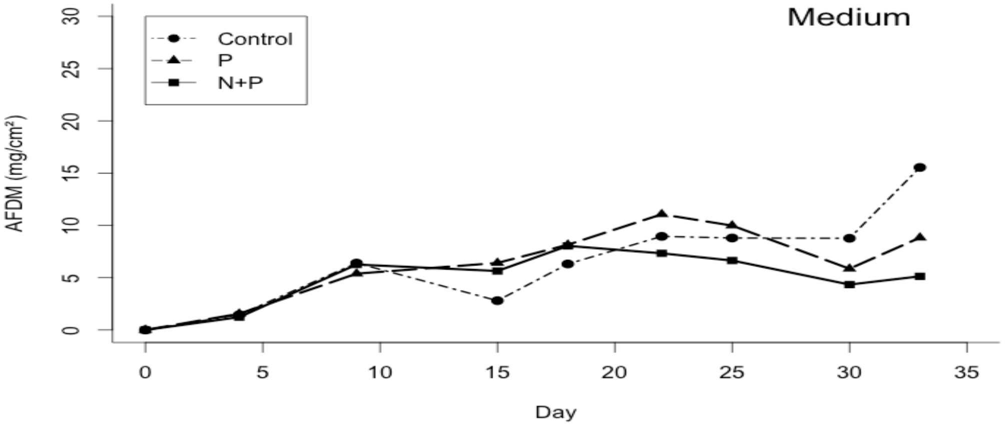
Figure 2.20: Nutrient concentrations for day 24 of sampling.



High
Control
P
N+P
AFDM (mg/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day



Medium-high
Control
P
N+P
AFDM (mg/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day



Medium
Control
P
N+P
AFDM (mg/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day

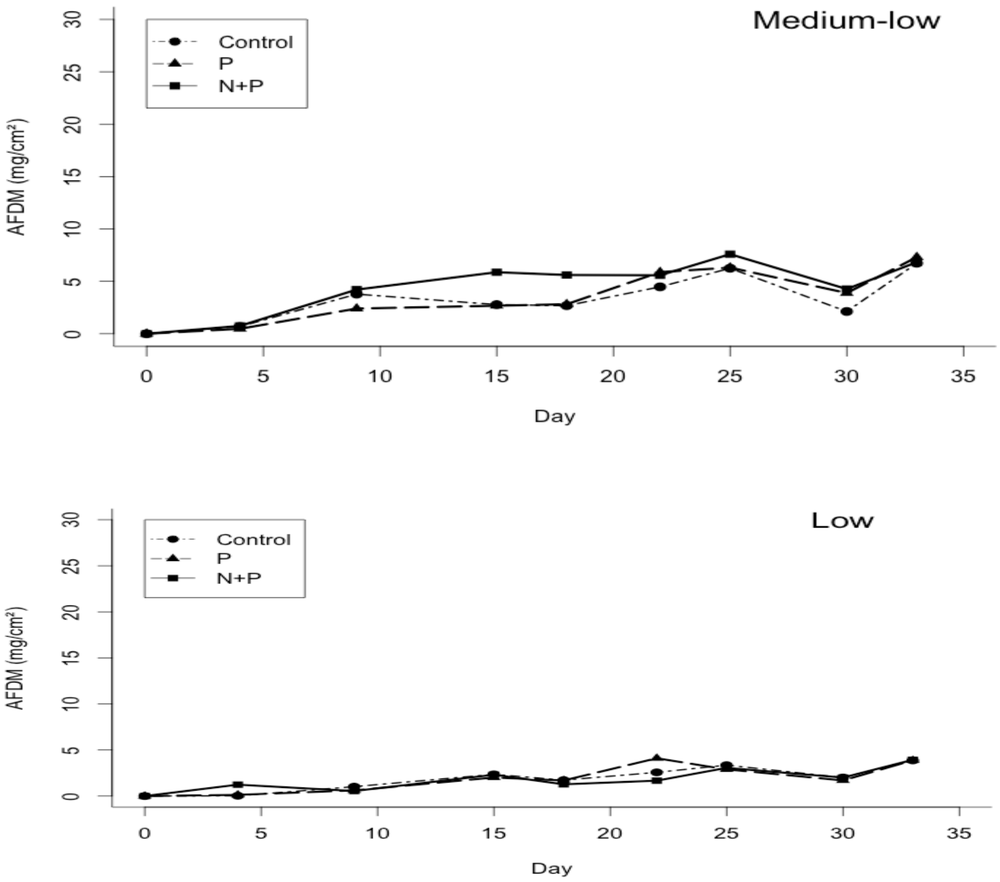
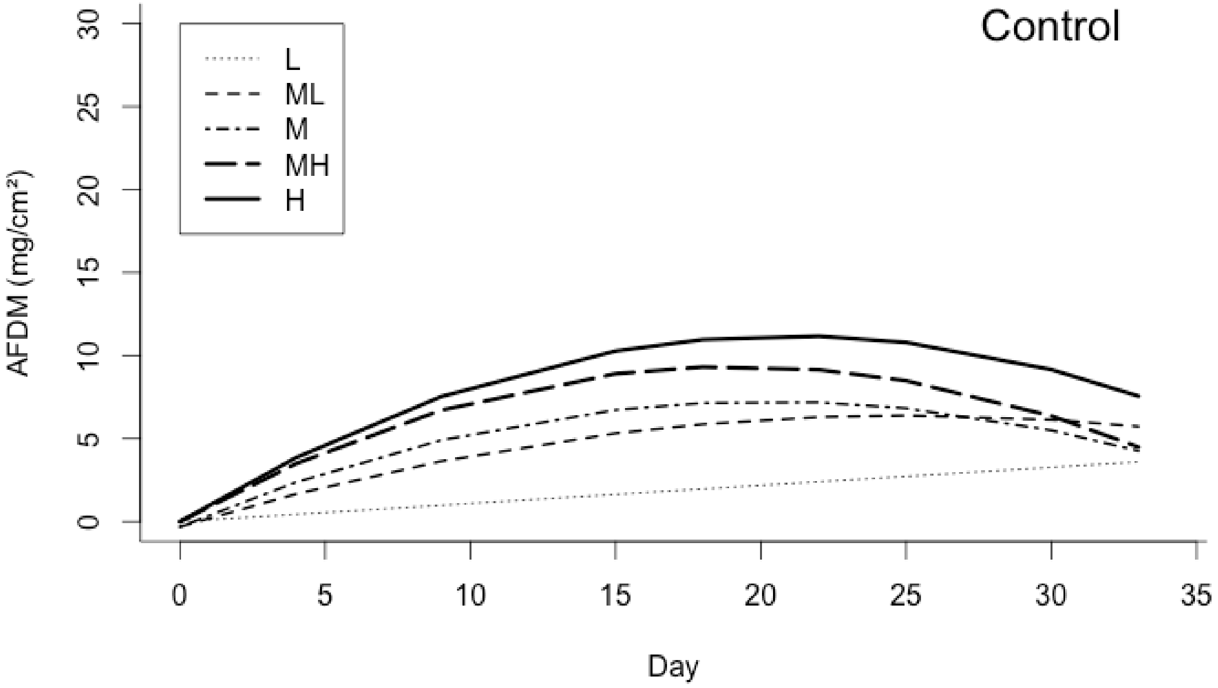
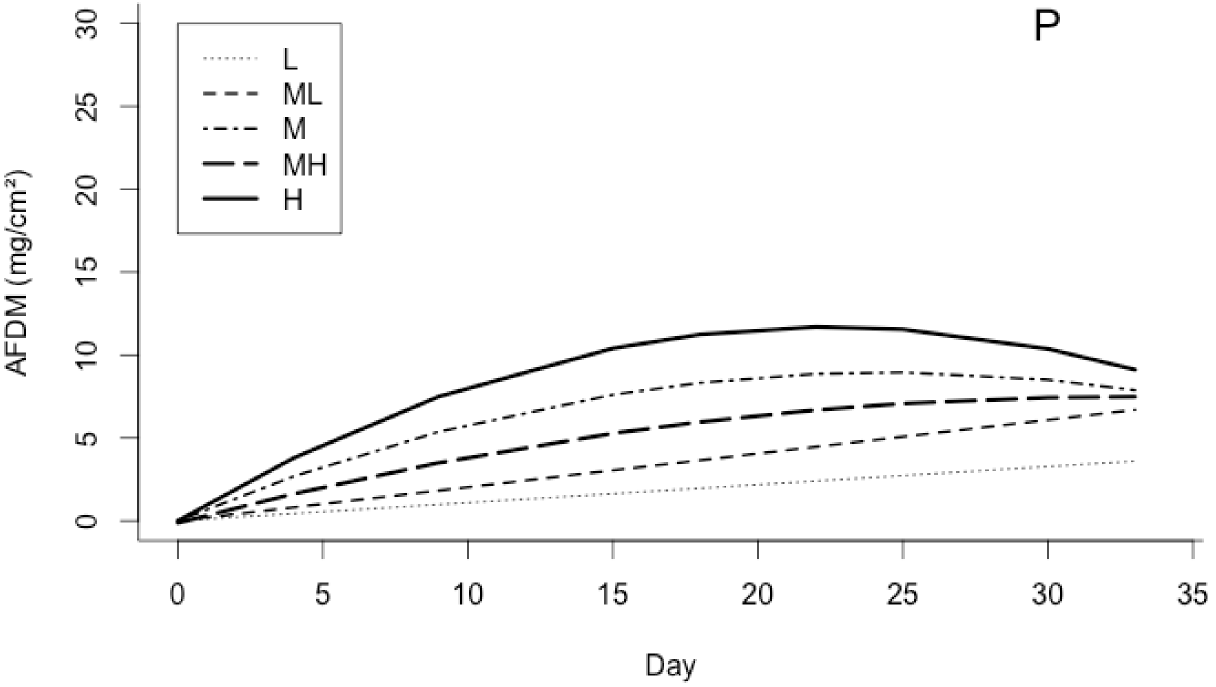


Figure 2.21: AFDM accumulation over time for discharge and nutrient combination treatment study.


Control
L
ML
M
MH
H
AFDM (mg/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day


P
L
ML
M
MH
H
AFDM (mg/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day

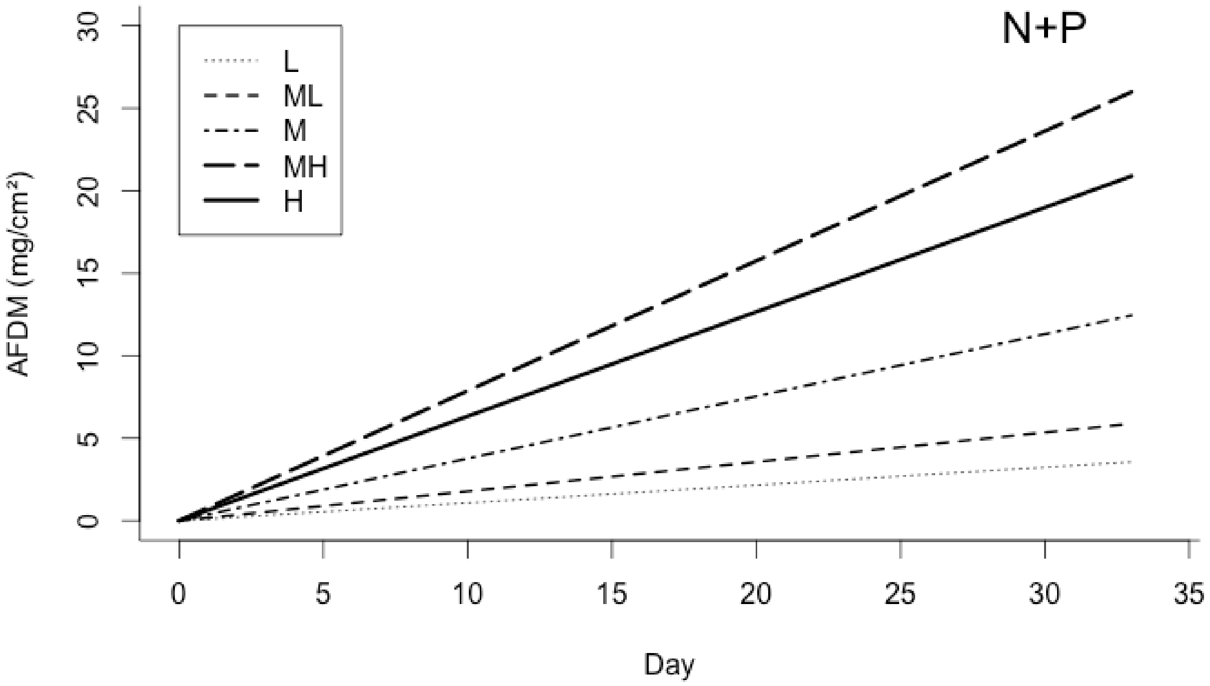


Figure 2.22: Regression models describing AFDM accumulation for discharge and nutrient treatment combinations.

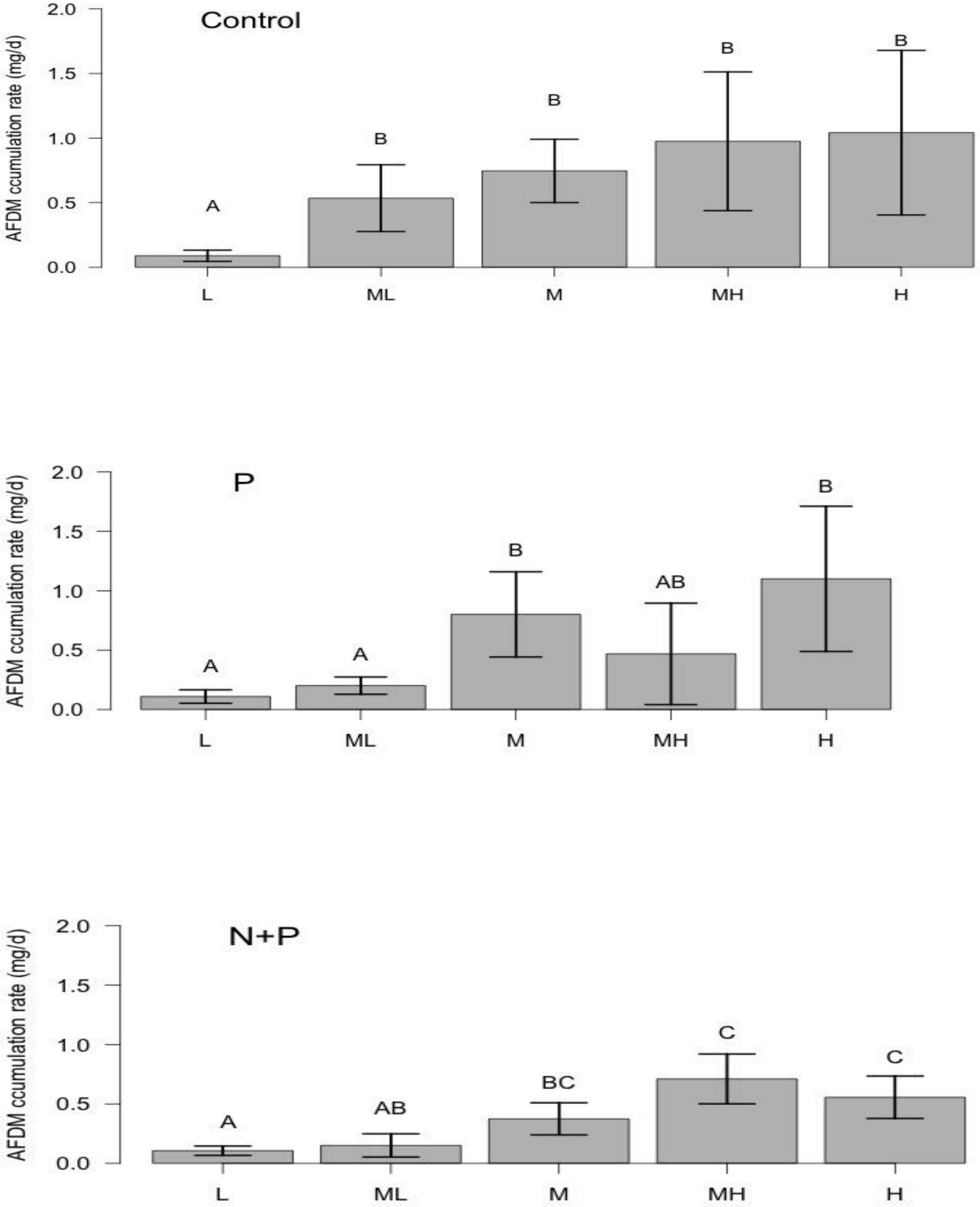


Figure 2.23: AFDM accumulation rates discharge and nutrient treatment with ± 95% CI. Discharge treatments with the same letters are not statistically different.

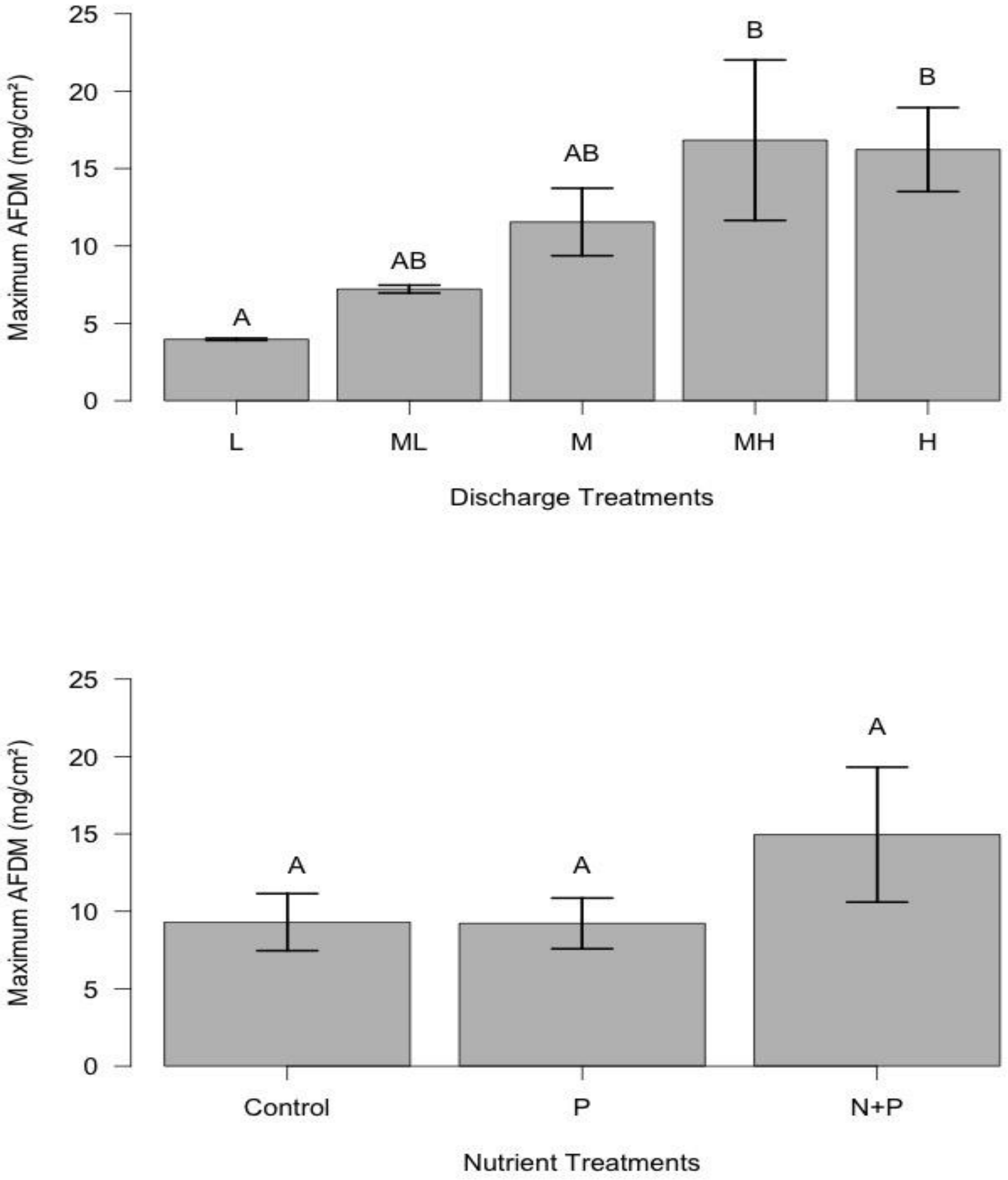
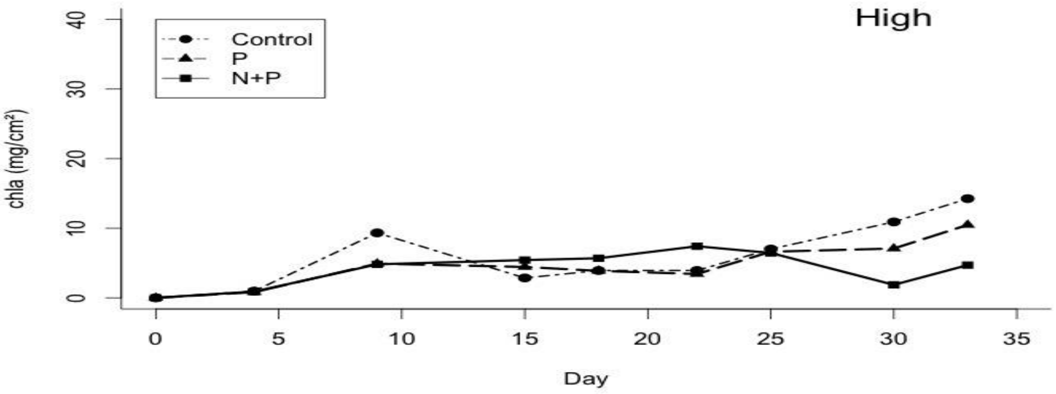
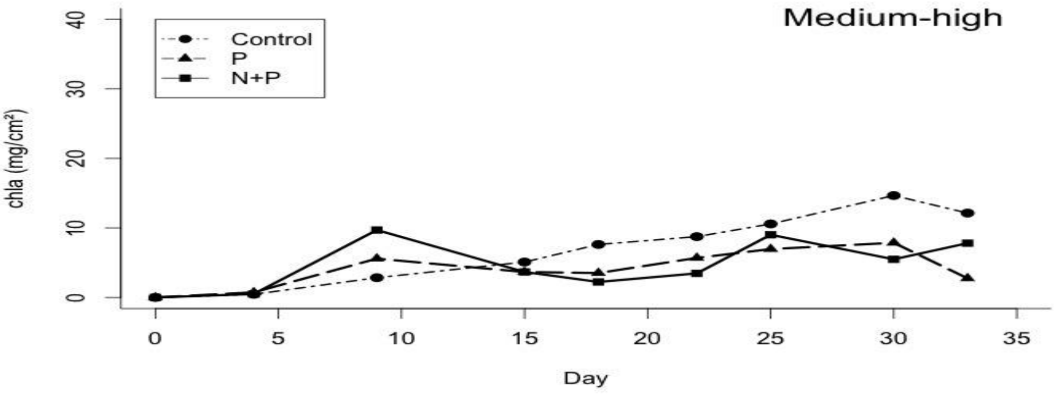
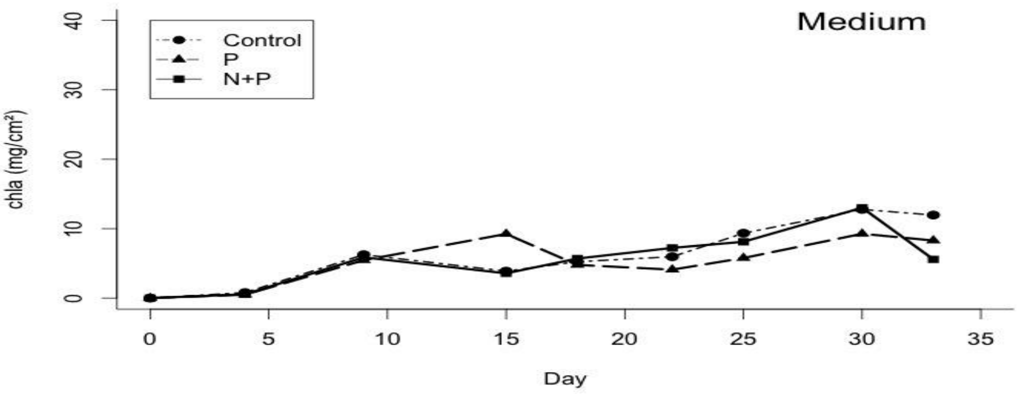


Figure 2.24: Maximum AFDM accumulation for different discharge and nutrient treatments. Discharge treatments with the same letters are not statistically different.


High
Control
P
N+P
chla (mg/cm²)
0
10
20
30
40
Day
0
5
10
15
20
25
30
35


Medium-high
Control
P
N+P
chla (mg/cm²)
0
10
20
30
40
Day
0
5
10
15
20
25
30
35


Medium
Control
P
N+P
chla (mg/cm²)
0
10
20
30
40
Day
0
5
10
15
20
25
30
35

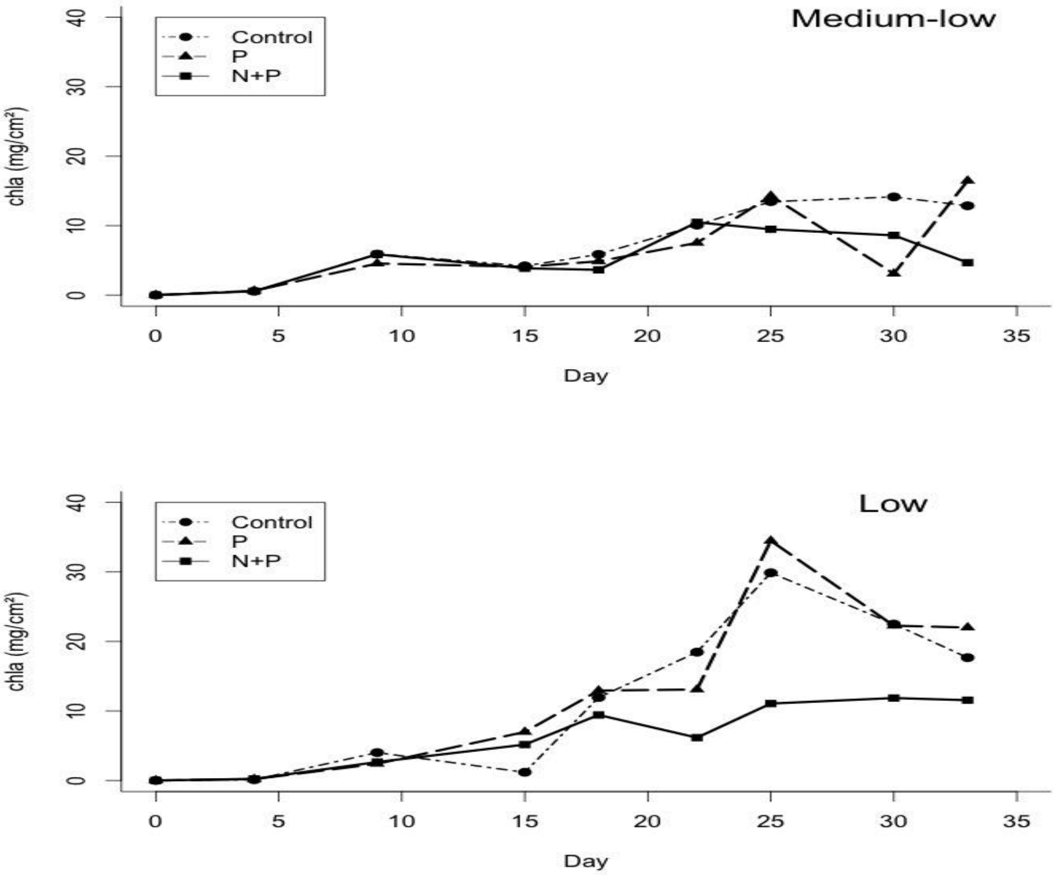
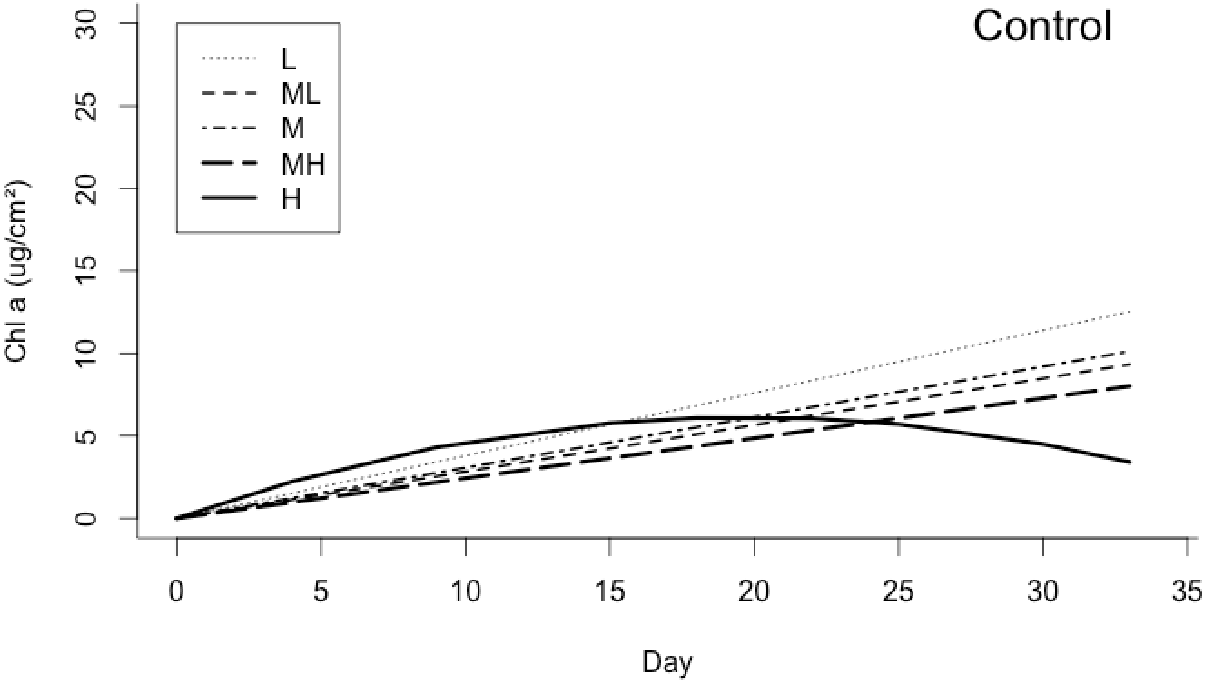
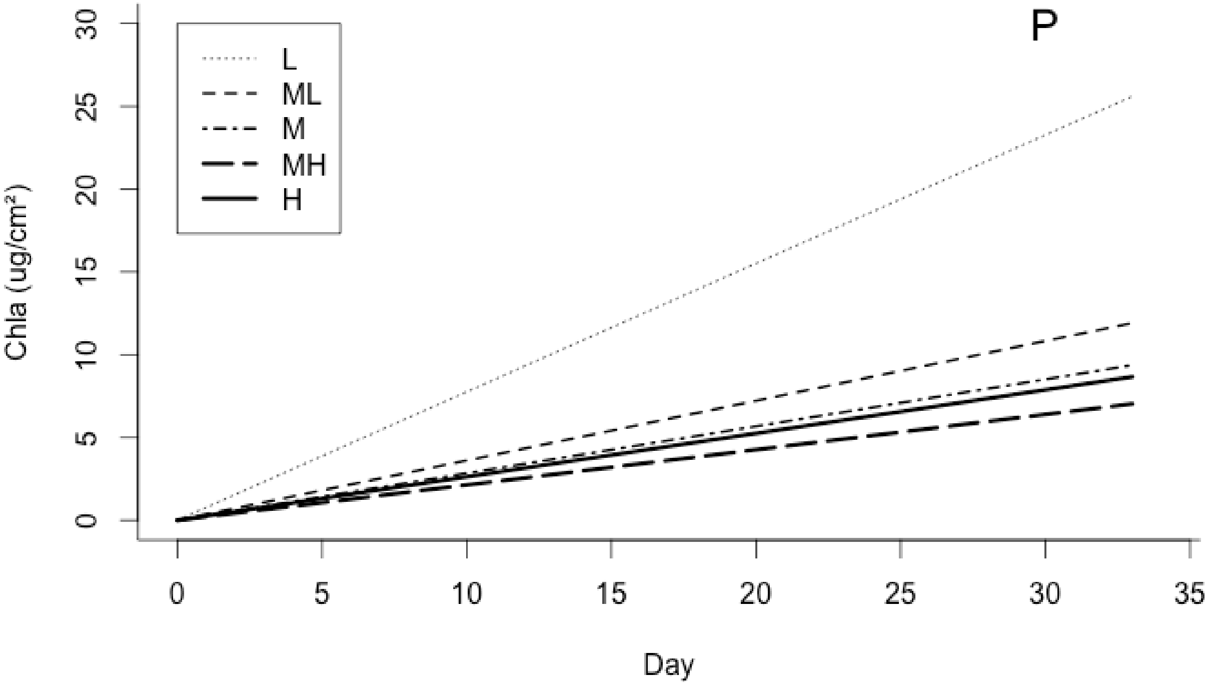


Figure 2.25: Chl a concentrations over time for discharge and nutrient combination treatment study.


Control
L
ML
M
MH
H
Chl a (ug/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day


P
L
ML
M
MH
H
Chla (ug/cm²)
0
5
10
15
20
25
30
0
5
10
15
20
25
30
35
Day

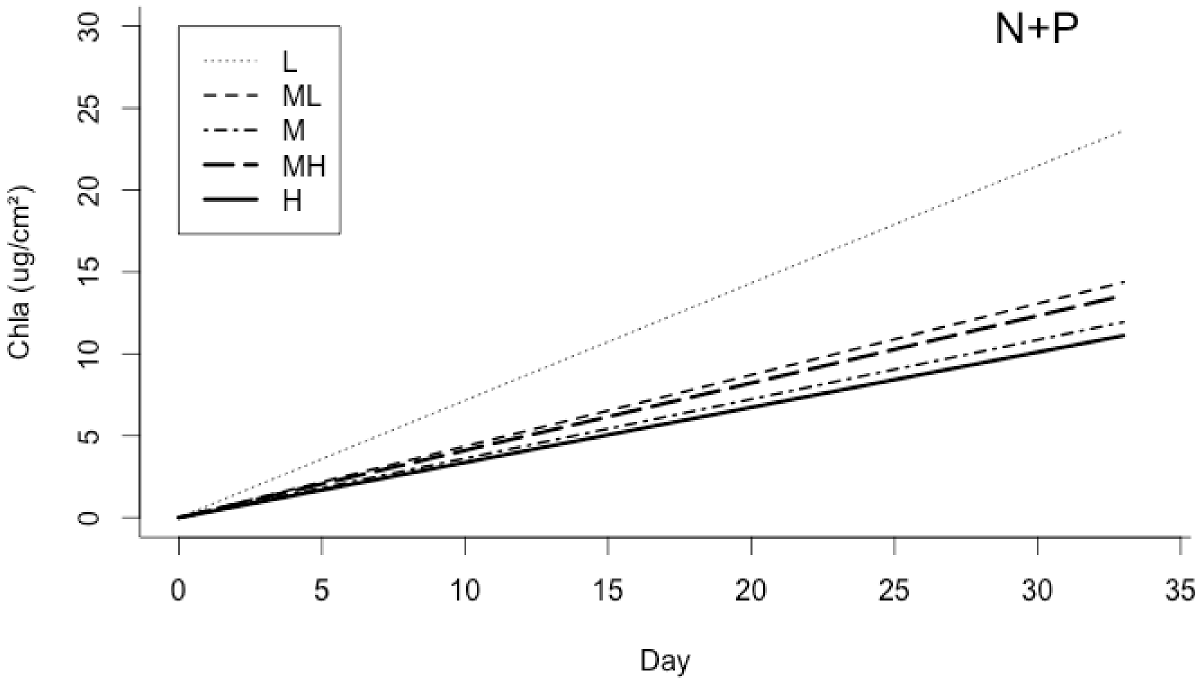


Figure 2.26: Regression models describing chl a accumulation for discharge and nutrient treatment combinations.

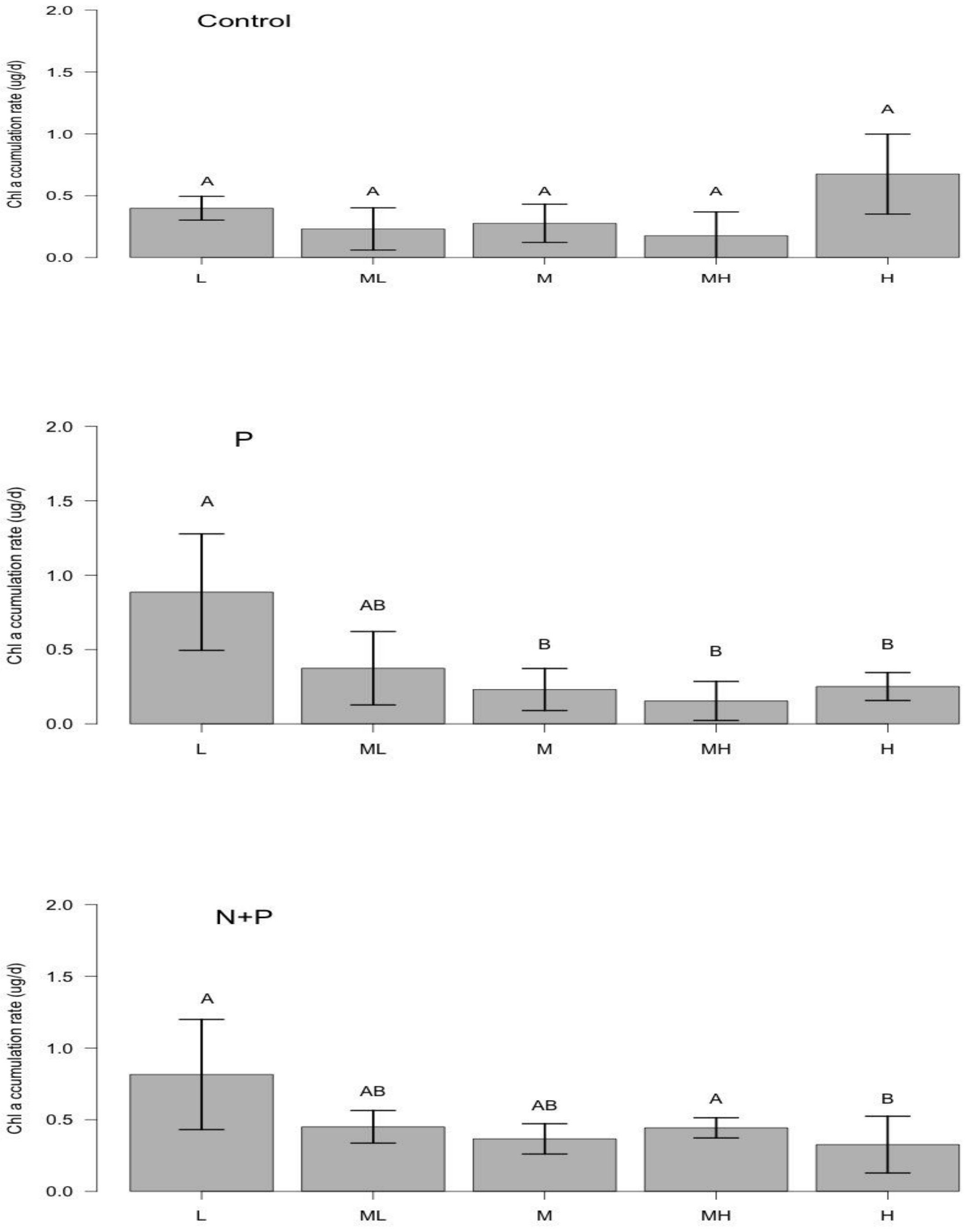


Figure 2.27: Chl a accumulation rates for discharge and nutrient treatment with ± 95% CI. Discharge treatments with the same letter are not statistically different.

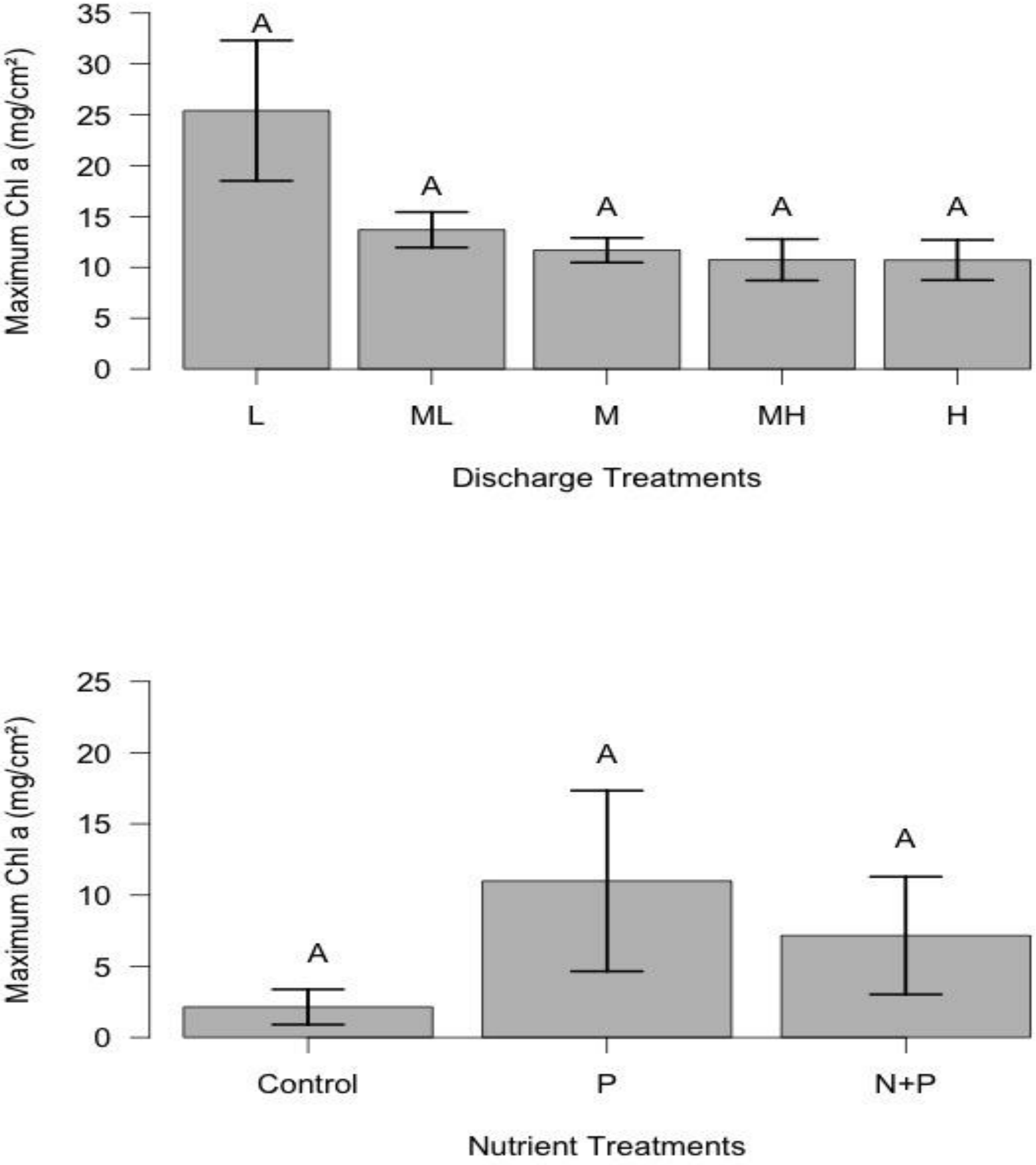


Figure 2.28: Maximum chl a concentration for different discharge and nutrient treatments.

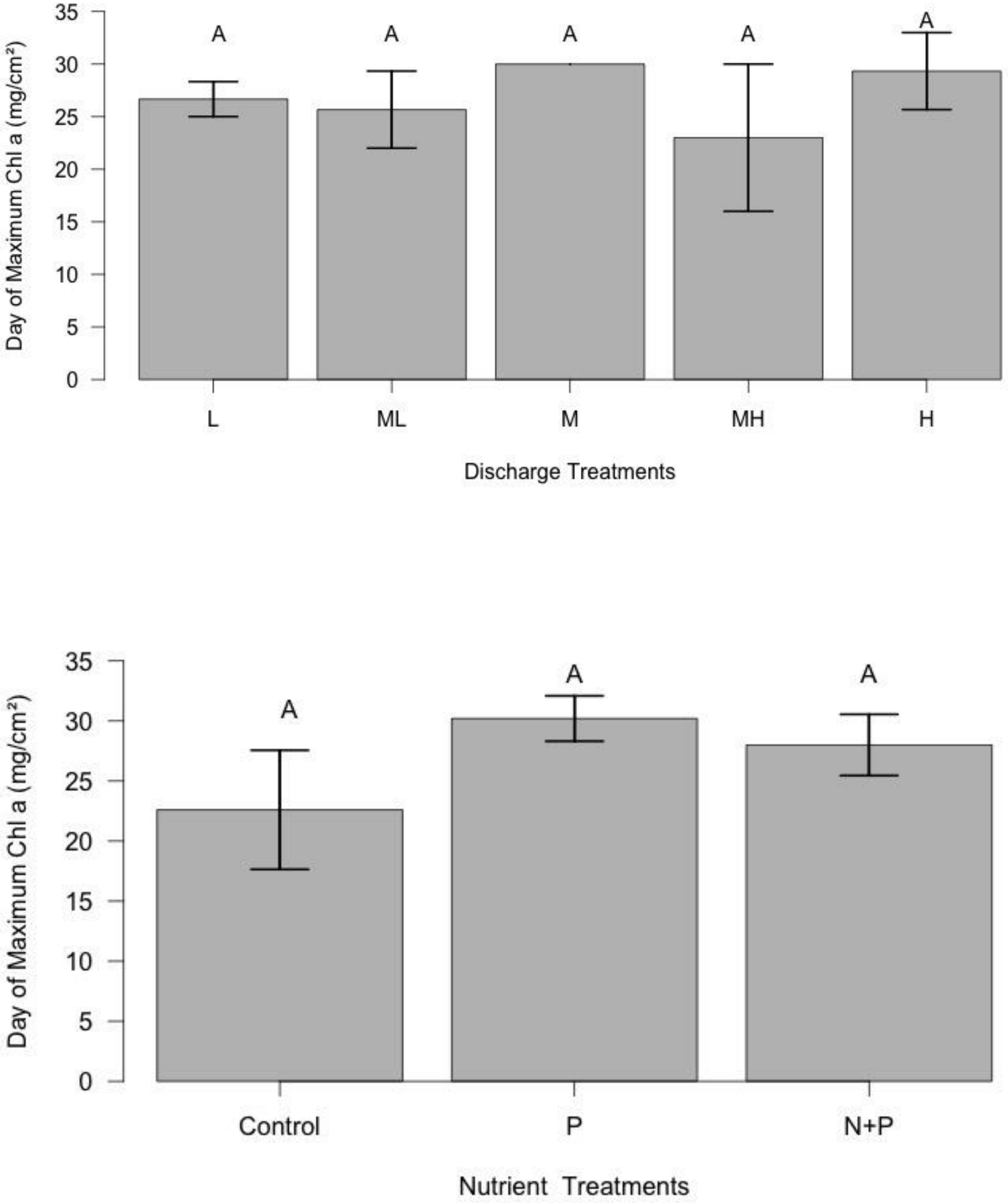


Figure 2.29: Day of maximum chl a concentration for discharge and nutrient treatments. Discharge treatments with the same letters are not statistically different.

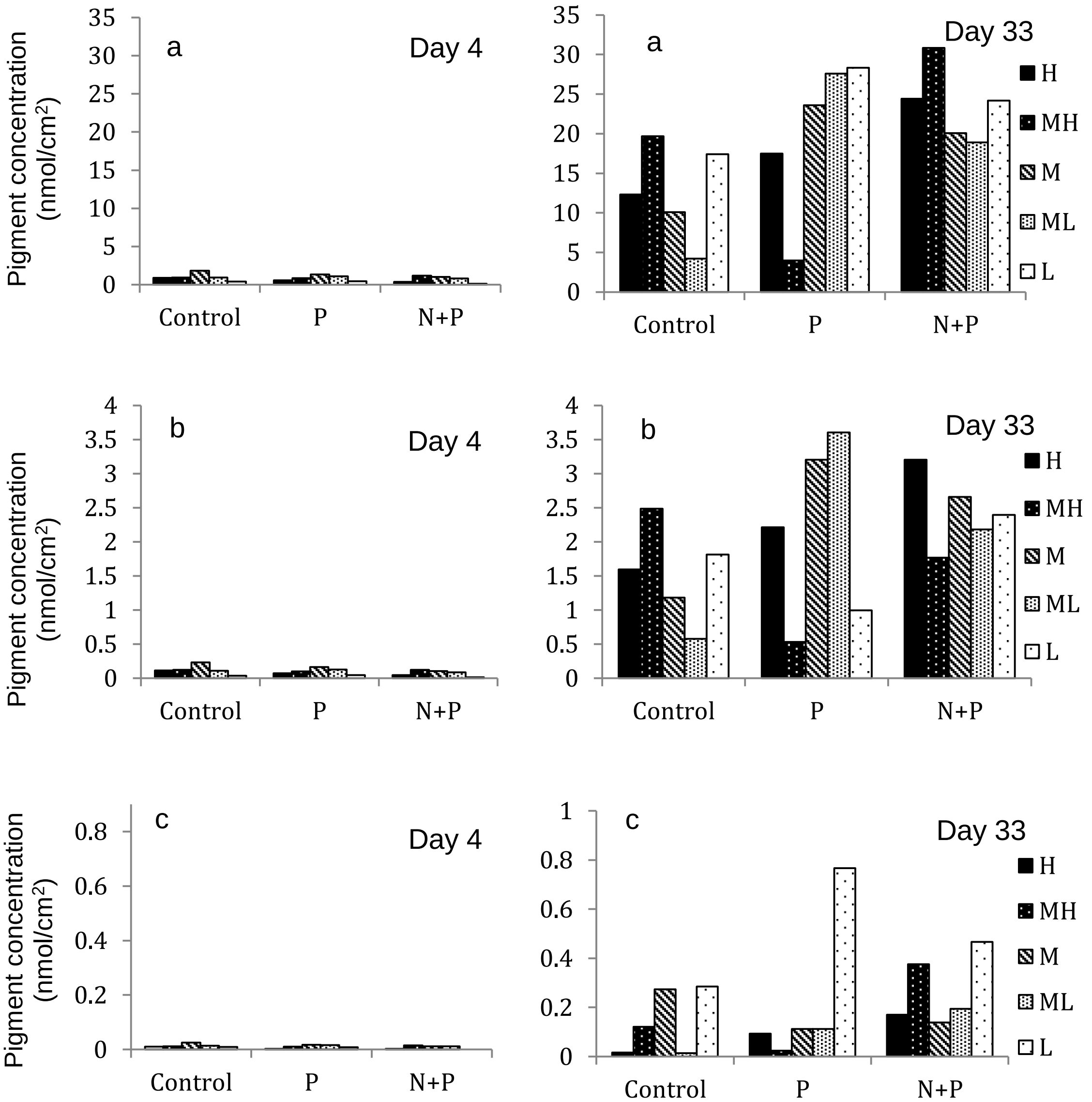


Fig 2.30: Concentrations of (a) total pigments (b) Siliceous algal pigments (c) Green algal pigments for the first and last day of the experiment.

CHAPTER 3

INTERACTIVE EFFECTS OF FLOW AND GRAZING ON PERIPHYTON BIOMASS AND COMPOSITION

Diaz, D.D., P.V. McCormick, and A.P. Covich, to be submitted to *Aquatic Ecology*

Abstract

Increasing human water demand coupled with predicted increases in the frequency and intensity of drought will likely result in declining stream flows in the southeastern USA with potential impacts on periphyton, an important basal energy resource in many streams. Examining how periphyton growth and composition is affected by alterations in discharge is vital in determining flow-ecology relationships, which are necessary to establish flows that protect aquatic systems and the services they provide to humans. We conducted controlled experiments to examine the effects of different discharge on periphyton biomass accumulation and composition and the ability of a common grazer to respond to and influence these changes in Ichawaynochaway Creek, a major Flint River tributary in southwest Georgia, during the summer and early fall of 2014. Total biomass (AFDM) and algal (chlorophyll a) biomass both increased with increasing discharge, and diatoms were the dominant algal group in all discharge treatments. Reductions in periphyton biomass caused by grazers increased with increasing discharge but snail growth rate decreased. These patterns may reflect increased metabolic demands on snails and the importance of periphyton loss due to physical disturbance in higher discharge environments. Increasing severity of drought and water withdraws could alter in stream conditions allowing for a shift to lower flow habitats with changes in algal accumulation and composition. Grazers may exert only a modest influence on these flow-induced changes in periphyton assemblages.

Introduction

Attached algae or periphyton are an important component of most streams and rivers. They can influence the chemical and physical habitat conditions for stream organisms and can regulate nutrients in lotic environments (Azim et al. 2005, Larned 2010). Additionally these primary producers are an important basal energy source for most stream herbivores or grazers (Heckey & Hesslein 1995, Saikia et al. 2015). Numerous factors control periphytic biomass, including nutrient availability, light levels, grazing, and water velocity (Dodds, Smith, & Lohman 2002, Liess & Hillebrand 2004).

Grazers play an important role in transferring energy and nutrients from algal biomass to higher trophic levels of stream food webs. The grazer-periphyton linkage can influence total ecosystem production, species distribution and biodiversity through effects on trophic cascades and habitat conditions (Liess & Hillebrand 2004). Stream grazers often control algal biomass and compositions and may have positive, negative, or neutral effects on algal accrual primarily depending on their density and consumption rates (Liess & Kahlert 2009**,** Hintz and Wellnitz 2013). In general, grazers decrease algal biomass through consumption and physical disturbance (Steinman, 1996). However, algal biomass can increase when grazer movement dislodges light-blocking sediment and stimulates algal photosynthesis (Haglund & Hillerbrand 2005). Likewise, grazers may also remove senescent over-story layers, thereby increasing periphyton production and growth in the underlying layer (Wellnitz & Poff 2006, 2012). Selective feeding by herbivores alters the taxonomic and physical structure of periphyton mats by promoting dominance by grazer-resistant taxa and growth forms (Dodds 1991, Dudley 1992, Tall et al. 2006).

Stream grazers often limit algal biomass but they may not be able to control algal accumulation when algal growth rates exceed herbivory rates (Lamberti et al. 1989, Ceola et al. 2013). Wellnitz & Poff (2012) found that increased grazer density was positively correlated with an increase in periphyton biomass as a result of apparent nutrient recycling. Increased nutrient supply and higher light availability represent ideal conditions for periphyton growth and biomass accumulation which could produce these types of algal growth rates (Francoeur 2001, Rosemond et al. 2000).

Changes in stream discharge can alter the benthic environment and may affect periphyton-grazer interactions (Poff et al. 2003). Increasing velocity has two opposing effects on biomass accumulation: a positive effect by increasing nutrient supply (Horner et al. 1990) and a negative effect by increasing shear stress (Biggs and Thomsen 1995). These changes to algal composition and accrual can impact grazer habitat and grazing capabilities (Hintz and Wellnitz 2013). Habitat availability for grazers and their distributions are both influenced by current velocity within lotic systems (Opsahl et al. 2003). In turn, these grazer and flow interactions can determine the distribution and abundance of periphyton in streams (Merten et al. 2010). For instance, Biggs and Hickey (1994) found that reduction in reduced shear stress at higher discharges altered periphytic mat composition and reduced habitat suitability for grazers that preferred higher-velocity environments.

Streams in the lower Flint River Basin in southwest Georgia USA exhibit broadly predictable seasonal discharge patterns that correspond with changes in underwater light availability and in-stream primary production. Discharge is typically highest during the winter and spring due to greater rainfall amounts that increase inflows of surface runoff and soil water. These water sources contribute dissolved organic matter content to the stream, resulting in high color values and low light penetration to the stream bottom. During the drier summer months, stream discharge is supported primarily by groundwater, resulting in low stream color and high light penetration. These periods of relaxation of light limitation can be associated with rapid periphyton growth and periods of high biomass in these streams. Anecdotal observations indicate that the biomass and composition of periphyton during these low-discharge periods is spatially heterogeneous and possibly related to variation in local hydraulic environments.

Our study was designed to explore the relationships between reductions in summertime stream discharge, periphyton growth rates, and the ability of grazers to control this growth and respond to increased biomass by exhibiting higher growth rates. Our objectives were to: (1) investigate effects of stream discharge on periphyton growth (2) investigate how a common grazer, the snail *Elimia albanyensis*, can limit periphyton biomass under different discharge conditions; (3) determine if higher periphyton growth rates results in increased grazer growth rates.

Methods

Site Description

This experiment was conducted in the lower portion of Ichawaynochaway Creek a 5th order tributary of the Lower Flint River in southwest Georgia, USA (Figure 3.1). The stream flows through a landscape where irrigated agriculture is a dominant land use and irrigation water is supplied by both groundwater pumping and direct withdrawals from the creek and its tributaries. These withdrawals contribute to reduced and variable stream flows during the summer growing season. The stream is characterized by coarse substrate including cobbles, boulders, and bedrock outcrops that can support periphyton growth under low-flow conditions.

Experimental Design

An artificial stream channel facility was constructed in an unshaded location adjacent to the stream to allow for controlled manipulations of water discharge. Channels were white plastic gutters (10-cm wide x 3.1 m long) that were capped at each end. (Figure 3.2). A solid cap was secured to the upstream end of each channel and a perforated cap was secured to the downstream end to allow for flow-through conditions with a channel water depth of approximately 6 cm. Channels were secured to a wooden frame that included a clear plastic (Suntuf ®) roof to protect the channels from heavy rain events that might cause scouring (Figure 3.3). Stream water was pumped through the facility using a pool pump, and discharge through individual channels (18 total) was controlled by spigots. After flowing through the channels, water was drained into a trough and returned to the stream. Temperature loggers were placed at the end of all the channels. Temperature ranged from 25°C to 10 °C with lower readings taking place at the end of experimentation.

A 2x3 factorial design was used for this experiment with two grazer densities (snails present or absent) and three discharges (low, medium and high flow). Stream water was pumped through 18 replicate artificial stream channels lined with ceramic tiles (2.5 cm x 2.5 cm) to provide Low (L), Medium (M), and High (H) discharge treatments (6 replicates/discharge treatment): Low: 0.95L/min Medium3.78 L/min; High:18.93 L/min. Snails (*Elimia albanyensis)* were collected from the stream and added to half of the channels within each of the three flow treatments at a density of 30 snails/channel, which was similar to the ambient density measured in the stream (Figure 3.4). Snails selected for use weighed between 6 and 7 g each. Each snail was individually marked with nail polish and measured for shell length and weight in order to quantify snail growth during the experiment (Figure 3.5). Grazers were added one week after flow was initiated to allow time for some accumulation of the periphyton food source. Barriers were placed between channels to prevent snail movement between channels. Snails were checked daily to ensure that none had escaped.

Channels were delineated into 4 sections from upstream to downstream, each containing the same number of tiles. One randomly selected tile was sampled from each section every 3 to 4 days until day 36, and periphyton from all the tiles was transferred to a Whirlpak® bag by scraping with a razor blade and brushing with a stiff bristle toothbrush. Scraped tiles were returned to the channel and turned over to avoid re-sampling. Sample bags were stored in the dark on ice and returned to the laboratory.

Initial processing of samples occurred within 2 hr of collection. Samples were homogenized using a hand blender to break up large aggregations of periphyton, and deionized water was then added to achieve a known volume. Homogenized subsamples were removed to quantify chlorophyll a, AFDM, and major groups of pigments indicative of different algal taxonomic groups. Chlorophyll subsamples were filtered onto a glass fiber filter, which was then folded, wrapped in aluminum foil, and stored in a freezer (-18°C) for no more than one month before processing. Chlorophyll a was extracted by grinding filters using a sonicator in 90% acetone using U.S.E.P.A. Method 445.0 (Arar & Collins, 1997) and quantified using a pre-calibrated Aquaflour® handheld fluorometer (Turner Designs, Sunnyvale, CA). AFDM samples were poured onto pre-combusted and pre-weighed foil cups (dried at 105° C for 24 h) and weighed after cooling in a desiccator. The samples were then ashed at 500 °C for 1h. A small amount of water was added to the ashed sample to account for water bound to clay particles before they were dried. The samples were then weighed a final time after 24 hours of drying. Periphyton AFDM was determined as the difference between dried and ashed sample mass (APHA, 2012).

The relative dominance of major algal taxa (i.e., diatoms, green algae, and cyanobacteria) was determined on days 4 and 33 by High Performance Liquid Chromatography (HPLC) following the methods of Leavitt and Hodgson (2001). Pigment analysis samples were extracted as described above and then centrifuged and filtered through 0.22 µm nylon syringe filters (Millex) to remove particulate matter. Samples were placed in an autosampler tray where they were mixed with an ion-pairing agent prior to injection into a Shimadzu HPLC system following the mobile phase and time sequence of Leavitt and Hodgson (2001). Pigments were separated using a C18 column. Pigment identification was made using retention times of known standards (DHI Lab Products, Denmark), and pigment-specific spectra were recorded by a photodiode array detector for carotenoids and a fluorescence detector for chlorophylls. Pigment concentrations were calculated by comparing peak areas against standards of known concentration.

Water discharge for each channel was measured during the experiment and adjustments were made to keep each treatment within 10% of the desired range. Temperature readings were also taken daily throughout the experiment. Temperature ranged from 25°C to 10°C throughout the experiment and were similar among discharge treatments.

At the end of the experiment, snails were collected, blotted dry, and processed for final weight and length. All but three snails were recovered at the end of the experiment.

Data analyses

Mean AFDM and chlorophyll a concentrations were calculated for each discharge and grazer treatment combination on each sampling date. Polynomial (linear and quadratic) equations were fitted to these data to estimate the average growth rates (linear coefficient) and to account for potential sloughing of biomass as the experiment progressed (quadratic coefficient). Linear coefficients were compared among treatments using 95% confidence intervals to test for significant treatment differences for growth rates. Log transformed maximum values of AFDM and chlorophyll a and the day they were achieved in each channel were determined and compared among discharge treatments using two-way ANOVA followed by a-posteriori multiple comparisons (Tukey's Honest Significant Difference test). Data were first log-transformed to better meet distributional assumptions. Autotrophic index (AI) ratios (AFDM divided by chl a, Feio et al. 2010) were calculated and compared among treatments for each sampling date using two-way ANOVA followed by Tukey's Honest Significant Difference test. Log transformed data of concentrations of total pigments and those indicative of siliceous algae, green algae, and cyanobacteria concentrations for each discharge and nutrient treatment combination were compared for the first and last day of experimentation using two-way ANOVA followed by Tukey's Honestly Significant a-posteriori test

Results

The accumulation of AFDM was positively related to discharge, and high discharge (H) channels maintained AFDM at levels as much as 10-fold higher than those in low discharge (L) channels throughout the experiment (Figure 3.6). Accumulation patterns in high (H) and mid (M) discharge channels were best modeled using a quadratic relationship ($p \leq 0.001$, $r^2 \geq 0.513$), with a significant downward trend later in the experiment, while AFDM in L channels accumulated in a linear manner ($p \leq 0.001$, $r^2 \geq 0.412$) (Figure 3.7). In the absence of snail grazing, accumulation rates were significantly different among all discharge treatments, and were 20 and 3.5 fold faster in H as compared to L and M channels, respectively (Figure 3.8). Grazed channels exhibited a similar pattern, although accumulation rates were not significantly different between M and H channels. There were no significant differences between grazed and unglazed treatments for each discharge treatment with the exception of the M treatment, with higher rates in the grazed treatment, based on comparisons of 95% confidence intervals**.** Maximum AFDM achieved during the experiment increased approximately 6-fold with increasing discharge (F=34.18, p<0.001) and was significantly different between all discharge treatments (Figure 3.9). Snail grazing did not significantly affect maximum AFDM (F=0.981, p=0.339). Neither discharge (F=0.850, p=0.449) nor grazer treatment (F=0.083, p=0.778) significantly affected the time required to achieve maximum biomass.

Chlorophyll a accumulation rates in ungrazed channels were similar among discharge treatments during the first part of the experiment but then diverged as concentrations declined in L and M channels to the 5-7 ug/cm^2 range but continued to increase in ungrazed H towards 15 ug/cm^2 (Figure 3.10**).** Accumulation patterns were best modeled using a quadratic relationship in all the discharge treatments ($p<0.001$, $r^2 \geq 0.691$) with the exception of the H ungrazed treatment which had a linear pattern ($p<0.001$, $r^2=0.911$) (Figure 3.11). In the absence of snail grazing, chl a did not differ significantly among discharge treatment, but L and M treatments displayed a decline in chl a later in the experiment as indicated by the significant quadratic coefficient (Figure 3.12). In grazed channels, accumulation rates were significantly lower for L channels as compared to the other discharge treatments with accumulation rates 1.5 and 1.6-fold slower than for M and H treatments, respectively. There were no significant differences between grazed and ungrazed channels in any discharge treatment. Maximum chlorophyll a concentrations did not differ significantly among discharge treatments but were reduced by grazing (F=6.346, p=0.025, Figure 3.13). Day of maximum concentration occurred earlier in the M channels (day 21) than in H channels (day 26) (F=8.091, p=0.005, Figure 3.14).

Autotrophic index (AI) ratios initially were higher with increasing discharge, but these differences gradually lessened as the experiment progressed (Figure 3.15). The AI of the H channels declined from >6000:1 to <4000:1 with time while M and L channels maintained an AI near or below 4000:1 throughout the experiment. There were significant differences in the AI among discharge treatments on any sampling day ($p \leq 0.058$). H channels were significantly higher than M channels on day 7 and 19 ($p \leq 0.031$) and than L channels throughout the experiment ($p \leq 0.001$).

Total pigment concentrations were similar among discharge and grazing treatments on day 7, but were positively related to discharge (F=5.930, p=0.014) and negatively affected by grazing (F=7.071, p=0.019) at the end of the experiment (day 33) (Figure 3.16). Grazing effects intensified with increasing discharge as indicated by a significant interaction term between these two factors (F=2.870, p=0.045). Chlorophyll a comprised roughly half of total pigment concentrations and was strongly correlated with chl a measurements obtained by fluorometry ($r^2=0.852$). Diatom algal pigments accounted for much of remaining pigment concentration and displayed patterns similar to those for total pigments, increasing with discharge (F=3.852, p=0.047) and declining with grazing (F=6.727, p=0.021, Figure 3.17). Lack of a significant interaction term indicated that grazing effects were consistent across all discharge treatments. Green algal pigment concentrations were low on both Day 7 to Day 33 and there was no significant difference across discharge and grazing treatments (Figure 3.18).

Snail growth decreased with increasing discharge (F=9.377, p=0.014), with a significantly higher mean wet-weight increase of 15% in L channels as compared to only 8% in H channels (Figure 3.19).

Discussion

Changes in stream discharge exert multiple influences on periphyton growth, including both stimulatory effects on cell metabolism and negative effects related to greater shear stress (Flinders & Hart 2009). The rate of AFDM accumulation was fastest at higher discharge, and while initial chlorophyll a accumulation rates were not significantly different among discharge treatments, the highest chlorophyll a concentration was also achieved at the highest discharge rate. Higher discharges can increase the nutrient loads into aquatic ecosystems, and decrease the diffusion boundary layer of algal cells, which in turn may increase algal growth rates (Kiffney & Richardson 2001). The negative influence of discharge was evidence by the quadratic growth patterns in the M and H treatments, indicating loss of biomass due to sloughing. Interestingly, chlorophyll a concentrations increased steadily and were highest at the end of the experiment in the H channels. These contrasting patterns for AFDM and chlorophyll suggest that senescent material accounted for much of the sloughed material and the loss of this material stimulated growth and division of active algal cells (Wellnitz & Poff 2012)

Diatoms were the dominant algal group in all discharge treatments and were more abundant at higher discharges as indicated by pigment concentrations. As already noted for total biomass indicators, we attribute higher diatom concentrations to increased algal metabolic rates at higher discharge where the diffusive boundary layer is thinner. This increased nutrient availability can increase metabolic rates, allowing for greater growth potential (Musielak et al. 2009). Our experimental results were consistent with observations we made in the stream itself, where higher discharge habitats had thicker diatom mats than lower discharge habitats. We also observed higher AI ratios at higher discharges which may be due to higher accumulation of mucilage as discharge increased (Biggs & Hickey 1994). Compared to our initial studies (Chapter 2), green algal abundance was low in all the discharge treatments. A decrease in temperature (dropping from 25°C to as low as 10 °C) as the experiment progressed may have limited the growth of green algae which is better adapted to higher temperature ranges (Piggott et al. 2015).

Our results are similar to other studies that demonstrate grazer control of algal biomass and growth (Hill et al. 1992, Steinman 1996). Grazer effects increased with higher discharge as evidenced by lower values of AFDM, chl a, total pigment concentrations, and diatom algal pigment concentrations in H channels that were grazed as compared to those that were ungrazed. We attribute a bigger difference between grazed and ungrazed channels to foraging and movement activities that can dislodge periphyton and increased algal export rates at higher velocities (Catteneo & Mousseau 1995, Alvarez & Peckarsky, 2005, Merten et al. 2010). The effect of grazing on biomass accumulation patterns was most evident at the highest discharge, where ungrazed channels experienced unabated linear growth while growth in grazed channels exhibited a quadratic growth pattern. However, snail growth rates decreased with increased discharge. This result may be attributed to increased drag in higher discharge channels that can lead to higher energy expenditures for activities, such as attachment and locomotion (Whal 1997).

Our results demonstrate that discharge has the potential to determine the composition and growth rates of periphyton during low flow conditions when light availability is high in this stream. In the stream itself, our observation that high biomass of periphyton was present at lower flows provided the basis for our initial hypothesis. We surmised that reduced flow and lack of scouring increase attached algal biomass by reducing sloughing of algal cells from substrata. In our experiment we found that higher discharges within our low flow treatments had greater AFDM and chl a accrual. At higher discharges, snail grazers have a limited ability to control periphyton biomass which may be due to flow conditions that promote high algal accumulation rates. Increasing frequency and intensity of drought coupled with an increased demand for water for irrigation in the LFRB, could lead to more frequent and prolonged low-flow conditions and more extreme low flows. This change in flow can directly and indirectly affect factors that control composition and abundance of periphyton within streams. Changes in this basal resource can affect habitats and food quality for stream organisms which can in turn affect food web dynamics in stream systems with flow alterations.

References:

Alvarez, M., & Peckarsky, B. L. (2005). How do grazers affect periphyton heterogeneity in streams? Oecologia, 142, 576-587.

American Public Health Association, American Water Works Association, and the Water Environment Federation. (2012).Standard methods for the examination of water and wastewater (Vol. 21). American Public Health Association. Washington, DC

Arar, E. J., & Collins, G. B. (1997). Method 445.0: In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence. United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory. Washington, DC

Azim, M. E., Verdegem, M. C., van Dam, A. A., & Beveridge, M. C. (Eds.). (2005). Periphyton: ecology, exploitation and management. CABI. Cambridge, MA

Biggs, B. J., & Hickey, C. W. (1994). Periphyton responses to a hydraulic gradient in a regulated river in New Zealand. Freshwater Biology, 32, 49-59.

Cattaneo, A., & Mousseau, B. (1995). Empirical analysis of the removal rate of periphyton by grazers. Oecologia, 103(2), 249-254.

Ceola, S., Hödl, I., Adlboller, M., Singer, G., Bertuzzo, E., Mari, L., & Rinaldo, A. (2013). Hydrologic variability affects invertebrate grazing on phototrophic biofilms in stream microcosms. PloS one, *8*, e60629.

Dodds, W. K. (1991). Community interactions between the filamentous alga Cladophora glomerata (L.) Kuetzing, its epiphytes, and epiphyte grazers.Oecologia, *85*(4), 572-580.

Dodds, W. K., Smith, V. H., & Lohman, K. (2002). Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences, 59(5), 865-874.

Dudley, T. L. (1992). Beneficial effects of herbivores on stream macroalgae via epiphyte removal. *Oikos*, 121-127.

Flinders, C. A., & Hart, D. D. (2009). Effects of pulsed flows on nuisance periphyton growths in rivers: a mesocosm study. River Research and Applications, *25*, 1320-1330.

Golladay, S. W., Watt, K., Entrekin, S., & Battle, J. (2000). Hydrologic and geomorphic controls on suspended particulate organic matter concentration and transport in Ichawaynochaway Creek, Georgia, USA. Archiv für Hydrobiologie, 149, 655-678.

Hecky, R. E., & Hesslein, R. H. (1995). Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society, 141, 631-653.

Hill, W.R., Boston, H. L., & Steinman, A. D. (1992). Grazers and nutrients simultaneously limit lotic primary productivity. Canadian Journal of Fisheries and Aquatic Sciences, 49, 504-512.

Hillebrand, H., Kahlert, M., Haglund, A. L., Berninger, U. G., Nagel, S., & Wickham, S. (2002). Control of microbenthic communities by grazing and nutrient supply Ecology, 83, 2205-2219.

Hintz, W. D., & Wellnitz, T. (2013). Current velocity influences the facilitation and removal of algae by stream grazers. Aquatic Ecology, 47, 235-244.

Hoffman, A. L., Olden, J. D., Monroe, J. B., LeRoy Poff, N., Wellnitz, T., & Wiens, J. A. (2006). Current velocity and habitat patchiness shape stream herbivore movement. Oikos, 115, 358-368.

Kiffney, P. M., & Richardson, J. S. (2001). Interactions among nutrients, periphyton, and invertebrate and vertebrate (Ascaphus truei) grazers in experimental channels. Copeia, 2001, 422-429.

Lamberti, G. A., & Moore, J. W. (1984). Aquatic insects as primary consumers. In: V.H Resh and D.M Rosenberg, The Ecology of Aquatic Insects. Praeger Scientific, New York, pp. 164-195.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., Steinman, A. D., & McIntire, C. D. (1989). Productive capacity of periphyton as a determinant of plant-herbivore interactions in streams. Ecology, 70, 1840-1856.

Larned, S. T. (2010). A prospectus for periphyton: Recent and future ecological research. Journal of the North American Benthological Society 29, 182-206.

Larned, S. T., Nikora, V. I., & Biggs, B. J. (2004). Mass-transfer-limited nitrogen and phosphorus uptake by stream periphyton: A conceptual model and experimental evidence. Limnology and Oceanography, 49, 1992-2000.

Lawrence, J. R., Scharf, B., Packroff, G., & Neu, T. R. (2002). Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. Microbial Ecology, 44, 199-207.

Leavitt, P. R., & Hodgson, D. A. (2001). Sedimentary pigments. In Tracking environmental change using lake sediments (pp. 295-325). Editor, Springer Netherlands.

Liess, A., & Hillebrand, H. (2004). Invited review: direct and indirect effects in herbivore–periphyton interactions. Archiv für Hydrobiologie, 159, 433-453.

Merten, E. C., Hintz, W. D., Lightbody, A. F., & Wellnitz, T. (2010). Macroinvertebrate grazers, current velocity, and bedload transport rate influence periphytic accrual in a field-scale experimental stream. Hydrobiologia, 652, 179-184.

Musielak, M. M., Karp-Boss, L., Jumars, P. A., & Fauci, L. J. (2009). Nutrient transport and acquisition by diatom chains in a moving fluid. Journal of Fluid Mechanics, 638, 401-421.

Opsahl, R. W., Wellnitz, T., & Poff, N. L. (2003). Current velocity and invertebrate grazing regulate stream algae: results of an in situ electrical exclusion Hydrobiologia, 499, 135-145.

Piggott, J. J., Salis, R. K., Lear, G., Townsend, C. R., & Matthaei, C. D. (2015). Climate warming and agricultural stressors interact to determine stream periphyton community composition. Global change biology, 21, 206-222.

Poff, L. N., Wellnitz, T., & Monroe, J. B. (2003). Redundancy among three herbivorous insects across an experimental current velocity gradient.Oecologia, 134, 262-269.

Power, M. E., & Rainey, W. E. (2000). Food webs and resource sheds: towards spatially delimiting trophic interactions. Ecological consequences of habitat heterogeneity (eds Hutchings, M.J., John, E.A. & Stewart, A.J.A.). Blackwell Scientific, Oxford,UK, pp. 291-314

Rosemond, A. D., Mulholland, P. J., & Brawley, S. H. (2000). Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Canadian Journal of Fisheries and Aquatic Sciences, 57, 66-75.

Saikia, S. K., Nandi, S., & Majumder, S. (2015). A review on the role of nutrients in development and organization of periphyton. Journal of Research in Biology, 3, 780-788.

Steinman, A. D. (1996). Effects of grazers on freshwater benthic algae, p.341-373. In R. J. Stevenson, M.L. Bothwell and R. L. Lowe [eds.] Algal ecology: Freshwater benthic ecosystems, Aquatic Ecology Series. Academic Press.

Tall, L., Cattaneo, A., Cloutier, L., Dray, S., & Legendre, P. (2006). Resource partitioning in a grazer guild feeding on a multilayer diatom mat. Journal of the North American Benthological Society, 25, 800-810

Tuchman, N.C., & Stevenson, R.J., 1991. Effects of selective grazing by snails on benthic algal succession. J. N. American Benthological. Society. 10, 430–443.

Wahl, M. (1996). Fouled snails in flow: potential of epibionts on Littorina to increase drag and reduce snail growth rates. Marine Ecology Progress Series, 138, 157-168.

Wellnitz, T., & Leroy Poff, N. (2006). Herbivory, current velocity and algal regrowth: how does periphyton grow when the grazers have gone? Freshwater Biology, 51, 2114-2123.

Wellnitz, T., & Poff, N. L. (2012). Current-mediated periphytic structure modifies grazer interactions and algal removal. Aquatic Ecology, 46(4), 521-530.

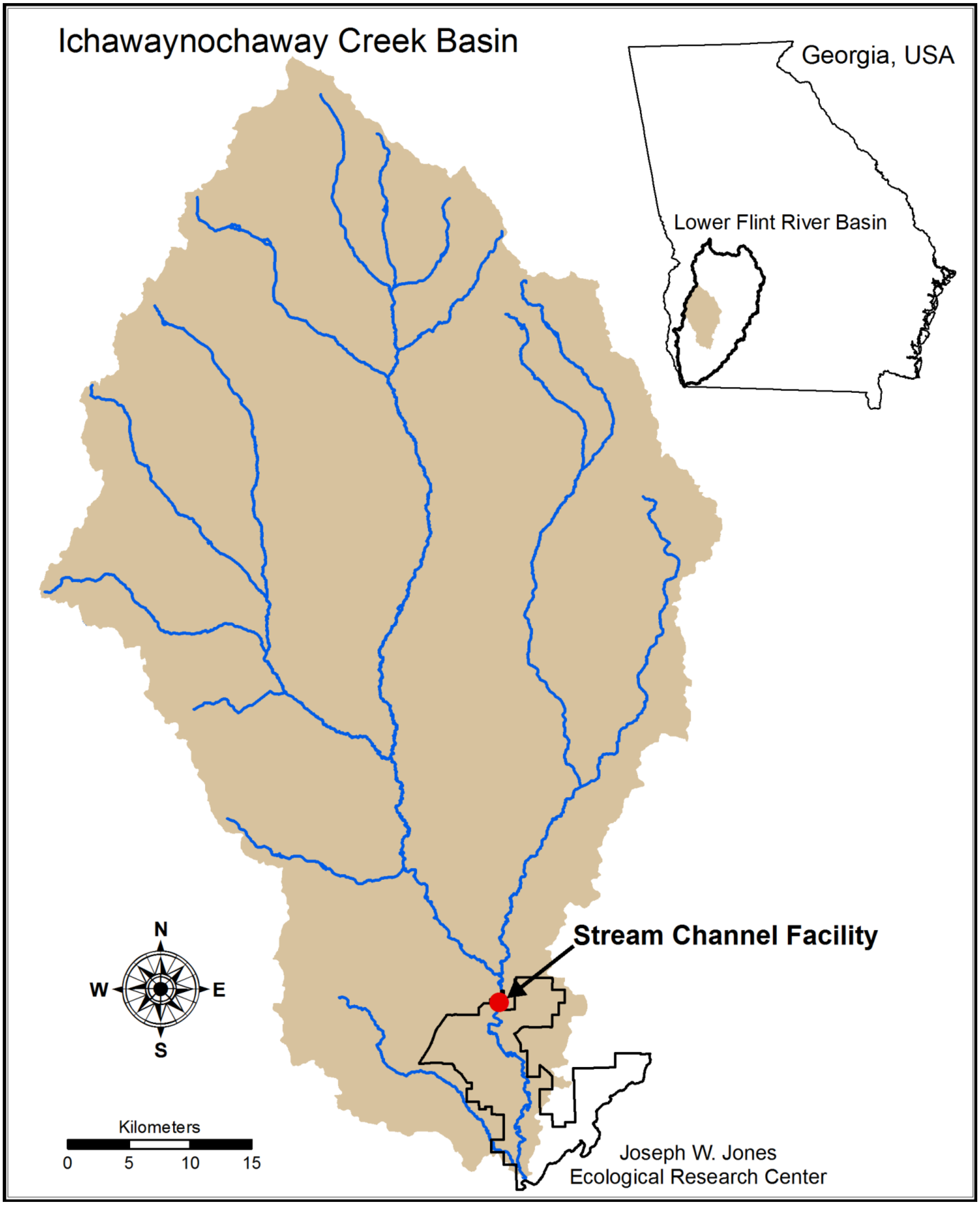


Figure 3.1: Map of study site located on Ichawaynochaway Creek (streams shown as blue lines and watershed shaded in brown) in the Lower Flint River Basin in southwest Georgia, USA.

Figure 3.2: Artificial stream channel facility channels made from plastic gutters.

Figure 3.3: Artificial channel system with clear plastic roofing.

Figure 3.4: Determination of ambient snail densities in the creek.

Figure 3.5: Nail polish was used to mark snails for the determination of growth rates in grazed channels.

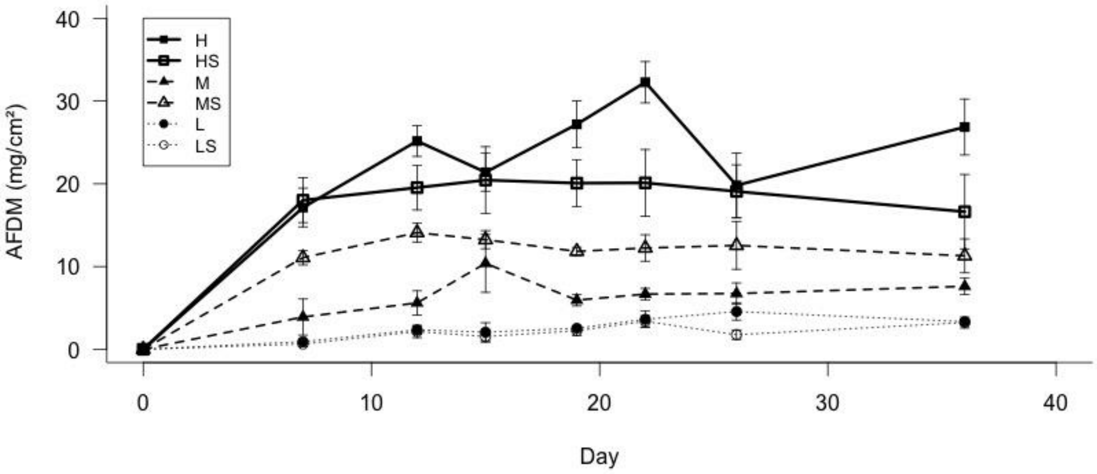


Figure. 3.6: Accumulation of AFDM in different discharge and grazer treatments. H-High flow, HS- High with Snails, M-Medium flow, MS-Medium flow with Snails, L-Low flow, LS- Low flow with snails. Each point represents the mean for 3 replicate channel discharge treatment ± one standard error

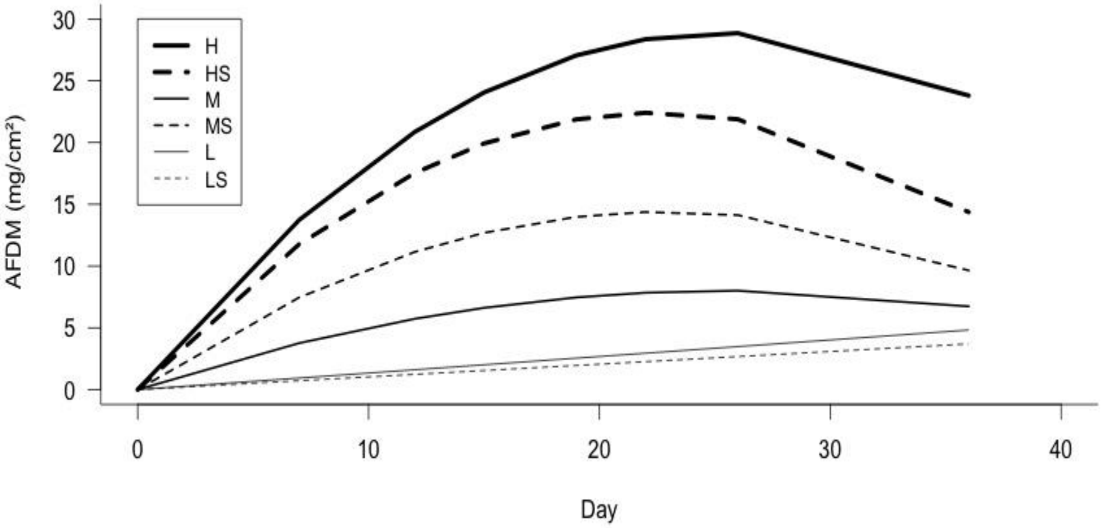


Figure 3.7: Regression models for AFDM accumulation for each discharge and grazing treatment.

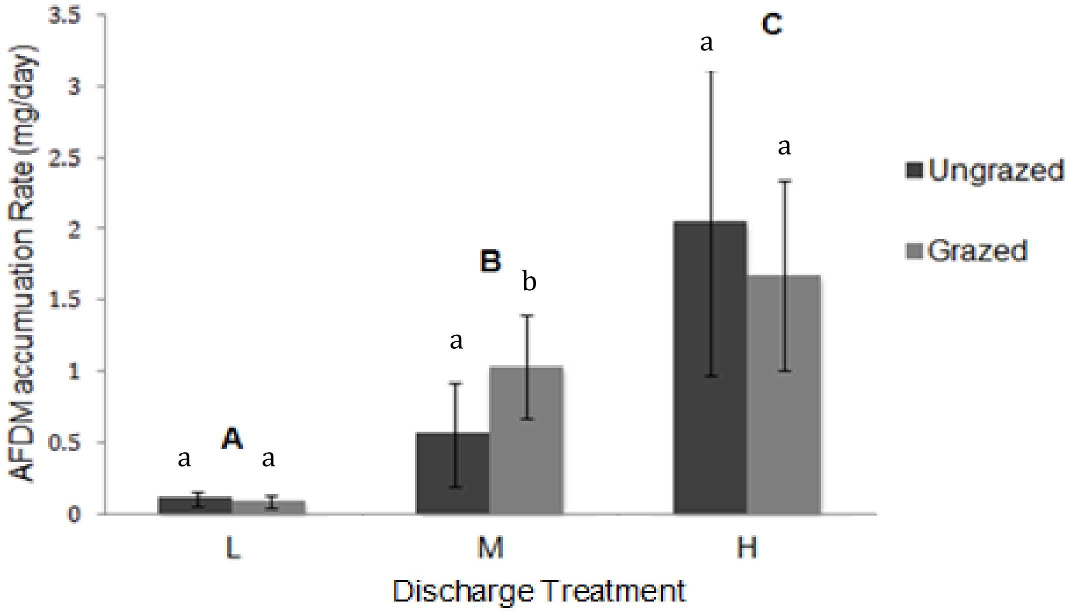


Figure 3.8: Mean AFDM accumulation rates for discharge and grazer treatment combinations. Bars are means of regression slopes for 3 replicate channels± 95% CI. Discharge treatments with the same letter are not statistically different.

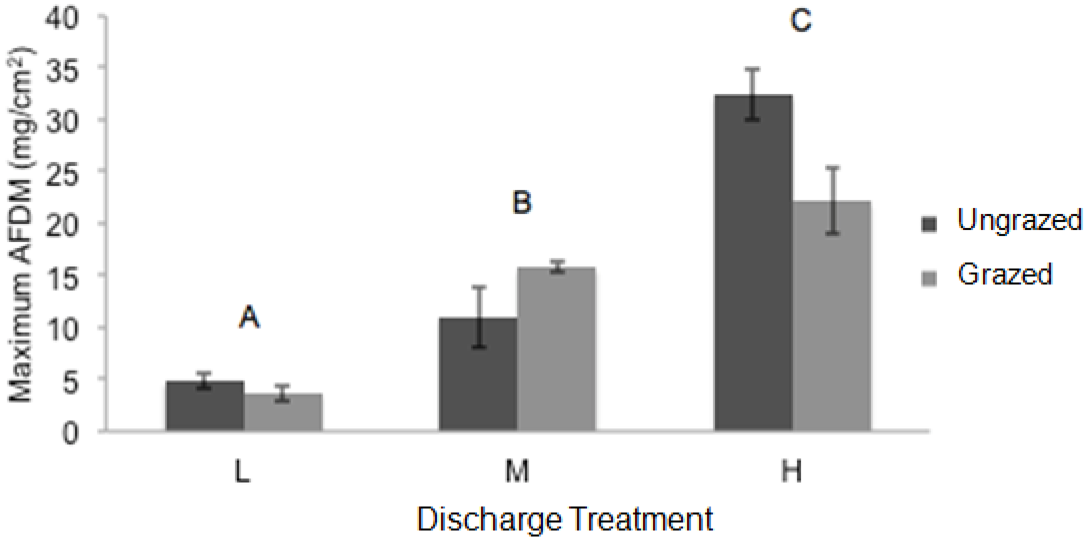


Figure 3.9: Maximum AFDM for different discharge and grazer treatments. Discharge treatments with the same letter are not significantly different. Bars are means ± 1 SE.

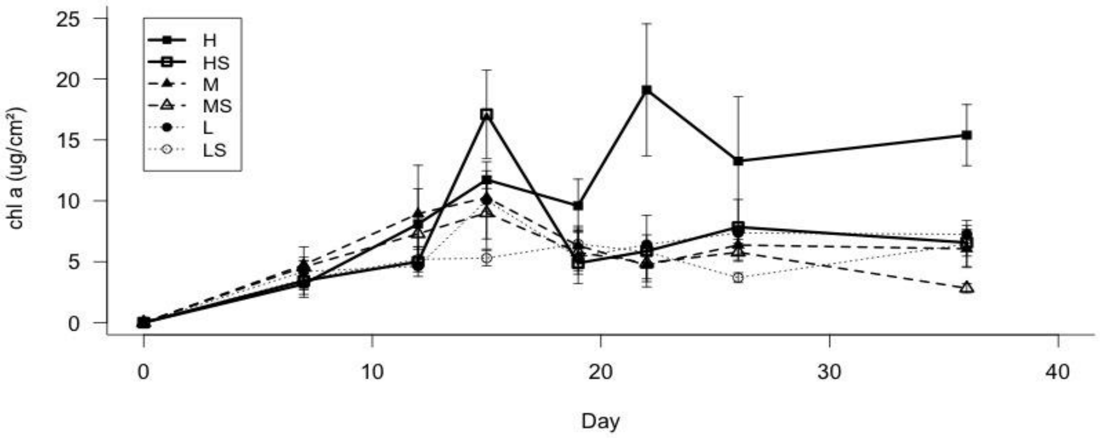


Figure 3.10: Chlorophyll a accumulation in different discharge and grazer treatment combinations. Points are means of 3 replicate channels ± 1 SE

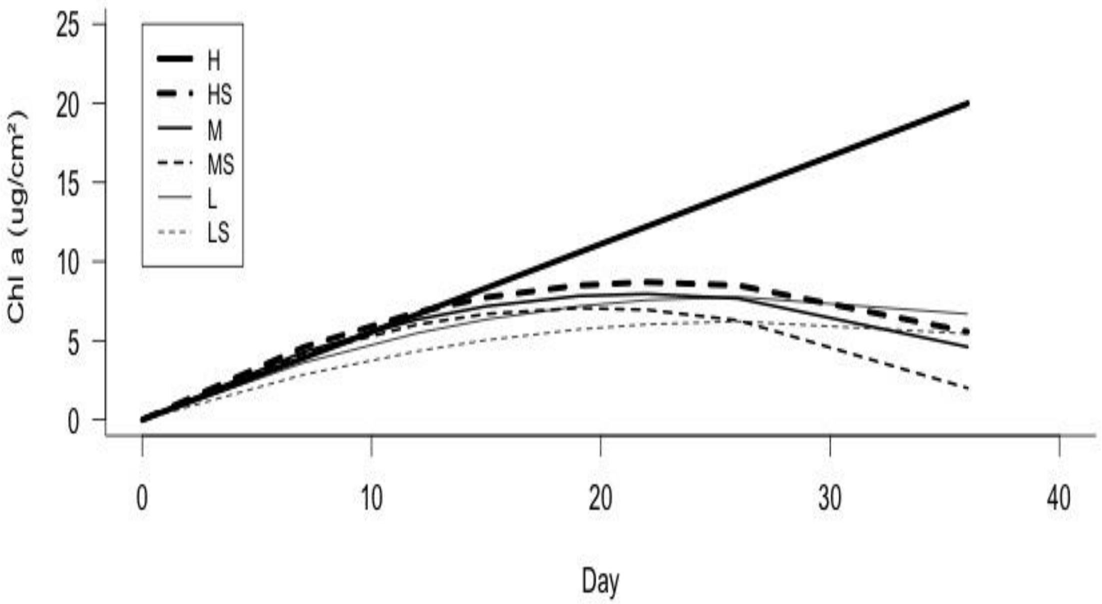


Figure 3.11: Regression models for chl a accumulation in different discharge and grazer

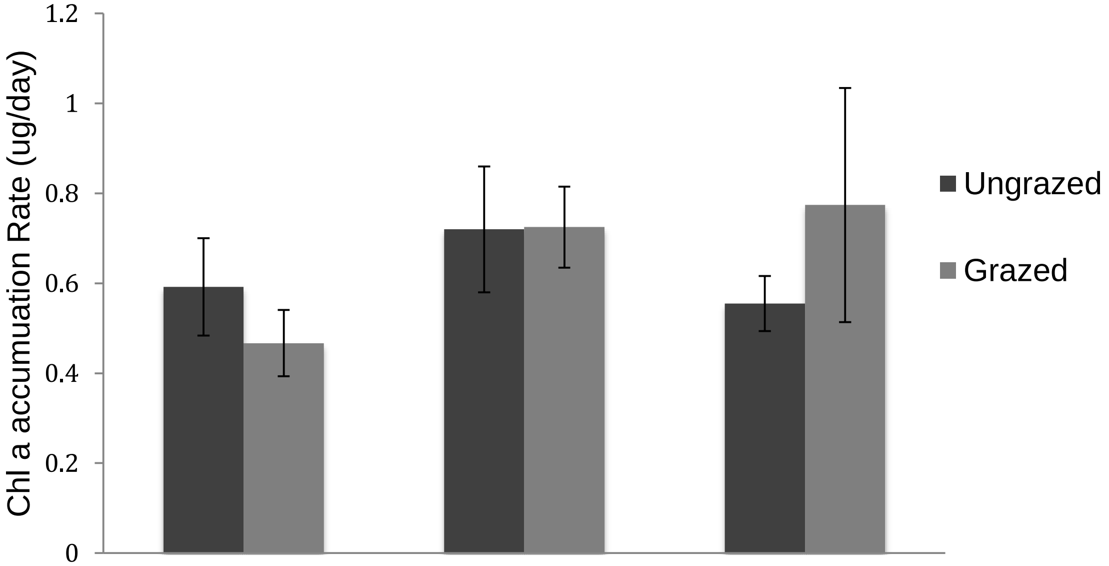


Figure 3.12: Mean chl a accumulation rates for discharge and grazer treatments. Bars are means of regression slopes for 3 replicate channels ± 95% CI.

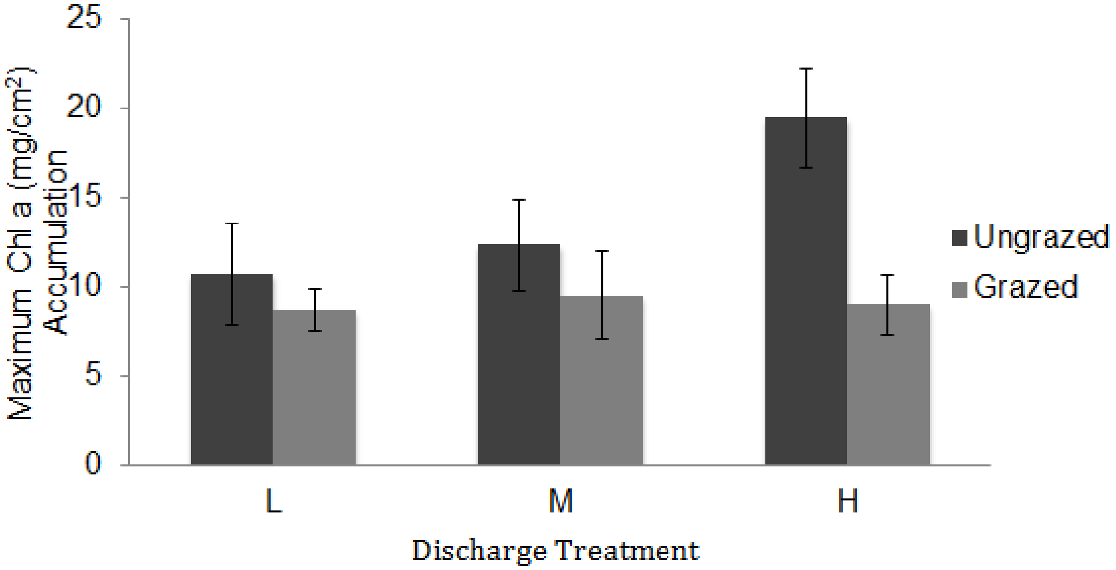


Figure 3.13: Maximum chlorophyll a concentrations for different discharge and grazer treatments. Grazer treatments with the same letter are not significantly different. Bars are means for 3 replicate channels ± 1 SE

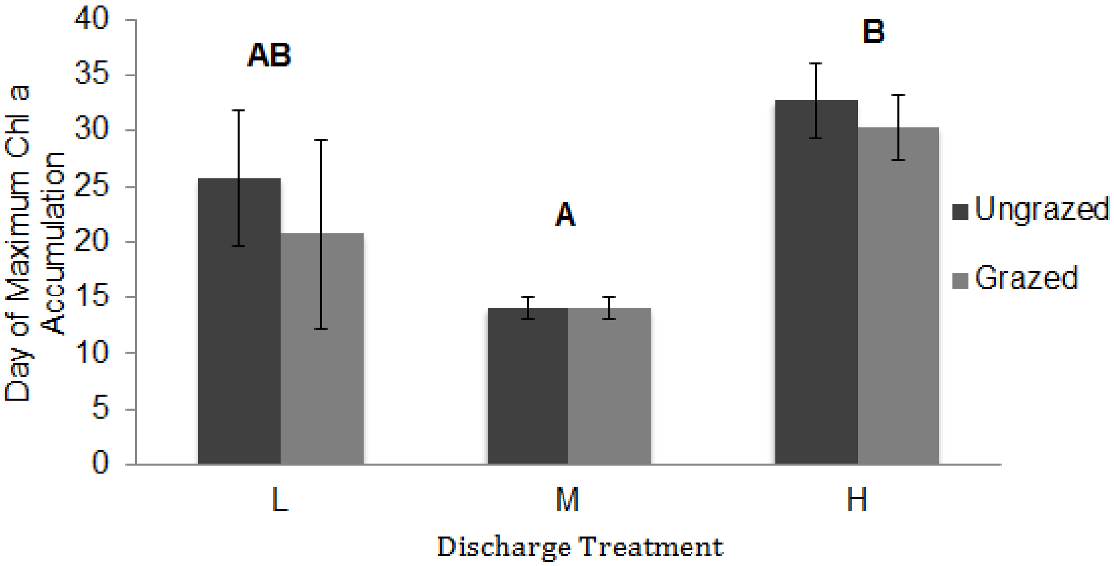


Figure 3.14: Day of maximum chl a concentrations for different discharge and grazer treatments. Discharge treatments with the same letter are not significantly different.

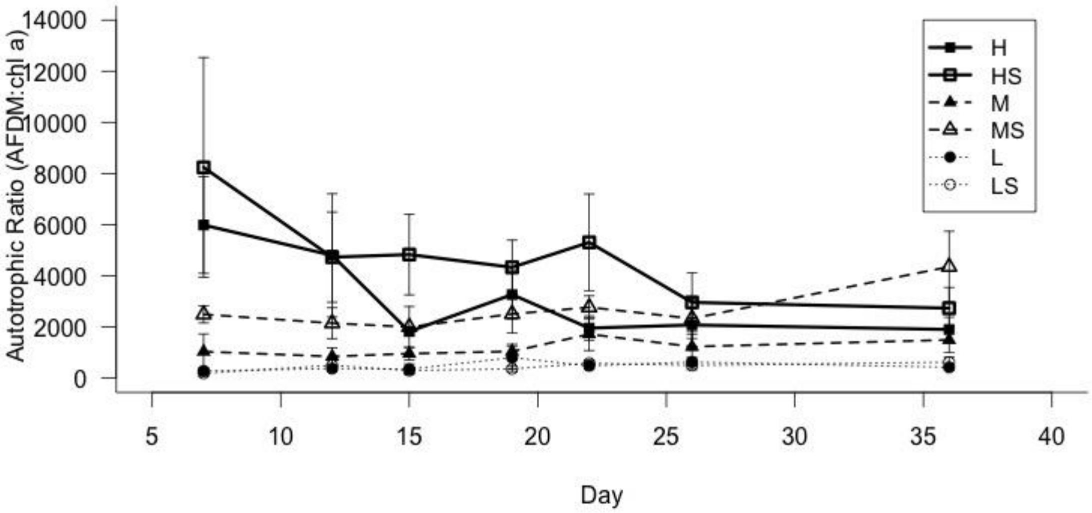


Figure 3.15: Autotrophic Index ratios for different discharge and grazer treatments.

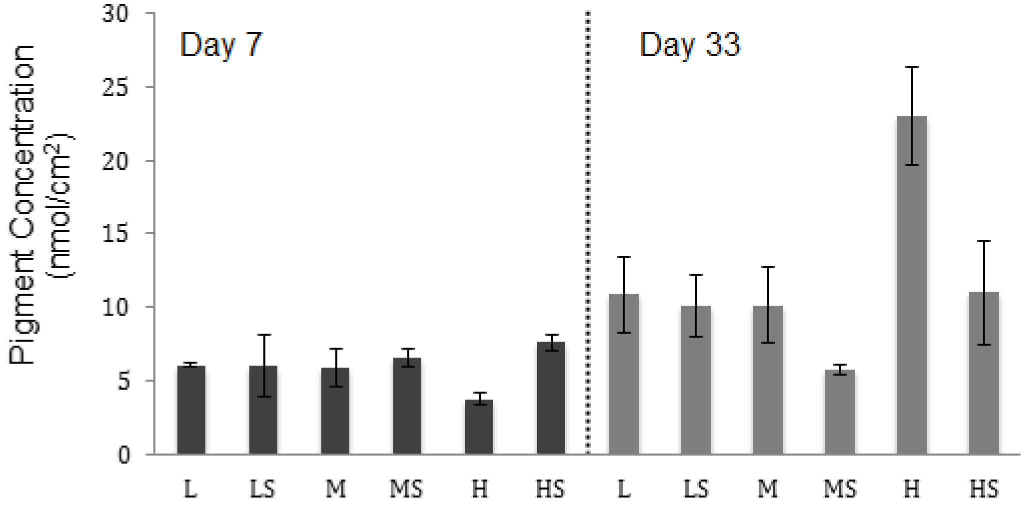


Figure 3.16: Total pigment concentrations for different discharge and grazer treatments on Day 7 and 33 of experimentation. Bars are means for 3 replicate channels ± 1 SE.

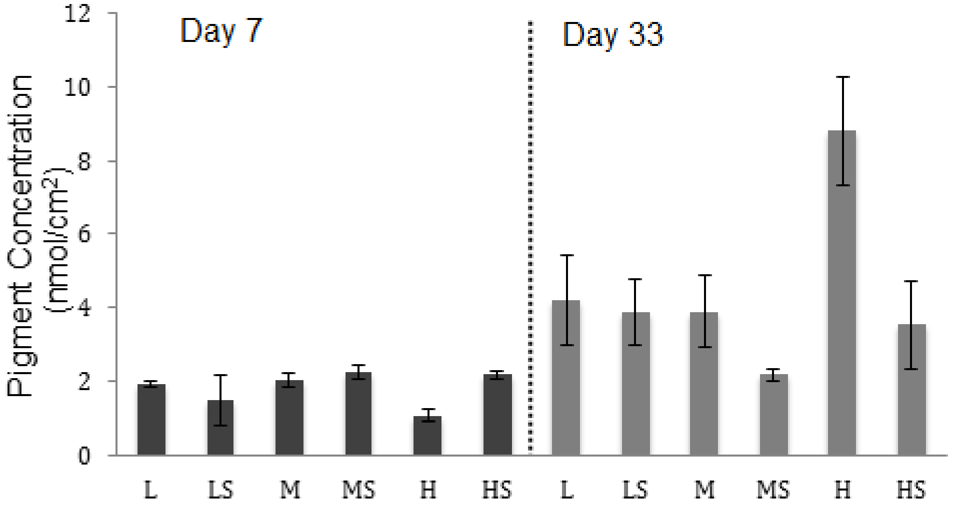


Figure 3.17: Siliceous pigment concentrations for different discharge and grazer treatments on Day 7 and 33 of experimentation. Bars are means for 3 replicate channels ± 1 SE.

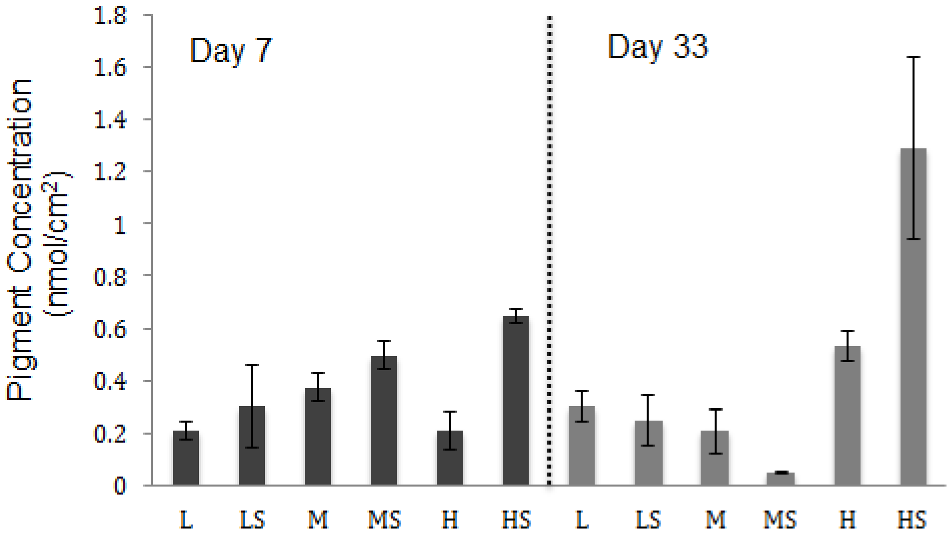


Figure 3.18: Green algal pigment concentrations for different discharge and grazer treatments on Day 7 and 33 of experimentation. Bars are means for 3 replicate channels ± 1 SE.

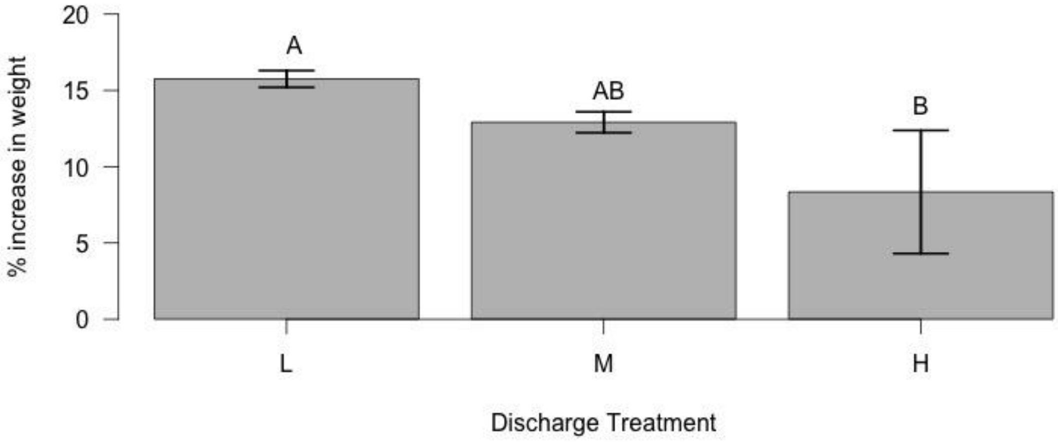


Figure 3.19: Snail growth rates in different discharge treatments. Bars are means for 6 replicate channels ± 1 SE

CHAPTER 4

CONCLUSIONS

Summary

The lower Flint River Basin is one of the most productive agricultural regions in the U.S (McKissick, 2004). With the advent of row-crop agriculture supported by center-pivot irrigation, this area has experienced an increase in growing season water withdrawals. Additionally, drought frequency is projected to increase in this region as a result of global climate change (Binita et al. 2015). The combined effects of increased human water use and drought have reduced summertime stream flows, and these reduced flows may in turn affect periphyton distribution, composition, and production. A better understanding of flow-ecological relationships, especially factors that promote rapid algal growth, can provide better understanding of the ecological impacts of human water consumption and low flows on lotic systems so that managers can better balance environmental flow requirements with the needs of society (Poff et al. 2010, Davies et al. 2014).

We used controlled experiments to quantify the effect of reduced flows on periphyton biomass and composition. Our study indicated that periphyton growth potential is high during the summer growing season. This may be due to favorable conditions within the stream, such as high light availability and a stable flow regime. Under these conditions, differences in the local discharge environment within the stream strongly influenced periphyton accumulation rates, maximum biomass, and taxonomic composition. For our first experiments we observed higher AFDM and lower chl a concentrations with increasing discharge throughout the course of experimentation while in our last experiment we observed both higher AFDM and chl concentrations with increasing discharge. Though these patterns are different, they both demonstrate that flow has an influence on both the biomass and photosynthetic capacity of periphyton. Differences in autotrophic community compositions can affect primary production and biogeochemical cycling within periphyton mats and influence their function in stream systems (Freeman & Lock 1995, Villeneuve et al. 2010)

Results from our nutrient experiment indicated that nutrient enrichment effects were greater at higher discharges than at lower discharges. Greater discharge, which generated turbulent flow conditions that facilitated nutrient transfer to algal cells through a decrease in the diffusive boundary layer, could provide an ideal scenario for algal growth (Biggs et al. 1998). This result suggests that in streams with greater nutrient availability, algae in higher flow environments could have a higher nutrient uptake potential than those found in lower flow habitats.

Flow alterations may also shift algal composition and consumption by stream grazers. We observed that at lower flows we had a greater accumulation of green algae, while diatom dominance was found in higher flows. We also observed that grazers exerted limited control over periphyton. Although at higher flows there was a greater grazer effect on periphyton growth, it appears that during low flow conditions periphyton growth rates may be higher than algal export rates due to consumption and dislodgement of algal biomass by grazers. Shifts in algal composition and accumulation can influence grazer-periphyton dynamics based on herbivore preferences in algae they consume (Hintz & Wellnitz 2013).

This study identifies some of the ecological consequences of increased severity and duration of low-flow conditions due to climate and human water use. In times of drought and heavy water withdrawals it is likely that there will be a shift from higher discharge habitats to lower discharge habitats along with changes in periphyton accumulation rates and composition. Flow alterations can, therefore, affect algal resources with potential effects on grazer habitat due to changes in periphyton biomass and growth forms (Dewson 2007) and food quality for aquatic consumers (Hessen & Andersen 2002). These shifts could affect food web dynamics based on flow alterations (Finlay et al. 1999, Jardine et al. 2013).

Future Research

Although we did not manipulate light levels in our experiments, we recognized during the course of experimentation that light availability exerts strong control over periphyton growth in colored streams (Carey et al. 2005) like Ichawaynochaway Creek. To further explore light availability and influence on periphyton growth, it is recommended that future studies manipulate light levels in order to quantify growth-light relationships. To learn more about flow's influence on grazer-periphyton interactions, it may be useful to apply a variety of snail densities within the stream's natural range in the experimental channels. Additionally stable isotope analysis could help provide a better understanding of food web linkages (Power & Rainey 2000) and the possible ecosystem impacts from changes in algal basal resources due to flow alterations. An inclusion of other invertebrate grazers and interspecies interactions on benthic algal assemblages and growth may be helpful to understand the full range of grazer-algae interactions in the creek (Wellnitz & Poff 2012).

Our study provides insight into the direct and indirect effect of low flows on periphyton through its influence on nutrient availability and the capacity of grazers to remove algae in benthic systems. This study is particularly important in highlighting the flow-periphyton relationship in agricultural areas where reductions and alterations of stream discharge due to human water abstraction and the influence of warmer climate is projected to be a prominent issue in the future.

References:

Biggs, B. J., Goring, D. G., & Nikora, V. I. (1998). Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology, 34(4), 598-607.

Binita, K. C., Shepherd, J. M., & Gaither, C. J. (2015). Climate change vulnerability assessment in Georgia. Applied Geography, 62, 62-74.

Carey, R., Vellidis, G., Lowrance, R., & Pringle, C. (2005). Nutrient enrichment and stream periphyton growth in the southern coastal plain of Georgia. *ASAE Technical Paper*, (05-2197).

Davies, P. M., Naiman, R. J., Warfe, D. M., Pettit, N. E., Arthington, A. H., & Bunn, S. E. (2014). Flow–ecology relationships: closing the loop on effective environmental flows. Marine and Freshwater Research, 65(2), 133-141.

Dewson, Z. S., James, A. B., & Death, R. G. (2007). A review of the consequences of decreased flow for in-stream habitat and macroinvertebrates. Journal of the North American Benthological Society, 26(3), 401-415.

Finlay, J. C., Power, M. E., & Cabana, G. (1999). Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. Limnology and Oceanography, 44(5), 1198-1203.

Freeman, C., & Lock, M. A. (1995). The biofilm polysaccharide matrix: a buffer against changing organic substrate supply?. Limnology and Oceanography,40(2), 273- 278.

Hintz, W. D., & Wellnitz, T. (2013) Current velocity influences the facilitation and removal of algae by stream grazers. Aquatic Ecology, 47(2), 235-244.

Hessen, D. O., Færøvig, P. J., & Andersen, T. (2002). Light, nutrients, and P: C ratios in algae: grazer performance related to food quality and quantity.Ecology, 83(7), 1886-1898.

Jardine, T. D., Hunt, R. J., Faggotter, S. J., Valdez, D., Burford, M. A., & Bunn, S. E. (2013) Carbon from periphyton supports fish biomass in waterholes of a wet–dry tropical river. River Research and Applications, 29(5), 560-573

McKissick J.C. (2004) The Economic Importance of Irrigated Food and Fiber Production: A Spotlight on Georgia’s Flint River Basin. The University of Georgia Center for Agribusiness and Economic Development, Athens, GA

Poff N.L., Richter B.D., Arthington A.H.et al. (2010) The ecological limits of hydrologic alteration (ELOHA): a new framework for developing regional environmental flow standards. Freshwater Biology, 55, 147, 170

Power, M. E., & Rainey, W. E. (2000). Food webs and resource sheds: towards spatially delimiting trophic interactions. Ecological consequences of habitat heterogeneity, 291-314

Villeneuve, A., Montuelle, B., & Bouchez, A. (2010). Influence of slight differences in environmental conditions (light, hydrodynamics) on the structure and function of periphyton. Aquatic Sciences, 72(1), 33-44.

Wellnitz, T., & Poff, N. L. (2012). Current-mediated periphytic structure modifies grazer interactions and algal removal. Aquatic Ecology, 46(4), 521-530.