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THE IMPORTANCE OF HEADWATER STREAMS IN PRESERVING WATER QUALITY:

A CASE STUDY OF THE KENNEBEC HIGHLANDS AND LONG POND





COLBY COLLEGE PROBLEMS IN ENVIRONMENTAL SCIENCE WATERVILLE, MAINE 04901 FALL 2013

Authors

This research was conducted by the students of the Environmental Studies 494 *Problems in Environmental Science* course at Colby College in Waterville, Maine during the fall semester of the 2013-2014 academic year. The advisers for this study were Professor Denise Bruesewitz and Teaching Assistant Abby Pearson.



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CHAPTER 1 INTRODUCTION

ABSTRACT: The Belgrade Lakes watershed contains a unique ecosystem and social environment. The landscape has experienced continuous change over the past several centuries due to evolving land use patterns and social contexts (Burgess & Nelson, 2009). The Belgrade Lakes are the centerpiece of the watershed, with most human activity revolving around tourism and sporting on and around the lakes. Therefore, the water quality of the lakes is critically important to social and economic systems in the watershed. Flowing into the lakes are several, often overlooked, headwater streams that support the ecological integrity of the entire watershed. However, these streams, along with headwater streams all over the world, are subject to increasing human impacts that could cause the ecological support they provide to be severely degraded. The goal of the Colby Headwater Stream Research Project is to assess the health of three protected, forested headwater streams that drain into Long Pong (one of the Belgrade Lakes), with the hope that information about these healthy stream conditions can be incorporated into watershed conservation strategies that affect more impacted streams throughout the Belgrade watershed.

1.1 The Belgrade Lakes Watershed

A catchment is an area of land that drains rainwater and groundwater into the same aquatic network. A catchment is delineated by a 'watershed', however we will use both catchment and watershed to refer to the area of land that drains into a lake, as is common practice. For example, while each of the Belgrade Lakes is a separate body of water, the entire network of lakes is connected through streams, wetlands, and an underground drainage system that collects water from all of these sources. Due to this strong connectivity of these water bodies, human disturbance to any aspects of these ecosystems or the land within the watershed can greatly disturb the function of the entire watershed (Fig. 1.1; Colby College, 2012).

The Belgrade Lakes region, located in central Maine, spans 180 square miles, 13 towns, and seven interconnected lakes (Burgess & Nelson, 2009; Colby College, 2012). However, the Belgrade Lakes watershed covers only 45 square miles (Fig. 1.1; Burgess & Nelson, 2009). The Belgrade Lakes include North Pond, East Pond, McGrath Pond, Salmon Lake, Great Pond, Long Pond, and Messalonskee Lake. While the most apparent connection, visually, in the lakes is between McGrath Pond and Salmon Lake, which intersect, there is a distinct flow within the watershed, from Great Pond

to North Pond to Long Pond to Messalonskee Lake. Beyond this inter-lake connectivity, there are many streams, rivers, and wetlands throughout the catchment that provide channels for water flow.



Fig. 1.1 a and b. a. Map of Maine with a red star indicating the location of the Belgrade Lakes watershed. b. Map of the Belgrade Lakes watershed in central Maine, including Long Pond, the focus of our study. Long Pond receives water from Great Pond, and the outlet of Long Pond flows into Messalonskee Lake. Images courtesy of Destination 360 and **Belgrade** Regional Conservation Alliance.



1.2 Belgrade Lakes History

The Belgrade watershed in central Maine has a rich history dating back to the Abenaki Indians, who resided in the area before European colonization in 1774 (Burgess & Nelson, 2009). As hunter-gatherers, the Abenaki tribe utilized the rich biodiversity in the pristine Belgrade Lakes region for food and shelter. After Europeans began colonizing the region in the late 18th century, the local population rapidly increased (Burgess & Nelson, 2009). The new residents cleared land for crop agriculture, particularly potatoes and apples, which quickly emerged as the region's leading industry (Burgess & Nelson, 2009). Milling operations, mostly grist and lumber, also grew in the area, as the lakes provided an excellent source of waterpower (Burgess & Nelson, 2009). Throughout this period of economic development and population growth, land was continuously cleared or modified to utilize natural resources.

One of the most unique aspects of the Belgrade community, in history and in present day, is the great influx of tourists every summer, which creates a large seasonal population in the region. Tourism in Belgrade became more prominent in the mid 1880s, partially due to the railroad and automobile revolutions and the nation's increasing respect and appreciation for the natural world (Burgess & Nelson, 2009). As tourism began replacing agriculture as the region's central industry, farms grew less common, and land was increasingly converted from farms to lakeshore cabins and homes. While this shift away from agriculture began to naturally reforest the land, the rapid surge in tourism and concurrent increase in the construction of railroads, roads, and cabins began to degrade the lake environment and threaten the stability of the watershed's ecosystem (Burgess & Nelson, 2009).

The negative impacts of tourism and land use are a problem that the Belgrade community still faces today. However, many conservation organizations have been established to help negate these impacts and conserve the unique Belgrade watershed ecosystem. All seven of the Belgrade Lakes have been placed on Maine's list of water bodies most at risk from development (Burgess & Nelson, 2009). Maine's Sustainability Solutions Initiative (SSI) funded by the National Science Foundation's Experimental Program to Stimulate Competitive Research (ERPSCoR) has the mission to advance economic and community development while protecting the environment. There is a Colby College team associated with SSI currently studying the effects of development on lake water quality in the Belgrade Lakes region (Fleming & Love, 2012). Many local conservation efforts have also emerged, which Fleming & Love (2012) speculate is due to the local residents' connection to the environment and consequent passion to preserve the place they have grown to love. Local conservation groups include lake associations, whose members are shoreline residents of the Belgrade Lakes, and the Belgrade Regional Conservation Alliance (BRCA), which has devoted substantial time and energy to promoting sustainable behavior among residents and tourists. The BRCA has partnered with the Maine Department of Environmental Protection and the U.S. Environmental Protection Agency to create a 10-year watershed management plan for Great and Long Ponds (Burgess & Nelson, 2009). By extending this conservation plan to the entire watershed, rather than just the lakes, the plan reflects a new focus on whole watershed protection and including wetlands and streams in conservation efforts. This shift in conservation policy is important because it means that headwater streams, which are a crucial part of

aquatic ecosystems and often overlooked, are finally beginning to receive the attention and protection they deserve (Lowe & Likens, 2005).

1.3 The Importance of Headwater Streams

Headwater streams are defined in many different ways, but fundamentally this means they are the smallest sized streams that flow into larger streams, river, and lakes. Headwater streams are first and second order streams, and they do not have any water flowing into them from other water bodies; water comes from landscape runoff or groundwater upwelling (Strahler, 1952; Meyer *et al.*, 2007). Freeman *et al.* (2007) define first order streams as intermittent or perennial streams with no temporary or perennial tributaries and second order streams as streams created by the junction of two first order streams. Poole (2010) more basically describes a stream community as an ecosystem that arise from, incorporates, and is dependent upon the factors of channelized water flow and the sediment transport dynamics. Furthermore, it has been concluded that headwater streams often compose over two- thirds, or 70%, of total stream channel length in the United States (Lowe & Likens, 2005; Benda *et al.*, 2005; Freeman *et al.*, 2007). This illustrates that while they may individually be small, headwater streams aggregately compose a major portion of aquatic ecosystems. Freeman *et al.* (2007) even go as far as stating that every important aspect of a river system starts in headwater streams.

Headwater streams provide crucial connectivity and linkages in aquatic ecosystems. These streams help maintain hydrologic connectivity by connecting all upland and riparian landscapes to the rest of the aquatic ecosystem (Allan, 2004; Freeman *et al.*, 2007). These linkages are crucial in providing movement corridors for biota, as well as for transporting water, sediment, nutrients, and organic matter downstream (Vannote *et al.*, 1980; Lowe & Likens, 2005; Clarke *et al.*, 2008). Headwater streams are connective arteries that transfer energy, nutrients, and organisms among terrestrial, river, lake, and marine ecosystems. They have even been described as "capillaries" of aquatic networks, which illustrates the idea that disturbing or severing these streams from the terrestrial environment or downstream ecosystems can drastically alter all downstream processes (Freeman *et al.*, 2007).

One of the crucial functions of headwater streams is processing terrestrial organic matter, such as leaves, branches, and woody debris. Organic matter is a critical energy source that serves as the base of the food web both in headwater streams and often in the rivers and lakes they flow into (McDowell & Fisher, 1976). Organic matter can enter streams as coarse particulate organic matter (CPOM) such as

leaves or sticks, fine particulate organic matter (FPOM), and dissolved organic matter (DOM) (Bilby & Likens, 1980; Tank *et al.*, 2010). Organic matter also can be created by primary production within the stream ecosystem (Fisher, 1977). In small streams, this algal and terrestrial organic material provides food for macroconsumers that directly shred and ingest it, as well as microorganisms that facilitate its decay and enhance its nutritive value by decreasing detrital carbon and nitrogen (McDowell & Fisher, 1976; Bilby & Likens, 1980). Beyond providing a direct food source for organisms that live in headwater streams, the organic matter that is not altered by respiration or consumption is stored in the stream or exported downstream, providing major energy inputs for the associated downstream river or lake ecosystem (Fisher, 1977; Webster & Meyer, 1997; Tank *et al.*, 2010).

Headwater streams also filter sediment and pollutants, maintaining high water quality and providing downstream habitats with inputs of clean water, sediment, and nutrients. Nutrient spiraling is a relatively new concept that considers the continuous downstream flow that carries nutrients in a lotic system (Newbold *et al.*, 1982; Palmer & Febria, 2012). Therefore, as nutrients are continuously processed in a given reach, the retention and reutilization of the nutrients depends on how quickly the nutrients are or are not taken up by organisms in the system. This distance between points where the nutrient is taken up by an organism, used, and re-released into the stream is often correlated with how small the stream is— implying that headwater streams have the tightest, most effective nutrient spiraling (Newbold *et al.*, 1982; Peterson *et al.*, 2001). Nutrients can be imported to the streams from groundwater, overland flow, riparian vegetation, or human sources (Gomi *et al.*, 2002). Headwater streams are crucial for downstream nutrient regulation because, assuming a stream maintains efficient nutrient spiraling, it will be able to filter out excess nutrients such as phosphorus (P) or nitrogen (N) before those nutrients enter and disturb downstream ecosystems (Lowe & Likens, 2005).

Headwater streams maintain high levels of biodiversity due to their unique habitats that provide refuge for many organisms, ultimately contributing to the biodiversity of the entire aquatic network. Small streams provide an array of microhabitats for microbes, plants, algae, and animals due to the complexity of the landscape—there is often both lentic (still water) and lotic (flowing water), rocks, woody debris, and many other factors that contribute to a diversity of habitats and environmental conditions (Meyer *et al.*, 2007). This level of habitat diversity creates niches for many different types of organisms, even headwater-specialist species of aquatic invertebrates (Lowe & Likens, 2005; Poff, 1997). Many organisms seeking refuge from predators, competitors, alien species, swift rapids, or even poor downstream water quality colonize headwater streams, contributing to their rich biodiversity

Introduction

(Meyer *et al.*, 2007). This high level of biodiversity is important not only for the stability of the headwater stream ecosystem, but it functions as a replacement for degraded downstream ecosystems (O'Hop & Wallace, 1983). Much like organic matter, sediment, and nutrients, invertebrates and other organisms also will travel downstream, so if any disturbance downstream—natural or human—depletes biodiversity, eventually organisms will drift downstream and recolonize the disrupted area, reestablishing ecosystem function as the water quality improves (Lamberti *et al.*, 2010). This illustrates that headwater streams can serve as a refuge for many organisms during periods of disruption.

Because of the strong interconnectivity among aquatic ecosystems in the Belgrade watershed, many headwater streams flow directly into the Belgrade Lakes, thereby impacting lake water quality. This strong connectivity in the Belgrade watershed means that the headwater streams hold a key position in the aquatic network. They have the power to process nutrients and sediment and serve as a biodiversity hotspot. However, the connectivity that allows them to perform these beneficial functions also means that if these headwater streams are impacted they could bring increased nutrients and sediment to the lakes. The health of headwater streams can impact the health of downstream ecosystems, the Belgrade Lakes in the case of this study.

1.4 The Colby Headwater Stream Research Project

Headwater streams play a critical role in the function of aquatic ecosystems by providing unique habitats and performing important many functions. Because headwater streams process inputs differently than larger streams do, they need to be studied differently (Gomi *et al.*, 2002). The Colby College ES494 Problems in Environmental Science 2013-2014 Capstone class, referred to as the Colby Headwater Stream Research Project in the rest of the paper, aimed to measure and understand the diversity of functions of these frequently overlooked stream ecosystems.

Tracking the movements of energy, nutrients, sediments, and organisms are fundamental parts of understanding any ecosystem, and there are several models for documenting stream ecosystem processess that are applicable to headwater streams in particular. One of the most useful is the River Continuum Concept, proposed by Vannote *et al.* (1980). They suggested viewing a river ecosystem as a continuous gradient of biological communities that change based on the physical characteristics of their habitat (Vannote *et al.*, 1980). In this model, downstream communities capitalize on the inefficiencies, or leakages, of upstream ecosystems, thereby minimizing the ecosystem's total energy loss (Vannote *et al.*).

al., 1980). In addition to using a theoretical model, monitoring the ecosystem in the field is essential to an accurate understanding of any headwater stream system's function.

1.5 Measuring Headwater Stream Ecosystem Function

Because headwater streams are a critical foundation to aquatic ecosystems and changes in them can have disproportionately large impacts on downstream ecosystem function, monitoring their health is important (Richardson, 2000). The process of measuring headwater stream health is far from perfect, and there is room, in particular, for improvement in the detection of nuanced changes (Richardson, 2000). Picking the measurements that are best suited to a particular site and situation is another challenge (Dahm *et al.*, 1995; Northington *et al.*, 2011). However, several measurements, such as stream metabolism, macroinvertebrate community quality, leaf decomposition rate, and nitrogen uptake length, have been established as accurate health indicators in most ecosystems (Gessner & Chauvet, 2002; Young *et al.*, 2008; Bernot *et al.*, 2010; Canobbio *et al.*, 2010; Clapcott *et al.*, 2010; Northington *et al.*, 2011). The primary reason these metrics are so effective is that they measure ecosystem function, rather than only assessing ecosystem structure.

The difference between structural and functional metrics and the information they provide is important. Historically, researchers studied stream ecosystems by characterizing ecosystem structure, quantifying ecosystem services, or comparing the ecosystem to a past state or reference site (Palmer & Febria, 2012). This approach is analogous to taking a snapshot of an ecosystem and basing the analysis of an ecosystem's state on those static data. However, a much richer body of information can be obtained if ecosystem functional dynamics are evaluated by taking dynamic, repeat measurements of key processes (Palmer & Febria, 2012). In fact, Peterson *et al.* (2001) showed that using functional metrics, in addition to structural metrics, can lead to discovering more details about ecosystem processes and completely change the resulting theories about how streams function.

Functional metrics are becoming increasingly popular within the river and stream ecological research communities, with researchers recommending such techniques to their colleagues (McGill *et al.* 2010). Researchers can particularly benefit from using functional metrics or a combination of structural and functional metrics. For example, Young & Collier (2009) found that functional metrics were especially useful for monitoring non-linear responses to disturbances and detecting early signs of degradation in high-quality habitat. Ryder & Miller (2005) point out that structural variables can be misleading when measuring progress on a restoration project, so additional functional metrics may be

necessary to see if a stream's functional capacities have recovered after a disturbance. The power of functional metrics lies in the fact that they require researchers to holistically consider the causal processes and relationships at play in an ecosystem (Norris & Thoms, 1999; Bunn & Davies, 2000).

The Colby Headwater Stream Research Project used a combination of structural and functional metrics to assess the impacts of three headwater streams on Long Pond water quality in the Belgrade Lakes watershed. Vannote *et al.* (1980) explain how upstream ecosystems affect downstream ecosystems, so we expected to find a clear relationship between the water quality of the protected headwater streams we studied and the water quality of Long Pond. The study also considers the role of anthropogenic activity in the headwater stream ecosystems of the Belgrade watershed and suggests ways that local residents can ensure that the ecosystems remain healthy in the future.

1.6 Anthropogenic Disturbance: A Conservation Lessons Preview

Logging activity, riparian buffer condition, and best management practices are all humancontrolled factors that have the potential to affect the health of headwater stream ecosystems. Logging is of particular concern to our project because important management decisions about logging in the Belgrade watershed will be made in the near future. These decisions could drastically impact headwater stream ecosystems. Likens and Bormann (1970) found that deforestation around a headwater stream increased its net nitrogen exports, raised its temperature, and shifted particulate matter export to contain a majority of inorganic particulate matter. Mallik *et al.* (2011) found that clear-cutting without a riparian buffer has long-lasting impacts on the closely linked headwater stream and riparian habitats for up to fifteen years following the logging event. Some studies have shown that while the popular forestry technique of selective logging may have less of an impact than clear-cutting, it still alters ecosystem function, and a significant riparian buffer is needed to mitigate logging effects (Huryn, 2000; VanDusen & Huckins, 2005; Winkler *et al.*, 2009).

One study that used only structural metrics claimed that riparian buffers completely annul the immediate effects of logging near streams, which again demonstrates the importance of incorporating functional metrics into any study (Jackson *et al.*, 2001). However, most scientists agree that riparian buffers can minimize, but not completely offset, the impacts of both clear-cutting and selective logging (Macdonald *et al.*, 2003; Wilkerson *et al.*, 2006; Mallik *et al.*, 2011). The disparity among these studies further shows the importance of using functional metrics and assessing ecosystem function over a period of time. Many communities now recognize the important role that riparian zones play in protecting

aquatic ecosystems, so Naiman *et al.* (2000) suggest that the goal going forward should be to establish watershed-wide networks of connected, healthy riparian zones, as opposed to disjointed sections of riparian zones separated by swathes of exposed, unprotected streams.

In order to successfully protect and conserve the Belgrade Lakes Watershed, policy makers and advocacy groups need to consider headwater streams and the concept of complete watershed management (Osborne & Kovacic, 1993; Alexander et al., 2007). The Kennebec Highlands Management Plan, written by the Maine Department of Conservation, is a unique plan for the Kennebec Highlands, a 6,000-acre public reserved land unit in the Belgrade Watershed (Maine Department of Conservation, 2011). This plan protects many headwater streams associated with the Belgrade Lakes, including two of our three study sites, by managing land use of nearby riparian habitat. However, in the next revision of the Kennebec Highlands Management Plan, policy makers should more specifically examine the headwater streams in the Belgrade watershed and the ecosystem services they provide. This measure would allow them to properly conserve the entire watershed as successfully and efficiently as possible.

1.7 Project Overview



Fig 1.2. A visual map of research topics, show as seen visually in headwater streams. As shown in the diagram, algae on organic matter, often a food source for macroinvertebrates, metabolizes energy from the sun. Throughout the stream nutrient spiraling, as well as sedimentation and erosion, occurs.

The question guiding our research this fall was: What ecosystem services do headwater streams provide to the Belgrade Lakes? In order to explore this question, we investigated the role of three



Fig. 1.3. Map of headwater stream study sites (indicated by red arrows) near Long Pond (thinly outlined in light blue). From the top of the map to the bottom are Whittier Stream, Beaver Brook, and Stony Brook. A reach of 100m was studied in each of these streams. The orange dots document disturbed stream study locations, which will be addressed in Chapters 5 and 8.

headwater streams in processing nutrients, carbon, and sediment before flowing into Long Pond. Additionally, we assessed the potential anthropogenic effects of riparian land use on headwater stream ecosystem services. Six research groups evaluated the structure and function of the three study sites, all protected headwater streams. Fig. 1.2 displays how each research topic manifests itself in a headwater stream, while Fig. 1.3 documents each research subject addressed, along with its chapter number in this report and its relationships with other research subjects. A brief overview of each group's research questions and some of the most fundamental articles they used to situate their research follows.

1.8 Study Sites

We examined three headwater streams within the Belgrade Lakes watershed that ultimately flow into Long Pond (Fig. 1.4). Two of these streams, Whittier Stream and Beaver Brook, are protected under the Kennebec Highlands Management Plan. The Kennebec Highlands is an approximately 6,000 acre public reserved land unit managed by the Maine Bureau of Parks and Lands (Maine Department of Conservation, 2011). This land is protected primarily for public recreational opportunities, but ecosystem conservation opportunities were also taken into consideration in the management plan (Maine Department of Conservation, 2011). Because this plan does not allow any public or private development near our two study sites, we consider these streams to be protected. Our third stream, Stony Brook, does not fall under the protection of the Kennebec Highlands Management Plan; however, we have been in contact with the property owner and are confident that this stream is also well protected from human disturbance.

1.9 Research Group Description: Nutrient Spiraling

The first research group investigated nutrient spiraling in the streams. Newbold *et al.* (1982) pinpointed uptake length of key nutrients, such as N and P, as an accurate indicator of stream functionality and health. Mullholland *et al.* (2002) explained this finding by demonstrating that uptake length increased when





streams were overloaded with nutrients, because once organisms have the amount of nutrients as they need, any extra nutrients in the water will continue downstream. The cycling of nutrients between water and substrate as they float downstream is called nutrient spiraling. Saturation of nutrients comes from large amounts of nutrient laden run-off flowing into the stream. Haggard *et al.* (2005) underscored this result when they concluded that polluted streams generally have longer uptake lengths than non-polluted streams. With this information in mind, the Nutrient Spiraling Team measured uptake lengths for NO₃, NH₄⁺, and P in each study stream and related those data to water quality in the headwaters and both lake basins of Long Pond.

1.10 Research Group Description: Metabolism

Stream metabolism is a way of holistically quantifying ecosystem function by examining its energy use. Metabolism is comprised of gross primary production (GPP), the conversion of energy into organic material, and ecosystem respiration (ER), the total energy inputs and outputs of all organisms in an ecosystem. Fellows *et al.* (2006) showed that GPP and ER are accurate indicators of stream health. Bernot *et al.* (2010) and Uehlinger *et al.* (2000) added that certain components of stream metabolic function, such as photosynthetic activity, may suggest changes in levels of human disturbance in the ecosystem if measured over long periods of time. The Metabolism Team members sought to integrate these primary literature findings into their research questions and methods. They investigated what metabolism indicates about headwater stream health, and ultimately Long Pond health, as determined by rates of GPP, ER. They also examined the relative contribution of streambed substrates to understand the controls on metabolism at each of the study streams.

1.11 Research Group Description: Organic Matter

The third research group investigated organic matter dynamics, which form the base of the headwater stream ecosystem food web. Wallace *et al.* (1997) emphasized the importance of terrestrial-aquatic linkages in headwater streams. They found that if they excluded riparian detrital inputs from streams, there was a decrease in abundance or biomass of organisms living there (Wallace *et al.*, 1997). This indicates that terrestrial-sourced detritus makes up a large portion of the food in headwaters and without in tact riparian zones, the food web in stream ecosystems is severely degraded. To complement this study, Callisto & Graca (2013) found that the more fine particulate organic matter they

added to a stream, the more organisms they counted in the stream. Coarse particulate organic matter is a more abundant food source in headwater streams, and only some organisms process it until it is broken down by abrasion, shredding invertebrates, fungi and bacteria (Callisto and Graca, 2013). This further demonstrates the strong linkages of stream food webs, with organic material being processed and used by different groups of organisms. According to Vannote *et al.*'s (1980) River Continuum Concept, the remaining organic matter is exported downstream and used by communities there. Structures such as debris dams and rocks can also impact the amount of organic matter reaching downstream ecosystems (Bilby and Likens, 1980; Díez *et al.*, 2000). They can reduce the total amount of organic matter reaching downstream ecosystems or increase the relative amount of FPOM reaching downstream ecosystems by retaining CPOM and allowing it to decompose (Speaker *et al.*, 1984). Based on this literature, the Organic Matter Team measured the leaf decomposition rates, CPOM retention, and amount of large woody debris in our three study streams. The team's ultimate goal was to characterize organic matter use and retention in our streams, and determine if the export of organic matter by the three study streams impacts the Long Pond ecosystem.

1.12 Research Group Description: Invertebrate Communities

Macroinvertebrate communities are a useful indicator of stream functionality because some sensitive taxa only exist in areas with high water quality, such has high dissolved oxygen and low nutrients (Fremling & Johnson, 1989). Biotic indices, particularly those based on macroinvertebrate communities, allow researchers to evaluate the functionality of the stream by looking at one of the ways that physical and chemical characteristics of a stream translate into biological activity. Niyogi *et al.* (2007a) confirm that high water quality causes high macroinvertebrate density and abundance. They go on to point out that disturbed areas have lower water quality, and therefore the macroinvertebrate communities are of lower quality, meaning they are smaller and less diverse (Niyogi *et al.*, 2007b). They suggest preserving riparian zones as a possible mitigation technique for conserving macroinvertebrate communities and water quality in disturbed areas (Niyogi *et al.*, 2007b). The Biotic Indices Team sought to discover if there was a difference between macroinvertebrate communities at disturbed sites, how macroinvertebrate communities relate to stream functions such as organic matter processing, and if macroinvertebrate communities are correlated with canopy cover or substrate types.

1.13 Research Group Description: Sediment and Landscape Interactions

Learning about the way the stream interacts with the landscape is critical for a large-scale understanding of ecosystem function. Suspended solids, both organic and inorganic particles, can impact water quality and biological communities, and sediment content and distribution of streams can affect nutrient spiraling (Bilotta & Brazier, 2008; Jarvie et al., 2008). In addition, storm disturbance events can influence water flow and dissolved organic carbon distribution (Hillman et al., 2004). These are just a few of the ways that sediment can affect headwaters. Erosion is a serious hazard to downstream water quality, and it is important to properly assess potential erosion threat and to be aware of what certain threat levels could mean for the future of a stream and downstream ecosystems (McIntosh & Laffan, 2005). The Sediment and Landscape Interactions Team measured the slope, depth and substrate of the stream to get a foundational understanding of the landscape at all three sites. The members of this team quantified erosion, and at one stream, used corn pollen to simulate and measure FPOM transport and Rhodamine dye to show movement of water (Georgian *et al.*, 1993). They determined the influence of hydrology and geomorphology on stream health and stability. In addition, they observed the relationship between stream-landscape interactions and sediment inputs, sorting, and outputs into downstream reaches. This information helped the team create a coherent picture of the current state of sediment and erosion regimes in the study sites.

1.14 Research Group Description: Lake Context

The headwater streams we studied eventually flow into Long Pond, and the ecological impacts that the headwater stream system has on the lake itself is of particular interest to the Long Pond community. Although shorelines have proven to be an important nutrient source for algae growth in some lakes, headwater streams also contribute nutrients to lake biological communities by exporting them downstream from the watershed (Alexander *et al.*, 2007; MacDonald & Coe, 2007; Dodds & Oakes, 2008; Vanni *et al.*, 2011; Alahuta *et al.*, 2012). The Lake Context Team sought to characterize the relationship between Long Pond and its headwater streams, especially with regard to nutrient loading, by taking water samples from the stream mouths and central points in Long Pond basins. This information allowed the team to evaluate the impact of the three study streams on Long Pond water quality.

1.15 Conclusion

Headwater streams are important resources to conserve and must be monitored closely and effectively. By using structural and functional metrics, the Colby Headwater Stream Research Team aims to understand several different aspects of stream health and functionality, including nutrient spiraling, metabolism, organic matter processing, invertebrate communities, sediment and landscape interactions, and the stream-lake dynamic (Fig. 1.3). This information will be used to make suggestions about conservation goals and techniques to local stakeholders, especially regarding logging practices and riparian buffer standards in the Kennebec Highlands. In its chapter, the Conservation Lessons Team explores the ways that people can be part of the solution to anthropogenic disturbance. Individuals can inspire their communities to participate in conservation efforts, thereby generating positive energy and tangible results, which keep the motivation high for people to partake in such activities. The Colby Headwater Stream Research Project hopes to deliver a rich body of scientific information to the Belgrade conservation community, so the valuable headwater streams of Long Pond and other lakes in the area can be protected for years to come.

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CHAPTER 2 NUTRIENT SPIRALING

Introduction

2.1 Background

Headwater streams are the primary source of water flowing into large, aquatic ecosystems such as lakes, rivers, and coastal oceans. Despite their small size, these streams account for the majority of channel length in river basins worldwide (Brookshire *et al.*, 2009). They are also responsible for cycling and retaining biologically important nutrients, which can lead to eutrophication of freshwater ecosystems if present in excess (Webster & Patten, 1979). Since eutrophication is one of the primary threats facing freshwater ecosystems today, maintenance of headwater stream function is of central importance (Haggard *et al.*, 2001).

2.2 Nutrients and Their Movements in Headwater Streams

Two of the most biologically important nutrients in freshwater ecosystems - nitrogen (N) and phosphorus (P) - are fundamental for cell growth, reproduction, respiration, bacterial activity, and are the ultimate base of the food web (Bott *et al.*, 2006). The cycling of limiting nutrients such as nitrate, phosphorus, and ammonium (NO₃⁻, SRP, NH₄⁺) is a key function of stream ecosystems (Webster & Patten, 1979). In streams, important nutrients, such as N and P, are constantly being exchanged between the biotic and abiotic portions of the ecosystem. One full cycle consists of a nutrient molecule being incorporated into aquatic biota from the water column via photosynthesis or other metabolic pathways and then returned to the water from the organism, either as waste or through decomposition of dead organisms. This exchange is referred to as a cycle because it occurs on a relatively static scale in lakes, the context in which the concept originated. However, streams are not static environments because the water flowing through them is constantly in motion. Therefore, normal nutrient cycling models are not applicable for the movement of nutrients in these systems.

Streams are unique ecosystems because water is constantly moving downstream, so they are never spatially static. This type of movement eliminates the potential to categorize nutrient movement as a cycle because water is moving downstream as the nutrients within it are assimilated. The term "spiraling" refers to the track of an individual nutrient atom being taken up into benthic biota, temporarily assimilated, and returned at a later period. Since streams are flowing, a nutrient introduced in one spot may be utilized by downstream biota (Brookshire *et al.*, 2009). To account for
this specialized water movement, headwater stream ecologists proposed a model for nutrient spiraling in streams (Webster & Patten, 1979) where the uptake length (S_w) of a nutrient is measured to provide an accurate assessment of the spatial journey of any given nutrient molecule as it progressed through a lotic ecosystem (Fig. 2.1). The central advantage of S_w is its ability to unite hydrologic and non-hydrologic processes into a single quantitative measurement (Runkel, 2007). S_w constitutes more than 90% of the length of the

nutrient spiral, so measuring this length allows for an accurate assessment of a stream's nutrient demand (Newbold *et al.*, 1983; Mulholland *et al.*, 1985).

Uptake length in streams can be correlated to the strength of nutrient limitation; shorter uptake lengths represent stronger nutrient limitation (Mulholland *et al.*,



Fig. 2.1. Nutrient spiraling in a stream. S_w (m) represents the distance it takes for a nutrient to be taken up by the stream biota. (Figure from: Newbold *et al.*, 1983)

2002). If a stream is nutrient limited, biotic growth is being controlled by lack of access to that essential nutrient, typically either P or N. Nutrient limited streams are less prone to algal blooms because there are not enough nutrients to support excess growth, which makes nutrient limitation a primary factor in maintaining stream water quality. Generally, the healthiest streams have the shortest uptake lengths, which means that nutrients are taken up quickly and there are lower concentrations present in the stream at any given time (Mulholland *et al.*, 2002). Severely disturbed streams, on the other hand, have much longer uptake lengths due to a surplus of suspended nutrients (Gücker, 2006). The excess nutrients no longer limit biotic growth because there is constant access to an abundant supply, which is demonstrated by a corresponding increase in uptake length.

2.3 Factors Affecting Uptake Length

Several factors affect differences in uptake lengths between streams. The type of vegetation in a stream catchment has the ability to affect nutrients, especially N. In desert streams, N storage is significantly less than in forested streams, with the majority (90%) of the N pool being autochthonous (originating in the stream) in nature (Grimm, 1987). The composition and age of the

forest surrounding the stream also affect N levels. In most hardwood forests, nitrate concentrations tend to be higher than in coniferous forests. This difference is associated with the decrease in forest floor biomass seen in hardwood forests (Binkley *et al.*, 2004). The age of the trees in the catchment also influences nutrient dynamics in streams. Younger forest stands are associated with higher levels of nitrate release into the surrounding environment (Martin, 1979; Cairns & Lajtha, 2005). Younger forests also tend to have more seasonal variation in nitrate than old-growth forests, because a larger amount of biomass is produced during the growing season (Cairns & Lajtha, 2005).

The metabolism rate of stream fauna can also be directly linked to differences in uptake length. Generally, increasing metabolism decreases uptake length due to the fact that an increase in microbial processes leads to a higher nutrient demand (Fellows *et al.*, 2006). This increase in demand decreases nutrient residence times in the stream because microbes utilize them faster. An increase in dissolved organic carbon also decreases spiraling length (Bernhardt & Likens, 2002). Because dissolved organic carbon is used in the metabolic processes of some microbes, an increase in the amount of dissolved organic carbon leads to a decrease in N (as nitrate) in streams. More available carbon means more biotic growth, which increases N demand and decreases spiraling length (Bernhardt & Likens, 2002).

The type of rock under a stream catchment also plays a role in nutrient dynamics. Streams in the Western United States flow over volcanic bedrock and have low uptake rates for phosphate, suggesting that there is a higher amount of phosphate leaching into the streams from the volcanic bedrock compared to granitic bedrock (Munn & Meyer, 1990; Binkley *et al.*, 2004). Conversely, streams in the Eastern United States flow over granitic bedrock and thus exhibit lower availability of phosphate, which exerts a strong biotic control. Carbonate, which releases P, was also limited. (Munn & Meyer, 1990).

The origin of bedrock is not the only geological factor affecting nutrient dynamics; the composition of the streambed (e.g. sand, cobble, boulders) also affects uptake length. As sediment size increases, there is a greater exchange between surface and groundwater, which increases the retentiveness of nutrients because they are removed from the thalweg (Carpenter *et al.*, 1998). A decrease in sediment size is also thought to decrease the uptake length for N because smaller substrate increases the surface area available for microbes, most of which utilize N in metabolic processes (Davis & Minshall, 1999). Whether nutrient dynamics are affected more by surface-groundwater exchange or increased microbial activity varies by stream. Stream biochemistry will

also influence the range of redox conditions in which nutrient transformation may take place. (Morrice *et al.*, 1997).

2.4 Human Impacts on Streams

Human activities also impact stream environments. Anthropogenic disturbance is the result of both point source (e.g. sewage treatment plants) and non-point source pollution (e.g. agriculture), with the latter being particularly devastating to streams across the globe. Non-point source pollution is variable in its location within a watershed and therefore more difficult to monitor and regulate than point-source pollution. It is generally accepted that removing agricultural land from a stream's catchment will improve net ecosystem function, as defined by water quality, species richness, and uptake length, while removing point sources will also initiate recovery but on a smaller scale (Newman *et al.*, 1996; Vitousek *et al.*, 1997; Daniel *et al.*, 1998; Mulholland *et al.*, 2002). Urban stream degradation also poses a significant threat, as over 50% of the world's population now lives in cities (Daniel *et al.*, 1998; Meyer *et al.*, 2005). Urban development near streams results in significant loss of forested catchment, increased loads of sediment and nutrients, and increased uptake length (S_w) (Roy *et al.*, 2003). This, combined with increasing industrial cultivation of rural areas, has resulted in widespread disruption of headwater stream function.

The central factor influencing this change in function is N loading in streams. The advent of the Haber-Bosch process in the early 20th century (which enables the artificial fixation of N for agriculture) has allowed global human populations to flourish by growing food with unprecedented efficiency. It has also increased the amount of N entering freshwater ecosystems by 6 to 50 times preindustrial levels (Carpenter *et al.*, 1998; Earl *et al.*, 2006). Almost all of our fixed N is used for industrial agricultural operations, and most of this is then washed into aquatic ecosystems (Yan *et al.*, 2003). Clearing trees for agriculture also increases nutrient loading because it increases erosion and sedimentation and eliminates the biggest storage buffer of all: trees. Despite their importance, buffers are rarely retained around streams as development progresses (Grimm *et al.*, 2009). The addition of P to streams can also affect water quality. As N increases in the stream, P becomes the limiting nutrient. Therefore, P loading can be just as harmful as N loading.

Nutrient-polluted streams have longer uptake lengths than undisturbed streams because the increased concentration of nutrients in the runoff entering the stream cannot be fully absorbed by the biota present (Haggard *et al.*, 2005). In one urban eutrophic stream outside of Berlin, Germany, the

uptake length of NO₃ (nitrate) in the winter was 167,763m, which is well above the 1,000m uptake length that is considered unhealthy (Gücker *et al.*, 2006). Physical and biogeochemical factors, such as transient storage areas, sediment, and catchment composition can affect uptake length as well (Valett *et al.*, 1997). Therefore, it is important to realize that there is more to S_w than input type and quantity.

It is the nature of healthy headwater streams to be low in nutrients, mainly because of geography and catchment. Headwater streams are often found in mountainous or hilly areas, and, depending on the region, are surrounded by forested catchments, which are extremely efficient at taking up and retaining nutrients (Ashkenas *et al.*, 2004). Headwater streams are also small; in a cumulative survey of over 607 streams around the world, the average discharge (q) for headwater streams was 191.27 L s⁻¹. The small size of headwater streams allows for maximal interactions between dissolved nutrients and the benthic biota that utilize them for growth, reducing the concentrations of nutrients in these streams at any given time.

When headwater streams become polluted, they lose much of their capacity to protect downstream ecosystems. There is a significant relationship between increasing S_w in first-order headwater streams and the nutrient loading (and subsequent eutrophication) of downstream sites (Haggard *et al.*, 2005). Eutrophication occurs when algae thrive on high nutrient levels, forming what are called 'algal blooms'. When this algae dies, it is rapidly digested by bacteria, which strip the water of dissolved oxygen (DO). Known as 'dead zones', the resulting hypoxic (low DO) and anoxic (no DO) conditions are prevalent throughout the world. The most famous and tragic example in the U.S. is the Gulf of Mexico, where excess nutrients from the Mississippi River have severely damaged water quality downstream (Rabalais *et al.*, 1996).

2.5 Local Context

In the Belgrade Lakes, protecting water quality is a priority for local residents and visitors to the region. The purpose of this research project was to investigate the status of three headwater streams in the conserved area of the Kennebec Highlands that drain into Long Pond. Our research question was: what are the uptake lengths of three potentially limiting nutrients (nitrate, phosphorus, and ammonium) in each of the three streams studied? If the streams in question represent streams within relatively undisturbed catchments, then they should experience strong nutrient limitation, which will give them short uptake lengths for each of the three nutrients studied. Within each stream, we should also get an

idea of which nutrient (P or N) is the limiting factor for growth. The shortest uptake length signifies the highest demand, which is the best indicator of a limiting nutrient. The uptake lengths will also indicate whether streams internally process nutrients or export them downstream. A short uptake length is characteristic of streams that export few nutrients.

Methods

2.6 Measuring Uptake Length

Uptake length of nitrate, phosphorus, and ammonium were measured within designated reaches of each of three headwater stream sites. All three streams flow into Long Pond and have similarly forested catchments. Whittier Stream had an average discharge of 115.6 L s⁻¹ and a stream velocity 0.32 m s⁻¹, which was the highest of the three streams. Beaver Brook was the largest of the three streams with a discharge of 158.3 L s⁻¹ but a relatively slow stream velocity, at only 0.12 m s⁻¹. Stony Brook was the smallest of the three streams, with an average discharge of 108.5 L s⁻¹ and stream velocity of 0.10 m s⁻¹. Both Whittier Stream and Beaver Brook are located in the conserved area of the Kennebec Highlands, and Stony Brook is within a relatively undisturbed forest patch on private property. The protected nature of these streams allowed for uptake lengths to be measured in a fairly undisturbed environment, although Whittier Stream had a road culvert located within the study reach.

A short-term solute injection of three nutrients (nitrate, ammonium, and phosphorus) was used to determine the uptake lengths. Prior to each release, the velocity and depth of the water was sampled every 0.1 m across a stream transect in three separate, random locations within the 100 m study reach at each stream. Discharge was calculated using the equation:

$$Q = \sum w * d * v$$

where *w* is the width of each measured segment (m), *d* is the average depth of the segment (m), and *v* is the average velocity (m s⁻¹). These measurements allowed for a rapid calculation of the amount of nutrients we needed to add so that we could begin nutrient releases.

To measure the uptake of nitrate/nitrite and phosphate, we used the technique outlined by Newbold *et al.* (2007) in *Methods in Stream Ecology*. Two 20-liter carboys of stream water from each stream were collected prior to the experiment. Approximately seven kilograms of salt (NaCl) were dissolved into one of the two carboys to act as a tracer throughout the first release process. Phosphorus and nitrate were also added to the carboy containing salt, but ammonium was run separately in the second carboy. The salt was monitored using a conductivity meter and served as a conservative tracer to measure the plateau point at which the concentration of the nutrients was highest in the stream.

Immediately before each release, background water samples (60mL) and background stream conductivity measurements were taken at each stream throughout the 100m study reach. Water samples were taken using a 60mL syringe, then filtered into acid washed containers. Five to six sampling sites were marked out at 20m increments along the stream reach, also before the release occurred. The nutrient release was conducted using a laboratory grade FMI pump (NY, USA) to deliver a constant flow (200mL min⁻¹) into the stream from the first carboy, which contained the salt, phosphorus, and nitrate. A timer was started as soon as the release began. Plateau conductivity was monitored at the last station. When plateau was reached, defined as constant conductivity for five minutes, the plateau conductivity and time elapsed were recorded and three filtered water samples were taken at each downstream sampling station. All samples were frozen until they could be analyzed in the lab.



Fig. 2.2. A conceptual plot of conductivity versus distance. The relationship between [N] and distance from the pump during the experiment, which allows for S_w to be calculated.

The same process was then repeated using the second carboy, which only contained ammonium. We did not add salt to the second carboy because we assumed that it would take the same amount of time for the ammonium to reach plateau as it did during the nitrate and P release, which was completed 30 minutes before the NH_4^+ release. We ran the ammonium release for five more minutes prior to collecting samples to allow for slight discrepancies between the two releases.

All water samples were analyzed using a Lachat Autosampler (Lachat Instruments, Loveland, CO). Uptake length was then calculated (in meters) using the equation:

$$S_w = \frac{1}{m}$$

where *m* is the slope of the graph of distance from injection site vs. $\ln(n/c)$, *n* is the concentration of the nutrient (mg L⁻¹), and *c* is the conductivity (µs) (Fig. 2.2). V_f (mm s⁻¹) was calculated from:

$$V_f = \frac{\frac{Q/1000}{w}}{S_w} * 1000$$

where Q is discharge (m³ s⁻¹), W is average stream width (m), and S_w is uptake length of the nutrient (m). Uptake rate, U, (mg m⁻² d⁻¹) was calculated from the equation:

$$U = \frac{(D*n)}{(S_w * W)} * (8.64 * 10^4)$$

where *D* is discharge (m³ s⁻¹), *n* is background nutrient concentration (μ g L⁻¹), *S_w* is uptake length of the nutrients (m), and *W* is average stream width (m).

2.7 Statistical Analysis

In order to understand the factors that affected nutrient spiraling, we ran linear regressions using Stata 13 to look for significant relationships. We rain correlations to explore relationships between variables that we thought would influence spiraling, but not in a statistically significant way. It was also useful for us to run an ANOVA to investigate similarities between the streams. We note that statistical analyses of in-stream nutrient spiraling metrics could not be performed due to lack of replication of the experiments at each site.

Results

2.8 Stream Dynamics

The three streams varied in their discharge values, ranging from 108.5 to 156.3 L s⁻¹ (Table 2.1). Background nutrient concentrations in all three streams were low. Of the three nutrients measured, background P concentrations were the lowest in all three streams and were below detection in all cases. Nitrate concentration varied from 0.20 to 0.45 μ g L⁻¹, and ammonium concentration varied slightly from 0.15 to 0.31 μ g L⁻¹ (Table 2.1). There was no difference in the background concentrations of any of the three nutrients between the streams (One-way ANOVA, p=0.58, p=0. 36, p=0.40).

							LWD	Background
		Background	Background	Background	Temperature		Volume	Conductivity
Stream	$Q(L s^{-1})$	$[SRP](\mu g \; L^{1})$	$[NO_3] (\mu g L^{-1})$	$[NH_4^+] (\mu g L^{-1})$	(°C)	Slope	(cm^3)	(µs)
Whittier Stream	108.5	-0.274	0.148	0.354	12.43	0.0408	1500	34.1
Beaver Brook	156.3	-0.075	0.447	0.251	13.74	0.068	13000	15
Stony Brook	115.6	0.065	0.197	0.311	13.52	0.015	0	11.5

 Table 2.1. Physical and chemical parameters for each headwater stream

2.9 Nutrient Retention

Nitrate uptake length was lowest in Beaver Brook and highest in Stony Brook. The uptake lengths of all three streams were comparable with values previously published in the literature (Table 2.2). Nitrate uptake lengths were 111 m, 32 m, and 217 m for Whittier Stream, Beaver Brook, and Stony Brook, respectively (Fig. 2.3). Uptake rates for nitrate were the highest in Beaver Brook (1.03 kg N m⁻² d⁻¹) and lowest in Stony Brook (3.88 g N m⁻² d⁻¹). The uptake rate in Whittier Stream was 6.03 g N m⁻² d⁻¹ (Fig. 2.3). The trend in the maximum transfer coefficient (v_f) was the same as that for uptake rate. V_f was lowest in Stony Brook (0.271 mm s⁻¹), followed by Whittier Stream (0.41 mm s⁻¹), and highest in Beaver Brook (1.43 mm s⁻¹) (Fig. 2.3).

Stream	Location	Q (L/s)	Sw (NO3) (m) Sv	w (NH4) (m) Sw	(SRP) (m) Source
Yellowbelly	Idaho	78	455 -		286 Arp & Baker 2007
Mack Creek	Oregon	52	1261	44 -	Ashkenas et. al. 2004
Sand Creek Tributary	Michigan	7	800	244	556 Bernot et. al. 2006
Pilgrim Creek	Wyoming	12	453	136 -	Hall & Tank 2003
Hubbard Brook	New Hampshire	29.3	9516	214	85 Hall et. al. 2002
Walker Branch	Tennessee	2.7	-	67	77 Mulholland et. al. 1990
Kye Burn N	New Zealand	1.7	501	76	324 Simon et. al. 2005
Upper Ball Creek	North Carolina	55.6	199	65	151 Tank et. al. 2000
Bear Brook	New Hampshire	4.4	102	14 -	Webster et. al. 2003
Gallina Creek	New Mexico	5.7	78	21 -	Webster et. al. 2003
Tawhekarere	New Zealand	8.8	29.5	80.6	204.1 Hoellein et. al. 2012
Whittier Stream	Kennebec Highlands	108.5	111.11	63.69	119.05
Beaver Brook	Kennebec Highlands	156.3	31.95	113.64	41.67
Stony Brook	Kennebec Highlands	115.6	217.39	200	175.44

Table 2.2. Literature values for streams throughout the world

P uptake length was also lowest in Beaver Brook and highest in Stony Brook. The uptake lengths obtained from our solute addition were 42 m, 119 m, and 175 m, respectively. The uptake rates for P were 9.08 g P m^{2 -1} d for Whittier Stream, 1.56 kg P m^{2 -1} d for Beaver Brook, and 1.80 g P m^{2 -1} d for Stony Brook. V_f followed the same pattern as well, being the highest (1.09 mm s⁻¹) in Beaver Brook, and the lowest in Stony Brook (0.336 mm s⁻¹). The maximum transfer coefficient was 0.383 mm s⁻¹ in Whittier Stream.

For ammonium, uptake length was lowest in Whittier Stream and highest in Stony Brook. The values for S_w , from lowest to highest, were 64 m, 114 m, and 200 m. Uptake rates in Whittier Stream and Beaver Brook were similar (10.8 g N m⁻² d⁻¹ and 9.18 g N m⁻² d⁻¹), and both were slightly higher than the uptake rate for Stony Brook, which was 7.17 g N m⁻² d⁻¹. The mass transfer coefficients for all



Fig. 2.3. Uptake length (S_w) , uptake rate (U), and the mass transfer coefficient (V_f) for the three study streams.

three streams were fairly close to each other; it was highest in Whittier Stream (0.716 mm s⁻¹) and lowest in Stony Brook (0.295 mm s⁻¹). It was 0.401 mm s⁻¹ in Beaver Brook.

When compared with literature values, the results from this study confirm that S_w increases as discharge increases (Fig. 2.4). Nitrate uptake length was the least correlated, with only 13% of the variation between streams being explained by differences in discharge (linear regression, $r^2=0.13$, p=0.13). Just under half of the variation in ammonium uptake lengths is explained by discharge (linear regression, $r^2=0.44$, p < 0.0001). P uptake length was the most positively correlated to stream discharge (linear regression, $r^2=0.59$, p=0.63).



Fig. 2.4. A meta-analysis of stream discharge and uptake length (S_w) for three potentially limiting nutrients. (Data from: Tank *et al.*, 2013)

2.10 Nutrient spiraling and metabolism

There was no significant relationship between metabolism and uptake length for any of the nutrients in the streams (linear regression, p>0.10). Gross primary production explained 98.5% of the S_w for nitrate and 94% of S_w for phosphorus—as gross primary production increased, S_w decreased (Pearson's correlation, n=3). Net ecosystem production (NEP) also explained 91.1% of phosphorus uptake length; as NEP increased, S_w decreased (Pearson's correlation, n=3).

Uptake rate for phosphorus and nitrate was dependent on the net ecosystem production in the streams (linear regression, p=0.0039, p=0.0053). Uptake rate for these two nutrients was also highly correlated with respiration, which explained 99.4% of uptake rate in both cases (Pearson's correlation, n=3). This was an inverse relationship, so as respiration increased, uptake rate decreased. Uptake rate of ammonium did not exhibit a strong correlation with metabolism.

The mass transfer coefficient for P depended on the net ecosystem production (linear regression, p=0.029). V_f for nitrate could be explained by net ecosystem production as well (Pearson's correlation, n=3, r = 0.995). Respiration was more correlated to V_f for P and nitrate than gross primary production (Pearson's correlation, n=3, r = -0.987, -0.977, respectively). The inverse relationship shows that as respiration increases, V_f decreases. V_f for ammonium did not show any correlation with metabolism.

2.11 Catchment and Riparian Characteristics

Relationships between S_w and different physical characteristics (see Chapter 6) within the stream catchments were also examined. Canopy cover was most strongly correlated with Sw for ammonium, explaining 86% of S_w (Pearson's correlation, n=3). As canopy cover increased, S_w for ammonium increased. An increase in slope was found to decrease S_w for P and nitrate (Pearson's correlation, n=3, r = -0.992, -0.998, respectively). Uptake length was not strongly correlated with substrate composition, but there is some evidence that as the size of the substrate increases, S_w decreases for P and nitrate (Pearson's correlation, n=3, r = -0.786, -0.667, respectively).

Discussion

Retention of all three of the nutrients tested was high within the three streams (Fig. 2.5). This retention was demonstrated in short uptake lengths of the nutrients at all nutrient sites. This finding also suggests that there is moderate to strong nutrient limitation in these streams (Mulholland et al., 2000). Theoretically, the limiting nutrient in each stream is the one with the fastest uptake rate, and thus the shortest uptake length (S_w) . Each stream had a different limiting nutrient: NH₄⁺ in Whittier stream, NO₃⁻ in Beaver Brook, and SRP in Stony Brook. Identifying the limiting nutrient is important in streams because any addition of this nutrient will lead to an increase in biotic growth within the stream.



Fig. 2.5. Schematic depiction of uptake length (S_w) for a) Whittier Stream, b) Beaver Brook, and c) Stony Brook.

There are several theories that address the difference in S_w among streams (Table 2.2). In addition to nutrient scarcity and healthy biota, uptake length is heavily influenced by stream morphology, e.g. physical and biogeochemical factors (Valett *et al.*, 1997). One morphological factor, transient storage space, refers to areas in a stream where discharge (q) and velocity (v) decrease due to physical barriers. These sections exhibit higher rates of uptake over shorter spatial periods, thus decreasing the measured S_w of the stream (Haggard *et al.*, 2001).

Of the streams studied in this experiment, Beaver Brook had different physical characteristics in comparison to the other streams. Rather than having a central channel and thalweg, Beaver Brook was characterized by a sprawling labyrinth of boulders and slow-moving pools of water, with no defined thalweg. The overall velocity of water in Beaver Brook was lower than in the other two streams, which led to an increase in the amount of time it took to reach plateau during nutrient releases (pers. obs.). The benthic surface area was higher in Beaver Brook because of a larger percentage of boulder substrata (83.7%) when compared with Whittier Stream (40%) and Stony Brook (30%). Beaver Brook also had the highest discharge (Table 2.1).

In addition to transient storage area, slope (the difference in elevation between the top and bottom of the stream reach) is generally positively correlated with nutrient uptake in the literature. A higher slope will result in a higher flow velocity, which, according to Haggard *et al.*, 2001, increases S_w . It takes longer for biota to take up dissolved nutrients that are traveling faster because there is less contact between the water and the substrate, and a higher slope generally causes the water to travel faster. In our data, slope was negatively correlated with uptake length. Beaver Brook had the highest slope (Table 2.1) but an average velocity of only 0.12 m s⁻¹. The discrepancy from the literature is probably at least partially a result of the slower movement of the water. Stony Brook was out of place with the lowest slope and velocity (0.015 and 0.10 m s⁻¹), yet the longest uptake length for all nutrients. This result is inconsistent with the literature, which suggests that decreased slope shortens uptake length (Haggard *et al.*, 2001). A possible explanation for this inconsistency is difference in stream metabolism (Fellows *et al.*, 2006). Respiration in Stony Brook is higher than in the other two streams, which means that there is less of a demand for nutrients in Beaver Brook.

The amount of organic matter retained in the stream could be a cause of the short S_w of nutrients in the three streams. Hotspots of organic matter allow increased surface area for benthic biota, which are responsible for the bulk of metabolism in headwater streams (Mulholland *et al.*, 1985). Certain areas where leaves and other organic matter accumulate are then responsible for a higher amount of uptake. In

all three streams, 60-99% of organic matter that was introduced into the stream was retained in the first 20m. Such a high retention of organic matter points to a large number of potential hotspots for nutrient uptake, which would decrease the uptake lengths. The seasonal variation within streams is largely a result of the influx of leaves in autumn, which provides a huge increase of surface area for biota habitat and as a source of organic matter (Mulholland *et al.*, 1985).

Additionally, decreased canopy cover increases photosynthesis while warm temperatures persist, as they did during our study period as the leaves began to fall and temperatures remained warm. There was a positive correlation between ammonium uptake length and canopy cover, suggesting that an increase in the percent canopy cover increases the uptake length. Thus, the uptake length of ammonium in these streams exhibits seasonal variation. Neither P nor nitrate could be linked to canopy cover, showing that changes in canopy cover do not affect their seasonal variation based on our limited sample size.

Metabolism (net ecosystem production = gross primary production + respiration) has been linked to nutrient uptake and spiraling length (Fellows *et al.*, 2006). Nutrients cannot be taken out of the stream if there is no metabolism occurring. Although S_w and metabolism were not significantly linked, P and nitrate were correlated with gross primary production. This suggests that while microbial activity is a fairly significant contributor to uptake length (98.5% and 94% respectively), it does not account for all of it. Net ecosystem production (NEP) also impacted the uptake rate for both P and nitrate. As respiration increases, microbes demand less of a nutrient, causing saturation (and a decreased uptake rate). An increase in respiration also significantly impacted the mass transfer coefficient of P and was correlated to that of nitrate. This suggests that the uptake efficiency of both of these nutrients is related to microbial activity as well. Less microbial growth (R > GPP) leads to a decrease in uptake efficiency because nutrients are no longer in demand. NO₃⁻ is lost from the stream through the process of denitrification, which is the disassembly of fixed N by bacteria for energy. Denitrification by bacteria can account for up to 50% of nutrient uptake in streams (Mulholland *et al.*, 2000).

Our research on nutrient spiraling has significant policy implications. Forested headwater streams have short nutrient S_w , and therefore fewer nutrients are transported downstream. Maintaining streams with higher nutrient scarcity will increase water quality in lakes downstream by reducing the total amount of incoming nutrients. Nutrients in excess lead to eutrophication and a breakdown of ecological structure (Mulholland *et al.*, 1985; Haggard *et al.*, 2005). The minimum policy for stream protection should be keep the forested catchment intact within 100m of headwater streams leading into

the Belgrade Lakes, because healthy forests store N entering the catchment (McIntosh *et al.*, 2005). Those interested in improving present water quality should focus on returning a forested buffer to parts of the stream that have been developed. The flow of point source pollution into these streams should be limited as well.

All three of the streams in the study site are protected in the Kennebec Highlands Conservation Area, so by analyzing these streams, we are able to provide an insight as to whether or not conservation in the area is successful and to provide a baseline for future study on impacted streams in the Belgrades. Our results show a short S_w, plenty of forested buffer area, and low nutrient concentrations in general. Even if we had just observed nutrient concentrations, we would have been able to assume that the stream was healthy due to the nutrient scarcity in each stream. However, by measuring nutrient uptake lengths we were able to look at the relative importance of NH4⁺, NO3⁻, and P in these streams. Continuous careful monitoring is important because if there is a disturbance upland that causes increased nutrient levels downstream, it would allow us to minimize the negative effects.

We can also offer some less conventional advice supported by the results we have collected. The principles stand that, with the exception of denitrification of $NO3^-$, almost all nutrients are taken up by benthic substrata (Runkel, 2007). One of the common effects of anthropogenic disturbances of streams is increased sediment deposition, which decreases surface area and raises the S_w. The main cause of sediment overload is deforestation of the catchment, especially for agricultural purposes, which results in erosion and sediment transport into streams.

While our headwater streams do not have increased sediment loads yet, we propose that in order to catalyze higher uptake, revitalization efforts should focus on the restoration of traditional substrata, which consists of a diversity of individual substrate types. Low-nutrient headwater streams are usually rocky because sediment is continuously removed and transported downstream. These rocks have ideal surface area for biota to settle on and for biofilms to develop, as long as they are not covered with sediment. Urban streams, where substrata is often covered by mud and silt, could especially benefit from restoration of substrata. Theoretically we could extend this hypothesis to include the introduction of artificial substrata (in streams where large rocks and wood may have been otherwise removed), although we do not recommend this in headwater streams because we are not sure how the disturbance caused by the introduction might affect the stream ecosystem.

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CHAPTER 3

METABOLISM

Introduction

3.1 Metabolism Background

Metabolism can be divided into two main components: gross primary production (GPP) and ecosystem respiration (ER). GPP is the total amount of energy produced in an ecosystem by autotrophs using light and chemical compounds. ER is the sum of all respiration, or the consumption of oxygen and release of carbon dioxide, by heterotrophs and autotrophs within an ecosystem. The term net ecosystem production (NEP) is used to describe the total amount of energy from primary production that is available after the costs of respiration are taken into account; in other words, it is the net accumulation of organic carbon. Most streams have higher rates of respiration than primary production (Mulholland et al., 2001). One important controlling factor of GPP and ER is light availability to the stream (Odum, 1957; Young & Huryn, 1999; Mulholland et al., 2001; Acuna et al., 2004). Thus, seasonal variation in light will cause rates of GPP and ER to change throughout the year (Uehlinger, 2006; Hoellein et al., 2009). In general, net ecosystem production is higher in the spring and fall than in the winter due to increased light availability (Odum, 1957; Roberts et al., 2007). Other factors that can control metabolism include riparian vegetation, water depth, temperature, dissolved organic matter, and nutrient levels, especially of phosphorus and nitrogen (Howarth et al., 1996; Acuna et al., 2004; Fellows et al., 2006; Frankforter et al., 2010; Halbedel et al., 2013). Streams with deeper water have lower rates of net ecosystem production (Howarth et al., 1996). In addition to increased light availability, respiration is typically higher in the fall season due to the influx of organic matter from trees shedding their leaves into the stream (Roberts et al., 2007).

3.2 Metabolism as an Indicator of Stream Health

Traditionally, indicators of stream ecosystem health have included measures of the biotic component of ecosystems, such as community composition (e.g. of invertebrates, algae, or microorganisms), or measurements of ecosystem structure such as water quality (Yates *et al.*, 2012). However, when evaluating stream health using purely structural measurements, the functional behavior of the stream may be overlooked. Such components, which include rates, trends, and ecological processes, are also important to use when assessing ecosystem health (Young, 2008; Young & Collier, 2009). Stream metabolism is one functional indicator that is becoming more widely used as a proxy for

the health of aquatic ecosystems, particularly when assessing the environmental impact of human activities (Yates *et al.*, 2012). Since stream metabolism is directly linked to many critical ecological processes, such as photosynthesis and nutrient cycling, it has been proposed to be an effective indicator of stream health and function (Bunn *et al.*, 1999; Houser *et al.*, 2005; Bernot *et al.*, 2010). Additionally, stream metabolism is relevant to all types of streams, responds to early stressors and subtle changes, and can be measured continuously (Izagirre *et al.*, 2008; Young & Collier 2009).

3.3 History of Metabolism Methodology

There is a wide range of methods used for measuring stream metabolism. Few studies have attempted to compare these methods and determine which ones are the best for addressing specific research questions (Staehr *et al.*, 2012). Many of the methods used to measure metabolism in streams could also be applicable to other water systems, such as lakes and oceans. There are two main methods used to measure metabolism: dark and light bottle incubations and whole system measurements. Both methods aim to measure changes in dissolved oxygen (DO) within the system and calculate the rates of ER, GPP, and NEP from these changes. There is debate over the accuracy of these methods, but with improved technology there will hopefully be more reliable methods to measure metabolism in the future (Staehr *et al.*, 2012).

In addition to measuring oxygen (O_2) and carbon dioxide (CO_2) production and consumption directly, the light-dark bottle method can also employ the ¹⁸O₂ tracer method for measuring oxygen production and ¹⁴C for carbon assimilation (Bender *et al.*, 1987; Staehr *et al.*, 2012). These tracers are used to quantify the change in dissolved oxygen or carbon concentration in the stream system. The changes in dissolved O_2 and other gases are measured in light-colored and dark-colored bottles to estimate rates of gross primary production and respiration, respectively. There has been much controversy over the reliability of this method. Some studies suggest that production is either underestimated or overestimated, while others suggest that this method is an accurate representation of metabolism in streams (Bender *et al.*, 1987; Swaney *et al.*, 1999; Aristegi *et al.*, 2010).

In the dark and light bottle method, specific substrates from the streambed are isolated in order to determine the biggest contributor to stream metabolism. By separating these substrates with water in closed jars and eliminating the flow of the stream, there can be error within metabolism measurements (Odum, 1957). However, substrate-specific experiments can demonstrate hotspots of GPP and ER;

Hoellein *et al.* (2009) found that wood and rocks are hotspots for GPP, while fine benthic organic matter (FBOM) is one hotspot for respiration.

Estimating metabolism in streams requires measuring the change in dissolved oxygen concentration of the water. Since metabolism is fairly consistent throughout large reaches of a stream, measurements can be taken at one or two locations along the reach and can be safely assumed to be representative of the conditions throughout the entire stream (Young *et al.*, 2008). These single-station studies can also be completed with relatively short study periods of about 1 to 2 days (Young et al., 2008). Using the single-station method, dissolved O_2 can be measured continuously over at least one 24-hour period and used to construct diel oxygen curves of daily metabolism rates, yielding reliable results (Swaney et al., 1999; Acuna et al., 2004). A diel oxygen curve is a graph that shows the change in dissolved oxygen over time, which is important to analyze the amount of consumption or creation of oxygen within these systems. Typically, DO will increase during the daylight hours due to primary production, and decrease during the night as respiratory processes continue. The single-station approach, with one sonde in the stream reach, is often used to determine the reaeration coefficient (Young & Huryn, 1999). The reaeration coefficient corrects for the reaeration rate, which is the rate of gas exchange in a stream system due to the natural, physical mixing of water and air. The two-station method measures dissolved oxygen and other metrics at a location upstream and at another location downstream. The minimum reach length to detect a significant change in dissolved oxygen is 20m in most streams (Riley & Dodds, 2013). The two-station approach can be more applicable to a greater range of terrains, such as along steeper slopes and provides more accurate reaeration values than the single-station method (Young & Huryn, 1999). Along steeper inclines, more stations can be set up and thus more measurements can be obtained for a wider range of habitats.

When measuring metabolism it is important to account for the reaeration coefficient (k) through one of two methods: mathematical modeling or a conservative gas tracer. For the latter method, a tracer gas (e.g. propane) is injected upstream and measurements of the gas are taken further downstream in order to determine the physical gas exchange rate of the system (Young & Huryn, 1999). Models are generally effective for determining the reaeration rate; however, their effectiveness depends on whether the equations involved accounted for all possible measurements of the factors that could contribute to the reaeration coefficient (Aristegi *et al.*, 2009; Riley & Dodds, 2013). Often these models overestimate or underestimate ERR and GPP (Aristegi *et al.*, 2009). The nighttime regression method, which includes respiration rates using values of dissolved O_2 obtained at night, has been proven to produce the most consistent and reliable results in reaeration modeling (Hornberger & Kelly, 1975; Aristegi *et al.,* 2009). Nonetheless, if limited resources are available, mathematical modeling of k is a reasonable estimation of reaeration coefficients.

3.4 Metabolism as a Measure of Whole Ecosystem Function

The current river paradigm describes a multi-level hierarchical organization of streams, comprised primarily of the stream catchment, segment, and reach (Frissell et al., 1986). According to this model, the structure and functioning of a stream is controlled by the physical landscape of its surrounding watershed. Catchment-scale parameters, such as land use, thus have a strong influence on the metabolism at the lower levels of the stream (Allan *et al.*, 2004). A growing body of work documents stream health on a broader scale, linking catchment land use with measurements of biological diversity and water condition (Allan, 2004; Young & Collier, 2009; Clapcott et al., 2010; Yates *et al.*, 2012). Land use has been demonstrated to be a controller of stream metabolism in a variety of habitat types and biomes (Lewis & Kerking, 1979; Bernot et al., 2010). Human activities alter a number of physical and chemical characteristics of the environment that may have a synergistic effect on the biological condition of streams (Allan, 2004). Disturbed stream catchments often consist of land used for agricultural purposes, or, to a lesser extent, for urban settings (Allan et al., 2004). Urbanization, however, has a significantly larger influence on stream health than does agriculture (Paul & Meyer, 2001). Land used for mining, forestry, and military purposes also affects nearby stream health, though these areas have been less extensively studied on a broad scale (Keppler & Ziemer, 1990; Hill *et al.*, 1997). Measures of stream metabolism, namely rates of GPP and ER, can be documented along with other functional metrics to determine the impact of changing land use on ecosystem processes. Rates of GPP and ER can also reveal the trophic state of an aquatic system (Fellows et al., 2006; Dodds & Cole, 2007). Using the P/R ratio, or the ratio of net photosynthesis to ecosystem respiration, communities can be classified as either autotrophic or heterotrophic (Odum, 1957). Determining the relative activity of autotrophs and heterotrophs is important to describe the structure and function of an ecosystem and, therefore, its health (Odum, 1957; Dodds & Cole, 2007).

3.5 Metabolism Study Goals

The purpose of this study is to evaluate the NEP, ER, and GPP rates of all three streams (Whittier Stream, Beaver Brook, and Stony Brook), as well as to determine the contributions of biofilms on different substrates that contribute to GPP and ER. This information will provide a deeper understanding of the ecosystem function of these forested headwater streams, a baseline against future changes in ecosystem management, and a comparison to other streams in the region. Our hypothesis is that due to the protected status of these sites, the GPP and ER values will be relatively balanced, meaning that NEP will be close to zero, reflecting a healthy stream system. The stream will be dominated by heterotrophic activity, as a result of high canopy cover, and leaf litter inputs which will contribute the most to respiration. Additionally, we hypothesize that the organic substrates, leaves and wood, are hotspots of heterotrophic activity while the biofilms on rocks are hotspots of autotrophic activity.

Methods

3.6 Open System Metabolism

To measure stream metabolism, dissolved oxygen (DO) and temperature were recorded every 10 minutes by an oxygen sonde (Hydrolab MS5), which was placed in the thalweg, or the site of highest flow velocity, of each of the three streams for a period of at least 24 hours. Photosynthetically-active radiation (PAR) was measured every 10 minutes as well using an Odyssey Data Recorder (Model Light Logger Z412), which recorded light data for a period of 5 days at each of the stream sites. The information collected by the PAR sensor was used to determine how much sunlight was available for autotrophs to perform photosynthesis. To account for the reaeration rate, ModelMaker was used to create a model that best fit the data and adjusted the GPP and ER to reflect the true amount of dissolved oxygen in the stream.

3.7 Substrate-Specific Metabolism

To identify the major contributors of net ecosystem respiration, five different substrates (a single rock, wood, leaves, sediment and macrophytes) and a control (stream water) were taken from the streambed and each put into isolated glass containers with lids (n=6 per substrate per stream). The relative amounts of each substrate obtained were approximately the same for each container. The rocks were about 10cm in diameter each, the wood pieces were about 10cm in length, the leaves amounted to

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about one-third of the volume of the glass containers, and the sediment amounted to a depth of 2cm in the jars. Aquatic macrophytes were taken from rock faces that were submerged under water and enough were collected to fill one-fourth of the glass container. The DO and temperatures of three of the controls were taken at the beginning of the experiment and averaged separately to obtain the initial DO and temperature values for all repetitions for that particular stream. Half of the jars were left in the light and half were covered with a black garbage bag to create a dark treatment. Light- and dark-exposed containers were used to estimate net ecosystem production and respiration, respectively. Three replicates were performed for each substrate for both the light and dark conditions. Jars were submerged in the stream and incubated for approximately one hour before DO and temperature were measured again. The substrates used in each experiment were then collected and stored in a refrigerator prior to massing. For the rock substrate, only the mass of the algae encrusting the rocks was considered, as this was the portion that was actively contributing to production. To remove algae from the rocks for massing, a toothbrush was used to scrub the surfaces of each rock and the material was rinsed off with water and collected. The dry mass of all substrates was measured in order to determine the relative biomass, excluding water weight, of each. To obtain the dry mass of the wood, macrophytes, leaves, sediment, and algae from the rocks, samples were put into a dry oven until all moisture was evaporated, and the final mass of the substrate was recorded.

3.8 Statistics

To compare the different substrates' effect on the stream's metabolic processes, either a one-way analysis of variance (ANOVA) or a Kruskal Wallis test was performed. The ANOVA was used if the data was normal (for the NEP of Whittier Stream and Beaver Brook). The Kruskal Wallis test was conducted for non-normal data; no transformations were done on the data. Any negative GPP values obtained in the statistical tests for substrate-specific metabolism were changed to 0; these negative values were the result of experimental error.

Results

3.9 Whole Stream Metabolism

Based on the diel dissolved oxygen data obtained from the sonde and the light data acquired from the PAR sensors, the three streams all had relatively low GPP and ER values. The lowest GPP rate was observed in Stony Brook with a value of $0.4218 \text{ mg O}_2/\text{g DM/h}$. Beaver Brook exhibited the highest value for GPP, with a value of $0.8862 \text{ mg O}_2/\text{g DM/h}$, yet the lowest value, -0.3008 mg O₂/g DM/h, for NEP. Whittier Stream had the highest value for NEP, -3.1394 mg O₂/g DM/h and the highest value for ER, -3.864 mg O₂/g DM/h (Table 3.1).

Table 3.1. The mean NEP, ER, and GPP for each stream based on the single-station method.

	NEP (mg O2/g DM/h)	ER mg (O2/g DM/h)	GPP (mg O2/g DM/h)
Whitter Stream	-3.1055	-3.8640	0.7586
Beaver Brook	-0.3008	-1.1870	0.8862
Stony Brook	-3.1394	-3.5612	0.4218

ER exceeded GPP across all three streams, and NEP was negative in each (Table 3.1 & Fig. 3.1). The net metabolism of the three streams was driven primarily by the activity of heterotrophs. In other words, the streams were all net heterotrophic.

Net Stream Ecosystem Metabolism



Fig. 3.1. The mean NEP, GPP, and ER of all three streams based on the single-station method

3.10 Substrate-Specific Metabolism

Table 3.2. The mean NEP, ER, and GPP for each of the streams based on combined rates of each of the substrates for the mean NEP, ER, and GPP.

	NEP (mg O2/g DM/h)	ER mg (O2/g DM/h)	GPP (mg O2/g DM/h)
Whitter Stream	-18.5115	-1.2640	-17.2475
Beaver Brook	-4.8535	-39.9569	35.1033
Stony Brook	-9.8836	-24.5344	14.6508

In the Whittier Stream, ecosystem respiration did not vary significantly among any of the substrates (Kruskal Wallis, p>0.05). Therefore, no single substrate can be designated the biggest contributor to respiration. In terms of net ecosystem production, there was a significant difference

among all of the substrates in this stream (ANOVA, p=0.007). The processes performed by the biofilm on the leaf substrate resulted in the lowest values for net ecosystem production (the most negative values), and was considered significantly different from all other substrates (Scheffe, p<0.05). There was a trend towards differences in GPP amongst substrates but due to abnormally distributed data, a nonparametric test could not be performed to determine whether there was a significant difference between specific groups of substrates (Kruskal Wallis, p=0.03). The general trend of the data, however, suggests that the GPP of Whittier Stream was dominated by the activity of microorganisms living in the biofilm formations on the rocks (Fig. 3.2a).



Fig. 3.2. The mean NEP, ER, and GPP for each the five substrates (leaves, macrophytes, rocks, sediment, and wood) of all three streams: (a) Whittier Stream, (b) Beaver Brook, and (c) Stony Brook.

In Beaver Brook, there was significant difference among substrates in terms of net ecosystem production (ANOVA, p = 0.001), ER (Kruskal-Wallis, p=0.04), and GPP (Kruskal-Wallis, p= 0.04). These results suggest that the contribution of microorganisms to metabolic processes varies. The highest NEP was produced by the bioflims on the leaves and was significantly different from all other

substrates (Scheffe, p<0.05)(Fig. 3.2b). GPP was dominated by the metabolic processes of the biofilm community on the rock. The highest ER value, produced by the biofilms on the leaf substrate, was - 14.75 g O_2/g DM/ h. No statistical test could be performed to determine significance between substrates, but the data suggests that the leaf and macrophyte biofilms were the biggest contributors to ecosystem respiration, due to the high respiration rates for those substrates. The overall NEP was negative, which suggests that metabolic activity was dominated by heterotrophs (Table 3.2).

For Stony Brook, there was a significant difference among substrates for NEP (Kruskal-Wallis, p=0.01) and ER (Kruskal-Wallis, p=0.02), indicating that the contribution of primary production and respiration differs among substrate type. No significant difference among substrates was found for GPP, suggesting that each individual substrate type did not contribute any more to production than the other substrates did (Kruskal-Wallis, p=0.06). Although there was no significant difference, the data suggest that the microorganisms living in the biofilms on the rocks were the most productive. No statistical test could be used to determine differences among the substrate types, but the general trend of the data suggests that the leaf and macrophyte microrganisms contributed the most to stream respiration (Fig. 3.2c).

Overall, for all three of the streams, the microorganisms present on the rocks, wood, and sediment contributed the least to net ecosystem production rates. Rates of GPP were usually highest on the rock biofilms, whereas rates of ER were highest on the biofilms of leaves and macrophytes.

3.11 Metabolism and Nutrient Cycling

Nutrient uptake rates of soluble reactive phosphorus (SRP) and nitrate explained the variance in NEP (R^2 = 1.0, F(2,1)=5061.913, p=0.00040)(R^2 =1.0, F(2,1)=13604.006, p=0.0050)(Fig. 4) (See Chapter 2). There was no significant relationship between NEP and NH₄ (R^2 =0.0047, F(2,1)=0.0047, p=0.96) (Fig. 3.3). Due to the small sample size of the experiment (n=3), the data are highly influenced by each individual data point, and more data would be needed to be collected for stronger conclusions to be made.





Fig. 3.3. Nutrient uptake rates of soluble reactive phosphorus (SRP), nitrate (NO₃), and ammonium (NH₄) compared to NEP.

Discussion

3.12 Metabolism and Relative Stream Health

The difference in mean NEP, GPP, and ER values obtained through two different methods demonstrates the necessity of conducting both types of experiments (Table 3.1 & 3.2). The light-dark bottle experiment highlights the specific substrates that contribute to stream metabolism but can often overestimate GPP and ER (Odum, 1957). The single-station method more accurately assessed GPP and ER but did not evaluate specific substrates, whereas the substrate-specific method allowed us to determine the major drivers of these processes. Therefore, the combination of these two methods is crucial to accurately measure stream metabolism. The results of this study are consistent with those from similar substrate-specific studies. Our data suggest that the microorganisms contained within biofilms on leaves and macrophytes, and rocks are hotspots for respiration and primary production, respectively. Hoellein *et al.* (2009) found that the highest rates of GPP are often the result of the presence of biofilms on rocks and wood.

Based on the negative NEP values of all three study streams (Table 3.1 & 3.2), metabolic activity at these sites is dominated by heterotrophic respiration on all substrates. An exception to this finding is

the activity of biofilms on rocks, which is autotrophic (Fig.2a & Fig.2c). This result is concurrent with findings from other respiration-dominated stream studies, suggesting that there is a high input of carbon from the areas surrounding the sites (Duarte & Prairie, 2005). Natural inputs of organic matter, such as wood and leaves, are important for the continuation of heterotrophic activity within the stream. Although no specific studies were conducted on the impact of roads and other impervious surfaces, the lower rates of primary production in Whittier Stream and Stony Brook may be attributed to the culvert and proximity of the stream to roads, respectively. Surface runoff from these impervious surfaces can result in an increase of sediment to the stream, thereby burying substrates and blocking light for photosynthesis, which can cause rates of production to decline. The beaver dam downstream of our study site in Stony Brook may have trapped organic matter, which we have determined to be a hotspot for respiration. Therefore, it is vital to continue research on the interactions between terrestrial and aquatic systems (Duarte & Prairie, 2005).



Mean GPP and ER of Literature Values and Current Study Values

Fig. 3.4. ER/GPP values from this study compared to the literature values.

The values of ER and GPP produced in this study were similar to the literature values obtained from Marcarelli *et al.* (2011). Our rate values aligned closely with the rates of streams identified as protected sites; the only streams that were characterized as unhealthy in this study had much higher rates of GPP and ER than did ours (Fig. 3.4). Such streams were characterized in this study by relatively uniform substratum and intact riparian vegetation, few open areas of cobble or gravel, minimal disturbance to the catchment, and net heterotrophic activity. In contrast, unhealthy streams were characterized by having a catchment dominated by agricultural and urban land use and had point-source pollution from wastewater treatment plants.

3.13 Metabolism and Nutrients

There was a direct positive relationship between the nutrient uptake rates of SRP and NO₃⁻ to NEP, while no association between NH₄ and NEP was found (Fig. 3.3). This finding suggests that SRP and NO_3^{-} play an important role in metabolic processes. The impact of nutrient concentration on net ecosystem production is best illustrated in headwater streams that drain agricultural sites. Since agricultural activities result in the delivery of non-point source pollution, an increase in sedimentation, and the removal of native vegetation and shading, agricultural streams often have greatly elevated GPP and ER levels as compared to their more pristine counterparts (Bernot et al., 2010; Griffiths et al., 2013). The main driver of GPP in prairie streams has been demonstrated to be human activities that increased nutrient inputs to the streams (i.e. livestock, wastewater treatment, and crop cultivation by fertilizers) (Yates et al., 2012). Land use for livestock and human wastewater treatment increases GPP in particular, since nutrients from these activities tend to be released in a form that is more readily taken up by autotrophs. The enhancement of nutrient concentrations by fertilizers within the stream also impairs respiration by negatively impacting aquatic invertebrates, thus decreasing litter breakdown by these organisms (Piscart et al., 2009). Since the three streams we studied were all within protected areas, disturbance from nutrient-adding human activities was minimal. We can thus conclude that some of the variation in NEP among our streams is due to the different nutrient levels at the sites.

3.14 Implications and Effects of Land Use on Stream Metabolism

Human development in the form of agricultural activities, urbanization, and timber harvesting has been demonstrated to impact stream metabolism by increasing erosion and sedimentation at the catchment level (Maloney *et al.*, 2005). Variability in stream metabolism may be caused by a

combination of factors including light availability, water temperature, storm flow dynamics, and desiccation – all of which can be affected by human activities in urban settings (Beaulieu *et al.*, 2013). Streams in more developed areas tend to have less riparian vegetation and thus are more exposed to light, leading to higher GPP levels than one would expect to find in a conserved site (Young & Hyun, 1999). Urban development also impacts metabolism by making high flow events more frequent due to a greater presence of impervious surfaces, such as paved roads, causing streambed disturbance and thus potentially impairing metabolic activities (Konrad *et al.*, 2005). In addition to an increased number of high flow events, higher rates of sediment deposition are a possible effect of anthropogenic activity. Disturbance from military reservations, for example, has been shown to affect stream metabolism by decreasing ER. These sites often experience high soil and vegetation disturbance where training exercises are held, resulting in a high input of sediments to the stream that can bury woody debris and benthic organic matter, thereby reducing their availability for algal colonization and stream respiration (Houser *et al.*, 2005).

Natural reforestation as a result of changing land use patterns has been observed to restore stream metabolism in regions that have historically been used for agriculture. Upon reforestation, stream metabolism can eventually recover to pre-agriculture states. This restoration would be indicated by a decrease in GPP to levels seen in forested streams (such as the streams in our study) and a shifting of net metabolism towards heterotrophy by limiting primary production and increasing shading (McTammany *et al.*, 2007). In one study, experimental removal of organic debris dams resulted in a decrease in ecosystem respiration by decreasing the retention of organic matter within the system (Bilby & Likens, 1980). This finding aligns with the light-dark bottle results in our study, which demonstrate that stream ecosystem respiration is partially attributed to the various substrates located along the streambed. Efforts taken to restore wood and debris dams and improve the heterogeneity of benthic substrates may also benefit agricultural stream metabolism by increasing organic matter availability (Giling *et al.*, 2013).

The impact of human activities occurring at greater spatial scales has important implications for stream conservation and management. The response of streams to anthropogenic disturbance may be influenced by a variety of factors acting in concert. Agricultural and urban land use may modify the regional characteristics of stream ecosystems, downplaying the unique ecosystem properties created by inherent differences in geography (Bernot *et al.*, 2010). The loss of ecosystem complexity resulting from human impacts on stream metabolism is thus a major concern to biodiversity across a range of

habitats. We propose that measurements of stream metabolism can be effectively used to assess the impact of human disturbance.

Conclusions

Based on comparisons of the metabolic data obtained from the single-station approach to literature values, Whittier Stream, Beaver Brook, and Stony Brook can be classified as healthy systems. Stream metabolic activity, as determined by our dark and light bottle experiments, is attributed primarily to rock biofilms for gross primary production and leave and macrophyte biofilms for respiration. Measurements of stream metabolism (i.e. rates of GPP, ER, and the P/R ratio) have been proven to be effective indicators of the extent of human disturbance occurring at varying levels along the stream (Garnier & Billen, 2007). Management efforts should thus focus on assessing stream metabolism along with other functional variables as measures of ecosystem health and the success of restoration efforts.

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CHAPTER 4 ORGANIC MATTER

Introduction

4.1 Coarse Particulate Organic Matter

Coarse particulate organic matter (CPOM) input from terrestrial sources comprises the base of stream food webs, and when disturbed, it can greatly reduce invertebrate activity within the stream system (Wallace *et al.*, 1997; Vannote *et al.*, 1980; Cummins, 1974). Even when CPOM is available in a stream, the conditions of the system must be appropriate for the organic matter to be processed effectively. The decay and retention of organic matter in streams are directly related to the biological utilization of organic matter (Bilby & Likens, 1980). Favorable stream conditions are those under which leaf matter can be colonized and processed by fungi and bacteria as well as consumed by macroinvertebrates (Hieber & Gessner, 2002). In order for those conditions to be possible, there must be a reliable input of CPOM from terrestrial ecosystems that is then retained by a variety of naturally occurring stream structures such as debris dams, rocks, and wood (Lepori *et al.*, 2005).

4.2 Organic Matter Retention

Headwater streams are the most retentive type of stream and are often more functionally stable, particularly to physical disturbances, than higher order streams (Minshall *et al.*, 1983). When impacted by human activities such as logging, loss of riparian buffer, and intentional channelization, stream retentiveness can be minimized, meaning organic matter more easily travels downstream rather than getting caught (Lepori *et al.*, 2005; Muotka *et al.*, 2002; Diez *et al.*, 2000; Gerhard & Reich, 2000). This loss of retained organic matter occurs because the aforementioned activities cause both the removal and decreased input of retentive structures. Retentive structures are obstacles to flowing particulate organic matter in the stream such as boulders, debris dams consisting of leaves and sticks, and large woody debris (Acuna *et al.* 2013; Kail *et al.*, 2007; Gerhard & Reich, 2000). Responses of retentive structures to human activities vary, though, as structural changes sometimes accelerate leaf decay rates whereas chemical additions to the system can speed or, occasionally, decelerate decay. In general, relative to undisturbed streams, impacted streams display a consistent decrease in organic matter retention but do not display any overall consistent response with regard to leaf decay (Walsh *et al.*, 2005, Gessner *et al.*, 1999).

Debris dams, which are defined as accumulations of organic matter which span the channel or extend part way across the channel but still form a major impediment to water flow and create a pool, are one of the most important factors in overall retentiveness in stream systems (Muotka *et al.*, 2002; Bilby & Likens, 1980). Not only do these structures contain nearly 75% of the standing stock of organic matter in first-order streams (Bilby & Likens, 1980), they also create vertical falls and pools in which slow water flows, allowing settling of sediment and organic matter (Díez *et al.*, 2000; Thompson, 1995). The more retentive structures or obstacles in a stream, the higher the probability will be that organic particles will be caught. The retention of organic matter gives time for this coarse particulate matter to be converted into fine particulate organic matter (FPOM), from which nutrients for uptake by the stream system are provided (Robison & Beschta, 1990; Speaker *et al.*, 1984). Removal of debris dams has significant impacts on the processing and retention of not only organic matter, but also as sediment retention and nutrient uptake lengths.

Large organic debris retention in streams varies seasonally. Raikow *et al.* (1995) studied the 'life cycle' of debris dams in third-order streams and found that while streams are generally most retentive in the summer due to base flow condition, debris dams are most retentive in autumn, and less retentive in winter and in the summer. Cobbles showed an opposite pattern. Focusing on nutrients, Warren *et al.* (2013) found that removing woody debris caused summer uptake velocities of nitrate to increase, again demonstrating seasonal variability.

Terrestrial leaf litter input is a vital component to stream food webs. Upon exclusion of this input, Hall *et al.* (2000) found that wood was the primary source of organic matter in the stream where litter has been excluded and that the stream had overall lower detrital flows. Conversely, the reference stream gained organic matter from sources such as leaf tissue, bacterial carbon, and animal prey (each ~25-30% of total secondary production from each). A decrease in quantity and variety of organic matter following leaf litter exclusion coincided with a decrease in the number of invertebrate taxa represented in the experimental stream. Wallace *et al.* (1997) found similar results, demonstrating that following an exclusion of terrestrial leaf litter inputs for a period of three years, the abundance and/or biomass of most invertebrate taxa declined as a result of a strong bottom-up trophic collapse, beginning with detritivores.

4.3 Organic Matter Decomposition

CPOM decomposition rates are commonly determined using the leaf pack method, which entails placing mesh bags of leaves throughout stream reaches and measuring loss in leaf mass over time. The effects of spatial factors, such as pool size, on leaf pack decomposition are important. The difference between effects of pools verses riffles is unclear, as pool leaf packs have significantly fewer invertebrates colonizing leaf packs than riffle leaf packs (Richardson, 1992) but middle pool patches have the highest rate of decomposition (Kobayashi & Kagaya, 2005).

There are different theories explaining why macroinvertebrates colonize leaf packs. Compson *et al.* (2013) studied the effect of slow-decomposing versus fast-decomposing leaves from two dominant trees of the same genus on emergent insects. They found that the difference in leaf type yielded the following differences: varying emergence patterns, different functional feeding groups by treatments and seasons, and that fast-decomposing leaves can support a more diverse, complex emergent insect assemblage during certain times of the year. At the conclusion of the study, the authors hypothesized that leaf species that decompose slowly provide habitat, while species that decompose quickly provide nutrients. Richardson (1992) conversely determined that food value is the primary determinant of leaf pack use for most shredders and non-shredders because shredders didn't colonize artificial leaf packs. Ultimately, leaf colonization for either habitat or food depends on the colonizing species and additional in-stream factors. In our study we placed leaf packs containing fast-decomposing leaves in pools. Because the leaf packs were placed in habitats with similar conditions, the decomposition rates for each of the streams are comparable.

4.4 Organic Matter Dynamics in the Kennebec Highlands

These variations in CPOM input, colonizing species, spatial effects, decomposition, and retention should be kept in mind when interpreting our autumnal study of organic matter dynamics in headwater streams. We addressed two main research questions; how quickly does leaf litter decompose in protected, forested headwater streams and how much organic matter is retained, as a baseline, in three conserved streams with intact riparian buffers and minimal human disturbance. Both of our research questions will provide insights into how effectively organic matter is retained and used in these protected systems rather than the streams allowing the organic matter to be transported downstream to Long Pond. Efficient storage and use of organic matter in the streams will prevent water quality degradation downstream and support the food web of the headwater streams.

Methods

4.5 Study Sites

Experiments were conducted during the fall season, from September through October 2013. This is an important time for organic matter inputs and processing in streams as there is a large influx of leaf litter falling from deciduous species along the stream banks. Whittier Stream, Beaver Brook, and Stony Brook were selected as the study streams, as all three are located in the Kennebec Highlands Management Area in Belgrade, Maine and have similar baseline characteristics.

4.6 Decomposition Study

Dried sugar maple (*Acer saccharum*) leaves were weighed and placed in onion bags. About four ounces of leaves were placed in each bag along with a numbered plastic bottle cap. The dry mass of the leaves was recorded for each leaf pack (mean = 4.13 ± 0.13). The 86 onion bags, including five control bags, were fastened using plastic onion bag fasteners. These fasteners were also labeled in Sharpie with a number 1 - 81.

In each of the three streams, three clusters of bags were placed at three different meter markings along the stream, with each cluster containing three bags (n=27 per stream). However, due to time constraints the study was ended early, so data from only 21 leaf bags were collected. The bags were fastened in the bottom of each stream using metal stakes and a mallet.

For seven weeks one bag was collected from each meter mark in each stream per week (n=3 per stream). The leaf litter bags were placed in individual, labeled Ziploc bags upon collection and transported to the lab. They were then either stored in the refrigerator or processed immediately for drying. Leaves were processed in the lab by emptying the onion bags one by one into a tray and gently spraying each leaf individually with a squirt bottle filled with tap water and/or placing the leaf in a water bath and gently rubbing the surface. Any macroinvertebrates found on the leaves or in the bags were collected and stored separately, by stream, in small glass jars containing 70% Ethanol. Rinsed leaves were placed into labeled brown paper lunch bags that were numbered to match their corresponding onion bags. These brown bags were left to dry for seven days and then the leaves were removed from each bag and the dry mass was recorded.

4.7 Retention Study

Dried Ginkgo (*Gingko biloba*) leaves that had been previously collected were counted and sorted into three groups of 1,000 individual leaves. The first two sets of 1,000 leaves were spray-painted neon pink and neon orange such that both sides of each leaf were completely covered in paint. Seventeen wooden dowels measuring about 1 foot in length were cut from larger dowels and spray-painted neon pink and orange to facilitate identification in the stream.

One leaf release and two dowel releases were conducted in each stream during the same day. The releases in Stony Brook were conducted first, followed by Beaver Brook one week later and Whittier Stream the following week. The leaf releases were conducted at a meter marking and then monitored for a 100 meter reach. In order to release the leaves, a basket of 1,000 wetted leaves was scattered across the width of the stream at the chosen meter mark and the leaves were monitored for movement and retention. In each dowel release, all dowels were tossed into the stream simultaneously at a chosen meter mark and then monitored for movement and retention throughout a 100 meter reach. Leaves and dowels that were retained by the stream were counted and the structure that retained them was categorized and recorded. The different retention categorizes were: rocks, roots, backwater, bank, wood, or debris dam. Any of these categories were further categorized as in a riffle, defined as flowing water, or a pool, defined as standing water. For this study a backwater was defined as a stagnant pool of any size that is backed up by an obstruction such as a log or debris. The bank was defined as the terrain on the edge of the water of the stream. A debris dam was defined as any sort of debris, such as a leaf pile up or pile up of other organic matter, creating a blockage of a sufficient size to disrupt water flow.

Leaf and dowel retention data was collected every ten meters throughout the 100 meter reaches. A net was placed along the width of the bottom of the 100 meter reach to stop any leaves or dowels that traveled through the entire reach, allowing for a measurement of export from the reach. Leaf retention counts for each stream do not add up to 1,000 leaves total because it was not possible to count and retrieve each leaf that was released.

4.8 Wood Volume

At each stream we measured the length and width of wood debris larger than 12.7 cm (5 inches) in diameter throughout a 100 meter reach to estimate the volume of large woody debris. Diameter of the wood was measured using a large pair of calipers. Only wood that was in the water at the time of the measurement was included, excluding wood that may be inundated during periods of high flow.

Results

4.9 Leaf Decomposition

Leaf mass in each stream decreased over the 49 day period to a final mass less than 50% of the original mass (Fig. 4.1). Leaves in Whittier Stream decayed 50.2% over 49 days, leaves in Beaver Brook decayed 61.6%, and leaves in Stony Brook decayed 66.9%. Each of the three streams had similar rates of decomposition through the leaf decomposition study (Whittier Stream= 0.0115; Beaver Brook = 0.0175; Stony Brook = 0.0191). Decomposition did not differ significantly between any of the streams (RM-ANOVA, F-ratio=0.115, p=0.892). The level of decomposition in each stream was almost entirely explained by the number of days the leaf pack was in the stream (Whittier Stream, r^2 =0.7887; Beaver Brook, r^2 =0.9724; Stony Brook, r^2 =0.8815) (Fig. 4.2).



Fig. 4.1. Leaf mass remaining out of 100% for Whittier Stream, Beaver Brook, and Stony Brook over a 49 day period.



Fig. 4.2. Rate of leaf decay over 49 days at Whittier Stream, Beaver Brook, and Stony Brook. Rates of decay(k), respectively = 0.0115, 0.0175, and 0.0191.

4.10 Leaf Retention

The leaf release experiment demonstrated that all three streams were very retentive. Only in Whittier Stream did any leaves make it to the end of the reach. Most of the leaves remained in the 0-10 meters traveled range, particularly in Stony Brook (Fig. 4.3). In Whittier Stream, 65% of the leaves released were retained by rocks. In Beaver Brook, the majority (64%) of the leaves released sunk to the bottom of pools in the first ten meters of the reach. The second most retentive structure in Beaver Brook was debris dams (14%). Leaves in Stony Brook were also retained primarily by rocks (87%).



Fig. 4.3. Leaf retention at each study stream. 1,000 leaves were released at each stream site, not all 1,000 leaves were counted and recollected with each release. All three streams were retentive and the majority of leaf matter was retained in the first ten meters of each reach.

4.11 Dowel Retention

The retentiveness of the streams, based on the dowel release experiments, showed some variability in both distance traveled and the structure on which the dowel was caught. Dowels released in Whittier Stream were retained primarily by rocks located in pools and riffles (58%) (Fig. 4.4). In Beaver Brook, rocks in pools and riffles were also the primary retentive structures (41%) and banks and debris dams retained the majority of the remaining dowels (Fig. 4.5). In Stony Brook, dowels were retained primarily by rocks in riffles and pools (85%) (Fig. 4.6). Despite the slight variability in distance traveled, all three streams appear highly retentive of small woody debris (Fig. 4.7).



Fig. 4.4. Dowel retentiveness of Whittier Stream (n=34). Dowels were retained primarily by rocks and in the first 20 meters of Whittier Stream



Fig. 4.5. Dowel retentiveness of Beaver Brook (n=34). Dowels were retained primarily in the first 10 meters of Beaver Brook.



Fig. 4.6. Dowel retentiveness of Stony Brook (n=34). Dowels released in Sony Brook were retained primarily in the first 10 meters of the reach but traveled farther than dowels released in Whittier Stream or Beaver Brook.



Fig. 4.7. Dowel retentiveness of each of the three streams. 34 dowels were released per stream. All three streams were retentive with most of the dowels retained in the first 10 meters.

4.12 Large Woody Debris

There were highly variable amounts of large woody debris in the wetted channel in the three streams. At the time of measurement, Stony Brook had no large woody debris, Whittier Stream had approximately 1,500 cubic inches and Beaver Brook had approximately 13,000 cubic inches (Fig. 4.8).





Discussion

The high retentiveness displayed by Whittier Stream, Beaver Brook, and Stony Brook is a positive indicator with regard to the functionality of these three protected stream sites. Though there is minimal large woody debris in both Whittier Stream and Beaver Brook and no large woody debris in Stony Brook, retention is still high throughout the reach. When retention in a system is high, less organic matter is exported to downstream ecosystems, which, in this study, is Long Pond. This limits the amount of carbon, nitrogen and phosphorous being transported. (Ostojic *et al.*, 2013). Minimal CPOM export reduces the potential for causing anoxic conditions in Long Pond.

In addition, when CPOM retention is high, it is available for processing by macroinvertebrates, fungi, and bacteria, and therefore supports a diverse food web. Because the majority of the leaves and dowels released were retained by rocks, rather than by debris dams, we can infer that these three streams are probably less reliant on large woody debris for retention than streams assessed in previous studies, at least during autumn (Muotka *et al.*, 2002; Webster *et al.*, 1990; Bilby & Likens, 1980). In the presence of higher flows, during the spring in particular, it is probable that the woody debris currently near the

stream, both hanging over the stream and along the banks, would play a larger role in retention in the study streams. These potentially important structures were not accounted for in our study because they were not physically in contact with the water.

Based solely on our results, it is of great importance to maintain large rocks and boulders in streams of the Kennebec Highlands because of their ability to effectively trap and retain flowing CPOM. When considering management and restoration plans for impacted streams in the region, it will be necessary to consider the addition of rocks and boulders to impacted streams in any instances where they have been previously removed (Warren *et al.* 2013; Muotka *et al.* 2002). This action will likely increase retentive functioning and the associated benefits for a low cost.

Looking further into our retention results, while we did not have enough replications to perform statistical analysis between the three streams, there were some observable differences in Whittier Stream. Whittier Stream retained fewer leaves and was the only stream that, during the leaf release, had leaves flow to the end of the 100 meter study reach. This stream is the only one with visible signs of anthropogenic disturbance, as a culvert is located in the middle of our study reach. In addition to this disturbance, this stream was formerly the site of a mill, which may have altered the flow dynamics of the stream. This observable difference in retentiveness between Whittier Stream and the other two streams speaks to the sensitivity of these headwater stream systems and the great value that should be placed on their protection from disturbance.

In an extensive review of litter breakdown studies, Gessner & Chauvet (2002) determined that the best way to compare decay rates across streams is to create a ratio of the decay rate in a disturbed stream to the decay rate in a reference stream in the same catchment. A comparison of stand-alone decay rates from streams with different habitat-types and in different ecosystems can be clouded by a variety of confounding variables. However, if the stressor being placed on the disturbed stream is known, it is possible to determine the severity of its impacts on CPOM decomposition by focusing on the magnitude of Gessner and Chauvet's ratio.

Future CPOM decomposition studies in the Kennebec Highlands management area will be able to create the Gessner and Chauvet ratio using the decay rate determined in impacted streams with the decay rates determined in this study. This will provide a basis for determining if the disturbed and protected streams differ in their decomposition. If it is found that they do differ, the differences may be traceable to one or more specific anthropogenic stressors. If it is found that they do not differ, it will be important to realize that this result indicates the current property owners should be commended for their personal management practices but that continued monitoring, especially if property owners change through time, will be important to maintain the streams high functionality.

Lacking a decay rate from a disturbed stream with which to create a ratio, we compared our results to a study of a woodland stream conducted from December to May using sugar maple leaves. Peterson (1974) found a coefficient of decay equal to 0.0107, meaning each day of exposure was associated with a 1% decrease in dry mass remaining. According to his classification system, sugar maple leaves are a fast decomposing species, taking less than 46 days to show 50% processing, and less than 107 days for 80% processing. Our decay rates ranged from approximately 0.01 to 0.02, which is close to Peterson's value. We also saw over 50% processing during our 49 day study, which fits perfectly with Peterson's prediction. This indicates that our results are similar to decay rates found in healthy streams.

While the three protected streams from this study show no signs of nutrient loading (see Chapter 2), it is possible that streams under less protection in the Kennebec Highlands management area might be suffering from higher nutrient loads from sources such as fertilizer. By increasing nitrogen and phosphorous inputs over time various studies have recorded related increases in leaf litter decay rates as a result of this nutrient loading (Greenwood *et al.* 2007). Future studies in the Kennebec Highlands may use the decay rates we calculated as a baseline for comparison with unprotected streams. If nutrient loading from runoff of the surrounding catchment is an issue, we would expect to observe decay rates higher than those from the protected streams in the unprotected streams. Again, it is suggested the ratio suggested by Gessner and Chauvet be used to help determine if there is a difference in decomposition rates between the study sites.

Another disturbance with proven impacts on organic matter decomposition and retention is logging. Lecerf & Richardson (2010) came to the conclusion that streams are highly sensitive to changes in forest cover caused by logging, observing a decrease in leaf decay most likely as a function of decreased fungal biomass and shredder richness. When considering selective logging in the Kennebec Highlands management area, changes in large woody debris and leaf litter input should be considered. A decline in large woody debris would normally be expected to decrease retention (Kreutzweiser *et al.*, 2008), but is less likely to impact these streams due to the low levels or absence of large woody debris in their current state. Therefore, maintaining inputs of coarse particulate organic matter in the form of leaf litter as well as an intact riparian zone along the stream bank should be focus of any conservation efforts. While our study does not speak to the current quality or quantity limitations of organic matter in

the stream, forest species removed and corresponding leaf litter quality, as well as leaf litter quantity, should be a key consideration before any form of logging is allowed.

It is also important to realize stream energy budgets are balanced in the long term, but not necessarily from year to year (Webster & Meyer, 1997). Our results would therefore be strengthened by repeated experiments in subsequent years, as well as increasing the parameters examined. The leaf decomposition study would be more accurate had we utilized sieves to determine what size leaves would be included as "remaining" mass, but this did not seem to greatly influence either the coefficient of decay or how well the days of exposure explain the decay seen. Overall, the organic matter indices in Whittier Stream, Beaver Brook and Stony Brook in the Kennebec Highlands indicate a highly functioning stream ecosystem.

Conclusion

Based on this seven-week decomposition study, as well as our single release retention study, both occurring during the autumn of 2013, we can classify Whittier Stream, Beaver Brook and Stony Brook as healthy stream ecosystems. The decomposition study is a functional metric showing the leaf litter present in the streams is being broken down at an equal rate, statistically speaking, to one another. This rate is comparable to decomposition rates found in the literature for healthy, protected, forested headwater streams. This decomposition rate will allow for utilization of organic matter but a large range of species, supporting a diverse ecosystem. The retention study is also a functional metric showing that organic matter, either leaf litter or woody debris, is not being exported to Long Pond and therefore will not be a contributor to anoxic conditions in the lake. When considering the Kennebec Highlands Management Plan or potential restoration efforts in the area, the preservation or addition of rocks and boulders is advised.

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CHAPTER 5 INVERTEBRATE COMMUNITIES

Introduction

5.1 Headwater Stream Water Quality

Headwater streams are vital components of an aquatic system because they affect all downstream systems. They are where organic matter is processed to supply nutrients to downstream bodies of water, like larger rivers or lakes (Vannote *et al.*, 1980; Meyer *et al.*, 2007). For this reason, water quality, a measure of how well a body of water meets the physical, chemical, and biological requirements of its associated biota, is an important characteristic of low-order streams (Karr & Dudley, 1981; Hammock & Wetzel, 2013). One key component of stream water quality changes seasonally as a result of varying levels of dissolved oxygen (DO). Cold water is more soluble than warm water, so oxygen is more available for consumption during the winter. Dissolved oxygen levels are also affected by current velocity and flow dynamics. Atmospheric oxygen is incorporated into water as it flows through riffles, leading to lotic habitats that tend to contain higher levels of dissolved oxygen than lentic pools (Genkai-Kato *et al.*, 2005). Stream properties may also be altered by natural disturbances, such as extreme weather events, including snowmelt, or anthropogenic disturbances (Arimoro *et al.*, 2012).

The water quality of a stream has a major influence on the breadth and extent of stream-dwelling macroinvertebrate communities (Karr & Dudley, 1981; Hammock & Wetzel, 2013). Nitrogen and phosphorus, as well as dissolved oxygen, restrict macroinvertebrate density. Minimum dissolved oxygen thresholds—approximately 10% saturation—exist even for the macroinvertebrate taxa most tolerant of hypoxic conditions. Any level of dissolved oxygen below this threshold is lethal (Connolly *et al.*, 2004). Another aspect of water quality that affects macroinvertebrate communities is nutrient concentration. It has been demonstrated that nitrogen acts as a limiting nutrient in aquatic ecosystems and that it is therefore beneficial for the system to limit nitrogen in order to restrict the production that may lead to eutrophication (Howarth & Marino, 2006; Atkinson *et al.*, 2013). Streams where water quality has been compromised due to excess nitrogen can support a greater abundance, but a lower biodiversity, of macroinvertebrates (Krueger & Waters, 1983; Feminella & Hawkins, 1995; Yuan, 2010). Similarly, in a phosphorus-loaded system, macroinvertebrate density increases but diversity decreases (Elwood *et al.*, 1981). Oxygen availability, along with both nitrogen and phosphorus, are the water quality factors that are most relevant to the structure of macroinvertebrate communities.

5.2 Habitat Quality

Like water quality, habitat type also has a major influence on the presence and composition of macroinvertebrate communities in a stream system (Friberg, 1997; Batzer *et al.*, 2004; Paillex *et al.*, 2007). The stream current has an effect on the amount of dissolved oxygen present within a stream, and it also has a physical impact on the ecosystem. The velocity of water flow within a stream is naturally variable across its length and width. In a relatively undisturbed headwater stream, a cross section of a stream will display both lentic and lotic zones. The thalweg, or the section of a stream width that has the highest volume of water flow, is inhospitable to all macroinvertebrates except for those that are adapted to cling to the substrate (Cortes *et al.*, 2002). The flow conditions of a stream contribute to other ecosystem functions as well. For example, drift, a phenomenon where macroinvertebrates engage in passive transport downstream in order to escape danger or find more resources, is only possible because of the lotic conditions of a stream system (Hammock & Wetzel, 2013).

Another influential aspect of habitat is the composition of the streambed. Benthic sediment can be categorized based on grain size (ordered by decreasing size: boulder, cobble, gravel, or sand). Depending on grain size, the sediment can support very different community types. Streams that have a predominantly sand and gravel bed can provide habitat for different organisms than streams with a bed composed of cobble and boulders. Macroinvertebrates that cling to substrate require large rocks. Sediment-dwelling burrowers, however, prefer finer sediment that they hide within (Cummins & Klug, 1979). Another substrate that provides unique niches in a stream is coarse woody debris. Climbers and sprawlers require woody debris and other falling plant material for their habitat. Woody debris, in addition to providing habitat, supplies the stream with energy (Vannote *et al.*, 1980). The placement of logs is a widely used strategy in stream ecosystem restoration because it provides another niche for macroinvertebrates with different modes of existence (Hrodey *et al.*, 2008).

Another habitat variable is canopy cover, which affects water temperature and algal growth, as well as eventually provides organic matter inputs to the stream (Vannote *et al.*, 1980; Sweeney, 1993; Bis & Higler, 2001). Because headwater streams are the furthest upstream origins of water, they must have a high input of organic matter. Inputs from headwater streams are ultimately carried downstream to supply some of the energy and resources to larger bodies of water such as rivers and lakes. Organic matter inputs are also vital within the headwater stream ecosystem itself. This is because there is generally no primary production that occurs in low-order streams. Primary production usually begins to

occur once the stream opens up to an order between 4 and 6. This means that riparian zone inputs are essential to providing energy to a headwater stream (Vannote *et al.*, 1980).

Large trees in the riparian zone provide stability for stream banks, lessening the potential impact of the erosion of fine sediment (Arimoro *et* al., 2012). Openings in the canopy can create microhabitats, which create more niches in the stream habitat (Bis & Higler, 2001). Some studies have shown that sites with a high percent canopy have higher macroinvertebrate diversity (Roy *et al.*, 2003). Other research suggests that closed canopy cover decreases the macroinvertebrate diversity and production (Riley *et al.*, 2009). Either way, canopy cover often influences the macroinvertebrate taxa present at a site (Arimoro *et al.*, 2012). Shade provided by a dense canopy prevents the overgrowth of algae in the stream. This finding is important because when there is too much algae in an aquatic ecosystem, the process of eutrophication within a body of water can be accelerated. The increased amount of respiration from decomposers as they break down the excess algae may decrease levels of dissolved oxygen within the stream. While shade acts as a bottom-up control of algae, macroinvertebrates that graze on algae as their main source of food also act as top-down controls (Sturt *et al.*, 2011).

5.3 Anthropogenic Impacts

Human activity contributes to water quality degradation and can make stream habitats unsuitable for macroinvertebrates. Humans most drastically impact stream macroinvertebrate communities though non-point source pollution that increases nitrogen and phosphorus levels (Roy *et al.*, 2003; Johnson *et al.*, 2013). Anthropogenic effects can also alter habitat characteristics (Waite *et al.*, 2010; Lewin *et al.*, 2012).

When limiting nutrients are in excess, macroinvertebrate community structures are reshaped and trophic dynamics are skewed. Macroinvertebrate communities subjected to poor water quality are dominated by the taxa most tolerant of environmental degradation. These taxa are all categorized within the same functional feeding group. This homogeneity in the community structure persists downstream despite changes in community composition predicted by the river continuum concept (Vannote *et al.*, 1980; Delong & Brusven, 1998). This homogeneity also threatens the entire stream system, because the ecosystem depends on macroinvertebrates efficiently breaking down organic matter derived from riparian vegetation. This efficiency cannot be achieved unless each macroinvertebrate functional feeding group is present and each functional role is occupied (Cummins, 1974; Vannote *et al.*, 1980). Normally, most secondary production is accomplished by macroinvertebrates that are sensitive to poor water

quality (Buffagni & Comin, 2000). Prior research suggests that non-point source pollution increases secondary production, but only as a result of an increased abundance of the tolerant taxa that often exist outside of the typical stream food web. The energy tied up in this secondary production therefore does not move through the stream food web because it cannot be utilized by stream predators (Johnson *et al.*, 2013). Excessive nutrients threaten macroinvertebrate communities not in terms of abundance, but instead by altering community structure and trophic dynamics.

In addition to changing the nutrient content of headwater streams, humans can impact these ecosystems by altering land use through the processes of logging, urbanization, and agriculture. The conversion of land for use in agriculture is an anthropogenic activity that causes intense disturbance, since it diverts water flow and inundates the watershed with excessive nitrogen and phosphorus (Brainwood & Burgin, 2006). Urbanization is another major cause of disturbance for streams. When developing land, stream burial and deforestation are common, especially in large cities (Bensted et al., 2003; Braccia & Voshell, 2006; Carroll & Jackson, 2008; Elmore & Kaushal, 2008; McGoff & Sandin, 2012; Palma et al., 2013). The installation of a dam results in restricted macroinvertebrate movement downstream and altered substrate composition (Boon, 1988; Hammock & Wetzel, 2013). The placement of dams may also interfere with macroinvertebrate drift (Genkai-Kato et al. 2005; Hay et al., 2008). Urban development in a stream's drainage basin increases the degree of non-point source pollution. Surface runoff water is reabsorbed into the earth less efficiently in developed areas because of the higher percentage of impermeable surfaces, and this surface runoff water introduces pollutants into streams as it drains into them (Basynat, 1999). Healthy macroinvertebrate communities are usually correlated with streams that have a forested riparian zone because surface water runoff is not inundated with urban pollutants, as is the case with urban streams (Roy et al., 2003). The capacity to buffer pollutants is the characteristic associated with forested riparian zones that most benefits macroinvertebrates (Mitsch, 1992; Roy et al., 2003).

In addition to having run-off effects, the development of land can also accelerate the process of erosion. When a slope becomes eroded, sediment is likely to be carried into the watershed because it is light enough to be moved by the flow of water. Bank sediment is generally rich in nutrients. Therefore, it can contribute to nutrient loading within a body of water (Extence *et al.*, 2013). When erosion occurs in a stream ecosystem, it has the potential to change the physical quality of the habitat as well as the water quality. Finer sediment often will begin to fill in the crevices between larger rocks. This habitat reduces niche diversity by making the only available habitat suitable for burrowers. Many burrowers are

members of the more tolerant taxa. This fine sediment can also work its way into the delicate gills of more sensitive taxa, such as mayflies and stoneflies, changing the community dynamic of the entire stream (Zimmermann & Death, 2002). Once taxa are lost from a macroinvertebrate community, it often takes several years for macroinvertebrate communities to recover, even if water quality is restored. The rate of recovery depends on the proximity of the nearest potential colonizing community (Langford *et al.*, 2009).

5.4 Macroinvertebrate Ecology

Merritt and Cummins (1978) were the first researchers to group macroinvertebrates into modes of existence within a habitat. These groups indicate habitat preference of different taxonomic groups and allow us to predict where each of these groups might be found within a stream. Burrowers, such as chironomid midges, live within the sediment of stream lentic zones. Climbers, such as certain dragonfly larvae, live on overhanging vegetation and woody debris. Sprawlers live on floating leaves or other elevated surfaces, similar to the climbers, in order to avoid suffocation by fine sediment. Clingers have adapted to live within rapidly flowing water and hold tightly onto a substrate, such as some mayflies. Swimmers use active transportation to change habitat based on temporary favorability. Macroinvertebrates categorized as skaters, divers, and planktonic are not generally found in streams due to their need for calmer, open waters (Merritt & Cummins, 1996; Heino, 2005).

Organic matter breaks down within a stream by physical processes related to the stream current, microbial communities, and processing by macroinvertebrates (Jayawardana *et al.*, 2010). Macroinvertebrate taxa are categorized into functional feeding groups based on their foraging and feeding behaviors. Taxa within a functional feeding group display similar behaviors in how they acquire their food (Baldy *et al.*, 2007). Because of this, food availability directly impacts which macroinvertebrate communities are present (Bis & Higler, 2001). Therefore, analyzing the representation of functional feeding groups is a meaningful way of characterizing a stream ecosystem (Cummins & Klug, 1979).

Cummins & Klug (1979) were the first to define functional feeding groups. Shredders have strong, sharp mouthparts for use in breaking down and chewing pieces of leaves and other organic matter; they have a crucial role because they condition food for other functional feeding groups downstream (Cummins & Klug, 1979; Baldy *et al.*, 2007). Collectors filter out the fine particles that have been prepared by the shredders. Scrapers feed off of algae that grow on submerged rocks. Piercers consume the juices from stream macrophytes. Finally, predators and parasites both have unique ways of consuming the tissue of living animals (Cummins & Klug, 1979). While defining these groups is a useful tool for the categorization of macroinvertebrates based on ecological function, a macroinvertebrate individual may not always fit strictly into one group. Shifts in functional feeding behavior can change from one stream to another, with changes in weather, with life stage of the invertebrate, or with seasonality (Paillex *et al.*, 2007). Anthropogenic impacts, such as fine particulate organic matter inputs upstream, may also have an influence on changing the functional group of a macroinvertebrate or changing the ratio of one functional group to another (Sturt *et al.*, 2011).

5.5 Biotic Indices

Because different taxonomic groups have different sensitivities within the stream system, it is possible to use analyses of macroinvertebrate communities to infer water quality (Beketov, 2004; Lewin *et al.*, 2012; Cortes *et al.*, 2013). Chemical assessments only describe water quality in terms of one specific parameter, but biotic indices use an approach that reflects dynamic interactions involving nutrient overloading, anoxia, and other stressors (Fremling & Johnson, 1989). Macroinvertebrate abundance is a poor indicator of ecosystem health because density often will increase as a result of poor water quality (Elwood *et al.*, 1981; Feminella & Hawkins, 1995; Yuan, 2010). Species richness, however, is a more reliable metric. Disturbances such as pollution or dam installation result in decreased species richness, as dominant taxa become more abundant and rare taxa are extirpated. Certain taxa are more tolerant of poor water quality than others. In impacted streams, these are the taxa that persist. Richness is negatively affected when less-tolerant taxa are lost from the ecosystem (Verberk *et al.*, 2006).

It is useful to assign tolerance values to macroinvertebrate taxa. These tolerance values quantify the ability of each taxon to survive in degraded ecosystems (Yuan, 2004). Water quality can be inferred from the Hilsenhoff Biotic Index (H.B.I.), which incorporates both taxa abundance and tolerance values to give a cumulative biotic index value that corresponds to a quantitative water quality metric. H.B.I. values that are close to zero will be composed primarily of the most sensitive taxa, indicating excellent water quality and a healthy ecosystem. Conversely, large values for H.B.I. are generally observed in degraded ecosystems dominated by taxa that are tolerant of poor quality water (Hilsenhoff, 1987).

The family Chironomidae, whose abundance increases with poor water quality, is one such taxon (Lenat, 1983; Hilsenhoff, 1987). Conversely, macroinvertebrates of orders Ephemeroptera, Trichoptera,

and Plecoptera are all sensitive to poor water quality. Because of this, their presence is often used as an indicator of good stream health (Fremling & Johnson, 1989; Usseglio-Polatera & Bournaud, 1989; Genkai-Kato *et al.*, 2005). Because these three families are all relatively common, easy to identify, and intolerant of poor water quality, the percentage of total macroinvertebrates comprised of Ephemeroptera, Trichoptera, or Plecoptera (%EPT) is a useful biotic index of stream quality. For the purpose of this index, we can conclude that the higher the %EPT among a community of macroinvertebrates, the healthier the ecosystem (Baker & Sharp, 1997; Crisci-Bispo *et al.*, 2007). When using biotic indices, it is always important to consider the unique habitat of each research site. It is possible for popular, widely used indices like H.B.I. and %EPT to vary in accuracy based on the site at which they are used. Therefore, the index appropriate for use should be determined with the particular site in mind (Waite *et al.*, 2010; Lewin *et al.*, 2012).

For our research, we studied three undisturbed headwater streams and seven disturbed streams of varying size. All undisturbed streams were located in or around the Kennebec Highlands protected area, which is within the towns of Rome, Mount Vernon, and New Sharon, Maine. All disturbed streams were within the boundaries of the Belgrade Lakes watershed within the towns of Oakland, Belgrade, Belgrade Village, Rome, and Smithfield, Maine. Our main objectives in this study were to (i) determine the health of these ten headwater streams using biotic indices, (ii) observe how macroinvertebrate communities correlate with site characteristics such as canopy cover and stream bed substrate, and (iii) understand the ecology of macroinvertebrate communities based on the categorization of functional feeding groups and the presence of sensitive taxa.

Materials & Methods

5.6 Study Sites

Study sites included seven disturbed and three relatively undisturbed streams, all within the Belgrade Lakes watershed. This research focused on the pristine headwater streams located mostly within the Kennebec Highlands protected area. In the Highlands, macroinvertebrate samples were taken from Whittier Stream, Beaver Brook, and Stony Brook. All three are low-order streams that flow into Long Pond. Samples were taken using four different collection methods every 20 meters along a 100-meter reach. Sampling from disturbed streams provided more of an overview for comparison and therefore included only two collection methods at one point in each stream. Disturbed streams were also more variable in size than the relatively uniform undisturbed sites. While not all seven disturbed streams

flow into Long Pond, they all are part of the Belgrade Lakes drainage basin. Disturbed stream sampling locations were at Messalonskee Stream off of Route 23 in Oakland, the dam between Great Pond and Long Pond at Lakepoint Real Estate in Belgrade Village, Robbins Mill Stream off of Route 225 in Rome, Great Meadow Stream in Rome, Rome Trout Brook in Rome, the Serpentine off of Route 8 in Smithfield, and the stream between Long Pond and Messalonskee Lake off of Route 8 in Belgrade.



Fig 5.1. The Belgrade Lakes watershed, with the protected streams represented in blue/green and sampling locations at the disturbed streams are represented in orange.

5.7 Macroinvertebrate Collection

In the 100-meter reach studied in each of three undisturbed streams, five macroinvertebrate samples were taken 20 meters apart. At each sampling point along each stream, a kick net collection and a hand collection were taken. Kick net collections were taken in pool locations at each sampling point. Macroinvertebrates were gathered by kicking vigorously into a downstream net for 30 seconds, putting

the net contents through a 500-micron sieve, and collecting sieve contents into a wide mouth sample jar. Hand collections were taken in the rocky thalweg of each sampling point. All rocks small enough to pick up within a 1-m² area were put into a bucket. Macroinvertebrates were scraped off rocks with paintbrushes, put through a 500-micron sieve, and then collected in a sample jar. Neither sample type was preserved in the field. Instead, macroinvertebrates were kept alive and stored at 4°C for ease of sorting.

5.8 Habitat Data

Densiometer measurements were taken from the each sampling point along the three streams to determine canopy cover at each site at the same time as invertebrate collection, prior to the majority of the leaves falling off the trees. Substrate type was surveyed along each sampling point as well to determine percent boulder, cobble, gravel, sand, and woody debris (see Chapter 6). Stream width was also measured at each sampling point. Miscellaneous qualitative observations were taken at each stream site.

5.9 Invertebrates and Colonization of Organic Matter

To investigate patterns in macroinvertebrate presence and organic matter, three leaf bags were deployed on September 9, 2013 in each stream. Every week after this date for 7 weeks, the three leaf bags from each stream were removed. Macroinvertebrates were removed from leaf packs and preserved in 70% ethanol.

5.10 Processing and Analysis

Sampled macroinvertebrates were stored in original sample jars at 4°C for no more than four days. Sorting of macroinvertebrates from organic matter and sediment was done in the laboratory. Macroinvertebrates were then preserved in 70% ethanol. Most macroinvertebrate individuals were identified under a microscope to the family level, but in several cases they were classified by order or genus. We used biotic indices based on the families of macroinvertebrates present to infer water quality of the ten sampled streams. Biotic indices used included % Ephemeroptera, Plecoptera, and Trichoptera (%EPT), the Hilsenhoff Biotic Index (H.B.I.), and taxonomic richness. An ANOVA was used to identify differences between %EPT values for each of the ten stream sites. Then, pairwise comparisons of means were taken in order to recognize which streams had statistically different %EPT values.

Results

5.11 Biotic Indices

At Whittier Stream, a calculated average Hilsenhoff Biotic Index value of 3.88 indicates water that is of "Very Good" quality. By the Hilsenhoff Biotic Index, low values indicate good water quality and high values represent poor-quality water. This was the highest average H.B.I value calculated for the three protected Kennebec Highlands streams. An average H.B.I value of 3.32 at Beaver Brook indicates "Excellent" water quality. "Excellent" water quality was also found at Stony Brook, where the average H.B.I was 3.0, the lowest value calculated for the protected streams (Table 5.2). Table 1 summarizes how each H.B.I value corresponds to a qualitative water quality metric. These results indicate that Stony Brook was the healthiest of the three Kennebec Highlands streams, but that all three are in relatively good health.

Table 5.1.	The water	quality	grade and	l degree	of organic	pollution	that co	orresponds	with each	different
range of H	.B.I values	(Hilsen	hoff, 198	7).						

H.B.I Values	Water Quality	Degree of Organic Pollution
0.00-3.50	Excellent	No apparent organic pollution
3.51-4.50	Very Good	Slight organic pollution
4.51-5.50	Good	Some organic pollution
5.51-6.50	Fair	Fairly sinificant organic pollution
6.51-7.50	Fairly Poor	Significant organic pollution
7.51-8.50	Poor	Very significant organic pollution
8.51-10.00	Very Poor	Severe organic pollution

Table 5.2a-c. A summary of the H.B.I values calculated for each sampling method at each of the five transects at Whittier Stream, Beaver Brook, and Stony Brook. The average H.B.I value for each stream is also provided, as well as the corresponding qualitative water quality metric. The average H.B.I at Whittier Stream was 3.88, indicating very good water quality. The H.B.I at Beaver Brook and Stony Brook was 3.32 and 3.0 respectively. Both values indicate excellent water quality.

Reach Point	Sampling Method	Taxa Richness	% EPT	H.B.I	Water Quality
0 m	rock	8	23%	4.85	Very Good
0 m	net	4	50%	2	Excellent
20 m	rock	19	45%	3.69	Excellent
20 m	net	12	27%	2.86	Excellent
40 m	rock	6	100%	3.4	Excellent
40 m	net	6	36%	4.5	Good
60 m	rock	5	15%	5.58	Fair
60 m	net	6	38%	4.13	Very Good
80 m	rock	13	74%	3.87	Very Good
80 m	met	0	0%		
Average		8	45.30%	3.88	Very Good

Table 5.2a.Whittier Stream

Table 5.2b. Beaver Brook

Reach Point	Sampling Method	Taxa Richness	% Ept	H.B.I	Water Quality
0 m	rock	9	88%	3	Excellent
0 m	net	4	50%	3	Excellent
20 m	rock	5	100%	2.78	Excellent
20 m	net	1	100%	2	Excellent
40 m	rock	3	50%	3.25	Excellent
40 m	net	3	75%	3.25	Excellent
60 m	rock	10	82%	3.41	Excellent
60 m	net	6	0%	1	Excellent
80 m	rock	17	95%	3.15	Excellent
80 m	net	0	0%		
Average		5.6	71.10%	3.32	Excellent

Reach Point	Sampling Method	Taxa Richness	% EPT	H.B.I	Water Quality
0 m	rock	14	71%	2.78	Excellent
0 m	net	8	55%	2	Excellent
20 m	rock	9	90%	3.32	Excellent
20 m	net	12	66%	2.66	Excellent
40 m	rock	14	89%	3.09	Excellent
40 m	net	11	84%	2.7	Excellent
60 m	rock	18	74%	3.76	Very Good
60 m	net	8	88%	3.64	Excellent
80 m	rock	7	92%	3.49	Excellent
80 m	net	8	46%	2.45	Excellent
Average		10.3	73.70%	3	Excellent

Table 5.2c. Stony Brook

5.12 Protected Versus Disturbed Streams Comparison

The %EPT at Stony Brook was the highest of the three protected Kennebec Highlands streams. 73.7% of all the macroinvertebrates sampled from Stony Brook belonged to either the family Ephemeroptera, Plecoptera, or Trichoptera. At Whittier Stream, %EPT was only 45.3%, the lowest percentage of the three protected streams (Fig. 5.2). The disparity in %EPT at Beaver Brook and Stony Brook was not significantly different (p=0.71), but Whittier Stream differed significantly from both Beaver Brook (p=0.035) and Stony Brook (p=0.013). These results mirror those of the H.B.I analysis because they indicate that Stony Brook is the healthiest of the three streams and that Whittier Stream is the least healthy (Fig. 5.2).



Fig. 5.2. The mean %EPT at all 10 streams; disturbed streams are indicated by orange color.

We measured %EPT at all 10 streams. The highest calculated %EPT was 88.5% at Robbins Mills Stream (RMS). The lowest was 0% at the Serpentine (SERP). Among the protected Kennebec Highlands Streams, the %EPT at Stony Brook (SB) of 73.7% was greatest, but at only 45.3%, %EPT at Whittier Stream (WS) was lowest.

There was a great deal of variation among the seven disturbed streams in terms of %EPT. The %EPT ranged from 0% at the Serpentine to 88.5% at Robin Mills Stream. Stony Brook, the protected stream with the best water quality, only differed significantly from two of the disturbed locations, Messalonskee Stream (p=0.037) and the Great Pond/Long Pond dam (p=0.002). Stony Brook did not differ significantly from the other disturbed streams in terms of %EPT.

Fig

5.13 Canopy Cover

Canopy cover is relatively consistent between Stony Brook and Beaver Brook. At Stony Brook, average canopy cover is 86.6%. At Beaver Brook, two average canopy covers were calculated because the stream is so wide; one was calculated at the stream bank and the other was calculated at the thalweg. At the stream bank canopy cover is 83.3%, and at the thalweg it is 81.5%. However, at Whittier Stream canopy cover is 59.8% (Table 5.3). Canopy cover measurements were taken relatively early in our sampling scheme while the majority of leaves were still on the trees.

	0 15	0	2	
Reach Point	Whittier Stream	Beaver Brook Rock	Beaver Brook Net	Stony Brook
0 m	69.7	77.48	70.2	80.6
20 m	64	74.36	73.8	77.3
40 m	77.5	87.36	85.3	90.9
60 m	23.9	88.92	92	92.5
80 m	64	88.4	86.3	91.9
Average	59.8	83.3	81.5	86.6

Table 5.3. All densitometer measurements are listed for each stream, as well as the average canopy cover values. Average canopy cover was highest as Stony Brook and lowest at Whittier Stream.

The mean % canopy cover was calculated along the stream banks where the net was used to collect samples and at the thalweg where sampledswere collected from rocks. When mean %EPT, calculated at the stream bank and thalweg of each stream's five sampling points, is plotted against the mean canopy cover, mean %EPT increases linearly. At Whittier stream, where mean canopy cover is lowest, the mean %EPT averaged between stream bank samples and thalweg samples was 45.3%. At Stony Brook, where mean canopy cover is highest, mean %EPT is 73.7%. The R-value corresponding to this linear regression is 0.7462, which indicates a relatively strong correlation between canopy cover and %EPT. The R²-value for this relationship was 0.5977. However, this trend is not significant (p=0.0885) (Fig. 5.3).



Fig 5.3. Mean % canopy cover from the three protected streams is plotted against the mean %EPT at the stream bank and the thalweg for each stream. There is a 75% correlation between these two variables, which is relatively strong although not statistically significant. Data shown ± 1 standard deviation.

5.14 Substrate Type

%EPT was compared with substrate type to determine if substrate type has any influence on the health of macroinvertebrate communities, assuming that healthier communities will be made up of mostly macroinvertebrates from either the Ephemeroptera, Plecoptera, or Trichoptera families. However, only one significant correlation between substrate type and %EPT was observed. The relationship between % sand and %EPT is characterized by a negative 56% significant correlation (R=0.5610, p=0.0296), meaning that %EPT taxa present decreases as the occurrence of sandy substrates increases in a stream. However, %EPT is not significantly correlated with any other substrate type. %EPT only has a 2% positive correlation with percent boulder (R=0.0225, p=0.9365) and a 14% positive correlation with percent gravel (R=0.1454, p=0.6052). There was a slight positive correlation between %EPT and percent cobble (R=0.3727, p=0.1712) There was also a very slight positive correlation between %EPT and % woody debris (R=0.2848, p=0.3035) (Fig. 5.4).



Fig 5.4a-e. % EPT is plotted against the percent of the reach substrate characterized by boulders, cobbles, gravel, sand, and woody debris. The only significant correlation observed from these five comparisons was between %EPT and sandy substrates (R=0.56; p<0.03).

5.15 Organic Matter and Functional Feeding Groups

Macroinvertebrates taken from the three leaf bags at each protected stream were collected and sorted into one of four categories of functional feeding groups. Every week the percent of the total macroinvertebrates taken from the leaf bags represented by each functional feeding group was recorded (Table 5.4).

Table 5.4a-c. The percent of each functional feeding group represented on the leaf bags throughout the temporal extent of the research. No pattern associated with changing percentages of functional feeding groups was observed.

Time (days)	Shredders	Collectors	Scrapers	Predators
7	0.00%	0.00%	71.00%	29.00%
14	0.00%	17.00%	83.00%	0.00%
21	12.50%	0.00%	63.00%	25.00%
28	0.00%	0.00%	88.50%	12.50%
35	0.00%	0.00%	33.33%	66.67%
42	0.00%	0.00%	92.00%	8.00%
49	0.00%	23.00%	54.00%	23.00%

Table 5.4a Whittier Stream

Table 5.4b Beaver Brook

Time (days)	Shredders	Collectors	Scrapers	Predators
7	0.00%	28.50%	43.00%	28.50%
14	0.00%	7.00%	80.00%	13.00%
21	25.00%	0.00%	25.00%	25.00%
28	0.00%	0.00%	62.50%	37.50%
35	0.00%	0.00%	33.00%	67.00%
42	0.00%	43.00%	43.00%	14.00%
49	0.00%	0.00%	75.00%	25.00%
Time (days)	Shredders	Collectors	Scrapers	Predators
-------------	-----------	------------	----------	-----------
7	0.00%	0.00%	98.00%	2.00%
14	8.00%	0.00%	72.00%	20.00%
21	0.00%	0.00%	77.00%	23.00%
28	0.00%	3.00%	94.00%	3.00%
35	0.00%	0.00%	100.00%	0.00%
42	0.00%	0.00%	95.00%	5.00%
49	0.00%	24.00%	72.00%	4.00%

 Table 5.4c
 Stony Brook

Generally, we found a substantial amount of scrapers over the entire sampling period. This abundance of scrapers indicates microbial colonization of the leaves, which generated a biofilm on the leaf surfaces. However, no pattern of changing functional groups emerged over time. The percent of each group represented was unpredictable.



Figure 5.5. The percentage of each of the four functional feeding groups found in the leaf bags changed unpredictably throughout the 49 days that the leaf bags were left in the steams.

Discussion

5.16 Biotic Indices

The Hilsenhoff Biotic Index is an efficient method of stream evaluation because it only requires family-level identification of macroinvertebrates. Using the Hilsenhoff Biotic Index sacrifices some accuracy in stream health assessment, but to identify to the species level would be very difficult and would only slightly increase the accuracy of the results. The Hilsenhoff Biotic Index is a useful measure of stream quality because it not only incorporates the abundance and ability to tolerate poor water quality of each family represented, but it also does not require this extensive identification time and effort (Hilsenhoff, 1987). It reduces the error associated with incorrect identifications because it is much simpler to identify to the family level than the species level. Another metric, family richness, is not very descriptive because unlike the Hilsenhoff Biotic Index, it does not incorporate the relative abundance of each sampled family or each family's ability to tolerate poor water quality. However, richness has been demonstrated to decrease as less-tolerant taxa are lost from ecosystems as a result of degradation (Verberk *et al.*, 2006). Family richness, while not the most accurate index for evaluating a stream, is still useful because it negatively correlates with stream degradation.

The results of the H.B.I analysis indicate that Stony Brook and Beaver Brook are both more pristine than Whittier Stream. Stony Brook and Beaver Brook both received water quality scores of "Excellent," the best possible category outlined by the Hilsenhoff Biotic Index (Hilsenhoff, 1987). However, Whittier Stream received a slightly poorer score of "Very Good." It is important to note that all three streams are in relatively good health. Of the seven possible categories of water quality associated with the Hilsenhoff Biotic Index, only the two healthiest categories were represented by the Kennebec Highlands streams.

Ephemeroptera, Plecoptera, and Trichoptera are three orders of macroinvertebrates that occur in oxygen-rich waters and are very sensitive to environmental perturbations. For an ecosystem to support these taxa, the system must be in good health. Therefore, these orders act as valuable indicator taxa (Baker & Sharp, 1997; Crisci-Bispo *et al.*, 2007). Assessing streams based on just the abundance of these taxa also serves to reduce the error implicit in macroinvertebrate identifications. Ephemeroptera, Plecoptera, and Trichoptera are all relatively distinctive and easy to identify (Hilsenhoff, 1987). Mistakes in identifying families within these orders are possible, but assessing streams with the % EPT index does not call for this level of identification – it is enough to merely recognize distinctions between orders. The results of the assessment of stream health using the % EPT index mirrors the H.B.I results.

Again, Whittier Stream appears to be the most degraded of the three Kennebec Highlands streams because only 45.3% of all sampled individuals belonged to either the orders Ephemeroptera, Plecoptera, or Trichoptera, whereas at Beaver Brook and Stony Brook the % EPT was equal to 71.1% and 73.7%, respectively. These results would confirm the result of the H.B.I analysis that Stony Brook is the healthiest of the three streams, but the disparity in % EPT between Stony Brook and Beaver Brook was not statistically significant (p=0.71). However, Whittier Stream differed from both Stony Brook and Beaver Brook in terms of % EPT.

Whittier Stream is ranked third in terms of stream health among the three protected Kennebec Highlands streams. The highest H.B.I value was calculated from samples taken from Whittier Stream, indicating a community populated considerably by the more tolerant taxa. Additionally, the smallest percentage of the macroinvertebrate community composed of either Ephemeroptera, Plecoptera, or Trichoptera was recorded. The Whittier Stream reach is unique from the other two study sites in the Kennebec Highlands because it contains a culvert within our study reach, which allows for the stream to flow underneath a road. This channel is very shallow and is characterized by a homogenous, flat substrate. Each benthic macroinvertebrate taxon is adapted to live on a specific substrate, so a homogenous substrate limits the number of taxa that can persist. The fewer substrate types represented in an ecosystem, the fewer macroinvertebrate taxa the ecosystem can support (Cummins & Klug, 1979). The culvert's homogeneity is likely partially responsible for Whittier Stream's tolerant macroinvertebrate community. Macroinvertebrate collections taken directly downstream of the culvert had very low %EPT Also, the flat substrate of the culvert also does not allow for atmospheric oxygen to mix with the water, so the dissolved oxygen levels at this point may not be sufficient to support intolerant taxa (Genkai-Kato et al., 2005). The culvert may interfere with macroinvertebrate drift (Hay et al., 2008). It is also possible that the road crossing over Whittier Stream is a source of fine sediment and road salt inputs, which would affect macroinvertebrate communities. The road crossing disrupts riparian vegetation, decreasing organic matter inputs from the tree canopy.

5.17 Protected Versus Disturbed Streams Comparison

All three Kennebec Highlands streams are in relatively good health. This is likely a result of their location in a protected area that minimizes negative anthropogenic impacts. The effects of non-point source pollution due to water runoff over impermeable surfaces are, for the most part, mitigated. The forested riparian zone benefits these three streams by providing organic matter inputs and a buffered

surface for runoff water (Roy *et al.*, 2003). Other than the culvert at Whittier Stream, the Kennebec Highlands streams were relatively unaffected by urbanization. The quality of the disturbed streams, however, varied quite a bit. The highest %EPT was measured at Robbins Mills Stream. At 88.5%, this %EPT was higher than the measurements taken from the protected streams, whereas the %EPT value at the Serpentine was 0%, the lowest measurement taken during this sampling. The healthiest of the protected steams as determined by %EPT alone is Stony Brook, but this %EPT value only differed significantly from two of the disturbed streams. This indicates that the disturbed streams in the Belgrade Lakes watershed are not much more degraded than the protected streams in the Kennebec Highlands. The lack of a disparity in water quality between protected streams and disturbed streams suggests that it is possible to preserve stream ecosystems despite the land use of the surrounding areas.

This range in %EPT values across the disturbed streams could be attributed to the fact that sample size was much smaller at each of these streams than at the protected streams. Samples were taken from just one point in the disturbed streams. This small sample size makes any results drawn from the data less reliable. Furthermore, these streams differed a great deal in width and substrate characterization because some were of higher orders than others. The assemblages of macroinvertebrate communities change longitudinally downstream, so streams of different orders would have different macroinvertebrate community structures (Vannote *et al.*, 1980). We compared disturbed streams by noting disparities in % EPT, but these taxa are more associated with low-order headwaters so they would be less abundant in higher-order streams even under healthy conditions (Cortes *et al.*, 2002). The streams in the Kennebec Highlands are protected from negative anthropogenic impacts, but each of the disturbed streams are affected by the decisions of private land owners who typically own only a small reach of the stream. One of our three study streams, Stony Brook, was also outside of the Kennebec Highlands, but the landowner provided excellent buffer and preserved the water quality. There is therefore more room for variation in the %EPT at the disturbed streams, because the health of these streams is determined by the commitment of each individual land owner to stream protection.

5.18 Canopy Cover

Our research suggests that, with increasing canopy cover, more macroinvertebrates from the orders Ephemeroptera, Plecoptera, and Trichoptera were present. The stream with the densest canopy cover, Stony Brook, also had the highest value for %EPT. This stream was comparable to Beaver Brook in terms of canopy cover. Additionally, the stream with the lowest percent canopy cover, Whittier Stream, was observed to have the lowest %EPT as well. This is a logical result, because the EPT orders

of macroinvertebrates are largely shredders and scrapers, which require high amounts of riparian vegetation inputs (Merritt & Cummins, 1996). The mean canopy cover in Whittier Stream was lower than the other two streams because there was a large gap in the canopy in the middle of the reach. This was the only stream out of the three relatively undisturbed stream sites that included a road within the sampling reach. Stream water passes through a culvert underneath this overpass, and transects at 40 and 60 meters had very low canopy cover. The substrate in these areas was covered in slick algae. This is not a surprising result because algae require sunlight from an open canopy to grow (Sturt *et al.*, 2011). The other two streams had consistently high canopy cover throughout the length of the stream.

A higher %EPT was observed in samples where macroinvertebrates were taken from rock surfaces during the hand collection. This means that kick net samples had lower values for %EPT. This difference was consistent with all three undisturbed stream sites. Because most EPT macroinvertebrates live somewhere on or around rocks, it makes sense that more of them were collected in the hand sample. Very few EPT families found in streams actually live beneath the fine sediment (Merritt & Cummins, 1996).

Canopy cover measurements were not taken quantitatively at the seven disturbed stream sites, but the percent canopy cover was clearly either zero or very close to zero. Messalonskee Stream, the Serpentine, and the dam between Great Pond and Long Pond were all medium-order streams and therefore would not be as influenced by the riparian vegetation even if there had been a high percent canopy cover (Vannote *et al.*, 1980). The other four streams were smaller, but they were located adjacent to major roads, within the yards of homeowners, or in disturbed forest sites with very few trees reaching the stream banks. Because %EPT values for disturbed streams were not significantly different from the %EPT of the undisturbed streams, it is possible that differences in private land management strategies can affect the health of a stream.

5.19 Substrate Type

Trends were found between %EPT and sediment grain size, although no significant differences were found. There was a slight correlation between %EPT and percent boulder, as well as between %EPT and cobble. The relationship between %EPT and cobble was stronger, which might be because cobble offers more surface area for clinger habitat than boulders do. There tend to be more crevices between cobbles than there are between boulders. There was also a slight positive trend between %EPT and coarse woody debris. This debris provides another type of habitat that would probably suit a variety

of macroinvertebrates with different modes of existence. There were slightly negative relationships between %EPT and gravel in addition to %EPT and sand. Smaller grain size provides habitat for different types of macroinvertebrates that are not part of the EPT group, such as most stream burrowers who require lower DO concentrations (Merritt & Cummins, 1996).

A possible reason that there were not very strong correlations between %EPT and substrate is that %EPT might not have been the correct index to use in this situation. It is possible that there might have been a correlation between substrate type and other orders of macroinvertebrates apart from the Ephemeroptera, Plecoptera, and Trichoptera. This index was chosen because the EPT orders are known to be composed of mostly clingers and scrapers. Another useful metric to look at in coordination with the sediment grain size would be the flow velocity of the stream. This might be more relevant than sediment type to the %EPT. While many EPT families specialize in clinging to submerged rocks, the flow of the stream may be more representative of the habitat than the rocky substrate itself. Another reason for the lack of correlation may be that our sampling method did not adequately account for all functional groups present at each transect.

Aspects of stream habitat characterize the ecosystem by creating niches for different taxonomic groups of macroinvertebrates. A high variety of niches will lead to a more diverse macroinvertebrate community composition, because it will provide opportunity for many different functional roles (Cummins & Klug, 1979; Vannote *et al.*, 1980; Friberg, 1997) (Fig. 5.6).



Figure 5.6. Conceptual diagram of different habitat types within a stream ecosystem. Macroinvertebrates require specific habitat types. Each zone depicted above provides a unique niche for a certain specialized group of macroinvertebrates. A stream that contains this diversity of habitat will have a more diverse community of macroinvertebrates.

5.20 Organic Matter and Functional Feeding Groups

The most common functional feeding group of all three streams was the scraper group, followed by predators. Beaver Brook had a relatively high number of collectors in addition to the scrapers that were present. Because we observed the decomposition of leaves over a forty-nine day long period, we expected that the macroinvertebrates present would be mostly shredders. However, because macroinvertebrates cannot actually be defined by just one category each, it is possible that the functional feeding group assigned to each of the identified taxa in the literature did not actually explain their ecosystem function in these three headwater streams. One reason why there might have been a high ratio of scrapers is because they feed on the biofilms growing on the surface of a substrate (Merritt & Cummins, 1996; Sturt et al., 2011). Setting up a leaf pack created a large surface area of new substrate into the ecosystem. It was likely colonized by bacteria and algal material, which was then consumed by the macroinvertebrate scrapers. There were probably a high number of predators because, just as the scrapers were attracted to the leaf packs, the predators were attracted to the high concentration of macroinvertebrates feeding off of the leaves. It would be interesting to measure the ratio of functional feeding groups within the stream over time without the bias of a leaf pack. In a headwater stream, we would expect that there would be a relatively high number of shredders, which was not observed here (Vannote et al., 1980). Because fieldwork took place during the autumn season, it is possible that

shredders were spread out over the many leaf inputs and therefore not as attracted to the artificially planted leaf packs. There was no change in presence of functional feeding groups from one week to the next within the leaf bags, except for that the percentage of macroinvertebrates classified as collectors rose from day 1 to day 49. This suggests that the availability of FPOM increased through the sampling period. It is difficult to draw conclusions from the organic matter data, because there was not a large enough sample size and because processing of the leaf packs was primarily for use in organic matter studies with a secondary emphasis on macroinvertebrate collection.

Macroinvertebrates provide essential ecosystem functions. They process organic matter and provide important trophic links. Therefore, it is crucial that macroinvertebrate habitat is protected. We have observed that the streams of the Kennebec Highlands are all in good health. Impacted streams in the area have very different levels of water quality, which suggests that individual management strategies are important. Protected, forested riparian canopies and diverse substrate types provides food habitat for a wide range of invertebrates. There is a potential concern over the negative anthropogenic impacts of road crossings because even though the stream may be protected and visually appear healthy, we noticed surprising differences in the macroinvertebrate communities between Whittier Stream and the other two protected streams.

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CHAPTER 6 SEDIMENT AND LANDSCAPE INTERACTIONS

Introduction

6.1 Fluvial Geomorphology and Stream Hydraulics

Headwater streams are the bodies of water that connect terrestrial and riverine ecosystems. Headwater streams are fluvially classified as having a stream order less than three and having riparian widths no larger than 10m (Benda *et al.*, 2005; King *et al.*, 2009). Benda *et al.* (2005) defines a headwater catchment as the area of a stream where its flow is controlled by runoff of the surrounding land. Precipitation provides a stream system with initial potential energy (Beaumont, 1975) that then turns into kinetic energy, which is ultimately used to transport sediment, scour the stream bed, and erode and undercut the stream banks (Beschta & Platts, 1989). Headwater stream systems have many rough boundaries, such as bedrock outcrops, heterogeneous substrate, and channel banks that dissipate stream energy.

6.2 Substrate and Stream Morphologic Features

The substrata of headwater stream channels consist of sands, gravels, cobbles, boulders, and rock outcrops (Gordon *et al.*, 1992). Sediment varies in size from sand particles less than 0.02cm across to boulders larger than 25.7cm wide (Table 6.1; Harrelson *et al.*, 1994). Fine sediment from the erosion of stream banks accumulates and fills the gaps between gravels and cobbles on the bottom of the stream channel. This concept is called sediment sorting

Table 6.1.	The four	categories of	of grain	size pr	esent in	headwater	streams	(Harrelson	et al.,	1994)
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Sediment Type	Grain Size (cm)
Sand	< 0.02
Gravel	> 0.02 and < 6.4
Cobble	> 6.5 and < 25.6
Boulder	> 25.7

Channel morphologies, such as pools, riffles, rapids, and chutes, shape and dictate the flow of a headwater stream. Pools are the deepest areas of a stream's channel; they are often formed from obstacles such as boulders or large woody debris, and they hold a large volume of the stream's water.

Riffles are shallow storage locations for sediment and bed material (Beschta & Platts, 1989; Hassan *et al.*, 2005): they are usually located between pools and contain fast moving water (Hauer & Lamberti, 2007). Rapids form over clusters of gravel or stone with a moderate gradient (Hassan *et al.*, 2005). A forced morphology is an obstruction that alters the stream's flow, displaces and stores sediment, and dissipates energy (Hassan *et al.*, 2005). Forced morphologies include boulder cascades, steps, and chutes. Together these characteristics determine the morphological structure of a headwater stream.

6.3 Suspended Matter and Discharge

The suspended load is the material that stays up in the central flow of a stream (Morisawa, 1968a) and is kept in the water column by the turbulent currents of the stream (Morisawa, 1968b & Hassan et al., 2005). The suspended load remains separate from the channel substrate until the stream loses enough energy that the material drops out of suspension. Transient storage describes the retention of solutes and other biological matter in a zone of standing or slow moving water that eventually moves back into the channel (Poole, 2010). Transient storage, or a pocket of slow moving water, is common in headwater streams. Subsurface hydrology, the flow and movement of water beneath the earth's surface, and the exchanges of water and nutrients through the hyporheic zone have an influential impact on ecological processes in a stream (Valett *et al.*, 1993). The hyporheic zone is the subsurface region of sediment where water within the stream exchanges and mixes with surface water and groundwater (Valett et al., 1993). It can be considered the "middle zone" that divides stream water and groundwater (Fig. 6.1). Many ecologists have found that the interactions between subsurface and surface water impact the chemistry and biology of streams (Jones Jr. & Holmes, 1996). The concentrations of dissolved oxygen, nitrate, ammonium, and phosphorous are linked to hyporheic water exchanges (White, 1993). Metabolism, nutrient cycling, and organic carbon cycling are also connected and influenced by the hyporheic zone (Hendricks, 1993).



Fig. 6.1. An image of the layers of a stream channel displaying the main water channel, the hyporheic zone, ground water and the impermeable stratum (White,1993).

Discharge is the volume of water over time moving through a cross section of a stream. It is measured in volume over time and fluctuates in a stream annually (Hauer & Lamberti, 2007). Tracers such as Rhodamine-WT (RWT), a fluorescent dye, are used in discharge calculation tests. The tracer can be continuously injected into a stream at a constant rate using a pump or added to the stream in one large dose, called a slug. Rhodamine is a useful tracer because it can be more accurate at detecting stream discharge than other halide tracers such as sodium chloride (NaCl), or common table salt (Bottacin-Busolin, 2009).

6.4 Channel Width and Depth

Stream channels are not uniform in shape, due to different flow conditions and variations in stream bed geology (Beschta & Platts, 1989). They widen due to an increased flux of water, such as during a flood, and narrow when sediment accumulates (Mosley, 1981; Friedman & Lee, 2002). The types of sediment and fluvial features that make up the streambed dictate width because widening will occur if the bank is an easily erodible material, and not if it is a more stable material such as boulders. The amount of vegetation on the banks of the stream also influences channel width. In forested streams the root systems of the abundant trees, moss, ferns, and other plants create a stable landscape that is not easily altered by stream flow (Mosley, 1981).

6.5 Land-Use and Erosion

Because headwater streams are closely linked with erosional and drainage processes due to their close proximity to the terrestrial environment, they are more susceptible to the effects of land-use changes such as roads, agricultural fields, increased urbanization, and forestry. Packed and impervious roads lead to increased runoff rates and contribute fine sediment to streams, the most detrimental input for water quality and the health of aquatic organisms (Reid & Dunne, 1984; Forman & Alexander, 1998). Fine sediments cause water quality issues, and coarse sediments build up in stream channels, reducing flow and leading to flooding and bank erosion. Furthermore, all sizes of sediment can carry toxic chemicals from anthropogenic sources into stream ecosystems (Nelson & Booth, 2002). Logging is one of the most destructive land-use practices impacting the sedimentation of streams. Mallik *et al.* (2011) found that recovery from the impacts of forestry on headwater streams could take 16-18 years, and some effects can still be seen up to 23 years later. There are multiple effects from forestry that affect the sedimentation of headwater streams, including the compaction of soil through the movement of

equipment, increased rainfall on soil surface due to the removal of the canopy, higher peak flows from decreased stability of stream banks, and a decrease in evapotranspiration of soil after removal of trees.

In watersheds that are already sensitive to erosion, such as watersheds with steep slopes, decreased vegetation, and thin soil cover, only a few millimeters of rain can lead to flooding events, such as landslides. Landslides are natural occurrences usually triggered by rainfall, but land-use changes can also be an underlying cause (Lyons & Beschta, 1983). Landslides cause a high sedimentation input directly into streams that is devastating for stream health. The large amounts of sediment and debris transported into the stream can quickly change stream channels and conditions. Landslides are somewhat rare events, but the scars left on the landscape by landslides can provide a channel for sediment transport for 1-2 years afterwards (Glade, 2003).

Many types of land-use occur around headwater streams, and while each type has slightly different impacts, many impacts are negative to the overall stream health.

6.6 Ecological Impacts of Erosion

Sedimentation has varied and often compounding impacts on water quality and the health of aquatic communities, affecting all stream biota from microbes to fish, as well as processes of primary/secondary production and nutrient cycling (Henley *et al.*, 2000; Relyea *et al.*, 2000).

Sediment inputs cause physical and chemical changes to the stream. Physical changes to a stream can include temperature increases due to less canopy cover and warmer runoff (Sponseller *et al.*, 2001; Moore *et al.*, 2005) and a decrease of the mean particle size in the streambed (Sponseller *et al.*, 2001). Chemical changes depend largely on what types of land-use are present in the landscape. Land-use changes and the consequent erosion can increase the stream's concentrations of inorganic nitrogen (Sponseller *et al.*, 2001) and soluble reactive phosphorus (Walling *et al.*, 2008). These nutrients can come from exposed soil in the catchment or from nutrient additions to the landscape, such as fertilizers. Sediment influx from a limestone-dominated watershed can increase the pH of the stream (Ryan, 1991), whereas erosion from deforested streams can decrease the pH of the stream (Likens *et al.*, 1970). Inputs of sediment with a high percentage of organic matter will increase microbial decomposition, often causing oxygen depletion (Bilotta, 2008).

Sedimentation of the streambed affects all levels of aquatic life: primary producers, macroinvertebrates, and fish. Sediment inputs can physically harm primary producers, impact their ability to photosynthesize, and destabilize or scour the substrate they attach to. Sediment inputs also increase turbidity, which results in a reduction of light attenuation. With less light reaching the bottom of the stream, it is harder for periphyton and rooted macrophytes to photosynthesize, which decreases the primary productivity of the entire ecosystem (Wood & Armitage, 1997; Bilotta, 2008). Macroinvertebrates are affected by increased flow and sediment that cause increased drift rates, without complementary upstream migration to make up for this loss (Bilotta, 2008). Fine sediment can clog the interstitial spaces between larger grain sizes—a key habitat for macroinvertebrates. Furthermore, the water running though this gravel is necessary for the exchange of oxygen and metabolic wastes between the benthos and the stream water (Ryan, 1991). Because many fish require gravel beds for spawning and incubating their eggs, many fish are negatively affected by sedimentation (Sutherland *et al.*, 2002). Sediment can also clog gills and rub off the mucus layer, making fish more susceptible to disease (Bilotta, 2008).

Since headwater streams are the beginning of the water system, they have no upstream water sources, and all impacts come from the surrounding landscape. Land-use changes can increase the amount of sediment flux into the stream. Inputs of water and sediment from the landscape affect the sediment structure, channel size, nutrient cycling, and aquatic organism diversity. The proper management of land-use in headwater streams is critical in order to protect the downstream system as a whole. For this project, we chose to investigate how the structure and function of the studied streams affect the health and stability of the ecosystem. The structural metrics that we investigated were slope, substrate, depth, and bank stability; functional metrics were discharge and suspension and settling of solids.

Structural Metrics

Functional Metrics



Fig. 6.2. A conceptual diagram of the concepts and methods investigated by the Sediment and Landscape Interactions Team.

Methods

6.7 Qualitative Flow Sketches

At several points in each stream, we sketched to-scale observations of the thalweg, minor flows, eddies, pools, substrates, and shoreline on graph paper, creating a visual representation of those flow dynamics.

6.8 Slope Estimation

Using handmade metric stadia rods with each decimeter marked off and a scope with a level in it, we measured the elevation gain in the landscape of each 10m interval of stream reach. One person stood downstream with one rod and the scope and then looked to see what level they could see on the upstream stadia rod. The vertical difference between the starting point of the person with the scope and the person upstream is the vertical rise over that 10m horizontal interval.



Fig. 6.3. An illustration of the slope estimation method. The vertical difference between upstream stadia rod and downstream stadia rod is the elevation change for each 10m interval.

6.9 Characterization of Channel Bed Sediment and Depth

For each stream reach we characterized the sediment on the bottom of the channel. At every 10m mark, the white lines in Fig. 6.4, we measured the channel width using a meter tape and divided it by ten to get ten equidistant intervals, the red dashed segments in Fig. 6.4, across the channel bed. We used a first touch approach where we put our hand into the stream, touched bottom, and whatever type of sediment we touched first within the transect interval characterized that entire interval. We did this touch test once for each interval across the stream's width giving us ten sediment characteristics at each meter mark of the stream's reach. Along with the sediment characterization within each interval, the red dashed lines, we recorded the depth of the stream using a meter stick. We took these measurements with the end goal of creating substrata maps.



Fig. 6.4. Schematic showing the channel bed sediment characteristics and depth transects. The schematic only shows the method for 30m of a 100m reach.

6.10 Erosion Quantification

While gathering sediment characteristics we also quantified the amount of bank erosion at each stream. For each 10m interval of the 100m reach we evaluated the amount of erosion present on each side of the stream in the stream bank (0-1m from the water's edge) and the buffer zone (1-6m). McIntosh & Laffan (2005) state that a six-meter riparian buffer can stop 99.5% of incoming sediment from reaching the stream. The streams of this study were forested headwater streams with small catchment areas and varied soils and slopes, much like the streams of our study. We ranked each area on a 1-3 scale where a 1 indicates there was no erosion present, a 2 indicates a potential for erosion, and a 3 indicates existing erosion. We also recorded causes of erosion such as undercut banks, steep slopes, or human impacts.

6.11 Total Suspended Solids Evaluation

At each stream, we collected three replicates of 1L water samples from the top of the 100m reach and the bottom of the reach, selecting a sampling area where the flow is concentrated in one area. Back at the lab, we filtered these samples onto pre-ashed, pre-weighed glass fiber filters. We dried and weighed these filters to calculate the total amount of sediment from each liter of stream water. To calculate the total organic matter in the sediment, we put them in the ashing oven at 500 degrees Celsius for three hours, burning all organic matter, and then re-weighed each sample. The difference in weights is the amount of organic matter that was in the sediment sample. We compared this mass of suspended solids from top and bottom of the reach to see if the sediment settles, thus decreasing the amount of suspended solids, or if the water picks up more sediment as if flows down the 100m reach. This approach is a simplification because settling may vary in different parts of the stream due to hydrologic differences, however, it gives us a basic comparative metric of sediment movement in our stream reaches. To estimate of sediment reaching the lake, we had the Lake Context Team take three 1L samples from the mouth of each stream that were put through the same analysis. We would have liked to use this suspended solids data from three sites per stream to calculate the amount of sediment exported from the reach, and from the stream into the lake, but the variability of the data made this calculation impossible. In small headwater streams the suspended solids load can vary hourly, making it difficult to get an accurate estimation (Meybec *et al.*, 2003). Additionally, the Conservation Lessons Team visited several impacted streams in the area and took samples, which we put through the same drying and ashing process to compare total suspended solids data from impacted and protected streams.

6.12 Measuring Discharge Using a Rhodamine Release

We used a non-toxic fluorescent tracer, Rhodamine-WT (RWT), to calculate the discharge of the streams. In our experiment we used a 20% concentration of RWT. To determine the amount of Rhodamine to use at each stream we used the discharge readings from a Marsh McBirney flow meter and a cross-sectional calculation of partial discharges used by the Nutrient Spiraling Team. In general we used more Rhodamine in the release where the discharge calculated by the Marsh McBirney flow meter was the highest. Before we launched the Hach Sonde instrument into each stream we preprogrammed it to collect readings of Rhodamine in micrograms per liter every 10 minutes for the entire duration it was deployed.

At each stream we put the pre-programmed Sonde 60m downstream from the top of the reach (Fig. 6). At the top of each stream's reach we added the predetermined amount of 20% Rhodamine, calculated specific to that stream, to a 15L carboy full of the test stream's water. The Rhodamine was pumped into the thalweg of the stream at a constant rate of 200 ml min⁻¹. This method is summarized in Fig. 6.5. In prior experiments we used a conventional tracer, sodium chloride (NaCl) and monitored the conductivity levels using a conductivity meter to estimate when the Rhodamine would pass through the stream. From the conventional tracer test we decided that after 45 minutes we would turn the pump off and wait another 15 minutes to allow the Rhodamine to flow through the stream system. We attempted five Rhodamine releases in total among the three streams: Whittier Stream, Beaver Brook, and Stony Brook, however, we were only successful at Beaver Brook. We only had one successful release due to technical difficulties with the pump the other four times we tried a release.

We plotted the concentration of Rhodamine over time, as recorded by the sonde, to create a concentration versus time graph for the data we collected from the Rhodamine release at Beaver Brook. To calculate the stream's discharge we used the average plateau concentration of Rhodamine as our final changed concentration value, C₁, and plugged it into a constant injection discharge equation (Appendix) (Gordon *et al.*, 1991).



Fig. 6.5. Schematic of constant injection Rhodamine release. The release was performed from the top of the reach and ended 60m downstream where a Hach Sonde instrument was deployed. The Sonde recorded Rhodamine concentrations over time.

6.13 Corn Pollen Release

Corn pollen grains were used as a proxy for natural sediment particles to determine the flow characteristics of Stony Brook. We chose to do a corn pollen release at only one stream due to time constraints on sample analysis, and because these three streams were all similar preserved, forested headwater streams. Stony Brook was chosen due to its bank accessibility and smaller stream width, which we thought would lead to a more successful use of the corn pollen technique. We modified methods developed by Dr. Emma Rosi-Marshall of the Cary Institute of Ecosystem Studies to take advantage of flow cytometry techniques to enumerate corn pollen grains. To begin, we performed a conductivity test on a 100m reach of the stream to determine the travel time for a parcel of water in the stream. This test was done with a sodium chloride release because salt and corn pollen move at similar rates in stream water. We used a conductivity meter to determine the conductivity peaks at the 30m, 60m, and 90m markers of the reach. Samples for the corn pollen release needed to be taken one minute

before the peak at 30m and two minutes before the peak at 60m and 90m to sample the corn pollen slug as it moved through the study reach. Once we finished the conductivity test, we filled a 4L bucket with stream water. We added 0.5g of corn pollen (Fisher Scientific) to the bucket. Teams of two people (one person to collect water samples and one person to record exact sample times) were stationed at 30m, 60m, and 90m downstream of the pollen release site (Fig. 6.6) Everyone synchronized times and the corn pollen release began at the ten-minute mark to give everyone time to get to their sample locations. We released the corn pollen rapidly into the stream while stirring the solution with a stick to keep the pollen suspended. The team at 30m took samples every 58.5s, the team at 60m took samples every 57.0s, and the team at 90m took samples every 55.0s. These samples began at the times determined by the conductivity test and were taken until all forty 250ml bottles were filled at each station. The intervals were determined by subtracting the start time from the time it took for the chloride slug to pass through the whole stream, divided by 40 samples.



Fig. 6.6. An illustration of the corn pollen release method, where the pollen is introduced at the top of the reach and then water is sampled at three sites downstream. The number of grains decreases because the pollen settles behind rocks, logs, and other in-stream features due to transient storage.

These corn pollen samples were analyzed in the laboratory with flow cytometry. We poured each bottle over 53µm mesh stretched over an embroidery hoop, which we then rinsed off with distilled water into a 400ml beaker. We measured the contents of the beaker with a graduated cylinder and noted the volume. Then the beaker was rinsed and the volume of water used was noted as well. We analyzed the combined sample and rinse water volume using a FlowCAM (Fluid Imaging Technologies, Inc.), using

flow cytometry, microscopy, and fluorescence detection. The FlowCAM automatically counts, images, and analyzes particles in a sample or a continuous flow. After each sample was run through the machine, the number of corn pollen grains per bottle was recorded and scaled up to the number of corn pollen grains per liter of stream water.

After the sample analysis, we estimated the uptake length of fine particulate organic matter in the stream, as well as its rate of deposition using corn pollen as a proxy. To begin, we found the number of corn pollen particles at each station by determining the area under the curve of corn pollen counts at each station (Georgian and Miller, 1992). We multiplied these numbers by discharge to get the total number of corn pollen passing through each station. We then plotted the natural log of the number of corn pollen grains passing over each station against meters down the reach to get the transport distance, which is the inverse of the slope of the line. From the transport distance we calculated the velocity of deposition.

These varied methods complement each other and highlight stream and landscape structure, as well as inputs and flows between the stream and landscape. This technique allows for a complete picture of how the stream interacts with the surrounding landscape.

Results

6.14 General Patterns of Stream Flow

Various geomorphic features affected the direction of flow by creating riffles, pools and runs in the stream. As can be seen Fig. 6.7 below, the three streams of this study differed in how water flowed through the streambed. Whittier Stream had a fairly regular pattern of pools and riffles bordered by large boulders on each side. This structure kept the stream narrow and prevented bank erosion from the flow of water in the stream (Fig. 6.7a). Beaver Brook was dominated by large boulders that caused the stream to flow around and between them, creating patterns of diffuse and concentrated flow as well as deep pools. The stream had a very large wetted perimeter and side channels at various points, making it hard to define exactly what counted as the stream and what does not (Fig. 6.7b). The streambed of Stony Brook was mostly smaller rocks and gravel beds that created small riffles and shallow pools. The stream banks were predominantly organic matter that led to undercut banks (Fig. 6.7c).







Fig. 6.7a-c. An aerial view of geomorphology and stream flow in one 10 m reach of: a)Whittier Stream. The channel was narrow and stabilized by large boulders, and the water flowed through a series of pools and riffles. b) Beaver Brook. Large boulders in the middle of the stream diverted water to both sides creating a wide wetted perimeter. c) Stony Brook. Mid-stream substrate was mostly smaller rocks, and stream banks were organic matter and soil.

6.15 Slope Estimates

The three streams of this study have drastically different slopes, falling 4.08m, 6.8m, and 1.5m for Whittier Stream, Beaver Brook, and Stony Brook, respectively, from the top of the 100m reach to the bottom (Fig. 6.8). Despite their similar discharge, this difference in slope will create different flow patterns and sediment distributions for each stream. Additionally, the differences in slope across the three streams reflect their proximity to Long Pond, the lowest point in the catchment. Our reach of Stony Brook was close to the outflow to Long Pond, whereas Whittier Stream and Beaver Brook were in the upland areas of the catchment (Fig. 1.4).



Fig. 6.8. The slope profile for the three streams. Whittier Stream's slope was 0.408, Beaver Brook's slope was 0.0680, and Stony Brook's slope was 0.0150.

6.16 Channel Bed Characteristics

For the overall composition of the streambed, Whittier Stream is about 15-20% each for sand, gravel, and cobbles, and 40% boulders, with the remaining 9% as the concrete culvert. Beaver Brook is 83% boulders, under 10% each of gravel and cobble, and no sand whatsoever. Stony Brook is almost even thirds of gravel, cobble and boulder, with about 8% sand. We compared these values from the three streams to see if the percent cover of each substrate differed (one-way ANOVA; Table 6.2). Whittier Stream and Beaver Brook differed in the percent cover of sand and boulders, but were not significantly different in cobbles or gravel. Beaver Brook and Stony Brook differed in percent cover of boulders, cobble and gravel, but not in percent cover of sand. Stony Brook and Whittier Stream did not differ significantly in any of the substrate classes (Table 6.2).

Table 6.2. Results from a one-way ANOVA comparing all three streams for each substrate class. Bold text indicates a significant p-value, meaning there is a difference between those two streams for that substrate.

Whittier Stream and	Beaver Brook and	Stony Brook and	
Beaver Brook	Stony Brook	Whittier Stream	
Boulders: p=0.000	Boulders: p=0.000	Boulders: p=0.719	
Cobble: p=0.353	Cobble: p=0.001	Cobble: p=0.057	
Gravel: p=0.096	Gravel: p=0.002	Gravel: p=0.391	
Sand: p=0.012	Sand: p=0.326	Sand: p=0.326	

Looking at each stream reach as a whole, the average substrate of Whittier Stream was covered equally by sand, gravel, and cobble. A large percentage of Whittier Stream's substrate was boulders, and a smaller percentage of the total stream reach was concrete due to the culvert that crosses the stream at the 50m mark. Boulders are the dominant channel bed sediment of Beaver Brook. By averaging the total stream substrate at Beaver Brook we learned there were small amounts of cobble and gravel and no sand. There was also a small percentage of organic matter covering the channel bed of Beaver Brook. Gravel and boulders were the co-dominant channel bed sediments at Stony Brook; a moderate percentage of the substrate was gravel and a small percentage was sand lining the channel bottom. (Fig. 6.9).



Fig. 6.9. Percentage of each channel bed sediment type for the entire reach of Whittier Stream, Beaver Brook, and Stony Brook.

Comparing various sections of the stream, boulders were the dominant sediment substrate at the top of each stream's reach. However, sand, gravel, and cobbles were all present at the top of Whittier Stream's reach, while the channel bed at the top of Beaver Brook's reach was 100% boulders. The channel substrate at the top of Stony Brook's reach was boulders, cobbles, and gravel. Halfway down Whittier Stream's reach (60m) the channel bed became predominantly sand with equal amounts of gravel and boulders, and a small percentage of cobbles. Halfway down Beaver Brook's reach the substrate was 90% boulders and 10% cobbles. Halfway down Stony Brook's reach the channel bed sediment was mostly sand and gravel with some boulders and cobbles. At the end of Whittier Stream's reach the substrate was mostly boulders, with equal percentages of gravel and cobbles and a smaller amount of sand. The sediment that made up the channel bed at the end of Stony Brook's reach the channel bed substrate was mostly cobbles and a moderate amount of gravel. (Fig. 6.10).

Looking at the distribution of sediment types across the width of the stream, Whittier Stream has a very heterogeneous distribution of channel bed sediments, without any obvious pattern down the stream. Each section of the stream we characterized had a mixture of sand, gravel, cobble, and boulders in different locations from the bank to the middle of the streambed. A concrete culvert interrupted the middle of the reach at the 50m mark (Fig. 6.11). Beaver Brook's channel bed was predominantly boulders (Fig. 6.9). For the first 30m, the streambed was all boulders with a small percentage of organic matter on stream islands. In the middle of the reach, from 30-80m, there were small areas in each transect with cobbles, gravel, and sand, but boulders remained the dominant substrate. The bottom of the reach returned to being entirely boulders with no other sediment types present (Fig. 6.11). Stony Brook also had a very heterogeneous distribution of channel bed sediments; however, we observed a pattern of sediment distribution between transects rather than the random sediment distribution seen in the other two streams. From 10m to 50m we saw a buildup of sand and gravel on the channel bottom near the left bank of the stream while the channel bed towards the right bank of the stream is cobble and boulders. Halfway down the stream this pattern flips: boulders and cobble cover the channel bottom near the left channel bank while sand and gravel cover the channel bottom near the right channel bank. At the last transect we characterized, the pattern switched again.

Beaver Brook's width was funnel-shaped, decreasing from 25m across at the top of the reach to 6.5m at the bottom of the reach. The width of Whittier Stream also varied by about 2m; the width of the stream created an hourglass shape ranging from 6m at the top of the reach to 5.5m at about the middle of

the reach to 7m at the bottom of the reach. Stony Brook was about 3m for the entire 100m reach expect for the last transect widened to 6m. (Fig. 6.11).



Fig. 6.10. The substrate distribution at the top, middle and bottom of each reach. All three streams were predominately boulders at the tops of their reaches, but then change throughout.







6.17 Erosion Quantification

The average amount of erosion in the stream bank (0-1m from water edge) and the buffer zone (1-6m) were similar to each other in two of the three streams in this study. Whittier Stream and Beaver Brook had similar levels of erosion in both areas, but Stony Brook had more erosion on the stream bank than in the buffer zone (t-test, Whittier Stream: n=40, p=0.71; Beaver Brook: n=40, p=0.32; Stony Brook: n=40, p<0.0001). For Stony Brook, the average erosion for the stream bank differed between the right and left sides (while looking downstream) of the stream. It was almost three times greater than the buffer zone for the left bank. On the right side, the erosion of the stream bank was about one and a half times more pronounced than the erosion in the buffer zone. Overall, Whittier Stream had the highest average level of erosion, followed closely by Stony Brook. Beaver Brook had the lowest average amount of bank erosion, but these values were not significantly different from each other (ANOVA, n=120, p=0.43).

Table 6.3. Average erosion values for each area of stream reach, based on our 1-3 visual classification scale.

Stream	Left Stream Bank 1-6 m	Left Buffer Zone 0-1 m	Right Stream Bank 0-1 m	Right Buffer Zone 1-6 m	Average for whole reach
Whittier Stream	1.5	1.8	2.1	2.6	2
Beaver Brook	1.1	1	1	1	1.03
Stony Brook	1	2.8	2.2	1.4	1.85

The erosion was highest for Whittier Stream due to several sources of erosion. This stream had areas of steep slope, a few places with undercut banks, and one area by the road culvert where the retaining wall was not effective enough at preventing erosion (Fig. 6.12 a). Beaver Brook had only one spot with undercutting and no erosion anywhere else in the stream (data not included). Stony Brook had a secondary channel and a footbridge crossing it, but it was well buffered and not very steep so the only significant erosion was from undercut banks (Fig. 6.12b).


Fig. 6.12a. A visual representation of our erosion scores for each section of Whittier stream. High erosion (red) was present where there were steep slopes or undercut banks. Areas with no erosion are shown in green.



Fig. 6.12b. A visual representation of our erosion scores for each section of Stony Brook. The most common issue was undercut banks. High erosion areas are shown in red, while areas with no erosion are shown in green.

6.18 Total Suspended Solids

As a whole, there was very little mass of suspended solids in the stream samples taken as base flow: most values are 0.001-0.002g sediment L⁻¹, with the highest at 0.009g sediment L⁻¹. Many of the values were negative, showing that the data was within the range of error of the scale. Within each stream, there was variability across the three sites (ANOVA, Whittier Stream, n=9, p=0.1250; Beaver Brook, n=9, p=0.574; Stony Brook, n=9, p=0.0002) though there was no consistent trend. Comparing the streams as a whole showed a difference between Whittier Stream and Stony Brook (ANOVA, p=0.043) but not between Whittier Stream and Beaver Brook or Stony Brook and Beaver Brook (ANOVA, p=0.458, p=0.772, respectively). Additionally, no difference in total grams of suspended solids was shown between the intensive sampling of the three protected streams of this study and spot sampling of the five impacted streams, all at base flow conditions (t-test, n=47, p=0.9890).

6.19 Discharge

The Nutrient Spiraling Team calculated the discharges of the three streams using the partial discharge summation method and found that the discharge rates for all three streams were similar. Beaver Brook had the highest discharge of 156.3 L s⁻¹, while Whittier Stream had the lowest discharge, 108.5 L s⁻¹; Stony Brook's discharge, 115.6 L s⁻¹, fell between the discharge values of the other two study streams (Table 6.4).

As stated in the method for measuring discharge with Rhodmaine, we used the Marsh McBirney flow meter discharge rates for each of the streams to determine how much Rhodamine to use in each stream's release. We had one successful Rhodamine release, which was at Beaver Brook. From our graph of concentration of Rhodamine, in μ g L⁻¹ over time (Fig. 6.13), we determined the final constant concentration of Rhodamine, C₁, and used it in the constant injection discharge equation (see appendix). We calculated the discharge of Beaver Brook to be 93.3 L s⁻¹. Our Rhodamine release discharge value, 156.3 L s⁻¹, was lower than the discharge determined using a Marsh McBirney flow meter. This may have been due to internal exchanges and transient storage zones within the stream that prevented the Rhodamine from reaching the sonde whereas the flow meter collected direct values from the stream's flow. This Rhodamine release allowed us to capture the overall stream discharge because it factors in fluvial processes such as transient storage, while the Marsh McBirney flow meter only measures stream behavior at a few specific locations.

Rhodamine Release- Beaver Brook



Fig. 6.13. A plot of concentration of Rhodamine in μ g L⁻¹ over time from a constant injection discharge procedure performed at Beaver Brook. The plateau of the graph is the final constant concentration of Rhodamine, Ct, the Sonde detected. This was used in a constant injection discharge equation to calculate Beaver Brook's discharge.

Table 6.4. Discharge calculations using Marsh McBirney flow meter and a constant inject Rhodamine release.

	Marsh McBirney (L s ⁻¹)	Rhondamine Release (L s ⁻¹)
Whittier Stream	108.5	N/A
Beaver Brook	156.3	93.3
Stony Brook	115.6	N/A

6.20 Fine Particulate Organic Matter Retention

The corn pollen release resulted in varied amounts of corn pollen at each station throughout the release as seen in Fig. 6.14. There are a few peaks early in the release at the 30m station, a distinct peak at the middle of the release time at the 60m station, and various peaks at the 90m station throughout the release. Based on Dr. Emma Rosi-Marshall's methods and her previous experiment, we assumed that corn pollen would travel as a defined slug down the stream, so the peaks would be distinct as it passed

by each station. There should have been a large peak towards the beginning of the release time at the 30m station, a slightly smaller peak at the 60m station near the middle of the release, and an even smaller peak at the end of the release at the 90m station. The gradual decline of the peaks would be due to the fact that the corn pollen would be more concentrated at the beginning of the release and would then start to settle on the substrate or get caught up in areas of transient storage. However, this was the first time we have attempted a corn pollen release and we only performed one of them in one stream. Repeated attempts at corn pollen releases in Stony Brook and releases in multiple streams may help to improve the process.



Fig. 6.14. The number of corn pollen grains per liter passing over each station over time, as determined by FlowCAM analysis.

We used the natural log of the total number of corn pollen grains per liter passing over the 30m, 60m, and 90m stations during the corn pollen release, as seen in Fig. 6.15, to determine specific flow characteristics of corn pollen, which can be compared to the behavior of fine particle organic matter in headwater streams. The downward slope of the line in the figure shows that the total amount of corn pollen in the stream decreased as the slug of corn pollen traveled down the reach, just as we predicted. The inverse of the slope of this line is the estimated transport distance of corn pollen in Stony Brook,

which was 62.7m. Because we used corn pollen as a proxy for fine particle organic matter, we can conclude that the average distance that fine particle organic matter travels through Stony Brook before depositing onto the substrate is 62.7m. From the transport distance, we found that the rate of this deposition was 0.567 mm/s.



Fig 6.15. A graph of the natural log total number of corn pollen grains per station. The slope of this line is used to calculate transport distance and deposition rate of sediment deposition.

Discussion

6.21 Stream Geomorphology Affects Flow

The structure of a stream—stream banks, channel beds, and flow rates—dictate the various functions of a stream such as the amount of in-stream and stream bank erosion, deposition of sediment, and sediment transport (Bąk *et al.*, 2013). The three headwater streams of this study vary widely in structure, despite their proximity and similar discharges. Whittier Stream has a moderate elevation gradient and an equal cover of the bottom sediment classes. Beaver Brook is steep and full of boulders that make up the channel substrate and divert the flow to a wide channel. Stony Brook is fairly flat, and has a mixture of bottom sediment substrates.

The vegetation on the banks and floodplain of a stream also dictate the overall shape and geomorphic characteristics (Mosley, 1981). The roots of trees and riparian vegetation provide support and stability to the upper layer of soil and sediment of the channel wall, however the bank material below the rooting zone is susceptible to undercutting and erosion (Beschta & Platts, 1989; Hassan *et al.*, 2005). Riparian vegetation on the banks of the stream also causes friction and slows stream water down, which allows sediment to deposit out of suspension. This process can make stream channels narrower (Friedman & Lee, 2002). This was seen in Beaver Brook which had less erosion than the other two study streams because its banks are mainly made up of boulders and supported by the roots of mature trees, and there are no sloping banks to induce erosion. At Stony Brook and Whittier Stream, despite the forested buffer, we noticed several areas of undercut banks. Although there is erosion, our streams are resilient and are able to adjust to sediment inputs and deposit them within the streams channels.

6.22 Sediment Settling Prevents Downstream Transport

In protected streams at base-flow conditions, a large portion of the total suspended sediment settles in the stream, preventing it from impacting downstream ecosystems, as shown by the corn pollen release. A study done by Miller and Georgian (1992) found that transport distances for corn pollen in a headwater stream in New York State ranged from 122m to 190m. These transport distances are roughly double our calculated transport distance of 62.7m. The average settling velocity of corn pollen in their study was 0.293 mm/s, which was a slower rate of deposition than our estimation of 0.567 mm/s. Our shorter transport distance and faster rate of deposition show that the sediment settles quickly, rather than remaining suspended, as demonstrated by the very low values for total suspended solids. In this case, these streams are highly retentive, preventing fine particles from flushing downstream. Like Stony Brook, the stream in Miller and Georgian's study (1992) was cobble-bottomed and bordered by a forested riparian buffer. However, they gave no explanation of what stream features lead to a faster or slower settling rate. Additionally the settling rate varied among the three months of their study, again with no explanation. This shows how understudied fine particulate matter retention is, and highlights the need for future studies of this important stream function.

Paul and Hall (2002), Jin and Ward (2013) found that fine particulate matter retention and deposition can be partially explained by transient storage. Areas of transient storage in our streams, such as studied in Stony Brook with our corn pollen release, allowed for high deposition rates.

6.23 Transient Storage Decreases Discharge and Increases Settling

Transient storage describes the retention of solutes and other biological matter in a zone of standing or slow moving water that eventually moves back into the channel (Poole, 2010). The presence of transient storage zones increases retention of nutrients, organic matter, and sediment and increases the diversity of habitats in a stream. Transient storage areas are created by boulders or debris dams that divert flow from the main channel (Hassan *et al*, 2005).

The discharge of streams is naturally variable and is affected seasonally and daily (Payn et al., 2009). D'Angelo et al. (1993) found that in Appalachian and Cascade Mountain streams, an increase in the stream's discharge and velocity increases transient storage within a stream. Therefore, streams with higher discharges had more water that created deeper pools and more areas of slow moving water that held up water and solutes. In addition, different measuring techniques will also cause variability in discharge values for a stream. Our three streams have similar discharge rates as measured by the Marsh McBirney flow meter, but our Rhodamine release at Beaver Brook had a lower value for the discharge. This disparity can possibly be explained by transient storage within the stream. Rhodamine could have been stored in pools, hyporheic regions, or other transient storage zones of the stream, which would inhibit the Rhodamine from reaching the Hach sonde. By comparing the cross-sectional discharge measurements with the Rhodamine release, we see that Beaver Brook has substantial transient storage zones. This is consistent with our observations and sketches. We would expect Beaver Brook to have the highest transient storage of the three streams because the boulders result in large, deep pools where water slows down, allowing for retention of nutrients and settling of sediment. In addition, the wide stream bed and various channels create more places of transient storage within the stream, rather than having one central avenue of flow like in Whittier Stream or Stony Brook.

6.24 Effects of Storms on Discharge, Erosion, and Sediment Sorting

During a storm, runoff from the landscape loads sediment into the stream that can result significant alterations to the stream structure. Long and heavy rainstorm events provide streams with extra energy that moves sediment within the stream and also erodes channel banks adding more external sediment to the system. This addition and movement of sediment changes the shape and fluvial geomorphology of the stream. Banks shift in location and internal bars, pools, and riffles relocate in the stream's channel as well. Streams often become much wider due to the excess water and movement of sediment. The increased water input in the river cuts and scars the banks of the stream. Once the storm

has stopped, the energy of the stream decreases and the excess material in suspension deposits out of the main water column and onto the channel bed. After a storm there is a distinct increase in sand particles in the channel: pools become erosional and riffles depositional. Streams try to regain balance after a dramatic amount of material has been input and transported; however, they are fluvial and geomorphologically changed by the event (Bak *et al.*, 2013).

A storm would affect our streams similarly. The added runoff from a storm would increase discharge and sediment inputs from erosion. Stream banks would be affected the most due to undercutting by the increased flow or water. Whittier Stream and Stony Brook would probably become wider because their banks are not stabilized by boulder or tree roots, such as in Beaver Brook. If the storm were severe enough, the in-stream sediment structure could also be moved by the increased water flow; however, this is unlikely because the buffer areas would slow water input before it enters the stream. Despite the buffer, we would still expect a larger amount of suspended solids in all three streams during a storm event due to increased sediment inputs and the mixing and uplifting of sediment within the stream. Our streams would become cloudier and carry more sediment downstream due to the new inputs and excess energy from the storm. However, this would only last for a short period of time during and after the stream since the streams have very quick settling rates.

6.25 Impacts of Human Disturbance on Headwater Streams

The streams in our study had relatively low levels of suspended sediments and were wellbuffered with riparian vegetation. However, if the Belgrade region is developed in the future without regards for stream protection, the health of the streams will decline from their present state. One potential development could be the construction of more roads near or even through our streams. Roads, both paved and unpaved, lead to increased runoff and fine sediment inputs, which negatively affect both the structure and ecology of the stream (Reid & Dunne, 1984; Nelson & Booth, 2002). The road crossing at Whittier Stream led to bank erosion on the side of the road not reinforced by a retaining wall or rip-rap. It also channelized the stream through culvert which caused the water to flow faster and kept sediment suspended the stream water. This sediment was deposited at the base of the culvert on the downstream side, as seen by the high amounts of sand in the transect just below the culvert. If more roads were constructed in the Belgrade region, the streams would become more channelized, have higher instances of bank erosion, and the transport distance of suspended sediment would increase. The current forested buffers are strengthening the banks and moderating the impacts of erosion, but if they are removed due to forestry, agriculture, or development of housing, the input of suspended sediment and organic matter into our streams would increase due to the destabilization of banks. Small headwater streams are especially important to conserve with buffers because their slow flows mean that sediment cannot be transported through them quickly to downstream ecosystems. This means that less sediment will reach these downstream ecosystems. (Kaplan *et al.*, 2008). Increasing sediment inputs from the landscape will increase exportation downstream, highlighting the need for riparian buffers along the whole length of the stream. However, buffers can only protect local reaches because land-use can affect sediment and nutrient characteristics at distances reaching 4000m. Truly effective stream health conservation must come from watershed-wide measures (Houlahan & Findlay, 2004).

Conclusion

The ecological health of headwater streams is based on functions such as nutrient cycling and sediment inputs, suspension, and deposition. These functions are regulated by stream structures, such as channel width and depth, substrate characteristics, and slope of the surrounding landscape. The three streams of this study are relatively healthy as can be seen by moderate bank erosion, minimal suspended solids, and quick sediment settling rate, which prevents sediment export to downstream water bodies. Continued monitoring of stream health and strengthening of riparian buffers will help mitigate the erosion that is occurring and allow for continued protection of stream health.

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CHAPTER 7 LAKE CONTEXT

Introduction

7.1 Headwater Streams and Lake Water Quality

Headwater streams, generally described as first or second order streams, account for most of the stream length in many watersheds and connect the terrestrial realm to downstream waterways. Land use in the catchments of headwater streams tends to be the most important factor in determining their water quality (Dodds & Oakes, 2008; Gomi *et al.*, 2002). Land use surrounding headwater streams also influences the water quality of the higher-order streams into which they flow (Johnson *et al.*, 2010; Alexander *et al.*, 2007, Meyer *et al.*, 2007). Headwater streams are important processors of nutrients, helping to prevent downstream export of nutrients such as nitrogen and phosphorus (Aguilera *et al.*, 2012; Vanni *et al.*, 2011; MacDonald & Coe, 2007; Peterson *et al.*, 2001). When these nutrients, especially phosphorus, enter downstream reservoirs such as lakes, they can cause lake aging, known as eutrophication. Symptoms of lake eutrophication include reduced water quality, algal blooms, and fish kills (Lopez *et al.*, 2008; Anderson *et al.*, 2002; Paerl *et al.*, 2001).

In accordance with the River Continuum Concept, we expect stream physicochemical makeup to evolve from lower order streams to higher order ones, through processes such as export, assimilation, dispersion and adsorption (Vannote *et al.*, 1980). Nutrient export of the key nutrients nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{-3}) is low in pristine, forested systems due to in-stream biological, physical, and chemical processes (Vanni *et al.*, 2011; MacDonald & Coe, 2007). Biological consumption of nutrients is mainly from algae and bacteria that take up, use, and transform nutrients based on various physical factors (Runkel, 2007). For example, in a small headwater stream, most algae and bacteria colonize the benthos, and the high ratio of surface area to volume in these streams facilitates the efficient uptake of these nutrients. Physical processes affecting nutrients include dispersion, advection, and transient storage. Dispersion is a similar concept to dilution, while advection refers to the movement of solutes by the stream water's bulk motion, and transient storage describes the temporary immobilization of solutes by geological structures (Runkel, 2007). Chemical processes, such as adsorption, also influence nutrient concentrations (Runkel, 2007).

Nutrient export is increased in human-altered systems (Freeman *et al.*, 2007; Alexander *et al.*, 2007; Billen *et al.*, 2007). In a river continuum study in Spain, Aguilera *et al.* (2012) found that NO_3^- removal in headwater streams was nearly three times the rate of total annual in-stream removal,

highlighting the importance of headwater streams in limiting nutrient export to downstream systems. Nutrient export is also correlated with fluctuations in stream discharge as a result of storm events, as Royer *et al.* (2006) found that more than half of the total NO_3^- and PO_4^{-3} export from a headwater system occurred during the greatest 10% of discharge values.



From Bottom Sediments: P

Fig. 7.1. A conceptual diagram depicting nutrient sources to a lake, lake processes and how they affect nutrient concentrations. Lake outflows additionally affect lake nutrient concentrations.

Nutrients, including NO₃⁻, NH₄⁺, and PO₄⁻³, can reach a lake via streams, terrestrial runoff, and lake bottom sediments (Gharibreza *et al.*, 2013; Anderson *et al.*, 2002; Kling *et al.*, 2000) (Fig. 7.1). The control of phosphorus export is especially important when the receiving waters are rivers, lakes, or the upper reaches of estuaries, as these areas tend to be phosphorus-limited (Correl, 1998). Whole-lake experiments have shown that increasing phosphorus loads increases phytoplankton activity and leads to algal blooms (Schindler, 2012). Algal blooms occur when there is an increase in the limiting nutrient, often PO₄⁻³ in lakes, which causes algae to grow faster than normal zooplankton grazing can control (Paerl *et al.*, 2001). This results in algal blooms.

Algal blooms can be catastrophic for lakes for several reasons. They can affect macrophyte populations (Lovendahl *et al.*, 2010) and copepod species (Uye *et al.*, 1994; Zhang *et al.*, 2011). When

the algal blooms die and sink to the bottom of the lake, the decaying matter consumes oxygen, creating hypoxic conditions that can lead to fish kills (Lopez *et al.*, 2008; UNEP, 2013). Hypoxic bottom water conditions also affect the bottom water chemistry and can draw even more nutrients into the water. For example, phosphate in the sediments is bound to iron in the presence of oxygen, but in the absence of oxygen this phosphate is released back into the water column. This internal loading of nutrients in turn promotes more intense algal blooms, completing a positive feedback cycle that is difficult to reverse once it has started.

In algal blooms, cyanobacteria, or blue-green algae, often outcompete other phytoplankton and can have an additional suite of effects on a water body. The toxins associated with these bacteria are poisonous to several mammalian species (Blaha *et al.*, 2009), humans and canines included. The proliferation of these bacterial populations can affect water supplies and damage local economies by forcing the closure of recreational lakes (Carvalho *et al.*, 2013). Because headwater stream health affects nutrient export, which can alter downstream ecosystems by promoting eutrophication and its associated affects, such as algal blooms, it is important to evaluate the role of protected headwater streams in maintaining lake water quality on a local scale.

7.2 Local Context

Long Pond, located in Belgrade Lakes, Maine, is a clear, low nutrient (oligotrophic) lake that consists of two connected basins. The population of the Belgrade Lakes region is well informed of the importance of lake conservation, and many efforts are in place to prevent lake eutrophication in Long Pond. A primary example of these efforts is the LakeSmart program, funded by the Maine Department of Environmental Protection (DEP). The program awards signs to shoreline property owners who meet a minimum standard in four target areas: driveway and parking areas; structures and septic systems; lawn, recreation and footpaths; and shorefront and beach areas. The main goal of the LakeSmart program is to promote lake health by stabilizing erosion areas, reducing chemical use, diverting overland flow into vegetated areas, and maintaining buffer areas of natural vegetation along the shoreline. As of 2011, 26 properties on Long Pond received LakeSmart Awards, accounting for 13% of Long Pond's shoreline (Maine Department of Environmental Protection, 2013). Long Pond has a similar proportion of LakeSmart certified properties to nearby Great Pond. This is just under the LakeSmart Gold Status standard, which awards Gold Status to lakes with greater than 15% of the shoreline LakeSmart certified. To date, 10 Maine lakes have achieved Gold Standard status (Maine Department of Environmental

Protection, 2013). Because of the success of the LakeSmart program on Long Pond and other lakes in the area, we expect that nutrient loading from surface runoff will be low, maintaining or improving lake health.

Another conservation initiative in the Belgrade region is the conservation of the Kennebec Highlands by the Belgrade Regional Conservation Alliance (BRCA). The Kennebec Highlands are a large area of relatively undisturbed land in the Long Pond watershed that the BRCA protects while providing public benefits such as hiking trails. The Kennebec Highlands Management Plan, outlined in the introduction, does not specifically focus on how protecting streams in the Kennebec Highlands contributes to maintaining lake health, and our research works to fill that gap.

7.3 Research Questions and Hypotheses

Our primary research question is whether the protection of the headwater streams is effective in mitigating the export of NO_3^- , NH_4 , and PO_4 into Long Pond. We also examined the role of these streams in exporting sediments and chlorophyll to the lake. We hypothesize that protected, forested headwater streams will be effective in preventing export of nutrients, sediment, and chlorophyll to the lake, thus maintaining lake water quality. Therefore, we also ask whether other sources of nutrients to Long Pond may be affecting the lake water quality, and we will use a GIS analysis of land use in the basin to determine what these sources may be.

Methods

7.4 Study Sites

Long Pond, located in Belgrade, ME, is an oligotrophic to mesotrophic lake. The lake has a surface area of 10347812 m², a total perimeter of 49.9 km. The average depth is 10.7 m and the maximum depth is 32.3m (Lakes of Maine, 2013). The lake has mixed shoreline land uses – mainly undeveloped forest and residential – and is divided into two main basins connected by a narrow channel. Our study sites included one deep-water site in each basin and three near-tributary sites. The deep sites located in the North Basin (44°31'48.9" N, 69°53'51.8" W) and South Basin (44°30'01.9" N, 69°54'47.7" W) coincide with historic and ongoing Maine DEP sampling sites, as shown in Fig. 7.2. We also sampled within 100 m of the mouth of each of the three studied tributaries: Whittier Stream (44°33'43.7" N, 69°54'43.7" W), Beaver Brook (44°32'53.1" N, 69°54'23.8" W), and Stony Brook (44°30'30.3" N, 69°55'39.3" W). Whittier Stream and Beaver Brook flow in the North Basin of Long



Fig. 7.2. Map of Long Pond. Names shown only for headwater streams included in the study, and approximate sampling locations are marked with a star (modified from Lakes of Maine, 2013).

Pond, while Stony Brook flows into the South Basin. Whittier Stream flows through Whittier Pond on its way to Long Pond. We sampled during the fall season, when the lake temperature and dissolved oxygen (DO) profiles showed no thermocline and the lake was beginning to mix.

7.5 Field and Laboratory Methods

We determined nutrient limitation status for each of the basins in Long Pond during the summer of 2013 using a nutrient limitation assay (Sterner, 1994). We treated samples taken from the North and South basin sites either as control or with additions of NO_3^- , NH_4^+ , PO_4^{-3} , or $NH_4^+ + PO_4^{-3}$. We collected initial levels of phytoplankton on a 0.7 µm glass fiber filter and kept the samples on a shaker table under fluorescent lights for approximately four days, after which we

collected the phytoplankton from each sample on a 0.7 µm filter. We were able to determine nutrient limitation status based upon which nutrient increased phytoplankton activity the most.

In the fall, subsurface samples (~1 m depth) were taken at each site, with additional bottom water samples taken at the North and South basin sites. We filtered half of the samples through a 0.7 μ m glass fiber filter and stored all of the samples in the freezer until they could be analyzed for nutrient content.

In the lab, we tested each sample for concentrations of phosphate (PO_4^{-3} , $\mu g L^{-1}$), nitrate (NO_3^{-1} , $\mu g L^{-1}$), ammonium (NH_4^+ , $\mu g L^{-1}$), total phosphorus (TP, $\mu g L^{-1}$) and total nitrogen (TN, $\mu g L^{-1}$). To determine the concentrations of TP and TN, we digested our unfiltered samples with a persulfate ($K_2S_2O_8$) reagent in an autoclave (Market Forge Sterilmatic; Gross & Boyd, 1998). The digestion brought phosphorus and nitrogen present in particles suspended in the water column into solution, so they could be measured as PO_4^{-3} and NO_3^{-1} , respectively.

We used a Lachat QuikChem Series 8500 to determine the concentration of all nutrients except TP. We determined the NH_4^+ concentrations using an indophenol blue method (QuikChem Method 10-107-06-1-Q), PO_4^{-3} concentrations using a molybdate reaction (QuikChem Method 10-115-01-1-B), and NO_3^- concentrations (including our digested TN samples) using a sulfanilamide reaction with UV light reduction (QuikChem Method 10-107-04-6-A). We ran our TP samples by hand using a molybdate method similar to the QuikChem method on a spectrophotometer (ID; Wetzel & Likens, 1991).

Following a protocol modified from EPA Method 445.0 (Arar & Collins, 1997), we collected subsurface chlorophyll-a samples at each site as a proxy for phytoplankton activity. First, we collected the phytoplankton from 200 mL of lake water on a 0.7 μ m glass fiber filter. We extracted the chlorophyll *a* pigment using 90% acetone and used a fluorometer (Trilogy, Turner Designs) to determine the concentration of chlorophyll *a* (μ g L⁻¹). We then acidified the samples and reran them to determine the concentration of phaeophytin (μ g L⁻¹), a chlorophyll degradation product, in our samples.

We collected sediment samples from the mouth of each tributary using a sediment corer. We collected the upper 5 to 10 cm of sediment from each core and froze the samples until they were analyzed for organic matter content. 5 mL of thawed, wet sample were weighed, dried, and ashed (ThermoScientific Lindberg Blue M) to determine the percent organic matter content of the sediment.

7.6 GIS Mapwork

A Maine state land use raster's information was confined to a shapefile of Long Pond's catchment area via the extraction by mask tool in ArcGIS's spatial analyst extension (Raster: 2012 ES Science Capstone; Shapefile: Lucy O'Keefe). Additional layers characterizing Maine's watershed basins and streams were individually intersected with the catchment area shapefile. GPS points collected every 10 m along the three studied reaches were also added. Land use was spatially represented and calculated as a percentage of total land use in the entire catchment and within 100 m of all mapped streams. Whittier Stream was not part of the GIS layer, and we did not have sufficient GPS coordinates along the waterway to add it. The resulting map is a spatial representation of the land use in Long Pond's catchment, including the catchment's waterways (with the exception of Whittier Stream) and basins.

7.7 Statistical Analysis

We conducted our statistical analyses using Stata 12.0. To determine the differences between tributaries and lake sites we ran t-tests, and to test for differences among the streams we ran ANOVAs

followed by Bonferroni tests. We used the Shapiro-Wilk Test to test our data for normality, and Levene's Test to test our data for equal variance. Data were log transformed to meet assumptions of normality and equal variance when necessary.

Results

7.8 Nutrient Limitation in Long Pond

The mean concentration of PO_4^{-3} in Long Pond was below detection, and the mean concentration of TP was $4.27 \pm 0.187 \ \mu g \ L^{-1}$. The mean concentration of NH_4^+ was $0.493 \pm 0.294 \ \mu g \ L^{-1}$, the mean concentration of NO_3^- was $62.8 \pm 8.82 \ \mu g \ L^{-1}$, and the mean concentration of TN was $824 \pm 11 \ \mu g \ L^{-1}$. The mean concentration of chlorophyll *a* in Long Pond was $1.66 \pm 0.26 \ \mu g \ L^{-1}$.

Both basins of Long Pond are phosphorus limited, while the North Basin also has a likely secondary limitation by nitrogen (Table 7.1, see Tank & Dodds, 2003 for statistical method). Although the secondary limitation by nitrogen is not statistically significant (p=0.0609), it trends towards secondary limitation, especially given our small sample size.

Nutrient	F-statistic	p-value		
South Basin				
Ν	0.22	0.6454		
Р	14.69	0.0028		
NxP Interaction	0.8	0.3889		
North Basin				
Ν	0.6	0.4561		
Р	6.16	0.0305		
NxP Interaction	4.46	0.0609*		

Table 7.1. Statistical Results of Nutrient Limitation Assay in Long Pond. Significant values in **bold**.

Table 7.2. Summary of ANOVA results for comparisons of nutrients between stream mouths. Significant values in **bold**.

Nutrient	F	df	р
PO4	4.32	2,6	0.0688
NH4	2.33	2,6	0.1785
NO3	3.02	2,6	0.1237
TN	0.14	2,5	0.8701
TP	8.32	2,5	0.0256
Chl-a	8.76	2,15	0.003

7.9 Nutrient Export by Headwater Streams

 NO_3^- concentrations at the stream mouth ranged from approximately 50 to 90 µg L⁻¹, NH_4^+ concentrations ranged from 6 to 15 µg L⁻¹, and TN concentrations ranged from 870 to 890 µg L⁻¹. TP concentrations ranged from 4.1 to 4.5 µg L⁻¹, and PO_4^{-3} was below detection at all sites. There was no

significant difference between NO₃⁻, NH₄⁺, PO₄⁻³, and TN concentrations at the stream mouths (Table 7.2). However, there was a significant difference in both TP and chlorophyll *a* concentrations at the stream mouths. Stony Brook had a significantly higher chlorophyll *a* concentration (5.04 μ g L⁻¹) than Beaver Brook (0.57 μ g L⁻¹; Bonferroni, p=0.003) and Whittier Stream (1.08 μ g L⁻¹; Bonferroni, p=0.035) (Fig. 7.3). Stony Brook also had a significantly higher TP concentration (4.48 μ g L⁻¹) than Whittier Stream (4.18 μ g ^{L-1}; Bonferroni, p=0.032). These results indicate that there may be a connection between TP and chlorophyll *a* concentrations.



Fig. 7.3. Average chlorophyll *a* concentrations by study site.

Because there was no significant difference in nutrient concentrations between the basins (Table 7.3), we concluded that there was sufficient mixing between the two basins and that the basin into which the streams emptied was not important in determining the effect of stream inflow on lake water quality. We found that stream mouths contained a significantly higher concentration of NH_4^+ than the lake centers (t-test, t_{16} = -9.31, p<0.0001) and a significantly lower level of TN (t-test, t_{18} = -2.55, p=0.02).

We examined differences in the

nutrient concentrations in the studied stream reaches, the mouth of each stream, and the central lake basin. The nutrient concentration decreased dramatically from the stream reach to the stream mouth, indicating that nutrient mitigation by these streams is effective (Fig. 7.4). For example, NH_4^+ concentrations decreased from about 300 µg L⁻¹ at the study reach in Beaver Brook to 6.43 µg L⁻¹ at the stream mouth. NH_4^+ concentrations decreased further from 0.586 µg L⁻¹ at the surface of the South Basin and 0.342 µg L⁻¹ at the bottom of South Basin.

Table 7.3. Summary of statistical testsfor connectivity between the North andSouth Basins of Long Pond.

Nutrient	t	df	р
PO4	-1.31	6	0.2351
NH4	0.123	10	0.9055
NO3	0.846	10	0.4176
TN	0.94	10	0.3695

7.10 Sediment

There was no significant organic matter content in our samples, and therefore particulate inputs from the studied headwater streams are predominantly non-organic sediment during base flow conditions. The amount of suspended sediment delivered from each stream was minimal.



Fig. 7.4. Spatial distribution of NH_4 (left) and NO_3 (right) concentrations from stream reach to lake center.

7.11 Historical Data

The Maine DEP collected long-term data in the summer months (May through September) from 1976 to the present. Measurements used were taken at a depth of greater than 6 m, as the most data points were available for these depths. Fig. 7.5 shows the available data, and there are many more data points closer to the present than there in the mid 1970s, as can be seen by the greater variation in the

data. Examination of historical Long Pond data shows a weak upward trend in chlorophyll *a* since 1976 (data courtesy of Whitney King). The positive trend is significant (linear regression, $r^2=0.1367$, p=0.048), and the rate of increase is 0.01743 µg L⁻¹ y⁻¹.





7.12 GIS Maps

Deciduous forests (28.7%) were the most common land cover in the catchment, and mixed forests (28.0%) were most common 100 m away from the mapped streams. Developed areas, agricultural areas, herbaceous zones and shrub zones were the least prominent both in the catchment and 100 m from the mapped streams.

Land Cover	Cover within 100m of mapped streams	Cover within Watershed
Cultivated Crops	0.0045	0.0161
Deciduous Forest	0.3796	0.2871
Developed, Low Intensity	0.0041	0.0084
Developed, Medium Intensity	0.0004	0.0005
Developed, Open Space	0.0221	0.0292
Emergent Herbaceuous Wetlands	0.0539	0.0072
Evergreen Forest	0.0845	0.1060
Hay/Pasture	0.0018	0.0226
Herbaceuous	0.0058	0.0045
Mixed Forest	0.2799	0.3349
Shrub/Scrub	0.0041	0.0100
Woody Wetlands	0.1593	0.0663

Table 7.4. Proportion land cover in the Long Pond watershed and within 100 m of mapped streams.

Discussion

7.13 Nutrient Limitation and Lake Trophic Status

The concept of nutrient limitation has three main components. First, we must understand that primary production in a given ecosystem, such as the lake, is regulated by the availability of one key, limiting factor (Smith, 1998). In Long Pond, the limiting factor in both basins is phosphorus. Secondly, because production is dependent upon this key nutrient, we expect production to increase proportional to increases in the limiting nutrient (Smith, 1998). Therefore, we would expect chlorophyll *a* in Long Pond to increase as phosphorus concentrations increase. Thirdly, because of the tight link between the limiting nutrient and primary production, control of lake eutrophication can be accomplished by controlling sources of the limiting nutrient to the ecosystem (Smith, 1998). In the Belgrade Lakes watershed, several organizations, such as LakeSmart and local lake associations, work toward this goal. Because of the multitude and variety of mitigation efforts, we expect lake eutrophication in Long Pond to be minimal.

We can determine lake trophic status based upon the concentrations of TN, TP, and chlorophyll *a* in each lake basin, following the trophic guidelines outlined in Nürnberg (1996). In both basins, the TN concentration is in the range considered "eutrophic", while the TP and chlorophyll *a* concentrations were in the range considered "oligotrophic" (Nürnberg 1996; Table 7.5). This indicates that the lake

trophic status is oligotrophic, as the lake is phosphorus limited and therefore total phosphorus is the more important indicator of lake water quality (Schindler, 2012).

0				
Frophic Status	TN (mg/L)	TP (µg/L)	chl <i>a</i> (μg/L)	
Oligotrophic	< 0.350	< 10.0	< 3.5	
Mesotrophic	0.350-0.650	10.0-30.0	3.5-9.0	
Eutrophic	0.650-1.20	30.0-100	9.0-25	
Hypereutrophic	> 1.20	> 100	> 25	

Table 7.5. Trophic status threshold levels for lakes based on total nitrogen (TN), total phosphorus (TP) and chlorophyll a (chl a) concentrations. Adapted from Nürnberg (1996).

7.14 Contributions of Forested Streams to Lake Nutrient Loading

Our study found the highest chlorophyll *a* and TP concentrations at the mouth of Stony Brook. However, the concentrations of all other nutrients were not significantly different across stream sites, regardless of photosynthetic activity. In the literature, nutrient concentrations in lakes, especially total phosphorus, are positively correlated to chlorophyll *a* values (Jones & Knowlton, 2005; Chen *et al.*, 2003; Canfield, 1983). Therefore, our study supports the existing literature on the relationship between TP and chlorophyll *a*, and confirms our expectations that our lake is phosphorus-limited. However, we would expect a similar relationship with PO_4^{-3} as well, which was not observed in this study because PO_4^{-3} concentrations were below detection at all sites. This suggests P is tightly cycled in Long Pond, with phytoplankton using organic species of phosphorus before it is mineralized.

If the forested headwater streams were contributing nutrients to the lake basin, we would expect to see a significantly higher nutrient concentration at the river mouth than at the lake center. This occurred with NH_4^+ , indicating that the headwater streams may be exporting ammonium into Long Pond. Other studies have found that the ratio of NH_4^+ to NO_3^- exported from forested streams can vary seasonally and between forested catchments (Cooke & Prepas, 1998), and more research is necessary to determine if any of these spatial and temporal factors influence NH_4^+ export in the Long Pond watershed. However, we found no significant difference in the other nutrients between the stream outlets and the lake centers, indicating that forested catchments are well equipped to remove NO_3^- and PO_4^{-3} from stream water before it reaches the lake. Even in the case of NH_4^+ , this hypothesis is supported by the drastic decrease in nutrient concentration between the stream reach and stream mouth (Fig. 7.4).

Seasonality and storm events may play a key role in nutrient loading to Long Pond, as stream flow increases during spring snowmelt and storm events, and decreases in the winter and during droughts.

7.15 Other Sources of Nutrients to Long Pond

Since the studied headwater streams were not a significant source of any nutrient except ammonium, which is not limiting in Long Pond, we used GIS mapping software to hypothesize other possible sources of nutrients to Long Pond. The watershed is predominantly forested (81.5%), and all anthropogenic uses combined in the watershed total 8.65%. Only 3.29% of the riparian buffer in the catchment is impacted by human activity. It would be important to study impacted streams since human activities have the potential to diminish nutrient mitigating capabilities in streams, by overloading them with nutrients or altering the stream ecology. However, the predominate sources of nutrients to Long Pond seem to be natural processes (Alexander *et al.*, 2007; Billen *et al.*, 2007; Freeman *et al.*, 2007).

Natural inputs of nutrients into a lake system include forested streams and other water from the catchment (groundwater and overland flow), lake bottom sediments, and the atmosphere. Our study found that forested streams were only a source of ammonium. It is possible that phosphorus input originated in lake bottom sediments, since the study was conducted during the fall mixing, when the breakdown of the thermocline allows lake nutrients to mix throughout the water column. Atmospheric deposition may also be an important source of nitrogen to the lake via nitrogen fixing bacteria.

Another important variable to consider is surface runoff. Our study did not examine the shoreline land cover of Long Pond, but shoreline runoff can be an important source of nutrients to lakes. Programs such as LakeSmart work to prevent anthropogenic nutrient inputs through the use of a lakeshore buffer, which consists of native vegetation that slows water and absorbs nutrients before the water reaches the lake (Maine Department of Environmental Protection, 2013). Due in part to this program, Long Pond's shoreline appears to be well buffered, but nutrients entering the lake from surface runoff could contribute to both nitrogen and phosphorus inputs. More research is needed to determine whether anthropogenic or natural inputs, and which subset of sources within these categories, are the most important contributor to nitrogen and phosphorus loading in Long Pond.

7.16 Future Impacts of Nutrient Loading

The threshold for a eutrophic chlorophyll *a* measurement is 9.0 μ g L⁻¹ (Nürnberg, 1996). At the current chlorophyll *a* increase rate, assuming no changes in behavior, we would expect Long Pond to

become eutrophic in approximately 2061. This is extrapolation outside the range of our data, and does not represent changing behavior, such as the increasing environmental consciousness of Belgrade residents. It also does not take into account potential future benefits of current practices. For example, a whole-lake nutrient study done by Schindler (1998) found that results from the reduction of nutrient loads were not detectable for several years. Thus, positive responses to recent changes, such as the increase in LakeSmart properties, may not be seen in this data.

If nutrients, specifically phosphorus, were to increase, we would likely see a quickly responding increase in chlorophyll *a* concentrations. Both of the lake basins are phosphorus limited, and current phosphorus levels in Long Pond are below detection, indicating that phytoplankton utilize phosphorus entering the lake almost immediately. Urbanization is a common source of both dissolved and particulate phosphorus, mainly from wastewater and fertilizer, but agricultural sources can also be important (Paul & Meyer, 2001). For example, a modelling study done on a tributary to the Thames found that 46% of the phosphorus import to the stream was attributed to cultivation of cereal grains, 13% to sheep farming, and 12% to cattle farming (Johnes, 1996). Currently, about 3.8% of the Long Pond catchment is categorized as "developed", and 1.8% is categorized as cultivated crops, and 2.5% is hay and pasture land for cattle. Meanwhile, 81.5% of the catchment is forested. The low level of urbanization and agriculture, as well as the high level of forest, may be maintaining a healthy lake ecosystem. Increases in both urbanization and agriculture, with a corresponding decrease in forest cover, could have a detrimental effect on the water quality in Long Pond by increasing nutrient loading to the lake.

Historically, development in the town of Belgrade has influenced the changes in lake nutrients. The increase in chlorophyll *a* in Long Pond from the mid-1980s to present coincided with a steady increase in the population of Belgrade and surrounding towns. Population growth in the area is associated with increased lakeshore development, removal of natural barriers, and an increase of nonpoint source pollution into the lake (Burgess, 2009). We cannot determine from our analysis which specific effects this population growth has had on the lake ecosystem, but the literature suggests that nutrient loading is a key result of urbanization, and future research should explore more fully the relationship between changing land use and water quality on Long Pond.

Since five of the lakes in the Belgrade Lakes watershed (East Pond, North Pond, Great Pond, Long Pond, and Messalonskee Lake) are connected by streams, it is important to control the effects of anthropogenic disturbance, such as urbanization and agriculture, not only in Long Pond's catchment but in the watershed as a whole. Nutrient loading in the lakes upstream of Long Pond could result in nutrient increases in Long Pond as well. Similarly, increased development in the Long Pond catchment and resulting nutrient increases could cascade downstream into Messalonskee Lake. Therefore, a whole-watershed approach is important in considering strategies for the management of Long Pond.

7.17 Current Conservation Approaches and Policy Recommendations

Because of the general good quality of the lake water in Long Pond, especially with regard to nutrients, current conservational practices, including the LakeSmart program and the conservation of the Kennebec Highlands, appear to be effective. The LakeSmart program focuses exclusively on preventing shoreline runoff, and therefore is outside the scope of our research. However, two of our three studied streams were protected within the Kennebec Highlands. Although the Kennebec Highlands are not conserved for the specific purpose of improving lake water quality by protecting headwater stream ecosystems, we have shown that this practice is effective in limiting nutrient exports to Long Pond (Belgrade Regional Conservation Alliance, n.d.). Because of the strong positive ecosystem services provided by headwater streams to the lake, future editions of the Kennebec Highlands Management Plan might consider adding measures to ensure continued protection of headwater streams as part of the effort to maintain Long Pond's water quality.

Conclusion

We have found that headwater streams in conserved forested watersheds are effective in mitigating nutrient exports into Long Pond. Initiatives such as the LakeSmart program have been effective in reducing nutrient inputs to the lake from terrestrial runoff, and the conservation of the Kennebec Highlands contributes to lake health by protecting the ecosystem services provided by the headwater streams. Although more research is needed to understand the effects of unprotected streams on lake health in the Long Pond catchment, current conservation policies in the area appear to be accomplishing their goal of preserving the water quality of Long Pond.

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CHAPTER 8 CONSERVATION LESSONS

Introduction

8.1 Watershed Characteristics

The conservation of streams at a watershed level is vital to protect all natural processes within the streams as well as the ecosystems to which they are connected. Watersheds are a combination of headwater streams, downstream waters, and hillslopes. The connectivity between streams and downstream waters allows for the exchange of energy, nutrients, sediment, and organisms (Nadeau & Rains, 2007). The health of a freshwater system is connected to its surrounding terrestrial ecosystems through land use, hydrology, runoff, sedimentation, chemical transportation, and hosting macroinvertebrate communities (Scott, 2006; MacDonald & Coe 2007; Fremier et al., 2010). Therefore, it is important to fully understand each component of the freshwater ecosystem in order to protect the watershed as a whole. Healthy streams are able to utilize and remove nutrients such as nitrogen and phosphorus, therefore decreasing the nutrient load flowing into downstream ecosystems (Elmore & Kaushal, 2008). This nutrient retention is important because it prevents the entry of excess phosphorus and nitrogen into the lakes, which can cause toxic algal blooms, dead zones, and fish kills (Carvalho et al., 2003). Poor stream health often leads to low water quality downstream, which consequently causes degraded habitat, alteration of essential metabolic processes, and changes in corridor structure (Elmore & Kaushal, 2008). Hence, degraded streams threaten the condition of whole river networks, signifying that stream water quality conservation is essential to preserve the health of entire watersheds (Meyer et al., 2007).

8.2 Anthropogenic Threats

Freshwater ecosystems are closely related to the surrounding land, which can be altered by humans through urbanization, among other processes (Scott, 2006; Bernot *et al.*, 2010). Landscape changes directly increase the amount of sediments and nutrients that enter streams through chemical runoff, sewer overflows, and eliminated stream buffers (Elmore & Kaushal, 2008; Jamwal *et al.*, 2008). The conversion of natural landscapes to urban areas also affects hydrological processes, channel structures, and organisms dependent on high water quality (Scott, 2006). Urbanized areas must take into
consideration surrounding watersheds and implement appropriate conservation practices in order to maintain stream health.

In addition to the urbanization of natural landscapes, the demographics of an area are also associated with disruption of headwater streams. Human population in a watershed is negatively correlated to stream chemical and biological health. In addition, poor socioeconomic status of local residents has a strong negative effect on river health (Jamwal *et al.*, 2008). Poor stream health in these communities can be caused by ineffective sewage systems, high levels of pollution, or little available conservation funding (Jamwal *et al.*, 2008; Hager *et al.*, 2013). Since poor socioeconomic status creates conditions that often lead to reduced environmental health, it is important to provide the social support needed in order to restore and conserve stream systems.

Similar to urbanization, logging greatly disturbs forest systems, altering overall structure, vegetation communities, and soil patterns, which in turn affect nearby streams (Choi, 2012). Lecerf & Richardson (2010) measured ecosystem disturbance eight years after logging had been completed in a study area and found that headwater streams remain extremely sensitive to logging for many years. Vegetation along river systems decreases the amount of sediment and nutrients entering streams by absorbing materials and nutrients from the soil. The removal of trees from the edges of river systems can alter water temperature, chemistry, hydrology, sunlight, retentive structures, and the amount and type of organic litter that enters the stream. Litter decomposition is particularly disrupted by tree harvesting, which decreases the amount of organic matter in the stream (Lecerf & Richardson, 2010), which is an important part of the food web.

Along with urbanization and logging, chemical practices can also have negative effects on the health of stream networks (Einheuser *et al.*, 2012). A predominant and ecologically harmful component of agriculture is the use of pesticides and fertilizer, which greatly impact stream ecosystem communities. These chemicals enter streams as runoff, with heavy storms causing the most damage. Pesticides and fertilizers, along with loss of habitat diversity, cause combined stress on the system (Rasmussen *et al.*, 2012).

Dams, another anthropogenic disturbance, alter the natural flow of the system and consequently have many affects on the health and hydrology of watersheds. Dams regulate the flow of rivers and lakes, which creates a static water system with flood pulses. When a large pulse of water flow is released after a long period of low flow, such as in a storm, it can cause low oxygen levels and salt intrusion with the sudden inflow of water momentum (Pearsall *et al.*, 2005). Dams also impact floodplain interactions,

juvenile fish, macrophyte seed development, and nutrient retention (Poff *et al.*, 1997). Conservation practices such as water-table management may allow the modification of stream channels in order to mimic natural stream hydrology (King *et al.*, 2009), decreasing the flow disturbance caused by dams.

8.3 Conservation Strategies

Researching streams is important in order to identify catchment water quality problems and create catchment-wide conservation solutions. As human disturbance increases, detailed conservation management plans must be put in place in order to combat anthropogenic stressors. This strategy is completed by educating community members, bridging science and policy through non-profits and other small conservation groups, and implementing policy change. Therefore, after collecting thorough information on stream health, it is vital to consider what conservation lessons can be drawn from our findings. It is very important for watershed communities to use the available scientific research to create appropriate conservation strategies for keeping local streams, and ultimately lakes, healthy.

8.4 Conservation Status of Whittier Stream, Beaver Brook, and Stony Brook

The three streams our project analyzed were Whittier Stream, Beaver Brook, and Stony Brook. All three streams are protected or in undisturbed areas. Whittier Stream and Beaver Brook are both within the Kennebec Highlands, a protected area owned by the Belgrade Regional Conservation Alliance (BRCA) that includes about 6,000 acres (Kennebec Highlands Management Plan, 2011). The reach of Stony Brook we studied is on the property of the executive director of the BRCA and is heavily buffered is also surrounded by the Kennebec Highlands. The Kennebec Highlands also include several mountains, streams, ponds, and wetlands (Kennebec Highlands Management Plan, 2011). In general, the Belgrade Lakes watershed is an important ecosystem for wildlife and natural resource conservation as well as a public resource for recreation (Kennebec Highlands Management Plan, 2011).

The Belgrade Lakes watershed already has a number of strong programs and strategies to maintain watershed health that involves various stakeholders, non-profit groups, and government agencies. The Kennebec Highlands Management Plan provides a summary of the protected land, planning processes, future resource distributions, and recommendations to improve the conservation of the Kennebec Highlands for the next 15 years (Kennebec Highlands Management Plan, 2011). There are also several non-profit organizations that are dedicated to preserving the land and water in the area, including the BRCA, the Belgrade Lake Association (BLA), the Maine Lakes Society (previously

known as the Maine Congress of Lake Associations), and Colby College. These groups work together at the Maine Lakes Resource Center (MLRC), located in the center of Belgrade Village. In addition to accommodating the organizations, the MLRC also provides a public gallery with displays of conservation techniques, local research, and information about the watershed.

8.5 Study Groups and Conservation Implications

In order to create effective conservation plans for the Belgrade streams, there needs to be a comprehensive understanding every part of the ecosystem. Streams provide many benefits for organisms migrating upstream from lakes such as refuges from high water flows, temperature changes, predators, and invasive species. Streams also offer habitats for reproduction, nurseries, and feeding (Fig 8.1). There is a huge diversity of activities that take place moving from upstream to downstream.

In the Colby Headwater Stream Research Project, five different research groups analyzed each study stream with different scientific focuses, allowing us to form conservation suggestions regarding several components of stream ecosystem health. We have a comprehensive understanding of the three streams in terms of their nutrient spiraling, metabolism, organic matter, invertebrate communities, sediment and erosion, as well as the influence of these streams on Long Pond. The key findings from each group have been used to determine the strengths and weaknesses of each stream individually, as well as all three protected streams as a whole. In addition, seven non-protected streams were sampled within the Belgrade Watershed. These streams are not within the Kennebec Highlands and are in close proximity to roads and houses. The water samples taken from these streams were then compared to equivalent samples from the three protected streams to investigate any significant differences. Understanding the strengths and weakness of the streams allowed us to determine lessons that can be used in the future to improve and protect stream health in the Belgrade region.



Fig 8.1. The different elements that affect the biology of headwater streams in river networks; factors on the right benefit species that are solely found in headwater streams and create the headwater habitat necessary for migrants downstream; the left are factors that provide biological elements to downstream ecosystems (*Modified from Meyer et al., 2007*).

Results

8.6 Summary Tables

Working with each research team, we summarized key findings from each group to derive important conservation lessons from their findings. For ease of comparison, Table 8.1 through Table 8.4 present these findings for Whittier Stream, Beaver Brook, and Stony Brook as well as an overall synthesis of the project. As a whole, Whittier Stream was healthy with signs of nutrient limitation, little signs of erosion and no signs of nutrient loading downstream. Although Whittier Stream was fairly retentive and water quality was determined to be "very good," it was determined to be the least healthy of the three streams, likely due to its proximity to human activity in the form of roads and a culvert (Table 8.1). Beaver Brook was determined to be healthy, with indications from all groups of excellent water quality. This stream makes an excellent model for a healthy stream ecosystem (Table 8.2). Stony Brook was determined to be healthy, with indications from all groups of excellent water quality (Table 8.3). This stream makes an excellent model for a healthy stream ecosystem. As a whole, the protected streams were all determined to be very healthy with high retention of organic matter, nutrient limitation, little signs of erosion and no signs of nutrient loading downstream. These three streams are all strong models for healthy stream ecosystems with Whittier Stream showing impacts of human activity in the area (Table 8.4)

Group	Key Findings	Conservation Lessons
Organic Matter	Only stream where organic matter reached 100m mark, therefore least retentive of the three streams	Organic matter not being retained as well as in other streams; may be caused by location near road and bridge
Nutrient Spiraling	P and NO3 uptake lengths show signs of nutrient limitation in stream	Shows signs of nutrient limitation, preventing the transport of nutrients downstream
Metabolism	Net Heterotrophic behavior, Negative EP	Rocks are valuable substrates for GPP. Leaves and organic material are valuable substrates for respiration
Biotic Indices	Lowest % EPT and water quality "very good"	Least amount of indication organisms; may be because runs under road
Sediment and Erosion	Little signs of erosion	Model for effective buffer zones
Lake Context	No signs of nutrient loading	Stream efficiently utilizes nutrients, no signs of nutrient loading from this protected stream

Table 8.1. The key findings and conservation lessons of each study group in Whittier Stream.

Group	Key Findings	Conservation Lessons
Organic Matter	Stream displayed strong retentiveness of organic matter	Large stream with many rocks and island areas that can assist with retention
Nutrient Spiraling	Shortest uptake lengths for P and NO3, within normal limitations of a healthy stream	Shows signs of nutrient limitation, preventing the n transport of nutrients downstream
Metabolism	Net heterotrophic behavior, Negative EP	Rocks are valuable substrates for GPP. Leaves and organic material are valuable substrates for respiration.
Biotic Indices	High % EPT and water quality "excellent"	Model for biotic indices
Sediment and Erosion	Low erosion levels	Model for effective buffers
Lake Context	No signs of nutrient loading	Stream efficiently utilizes nutrients, no signs of nutrient loading from this protected stream

Table 8.2. The key findings and conservation lessons of each study group in Beaver Brook.

Table 8.3. The key findings and conservation lessons of each study group in Stony Brook.

Group	Key Findings	Conservation Lessons
Organic Matter	Stream displayed strong retentiveness of organic matter	Stream had a strong presence of rocks and large woody debris that may contributed to retention.
Nutrient Spiraling	P and NO3 uptake lengths show signs of nutrient limitation in stream	Shows signs of nutrient limitation, preventing the transport of nutrients downstream
Metabolism	Net heterotrophic behavior, Negative EP	Rocks are valuable substrates for GPP. Leaves and organic material are valuable substrates for respiration.
Biotic Indices	Highest % EPT; Highest taxa richness and lowest HBI; Water quality "excellent"	Model for healthy biotic indices
Sediment and Erosion	Low erosion levels	Model for effective buffers
Lake Context	No signs of nutrient loading	Stream efficiently utilizes nutrients, no signs of nutrient loading from this protected stream

Table 8.4. The overall key findings and conservation lessons of each study group, considering all three streams studied.

Group	Key Findings	Conservation Lessons
Organic Matter	Strong retention of organic matter in all 3 streams	Models for organic matter retentiveness. All streams had strong presence of rocks and large debris that may assist in organic matter retention.
Nutrient Spiraling	Significant correlation between net ecosystem production and VF (uptake efficiency) for phophorus; uptake rate and net ecosystem production significantly correlated for phosphorus and nitrate; nutrient-limitation observed in all streams.	Models for healthy nutrient spiraling and prevention of nutrient loading downstream
Metabolism	Net heterotrophic behavior, negative EP	Rocks are valuable substrates for GPP, leaves and organic material are valuable substrates for respiration
Biotic Indices	Slight positive correlation with cobble and woody debris & negative correlation with sand; Whittier Stream least healthy	Beaver Brook and Stony Brook models for healthy biotic indices; cobble and woody debris should be encouraged as streambed composition
Sediment and Erosion	Low suspended sediment load, little signs of erosion on shoreline	Model for effective buffers
Lake Context	No signs of nutrient loading	Three streams efficiently utilize nutrients, no signs of nutrient loading from protected streams

Discussion

8.7 Overall Findings

It is critically important to understand not just how human activities contribute to environmental degradation, but how they can also be part of the solution. Anthropogenic impacts caused by activities such as urbanization, agriculture, and logging have had severe consequences for aquatic ecosystems (Wang *et al.* 2013; Zhang *et al.* 2013). Human disturbances lead to decreased biodiversity of benthic macroinvertebrates and an overall decline in stream health (Greenwood *et al.* 2012; Schmera *et al.* 2012). The major impact observed on the protected Belgrade streams from urbanization was the culvert on Whittier Stream. In addition, this stream was added to the Kennebec Highlands most recently by the BRCA, so it may have been protected for less time. There are also homes on one side of Whittier Stream, while it is still fully protected on the other side. Although this stream was overall very healthy, it was determined to be the least healthy of the three, which may be attributed to the road it flows under

as well as its recent protection. Along with protecting more land, this suggests that streams running under roads should receive more buffers and other better management practices.

A constantly growing human population is increasing the demand for stream resources such as clean water and retention of sediment and nutrients. Therefore, it has never been more critical to address issues endangering stream health with conservation action. A dynamic and multifaceted approach to conservation lessons can engage the public in a way that catalyzes progressive environmental change. Stream conservation issues must be addressed on individual and community scales along with the use of strategic partnerships among local groups and effective policymaking. We believe that this combination of actions could lead to catchment wide preservation of healthy stream networks in the Belgrade Lakes catchment, leading to improved water quality in the lakes.

Various conservation methods can be implemented along and within streams to prevent the downstream movement of nutrients and organic material. A buffer zone prevents the runoff of nutrients from shore, helps to retain the integrity of the soil, and is a source of organic matter to the stream. Within a stream, a riprap zone consisting of boulders and other natural substrates can assist with the retention of organic matter. A winding path connecting properties to streams assists in redirecting surface water runoff through vegetation rather than directly into the stream environment (Fig. 8.2).



Fig. 8.2. Types of conservation actions that can be implemented on headwater streams.

8.8 Lessons for the Individual

Individual acts of conservation should be emphasized as a way to involve communities in protecting their natural areas. The cumulative effect of these small-scale conservation efforts can significantly benefit the environment. Personal conservation efforts have the potential to resolve some of the most important problems in the aquatic environment. One of the most effective actions that can be taken by individuals is to add buffers on the stream bank. Buffers are usually various forms of vegetation, but can also be large rocks, such as riprap, positioned at the stream bank. Riparian buffers mitigate many of the adverse affects of harmful catchment land use, including nutrient runoff and stream bank erosion (Wahl *et al.*, 2013). Buffers also prevent both point source and non-point source pollution from entering a body of water, making them an effective and efficient method for decreasing pollution. Natural riparian buffers largely contribute to reduced erosion and sediment loading at each of our three stream sites.

In addition to adding riparian buffers, there are other techniques individuals can implement to help improve stream quality in certain situations. On agricultural land, a reduction in the volume of chemical inputs will ultimately lead to less nutrient loading in streams (Januchowski-Hartley *et al.* 2012). All three of the streams studied were in well-protected areas and therefore did not display detrimental affects caused by chemical runoff. However, the application of fertilizers, other agrochemicals, and winter road salt is commonplace in many unprotected stream reaches, and the limitation or control of this chemical application should be taken into consideration when developing a conservation plan. A stream conservation method that can be adopted on an individual scale is decreasing the amount of chemicals and fertilizers used on individual properties. Another technique that farmers can use to protect streams on their land is conservation tillage, which decreases the amount of soil runoff by slowing water movement with the retention of a certain amount of crops (Sheeder & Lynne, 2011).

8.9 Lessons for the Community

Story & Forsyth (2008) found that public awareness of watershed issues was the most important factor in establishing conservation behaviors within communities. Creative and interactive education efforts that take a multilateral approach to solving problems will most successfully resonate within the community and will ultimately be the most effective method for this scale of conservation. This study also suggested educating the community on the local watershed ecosystem, because many community

members do not realize that land not visually recognized as wetlands but within the boundaries of the watershed impacts the local water quality (Story & Forsyth, 2008). This common misunderstanding could be prevalent in the Belgrade watershed, which includes seven lakes and spans several towns. Therefore, it is essential that everyone in this large area understand the implications of their communal actions on the Belgrade watershed. For example, while the LakeSmart program currently focuses on shoreline properties, conservation practices need to be emphasized to all the other owners within the watershed. This is one of the next critical steps toward improving water quality in Belgrade.

Involving watershed communities through outreach activities is another great way to get the public excited about conservation. Large-scale outreach efforts, such as organized stream clean ups, can act as focusing events that emphasize the importance of a particular issue (Goodwin *et al.*, 2012). Citizen-science, which allows students or landowners to assist in data collection, is also a common practice within many communities, (Nerbonne & Nelson, 2008; Oberhauser & LeBuhn, 2012). The opportunity for local citizens to assist in scientific research is a great way to connect the community to the science aspect of conservation. This research project is a great an example of student engagement in the Belgrade region, as is the educational outreach event at the MLRC with 80 sixth grade students that we organized and hosted this past fall. These engagement projects and events allow students to further understand watershed and stream conservation, and this particular research project, conducted every year at Colby College on various topics related to the Belgrade watershed, has resulted in years of data collection and ongoing public interest in the research projects.

Volunteer monitoring is a well-known method of increasing public awareness, collecting large amounts of data, and providing the scientific knowledge to encourage policy change (Nerbonne & Nelson, 2008). Volunteer programs for the monitoring and eradication of the invasive plant milfoil are currently present in Belgrade, and this type of citizen-science could be applied to other conservation areas as well. A tactic that could be specifically applied to streams in the Belgrade area is a volunteer program that utilizes volunteers' private property for water and macroinvertebrate samples. This program would allow local scientists to have a much better understanding of stream health in Belgrade, and it would provide another opportunity to involve the community in local conservation. The most successful forms of outreach involve entire communities, as they rely on the innate curiosity of citizens and their willingness to learn about the environment around them.

8.10 Lessons for Collaborators

The community members of the Belgrade Lakes watershed are vital players in maintaining the health of the watershed, and can be encouraged to participate by local organizations. One way the public can become involved with conservation efforts is through LakeSmart Certification, a program designed to encourage property owners to create lake-friendly waterfronts by adding buffers, rain gardens, and other "best management practices" (BMPs) that intercept runoff before reaching the lakes. This program has proven to be extremely effective in the community and has the potential to set a precedent for other programs, which could potentially include streams. Looking to LakeSmart as an example, it may be effective to create a StreamSmart program, which would target landowners along streams. A subprogram like this could also encourage the public to consider the Belgrade Lakes watershed a whole ecosystem, rather than thinking of the seven lakes as individual entities. The Belgrade Lakes watershed is unique because it includes seven individual lakes that are all interconnected by streams. Because of this interconnectivity, the health and water quality of each lake has an effect on all the others, since the water flows throughout the watershed via the stream network. Conservation of the Belgrade streams should therefore be as equally emphasized as the lakes, as the stream health is strongly influential on lake health; it therefore makes sense to extend the LakeSmart program to include streams so that the public is aware of their importance.

Through collaboration with non-governmental organizations (NGOs) and non-profit conservation groups, community leaders and decision-makers can significantly broaden their audience and resources. Local conservation groups are a very effective way to get regional stakeholders involved in local efforts (Freeman & Ray, 2011). These associations need to consider the entire watershed when assisting policymakers, while focusing their activities and education within the community on a local scale (Freeman & Ray, 2011). Our study found that the three protected streams had very high water quality, and the seven impacted streams had huge variation in terms of their health based upon the invertebrate community sampled at each stream. Therefore, it may be beneficial for the large number of non-profit groups devoted to watershed conservation in the Belgrade area to collaborate on a stream conservation project. Collecting more data on both impacted and protected streams is vital in order to get a stronger understanding of stream health and potential contributions to downstream water quality problems.

8.11 Lessons for Policymakers

For policymakers, there are many approaches to regulating the use and treatment of stream resources. One approach is "Streamside Management Zones (SMZs)": vegetation buffers placed along stream banks to reduce nutrient overflow, provide shade, and regulate water temperatures (Choi, 2012). In addition to being applied by individuals within the community, an organized buffer system can also be created through policy measures. Some communities have created "Freshwater Protected Areas," which focus on whole-catchment management, preserving natural stream-flow, and eliminating invasive species (Saunders *et al.*, 2002). Whole catchment management strategies have been identified as the preferred method of management because they take into account the interconnected nature of the watershed rather than single, isolated subsections (Saunders et al., 2002). If whole-catchment management is not possible, other strategies can be used such as multiple-use modules, which have a strongly protected center and are surrounded by buffer zones that allow various amounts of human activity (Fig. 8.2) (Saunders et al., 2002). The river continuum concept promotes the protection of headwater streams, since these reaches have a huge effect on downstream areas (Fig. 8.2; Vannote et al., 1980; Saunders et al., 2002). Finally, vegetation buffer strips with widths ranging 10-50m have been shown to control stream temperatures and prevent sediment and nutrient runoff (Saunders et al., 2002). "Selective logging" has been attempted on forested areas, although it did not seem to improve the stream status (Lecerf & Richardson, 2010), making buffers currently the most effective way of protecting streams from logging in the catchment. Increasing buffers along riparian systems helps to improve disturbances relating to urbanization, logging, and agriculture, making it an essential conservation action.



Fig. 8.3. Approaches for protecting streams from land-use practices: a) whole-catchment management, b) multiple-use modules, c) river continuum concept, and d) vegetated buffer strips (*Reproduced from Saunders et al., 2002*).

Although the three streams that we studied are healthy and in a protected environment, the Belgrade Lakes watershed as a whole is largely unprotected and vulnerable to degradation at many different locations. A Stream Protected Area (SPA) program could potentially designate critical stream habitats, apply multiple-use methods, and consider the river continuum concept in all management decisions (Pearsall *et al.* 2005). This varied approach to management would ensure that all aspects of stream conservation are taken into consideration. Stream protected areas would also prevent impacts such as logging in designated areas. This is vital because tree harvesting decreases canopy cover, which has been found to decrease the presence of sensitive macroinvertebrate taxa, as well as decreasing organic matter. These management strategies are important to understand and implement because most of the Belgrade watershed does not lie within protected areas; therefore, it is important to consider applying broader conservation practices.

Dam management is another topic that needs to be addressed among policymakers. Many of the small streams in Belgrade were used in the past for small mills, and therefore may have been dammed. Whittier Stream has noticeable remains of a dam, which may have had an impact, resulting in the slightly lower water quality found in terms of indicator species. The general historic trend of Belgrade dams may still have impacts on the streams.

Ideally, improving the environment surrounding one's community would be the only necessary incentive for conservation. When this is not the case, it is often helpful to incentivize conservation behavior among local communities. In order to make these previously mentioned methods of mitigation appealing, a strong emphasis on financial and personal gains from conservation should be stressed. Many landowners can be motivated by a sense of stewardship and similar internal factors other than financial incentives (Januchowski-Hartley *et al.* 2012). In a suburb of Cincinnati, Ohio researchers used a reverse auction to help landowners recover the opportunity cost of land that they had forfeited by installing rain gardens and collection barrels to mitigate storm water runoff (Green *et al.*, 2012). This small financial incentive. These incentivized programs create a culture of conservation and motivate individuals within the community by making conservation the status quo (Suter & Sammin, 2013). The Maine Lakes Resource Center slogan "Making conservation a tradition" is an excellent representation of this in Belgrade.

When a conservation plan requires a significant portion of profitable land to be altered, financial incentives are particularly necessary (Jellinek *et al.*, 2013). This costly transition can be overlooked if there is reassurance that the financial loss will be recovered. Incentives of both social and financial benefits play an integral role in the process of conservation and are often key motivators towards social change.

In order to successfully conserve the Belgrade streams and lakes, stakeholders of all levels need to be included. In order to bridge science and policy, ecological problems need to be identified from research in order to create conservation plans, which can then be carried out by policy and local non-profit groups. The progress also needs to be effectively communicated to the public to create community involvement (Fig 8.3). When conservation is understood on the individual, community, small organization, and policy levels, all stakeholders can work together in order to efficiently preserve headwater streams.



Fig. 8.4. A comprehensive diagram displaying the steps to connect scientific findings to conservation actions.

8.12 Future Studies

There are many future stream conservation studies that would be useful to complete in the Belgrade Lakes catchment. It would be interesting to collect detailed data on impacted streams in comparison to the protected streams. The impacted and protected streams could then be compared on the basis of water quality and the various land-use surrounding the streams. This study would provide a more comprehensive understanding of the specific stream health issues the Belgrade region may have. Our study only surveyed biotic indices for seven impacted streams, which all showed to have very different levels of indicator species. This suggests that individual management, such as on private properties, have direct impacts on the stream health, since the seven impacted streams were all most likely managed differently from one another. It is vital to better understand this relationship between different land-use practices and stream health. Meaningful conservation lessons could then be determined and changes could be made in order to improve any weaknesses in management plans.

Another future study could be a survey project conducted on the Belgrade Watershed residents to investigate their understanding of streams' role within the watershed. Since community members do not always understand the boundaries of watersheds, it would be interesting and important to gauge the understanding of Belgrade residents. This understanding is essential in recognizing the role streams have on the nearby lakes and the importance of their conservation. This is a point we emphasized to the 6th grade students who participated in our outreach event (see Appendix for more information).

Conclusion

8.14 Headwater Stream Project Conclusion

Protecting the health of headwater streams is essential in conserving the Belgrade watershed because they connect the terrestrial and aquatic environments. There is very little knowledge on environmental status of the streams in the Belgrade region, suggesting the need to increase scientific research in these areas. More detailed information on the health of the headwater streams will allow local scientist, conservation organizations to identify ecological problems and create possible conservation plans. The local organizations can then suggest conservation strategies policymakers, who have the political power to initiate changes that can improve headwater stream condition, and therefore the health of the entire watershed. Community involvement is already extremely committed in Belgrade, and needs to expand to include streams, in addition to the lakes, in its conservation targets. Stream protection is an important step toward improved health and functionality of all areas in the Belgrade watershed.

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Appendix

Outreach Event at the Maine Lakes Resource Center

The goal of the outreach event was to explain the influence of stream health on lake and watershed health to the students. The students will also learn the research and conservation methods applied to headwater streams. A total of eighty-four 6th grade students came to the Maine Lakes Resource Center for our education and outreach event. We split the students into four groups of about twenty students each and rotated them through four thirty-minute stations covering the various field research activities we completed at our study sites.

9:30-9:45: Introduction/Split up groups/explain where to go 9:50-10:20: Station 1 10:25-10:55: Station 2 11:00-11:30: Snack Break 11:35-12:05: Station 3 12:10-12:40: Station 4 12:45-1:15: Lunch 1:15-1:30 Conclusion

4 Stations (2 outside, 2 inside; 30 minutes at each):

Outside

- 1. Stream Nutrient Spiraling and Erosion
 - a. Send down food dye "nutrient release" (split into smaller groups, each group can have a different color)
 - b. Use slinky to explain nutrient spiraling
 - c. Send stick down stream to demonstrate organic matter movement/retention
- 2. Catching Bugs
 - a. Catch invertebrates with nets and put in jars to bring inside to lab
 - b. Identify various invertebrates
 - c. Discuss indicator species and their role in streams

Inside

- 1. Sediment, Erosion, Landscape, Lake Context
 - a. Start with interactive drawing- what kids think a stream/lake friendly property looks like
 - i. Repeat at end of lesson to observe the change in perspective of what a stream friendly property actually looks like
 - b. PowerPoint showing pictures & impacts of Nitrogen and Phosphorus
 - c. Discussion of mitigation methods (buffers etc.)
- 2. Stream Organic Matter and Bugs
 - a. Microscopes
 - i. Show slimy/dry leaves (talk about metabolic processes)
 - ii. Observe and identify bugs
 - b. Bag of Leaves to show method for organic matter study
 - c. Look at preserved bug specimens and discuss role in aquatic environment

The event was received well by all involved. The students were engaged and excited to participate in the activities, the teachers were well organized and helpful with coordination, the MLRC was extremely

welcoming and helpful with facilitating all of the spaces and resources needed, and all of our volunteers were well prepared and enjoyed the event. With early planning and coordination with the parties involved, this event could easily be something that is repeated annually. In the future, it may be helpful to have a smaller class size attend.