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# Anthropogenic Impacts on Headwater Streams: A Case Study of Three Streams in the Belgrade Lakes Catchment

Colby College

Problems in Environmental Science course (Biology 493), Colby College

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# Anthropogenic Impacts on Headwater Streams:

## A Case Study of Three Streams in the Belgrade Lakes Catchment



**Colby College**
**Problems in Environmental Science (ES 494)**
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This report was the culmination of a semester-long research project in Environmental Studies 494: *Problems in Environmetal Science* course at Colby College in Waterville, ME during the fall semester of the 2014-2015 academic year. The course instructor was Denise Bruesewitz.



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3. Organic Matter: Caleb Harris and Sydney Morison
4. Nutrient Spiraling: Bea Smith and Savannah Judge
5. Storms and Fluxes: Peter Quayle and Anne Schechner
6. Riparian Zone: Natalie Cecere and Olivia Rivera
7. Macroinvertebrates: James Lucas and Caroline Keaveney
8. Pharmaceuticals in Streams: Tom Abare, Carol Walker, and Olivia Avidan

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**CHAPTER 1**
**INTRODUCTION**

## 1.1 Introduction

### 1.1.1 Conservation of the Belgrade Lakes Region in Central Maine: A Historical Reference

Prior to European colonization, the Belgrade area, like most of northern New England was inhabited by the Abenaki tribe. Philip Snow became one of the first white settlers to establish a farm in the area in 1774. His cabin was near what is now Messalonskee Lake. As the region grew in popularity, more farmers came to clear the land for crops, and livestock (namely sheep for woolen mills). The Maine Central Railroad was established in 1849 so that farmers could export their goods. In addition to facilitating trade, the railroad system, with help from the boom in the automobile industry, ushered in the "Golden Age of Tourism". Members of the upper middle class traveled to Belgrade to escape their urban environments and enjoy the beauty of the natural world. In order to accommodate these seasonal tourists, homeowners rented out their own properties or built cabins and cottages while developers and city planners built hotels and inns (Belgrade Historical Society 2014).

Year-round residents watched this all unfold and noticed the many changes made to the landscape of the Belgrade Region. They realized that the increase in

human activity was putting a great amount of pressure on the ecosystems that they highly valued. As a result, a number of conservation groups were formed in the area. The Belgrade Lakes Association (BLA) was founded in 1909 by people interested in maintaining annual reports on the state of Belgrade fisheries and tracking changes in the lakes (Belgrade Lakes Association 2014). Today, the Water Quality Committee of the BLA continues to monitor the health of the watershed. They share information and resources with Maine's Volunteer Lake Monitoring Program to help record changes in water chemistry and clarity. They also coordinate efforts with other local lake organizations to remove invasive plants species.

Founded in 1988, the Belgrade Regional Conservation Alliance (BRCA) is an umbrella organization that connects the five Belgrade lakes organizations, including the BLA. It is comprised of a 7000 acre land trust (6000 acres is the Kennebec Highlands) and a lake trust for the purpose of conserving land and improving water quality. Their public outreach efforts focus on how land use decisions affect the health of the Belgrade watershed. The BRCA also manages the Milfoil Committee (invasive plant species removal), provides courtesy boat inspections and offers activities like public lectures and guided hikes to members of the Belgrade community.

**1.1.2 Our Conservation Research of Headwater Streams**

Members of BLA, BRCA, and Colby College faculty came together to found the Maine Lakes Resource Center in 2011. The purpose of the MLRC is to facilitate community outreach and connect people with ecological knowledge. Their goal is to provide the Belgrade area with a general community center and opportunities for water quality education. The motto of the MLRC is "Making Conservation a Tradition." By providing a space for both regular community events and conservation initiatives, they show how taking care of the watershed can simply be part of the local culture (Maine Lakes Resource Center 2014). Our research project is a part of this tradition. This report focuses on understanding the role of headwater stream ecosystems impacted by human activities in the Belgrade watershed.

**1.2 The Role of Headwater Streams in the Watershed**

**1.2.1  Conservation Context of Research**

Within a catchment area (Figure 1), headwater streams are of first, second, and third order while streams of the fourth order and higher are considered large streams or rivers (Horton 1945; Strahler 1957).
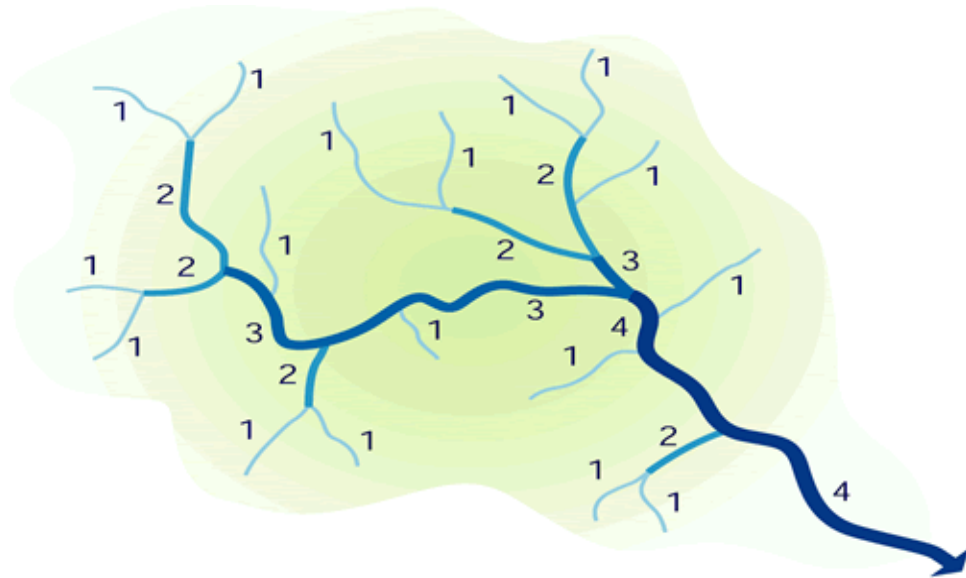
**Figure 1.1:** *Diagram of the size and relationship between streams of different orders, with small, headwater streams feeding into larger streams and rivers. Source: http://www.fgmorph.com/*

Stream networks are comparable to the circulatory system; headwater streams are the small capillaries that converge to supply larger arteries and veins or higher order streams (Minshall et al. 1985; Vannote et al. 1980). Headwater streams may not flow year round and are often unnamed. They may not appear in official maps, but they are important because they serve as the beginnings of large river networks. These streams make up approximately 80% of the United States' stream network length and occur across the entire range of climatic, geologic and biogeographic settings of the country (Meyer et al. 2003; Meyer et al. 2007). Due to the connectivity of water systems, a decline in ecosystem health upstream affects the water quality downstream.

Headwaters play a crucial role in promoting and maintaining the health of downstream systems including lakes, rivers and estuaries. Understanding the physical roles (i.e. hydrology and geomorphology) as well as the chemical and biological dimensions of headwater streams, is key to comprehending the health of the watershed and its water quality (Gomi et al. 2002; Minshall 1988). They also help us to understand the condition of the watershed's biodiversity and heterogeneity (Palmer & Poff 1997). Biodiversity is the diversity and abundance of organisms in an area while heterogeneity encompasses their interactions.

For the purposes of this study, we examine the importance of streams into five categories that illustrate the dynamic nature of these systems and their influence on downstream processes. These categories are the relationship between surrounding land and streams, downstream biodiversity, water quality and ecosystem services, and conservation issues. Headwaters are the source of life in all water systems and their preservation is of utmost importance to the ecological and human communities that depend on them (Lowe & Likens 2005).

Our overarching goal as a research team is to examine the ecosystem structure and function of headwaters in order to evaluate the effects of road crossings and land use on stream health in the Belgrade Lakes watershed. Ecosystem services describe how streams support the lives of organisms in and around them.

This includes facilitation of species reproduction and migrations, removal of pollutants, flood management, and decomposition of leaves/waste.

We focus on several different measures of ecosystem functions. Ecosystem functions measure ecological processes over time, such as nutrient movement or use of organic matter. Our investigation is divided into eight research subsections that consider different aspects of headwater stream ecology. This research effort is embedded in a long-term evaluation of local stream conditions, which will allow for the long-term measurement of stream function in the Belgrade region. By gaining a better understanding of stream conditions, we will be better able to assess how human development impacts the small streams of Belgrade, and ultimately the lakes into which they flow.

## 1.2.2 The Terrestrial-Aquatic Linkage: Headwaters and Their Surroundings

In order to understand the role of headwaters in the greater watershed ecosystem, it is essential to learn about the origins of streams and the land surrounding them (Frissell *et al.* 1986; Lotspeich & Platts 1982, Wipfli *et al.* 2007). Most small streams are fed by groundwater and runoff sources. The volume of water in headwaters depends on the geology, topography along with soils and vegetation, of the surrounding areas (Dodds & Whiles 2010; Leopold *et al.* 1964). These processes and features dictate water storage patterns and the actual flow

paths in the streams (Soulsby *et al.* 2006). It is essential to understand that the structure and dynamics of stream habitat is determined in part by the surrounding land. It shapes the abundance and types of habitats available for stream organisms by controlling, for instance, light through canopy cover and organic matter inputs from surrounding plants (Hynes 1975).

### 1.2.3 Role of Headwaters in Promoting and Maintaining Downstream Biological Diversity

An important consequence of the close link between streams and surrounding terrestrial ecosystems is that their biological communities are often heterotrophic. This means that they are based on carbon that comes from the surrounding land rather than from algal carbon (Cummins 1974). The heterotrophic communities of headwater streams depend on terrestrial inputs like dissolved nutrients (N and P) and particulate matter (i.e., leaves) to meet their energy requirements (Likens & Bormann 1974) and to regulate their composition and productivity rates (Wallace *et al.* 1997). A great example of this interaction has been documented in Bear Brook, a first-order stream in New Hampshire. Researchers found that 99% of the brook's energy supply (particulate and dissolved organic matter) came from the surrounding vegetation (Fisher & Likens 1973).
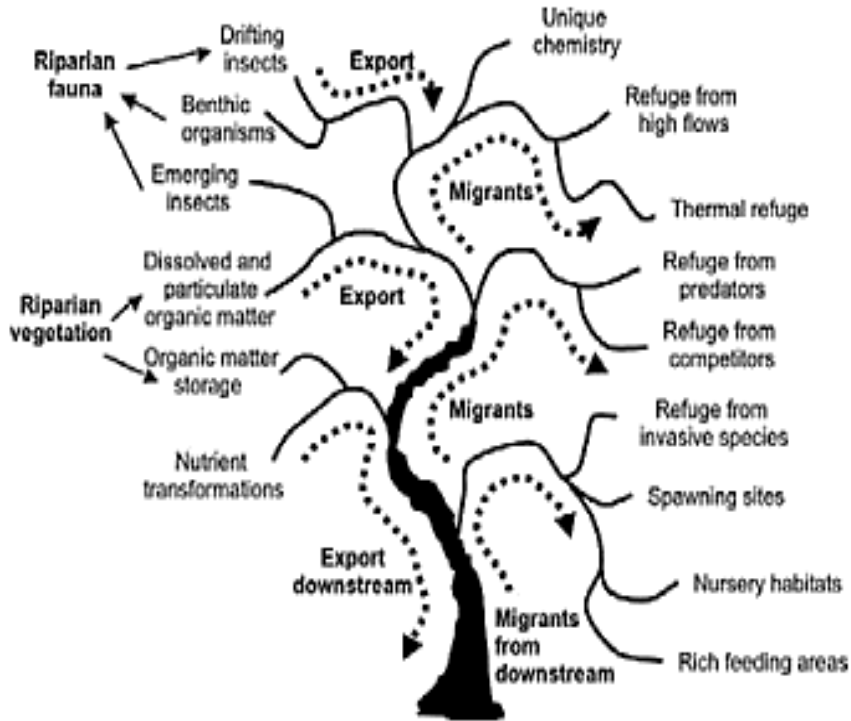
**Figure 1.2:** A visual representation of the role of headwater streams in promoting and maintaining downstream biological diversity. The factors contributing to higher order stream networks are both structural (right side of the diagram) and functional (left side). This diagram was taken from Meyer *et al.*

### 1.2.4 Role of Headwaters in Maintaining Downstream Water Quality

The second major role played by headwater streams in the watershed context

is their influence on water quality in downstream lakes, rivers and estuaries. Even

isolated wetlands, wetlands without surface water connection to any major river or

lake, are impacted by the water quality of surrounding bodies and affected through

underground pollutants and nutrient movements (Leibowitz 2003; Whigham &

Jordan 2003). The water quality of downstream river networks is heavily influenced

by headwater streams, because they transport nutrients both at underground and

surface levels. Headwaters affect the supply, transport, and fate of water and

solutes, including nutrients, in watersheds (Alexander *et al.* 2007).

For example, a study in the Northeastern United States revealed that nitrogen

pollution affects both downstream and upstream aquatic systems (Driscoll *et al.*

2003; Grimm *et al.* 2003). Similarly, other researchers concluded that headwater

streams exert control over nutrient exports to higher order streams in a

disproportionately large way (Peterson *et al.* 2000). This phenomenon is explained

by the higher efficiency of headwaters in processing and retaining nutrients like

nitrogen compared to larger streams (Alexander *et al.* 2000). In summary, when

headwater streams are altered, their ability to process nutrients is decreased, and the

nutrients will travel downstream in unnatural manner.

## 1.2.5 Headwater Stream Ecosystem Services and Anthropogenic Sources of Impact

Keeping headwaters healthy is crucial to maintaining riparian and aquatic

biodiversity and water quality. A third dimension of examination incorporates

humans as both the beneficiaries of headwater stream processes and the sources of

major impact on stream ecosystems. An ecosystem service is the "benefit that

humans receive from the functioning of natural ecosystems" (Meyer *et al.* 2003).

Essentially, ecosystem services refer to the same underlying processes of ecosystem functions, but the former is viewed from a human perspective.

Some ecosystem services provided by headwaters are drinking water, waste removal, renewable energy and recreation (Allan & Flecker 1993; Lowe & Likens 2005). In urban landscapes, streams provide services such as microclimate regulation, decontamination and sentimental value (Bolund & Hunhammar 1999). A number of studies have detailed the importance of streams in urban settings, stressing how they provide various ecosystem services that are compromised by direct degradation of the stream channel and inputs of overland flows that contain sediment, nutrients and pollutants (Klein 1979; Booth 1991; Paul & Meyer 2001). This is particularly important as the world population and the extent of urbanization rapidly expand.

Nonetheless, in rural landscapes such as the Belgrade Lakes region, the anthropogenic impacts on headwater streams are also substantial, especially when considering the influence of land use on stream networks (Allan 2004). Rural landscape streams exposed to road crossings often have more intense floods (Jones *et al.* 2000). Unlike the forest floor, roads are impervious surfaces and cannot soak up water. During a storm, most of it rushes off of the road and into the stream causing more intense floods. Similarly, during a storm, in addition to the increased volume of water, loose dirt, rocks, and sand would be washed into the stream. In forested

areas, road construction can lead to an increase of sediment in the stream, which may result in changes to its physical structure. (Beschta 1978; Bilby *et al.* 1989).

## 1.2.6 Conservation Implications

At present, headwater streams are overlooked in conservation efforts and they are not protected under the United State's Clean Water Act. They must be integrated into science and policy undertakings in order for humans to achieve sustainable water resource management, conservation of biological diversity, and maintenance of downstream water quality (Nadeau & Rains 2007).

Stream systems are hierarchical and complex (Abrahams 1984; Strahler 1964). Stream networks are assemblages of branching water sources that join to form larger, higher-order streams (Tarboton *et al.* 1988). Consequently, conservation efforts focused on preserving and maintaining watershed health should consider the fractal nature of stream networks and assign headwaters with corresponding high importance within the watershed hierarchy (Lowe & Likens 2005). In this way, management and conservation efforts conducted at headwater streams will more effectively extend to the entire watershed and inform design considerations for stream-based protected areas (Lowe *et al.* 2006; Saunders *et al.* 2002). Holistic approaches to stream network conservation are required to ensure the maintenance

of biodiversity and water quality at the watershed level, and to protect related ecosystem services that are so essential to human life and biodiversity.

## 1.3 Overview of the 2014 Colby Environmental Science Capstone Investigation

### 1.3.1 Research Area

In this report, we investigate the impact of roads and land use on streams. Seven research groups looked into various structures and functions of three different headwater stream sites that are tributaries to Belgrade Lakes (Figure 3). Robbins Mill Stream and Rome Trout Brook are near road crossings and have culverts to continue the respective flows beneath the roads. Both are in areas that have been affected by agriculture and small logging operations. Whittier Brook, situated in the Kennebec Highlands, is undisturbed by human activities because it is protected by the Kennebec Highlands Management Plan (Maine Department of Conservation 2011). Stream health is essential to watershed health and both Robbins Mill Stream and Rome Trout Brook flow directly into Great Pond. Whittier Brook feeds into Whittier pond, which empties into Long. Below are short introductions to the studies conducted on the three streams as written by each research team, and a visual representation of how the projects are connected (Figure 4).

**Figure 1.3:** *Geographic context of our research. Red points show exact locations of research sites at three Belgrade Lakes low order streams.*

### 1.3.2 Sediment and Hydrology Team

We set out to answer four key questions about headwater streams in the Belgrade Lakes Watershed: 1) How does the morphology and incidence of humans affect stream channel hydrology? 2) How does the presence of road crossings affect the sediment load? 3) How will the presence of roads affect substrate composition? 4) How does sediment sorting near noted features of erosion compare to the sediment composition of the entire stream?"

We have found that the hydrology of Whittier brook is different from the other two, most likely due to its boulder-bed geology, as opposed to the

sand/gravel-bed geologic context of Rome Trout Brook and Robins Mill Stream. We found the sediment load to be highest in the lower reach of Rome Trout Brook, where there are several noted points of erosion from the road crossing. Robins Mill also had significantly higher sediment loads than Whittier, confirming that road crossings are associated with higher concentrations of suspended solids. Substrate composition was linked to road crossings. Our impacted streams had significantly greater proportion of silt than Whittier, confirming that the roads contribute to more fine sediment input, usually from erosion. At each noted point of erosion, results varied due to flow rates that control deposition of fine sediments that may be input via erosion. We can conclude that although the relatively unimpacted stream differs hydrologically from the impacted streams, the signature of human impacts from road crossings is seen with sediment deposition and suspended sediment loads due to erosional input of silts.

### 1.3.3 Organic Matter Team

Organic matter is important because it comprises the lowest level of the food web in streams and provides habitat to organisms. In order for the organic matter to be used, it must be retained. Streams that fail to retain organic matter consistently are energy-impaired and may have issues with excess nutrients being exported downstream. Organic matter, containing carbon, and nutrients is added to the

stream and used by stream biota through decomposition. Thus, we studied the

retentiveness and organic matter decomposition rates at the Belgrades. Through this

we hoped to see whether streams with more human impacts had altered

decompositional rates or decreased retention. We hypothesized that urbanized

streams would have less retention due to less organic matter in the riparian zones

and that decomposition would be higher in more urban streams due to higher

nutrient levels.


### 1.3.4 Nutrient Spiraling Team

Nutrients, such as phosphorous (P) and nitrogen (N), are chemical elements

that are common limiting nutrients in aquatic systems. N and P are taken up and

recycled by living things in freshwater environments. In ponds and lakes, nutrient

cycling is conceptualized as a circular pattern where nutrients in the water are taken

up and remineralized by organisms in the ecosystem. However, streams have

another dimension: flow. Nutrients are cycled while simultaneously moving

downstream, creating a theoretical "spiral" (Webster & Patten 1979). Nutrient

spiraling is particularly important for understanding headwater streams because

these streams help control the amount of nutrients exported to lakes by processing

and using nutrients in streams (Peterson *et al*. 2001; Hall *et al*. 2002). The goal of our

research was to determine how far nutrients travel downstream (i.e. uptake length),

and whether road crossings influence how quickly nutrients are used in the environment. Our hypothesis was that impacted streams would have longer uptake lengths due to nutrient loading and the presence of culverts, which may facilitate nutrient transport downstream and inhibit uptake of nutrients by bacteria and algae.

Our results indicate that impacted streams do, in fact, have longer nutrient uptake lengths, as well as higher overall background concentrations of nutrients. Sediment uptake experiments show that streambed composition is affected by road crossing, altering nutrient uptake capability. These results present vital information for homeowners and others associated with the Belgrade watershed, and help to understand the relationship of anthropogenic land development and water quality.

### 1.3.5 Storms and Fluxes Team

We looked at how different patterns of precipitation cause changes in nutrient fluxes within streams and the effects of anthropogenic influence. Fluxes simply refer to the changes in nutrient levels moving in the stream. We specifically examined how water moves from where precipitation touches land to where it gathers in the stream, and how differing stream characteristics influence the discharge and flux peaks for various nutrients. We expected that the Whittier stream would be slowest to respond to precipitation with increased discharge, and we still believe that to be the case despite confounding results. We also looked into

*E. coli* which was at safe levels in all three streams. As expected, *E. coli* levels were somewhat higher in the anthropogenically influenced streams than in the reference stream. Nutrient fluxes following storms provide us with a tool to examine the influence of anthropogenic inputs in a way that cannot be assessed during basal flow periods.

**1.3.6 Riparian Zone Team**

The goal of our study was to create a general characterization of riparian vegetation and environmental factors, such as light intensity, soil moisture and organic matter. The riparian zone is an essential component of the stream ecosystem, as it provides numerous services ranging from nutrient input regulation and stream bank morphology maintenance. Due to its stream-side location and rich biological diversity, the riparian zone is often extremely vulnerable to anthropogenic impacts. Such impacts have many implications for the future water quality of their corresponding streams, as well as the larger downstream bodies of water. By providing a baseline description of biotic and abiotic factors in the riparian zone of three streams within the Belgrade Lakes region, future studies regarding the effects of anthropogenic development will have the opportunity to generate comparisons over longer time periods.

### 1.3.7 Biotic Indices Team

Macroinvertebrates are a major component of stream communities across ecosystems and play an essential role in nutrient cycling. A macroinvertebrate is defined as an organism without a backbone that is greater than 500 microns in size (Hauer & Lamberti 1996). Benthic invertebrates serve as indicators of stream health, thus measures of species diversity, composition and behavior are important when evaluating a stream ecosystem. Biotic indices and measures of richness and diversity can be used to characterize the macroinvertebrate community in relation to overall stream condition. We characterized the structure and diversity of macroinvertebrate communities in our three study streams in the Belgrade Lakes watershed. We also investigated how other stream measures, such as dissolved oxygen or canopy cover, correlate with biotic indices. We anticipated that healthier streams, ones with low anthropogenic impact, would support a higher level of macroinvertebrate diversity and register a lower Hilsenhoff Biotic Index (HBI) value. Additionally, macroinvertebrates serve as a good education tool and provide a tangible means of supporting conservation initiatives in aquatic habitats.

### 1.3.8 Pharmaceuticals Team

Pharmaceuticals can be defined as any chemical substance that contains a single pharmacologically active ingredient, any substance that causes therapeutic

effects as well as the side effects of a drug, intended for human or veterinary medical treatment or disease prevention. The issues surrounding the presence of pharmaceuticals in the environment is becoming an increasingly explored topic in the scientific community, and is one that raises questions regarding the potential impacts of these compounds on the ecosystems and human communities that draw from these environments. It was therefore the purpose of our research to examine these impacts in the freshwater streams of the nearby Belgrade lakes watershed. While we did not test for the presence or absence of pharmaceuticals in these streams, we sought to understand which pharmaceuticals generate the greatest effect on the microbial communities that inhabit them, as their role in the ecosystem is a pivotal one. In addition, we sought to determine if pharmaceutical impacts on microbes were a function of stream proximity to anthropogenic influence, as well as whether or not the effects of a cumulative mixture of pharmaceuticals in the environment are additive. From these research questions we hypothesized that antibiotics would have the most detrimental impact on microbes, that the effects of a pharmaceutical mixture in the environment would be additive, and that those streams more closely located to human influence would yield the highest levels of impact on these bacterial communities due to existing stress on the microbial community.

**1.3.9 Conservation Lessons and Community Outreach Team**

Scientific research must be properly communicated to the local actors where the investigation is taking place in order to keep them informed and maintain a dialogue about conservation. The conservation lessons team coordinated ways in which to make our research available to local community members. Our work mainly focused on conveying to the Belgrade community how important headwater stream health is to the health of the Belgrade Lakes. We held a stream ecology field trip at the Maine Lakes Resource Center, for a group of over 80 6th grade students. They were invited to participate in an interactive day of learning not only about stream ecology but also ways in which humans can decrease their impact on stream. We also created a short documentary that presents the importance of conserving headwater streams in Belgrade. In this video we interviewed members of the BRCA, Maine Lakes Society, as well as a local independent wetland scientist in order to gather the perspectives of local conservation actors and share them with the larger community.

**1.3.10 Team Connections**

Stream ecosystems are complex and naturally, our research foci are interconnected. Below is a diagram that illustrates the many aspects of stream health and how they are related to one another. As you will see in this report, these

connections will make themselves visible. We encourage you to periodically return

to this visual to better understand these connections.
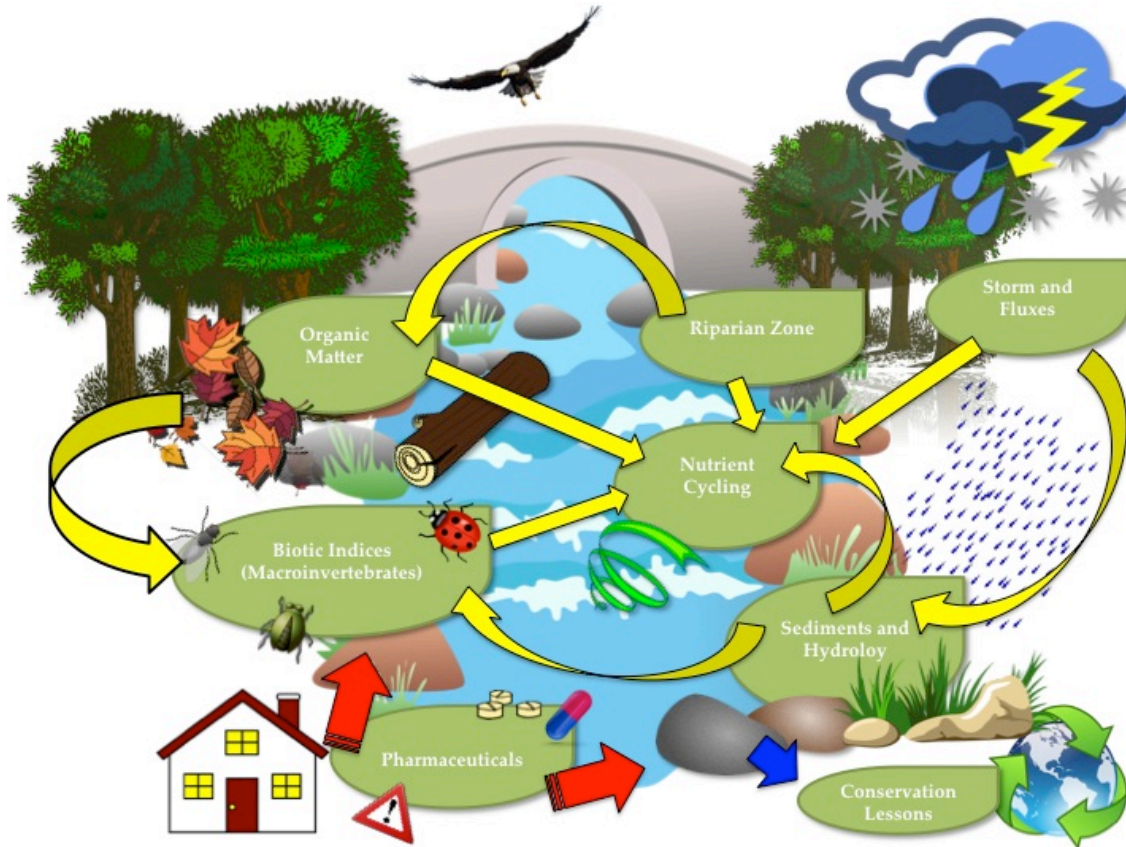


**Figure 1.4.** *Animated connections among the different research teams.*

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# CHAPTER 2
# SEDIMENT AND HYDROLOGY

## 2.1 Introduction

### 2.1.1 Physical Structure Of Streams

### 2.1.1.1 Hydrology

Headwater streams exhibit all the complex aquatic biogeochemical processes that are found in lentic environments (i.e. lakes and ponds), but with the added variable of water flow. It is important to understand and accurately measure stream hydrology as a component of assessing the condition of a stream ecosystem (Statzner *et al*. 1988). These lotic systems can be defined by many parameters, the most basic of which is stream discharge (Harrelson *et al.* 1994). This measurement, expressed in volume per time, along with more complex parameters, like particle retention, that are key to characterizing a stream. Important hydrology parameters change predictably when moving from small headwater streams to larger rivers (Figure 2.1). Some fundamental hydrologic features are pools and riffles. Riffles are shallow areas with a high flow velocity, and the sediment is usually mixed gravel and cobbles. Pools are deeper areas with slow flow and finer sediment particles (Figure 3).
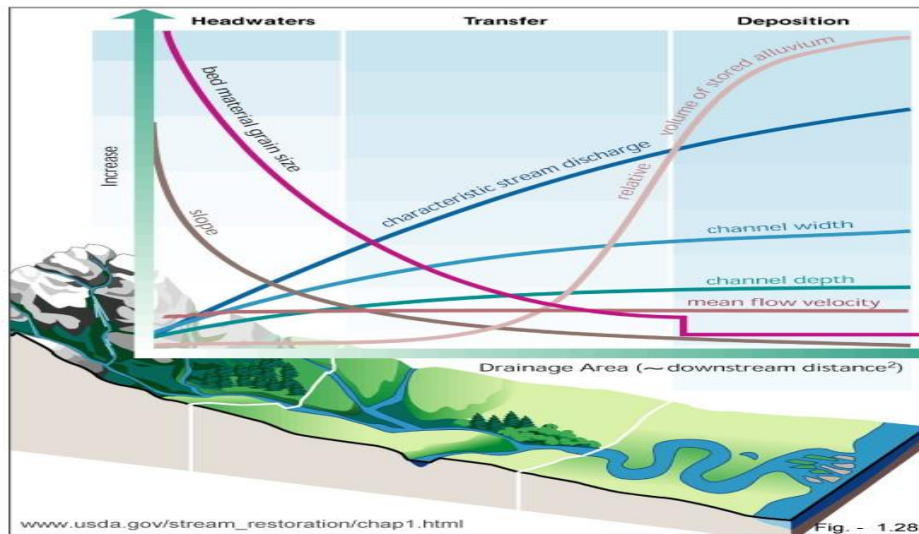
**Figure 2.1** *Conceptual diagram of headwater streams and seven key parameters of geomorphology as they typically to change downstream.*

Hydrology is primarily determined first by rainfall, and can be heavily influenced groundwater exchange, slope and geology. Hydrology is also highly susceptible to climate change (Campbell *et al.* 2011; Curran & Cannatelli 2014). Flow regimes of headwater streams in the Northeast have been altered by changes in rainfall patterns (Navrátil *et al.* 2010; Campbell *et al.* 2011; Gupta *et al.* 2011) and land conversion, particularly deforestation (Kalantari *et al.* 2014b). These studies show drastic changes in hydrographs and volume of peak discharge in recent years.

### 2.1.1.2 Sediment

Streams are also influenced by sediment, which generally is classified as being suspended in the water (suspended sediment), or as part of the benthic substrate. The interactions between stream flow and channel sediment plays a role

in determining the health of the stream (Gordon *et al.* 1992; Allan *et al.* 2007).

Streambed material is transported downstream when the flow velocity is strong

enough to move a specific particle size. The critical erosion velocity is the lowest

speed at which sediment of a specific size can be moved. Sand has a critical erosion

velocity of 0.2 ms$^{-1}$, while coarse gravel has critical erosion velocity of 1 ms$^{-1}$.

Particles smaller than sand, like silt and clay, have increased cohesion and require a

higher critical erosion velocity to transport downstream (Table 2.1; Allen *et al*. 2007).

**Table 2.1** *Sediment particle grain sizes in millimeters and their respective categories. Larger particles generally have a higher critical erosion velocity.*

| Category | Particle Size (mm) |
|---|---|
| Boulder | >256 |
| Cobble | 63-256 |
| Gravel | 2-64 |
| Sand | 0.0625-2 |
| Silt | <0.0625 |
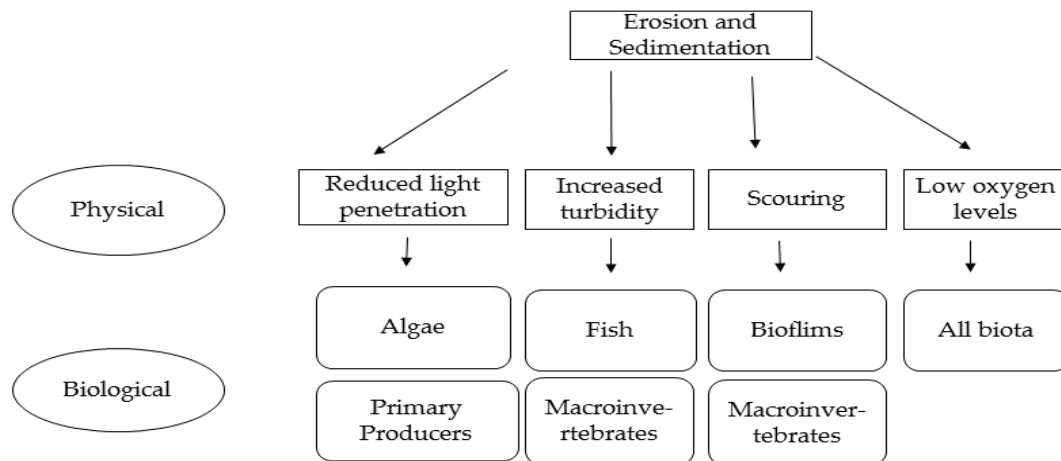


**Figure 2.2** *Conceptual model of stream erosion effects on physical factors and biological factors in the aquatic ecosystem.*

The suspended sediment or diffuse load, moves through the stream reach without settling. Suspended sediment in the stream channel reduces incoming light and increases turbidity and scouring, which affects stream ecosystem processes, such as primary production (Allan *et al.* 2007). Sediment is important to the health of streams and the surrounding watershed because it influences the channel dynamics as well as the quality of habitat for biotic organisms (Figure 2.2; Allan *et al.* 2007).

**2.1.2 Natural Forces Effecting Streams**

**2.1.2.1 Changes in Geomorphology**

A stream typically has a regular width and distance between pools and riffles as well as a meander shape which forms a dynamic equilibrium. This ideal morphology and energy in a stream is used as a reference for streams impacted by human activity (Dodds & Whiles 2010).  Interactions between water and sediment from the pool-riffle sequence (Figure 2.3) are common in medium gravel bed streams. (Lofthouse & Robert 2008; MacVicar & Roy 2011). Undercuts and oxbows are sources of morphological change due to erosion, and can lead to channels cutting off bends and straightening (Morisawa 1968).This riffle-pool sequence is formed by sediment particle sorting of different size classifications (Allan *et al.* 2007). Step-pool streams are also constantly changing despite their boulder and bedrock substrates.

Pools represent slow geomorphological change, and decreasing gradient (Chin 1998).

## 2.1.2.2 Woody Debris

Woody debris play an important role in channel morphology because they form habitat, pools, and catch sediment (Wallerstein & Thorne 2004). Ryan *et al*. (2014) observed the volume of sediment behind woody debris steps was an order of magnitude higher than the volume exported. When sediment was further explored, they observed woody debris features were more effective at storing coarse sediment and usually did not trap fine sediment. These obstacles and others play important roles in changing flow and sediment characteristics.
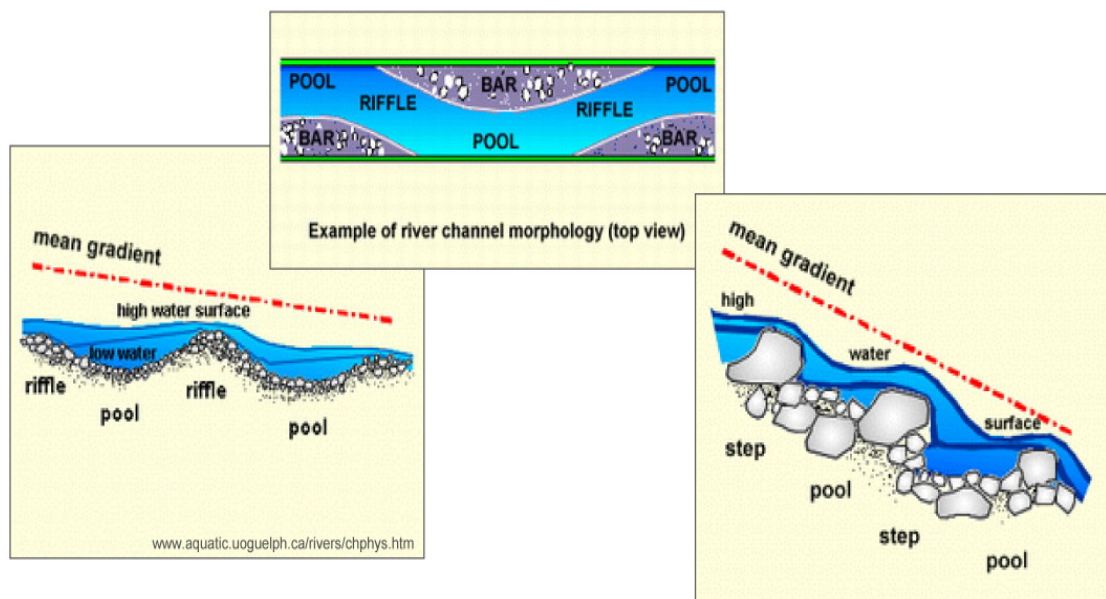


**Figure 2.3** *Conceptual diagram of pool-riffle sequences (left), these with a meander (center) and step-pool sequence (right).*

### 2.1.2.3 Beaver Dams

Beaver dams can often change channels by increasing silt deposition on riparian floodplains, and vastly expanding ponds and wetlands (Stefan & Klein 2004; Westbrook *et al*. 2013). Perhaps the most important and fundamental action of beaver dams is the trapping of sediment – especially fine grain sediment – upstream of them. Downstream of existing dams, fine sediment is less prevalent but is sporadically deposited when dams breach (Pollock *et al.* 2007; Levine & Meyer 2014). By re-introducing beavers to a watershed, conservationists expect to see increased channel stability, more fine grained particle retention within reaches, and increases in wetland, stream, and riparian life (Curran & Cannatelli 2014).

### 2.1.3 Human Impacts On Streams

Human development is linked to recent changes in stream morphology. The most dramatic of these impacts is dams. Even small dams have large impacts on the sediment regime, nutrient load, salinity, flooding, and erosion regimes of rivers (Skalak & Pizzuto 2005; Merritts *et al*. 2011). However, the negative effect of dams is highly controversial. For instance, a recent study showed that there is no significant difference in sediment loads upstream versus downstream of dams (Csiki & Rhoads 2014).

Sediment is also heavily dependent on erosion. In 1998, an estimated 13% of all rivers and 40% of impaired rivers were subject to heavy sedimentation (Allan *et al.* 2007). Sediment in streams often comes from banks and runoff, but has been proven to be reduced with increased riparian bank vegetation.

*Land-use*

Expansion of human population has led to land conversion and there are growing concerns about its effect on stream ecosystems (Jones *et al.* 2001). Changes in land use have a large effect on stream degradation and biota such as macroinvertebrates (Villeneuve *et. al* 2014). Generally, land use has changed from forested to agriculture, logging plots and urban areas.

Urbanization near streams causes pollution to move slowly downstream and increased peak flows compared to in forested areas (Dere *et al.* 2006). Additionally, streams near higher percentages of urban areas had larger peak discharges than near similar percentages of undeveloped land (Poff *et al.* 2006). Agriculture still altered stream systems because it reduces bank vegetation, and cause erosion. Gross *et al.* (2014) found forest fragments near stream banks can reduce the impacts of agriculture on streams. In logging areas, noticeable increases in diffuse sediment load causes eutrophication and higher turbidity (Ahtiainen & Huttunen 1999; Burns 1970). Roads are used in agriculture, logging, and urban areas and are ubiquitous in many watersheds (Trombulak & Frissell 2000).

**2.1.4 Road-Crossing Impact On Streams**

**2.1.4.1 Culverts**

Many roads cross over streams via culverts, which are typically a steel corrugated pipe buried under the road. They are commonly discussed in the context of fish passage because their high flow velocity can bar anadromous fish from passing upstream (Clark & Kehler 2011). The inlets of these culverts, specifically corrugated steel culverts, have a central thalweg of high velocity, surrounded by slower jets of water and recirculation zones (Hunt *et al.* 2012). This serves to make culverts – and thus road crossings – difficult for fish and other organism to cross, effectively fragmenting the stream habitat. On the outlet side of a culvert, there is often deep scouring. This is caused by fast flowing jets within the culvert (Day *et al.* 2001), which vary according to upstream blockage. Debris from the road crossing above a culvert can partially block culverts, affecting the location and depth of the tailwater scouring (Sorourian *et al*. 2014). Culverts also face the problem of being overwhelmed and damaged by large positive fluxes in discharge. Recently, a modeling study demonstrated that a culvert could be insufficient to deal with the amount of predicted positive flux caused by larger and more frequent storm events induced by climate change. This is especially relevant to Maine and New England where weather records already indicate climate change is increasing rainfall and leading to increases in peak discharge for many headwater streams (Navratil *et al.*

2010; Campbell *et al.* 2011; Gupta *et al.* 2011). This underscores the potential damage

of culverts given our current management strategies.


**2.1.4.2 Runoff and Erosion**

Road crossings often cause erosion and subsequent hydrologic effects.

(Forman & Alexander 1998; Paul *et al.* 2001). Sediment inputs that are associated

with roads are due to road surfaces, ditches, bridges and culverts (Forman &

Alexander 1998). Evidence shows that roads are linked to higher peak flow (Jones *et al*. 2000; Foreman & Alexander 1998) and erosional inputs in streams. Road

construction can be especially damaging to streams as illustrated in a study on road

crossings in the Italian Alps showed that the impacted stream had a sediment load

of 116 $T/km^{-2}yr^{-1}$(of catchment area) per year, as opposed to the 14 tons in the

unimpacted streams (Pelacani *et al.* 2010). The proportion of impervious surfaces

showed positive correlation with degradation of macroinvertebrate communities,

fish communities and the stream channel (Walsh *et al*. 2001). With this past research

in mind, we decided to investigate the direct impacts of road crossings on

headwater stream hydrology and geomorphology.

**2.1.5 Research Questions and Hypotheses**

We studied three headwater streams in the Belgrade Lakes watershed. Two

streams, Robins Mill Stream and Rome Trout Brook, were impacted by road

crossings. Whittier Brook acted as our unimpacted reference stream. Our main

objective in this study was to characterize the flow regimes and the sediment

composition of each of the stream reaches. We explored four research questions. 1)

How does the morphology and incidence of human land-use affect stream channel

hydrology? 2) How does the presence of road crossings affect the sediment load? 3)

How will the presence of roads affect benthic substrate composition? And, 4) How

does sediment sorting near noted features of erosion compare to the sediment

composition of the entire stream? We hypothesize that finer particles will be more

prevalent in impacted streams, particularly in proximity to the roads, while

hydrology will vary based on stream morphology.

**2.2 Methods**

**2.2.1 Characterization Of Channel Width, Depth, And Flow**

At each 100m stream reach we set up transects every 10m across the stream,

perpendicular to the main channel. At each transect we measured the stream width

using a transect line, depth every 0.5m with a meter stick, and flow every 0.5m using

a flow meter (Marsh McBirney). Discharge is calculated as Q=depth(m) x width(m) x flow(ms$^{-1}$).

## 2.2.2 Bed Sediment Characterization

At each transect in each stream reach we took three approximately  sediment core (top 5 cm) samples from the left, the middle, and the right side of the stream channel as well as from noted morphological features such as distinct pools or sand bars. We used an automatic sieving machine to separate the samples into eleven grain sizes ranging from >=1.41mm to <0.0625mm in diameter and recorded their mass. These grain sizes were then aggregated to categories of gravel (>=1.41mm) sand (1.41-0.0625mm) or silt (<0.0625mm).

## 2.2.3 Suspended Solids

At each stream we collected two 1L water samples from the top of the reach, from the middle of the reach, and from the lower end of the reach. We vacuum filtered the samples through pre-ashed glass fiber filters and dried them.  We then weighed each filter to find the increase in mass, over the average initial filter mass, to measure the mass of inorganic solids per liter of stream water.

**2.2.4 Erosion Identification**

For each stream reach we stood at the bank at each transect and sketched points of erosion. We looked for washouts, oxbows, undercuts, and major sources of erosion. We used ArcGIS 10.2 to map the stream reaches and points of erosion.

**2.2.5 Statistical Analysis**

We converted sediment masses to proportions for each core, and aggregated them by stream or transect as needed. Using Stata, we then compared suspended solids and grain sizes using ANOVAs and Scheffe multiple comparison tests. We used a significance level of $\alpha$=0.05. Graphical figures were made using Sigma Plot and Microsoft Excel.

**2.3 Results**

**2.3.1 Hydrology**

Stream width, depth, and flow measurements varied at each transect for Robbins Mill Stream, Rome Trout Brook and Whittier Brook. We graphed three transects from each stream as a representation of the changes in measurements in the 100 meter reach (Figures 2.4 to 2.6). The location of each transects thalweg relative to the middle of the stream varied between all transects, as did maximum

channel depth and width. In Whittier Brook the depth profile varies more than the

other two streams across each transect illustrating its boulder-bed morphology.
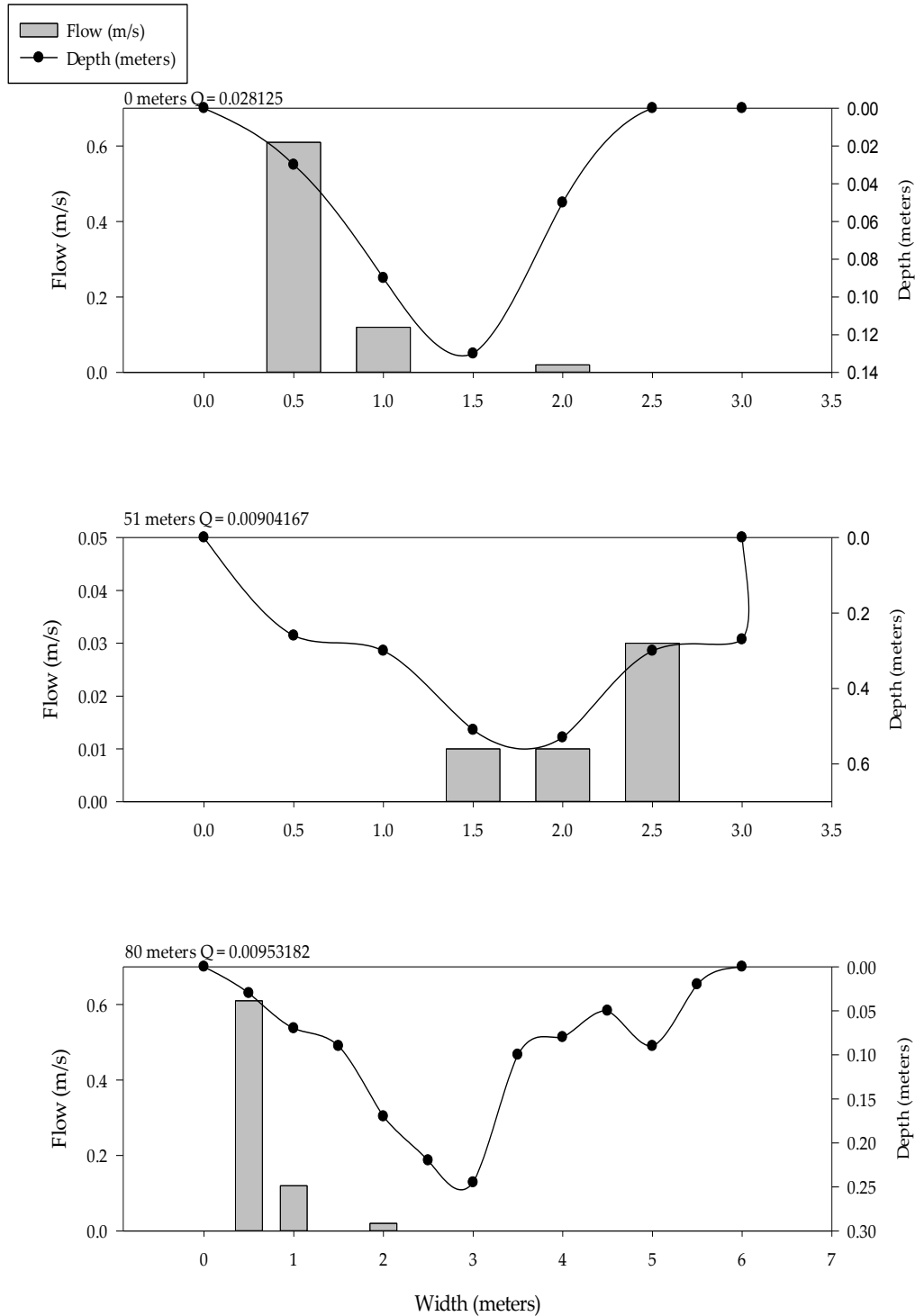


**Figure 2.4** *Stream depth (m) and flow (m/s) at 0 meters, 51 meters and 80 meters at Robbins Mill Stream. Discharge: Q=L s⁻¹*
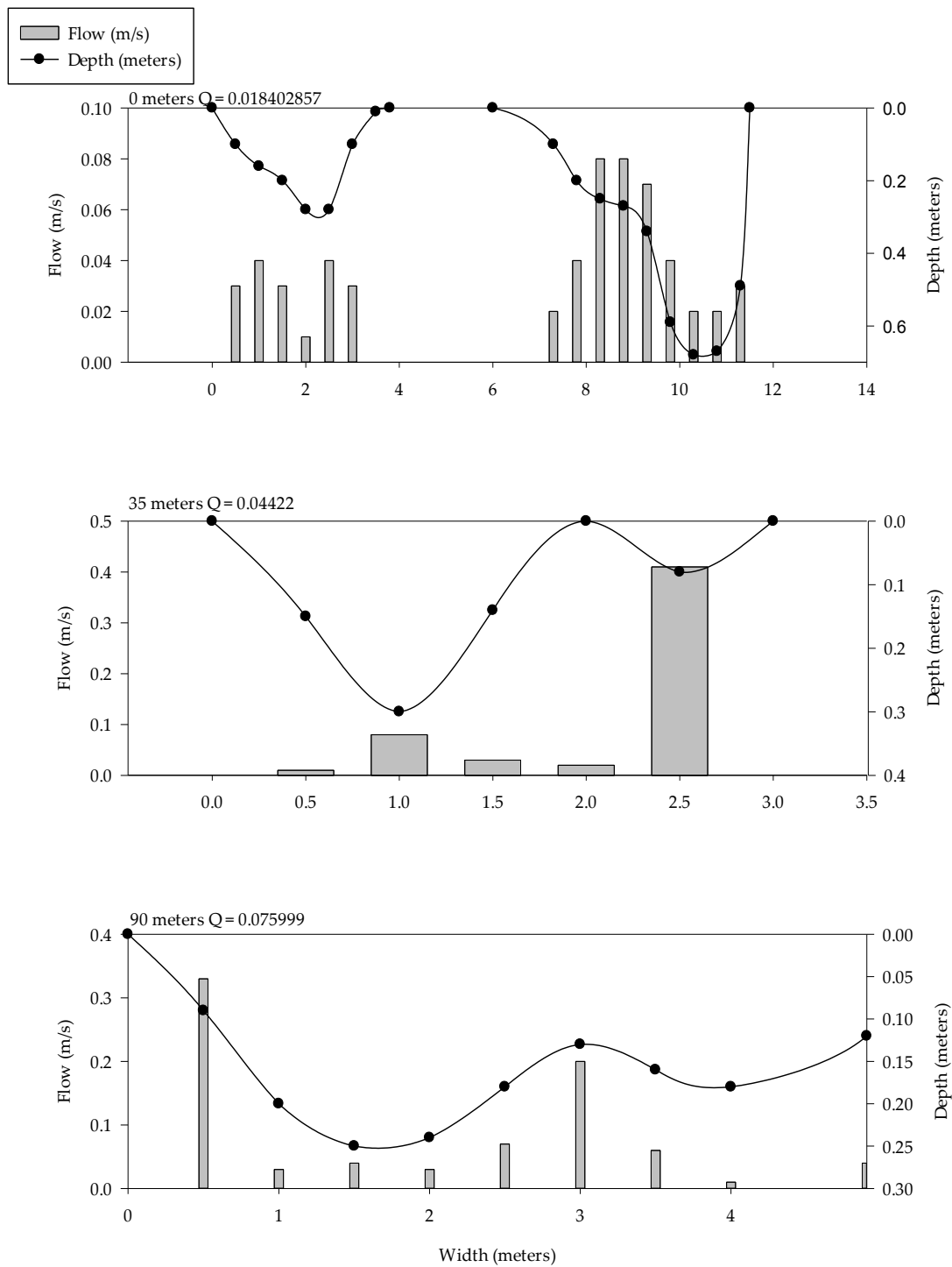
**Figure 2.5** *Stream depth (m) and flow (m/s) at 0 meters, 35 meters, and 90 meters at Rome Trout Brook. Discharge: Q=L s$^{-1}$*
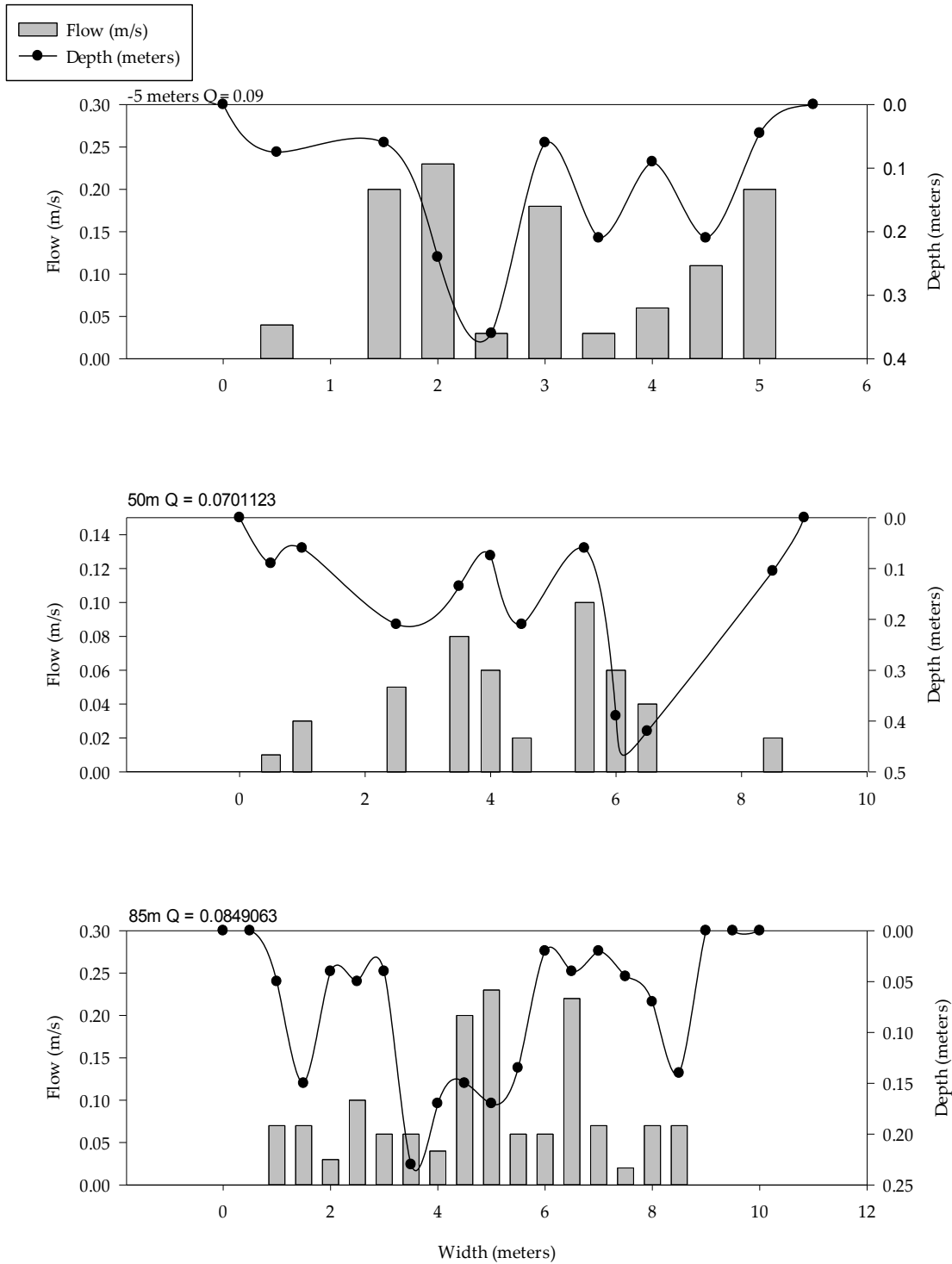
**Figure 2.6** *Stream depth (m) and flow (m/s) at -5 meters, 50 meters and 85 meters at Whittier Stream.*
*Discharge: Q=L s⁻¹*

The average discharge calculated from the Marsh Mcbirney flow meter was different for each stream. Whittier Brook had the highest average discharge of 71.2 L s$^{-1}$ followed by Rome Trout Brook (52.8 L s$^{-1}$) and Robbins Mill Stream (28.8 L s$^{-1}$) with the lowest discharge (Table 2.2).

**Table 2.2**. *Discharge rates for each stream measured with the cross- sectional method and a Marsh McBirney flow meter.*

| Stream | Discharge (L/s$^{-1}$) |
|---|---|
| Robbins Mill Stream | 28.8 |
| Rome Trout Brook | 52.8 |
| Whittier Brook | 71.2 |

**2.3.2 Suspended Solids**

Suspended solids were significantly variable with and among streams (Scheffe, $p < 0.05$). Rome Trout Brook's lower reach sample (downstream of the road) had the highest concentration of sediment load at 25.88 mg/L$^{-1}$ (Table 2.3). This sample was significantly higher than the Rome Trout Brook mid reach sample (Scheffe, $p = 0.0164$). There was a significant trend of increasing load downstream with the exception of the decrease from mid to lower reach in Robbins Mill Stream (Scheffe, $p < 0.05$).

**Table 2.3**. *Suspended solids from the upper reach, middle reach, and lower reach of Whittier Brook, Rome Trout Brook and Robbins Mill Stream.*

| Stream | Zone | Suspended Solids (mg/L$^{-1}$) |
|---|---|---|
| Whittier Brook | Upper | 0.380 |
| | Middle | 0.980 |
| | Lower | 2.38 |
| Rome Trout Brook | Upper | 1.58 |
| | Middle | 9.48 |
| | Lower | **25.88** |
| Robbins Mill Stream | Upper | 2.00 |
| | Middle | **6.90** |
| | Lower | **5.50** |

## 2.3.3 Noted Points of Erosion

### 2.3.3.1 Robbins Mill Stream

Below we describe the composition of sediment cores near specific points of erosion in Rome Trout Brook and Robbins Mill Stream. There were no points of erosion at Whittier Brook (Figure 2.11).

We identified a sediment deposit on the right side of transect 20, just before the culvert. The sediment core here had a significantly higher gravel proportion than the stream average (Scheffe, $p<0.0001$). The proportion of sand was not significantly different (Scheffe, $p=0.39228$) and there was significantly less silt than the stream average (Scheffe, $p=0.010503$).

The sediment samples from the right and left side of transect 40 were below noted bank erosion on either side of the culvert. The left side sample had significantly less gravel than the stream average (Scheffe, $p=0.031837$). There was a

significantly higher proportion of sand (Scheffe, p<0.0001) and a lower proportion of silt (Scheffe, p=0.006429). The gravel in the right side of transect 40 did not vary significantly from the stream average of gravel, but there was significantly more sand and significantly less silt (Scheffe, p<0.0001; p=0.003284).

The core from the sediment deposit at 80 meters didn't have a significantly different proportion of gravel from the stream average. There was significantly more sand and silt at the sediment deposit (Scheffe, p<0.0001). See Figure 2.7 for noted points of erosion along stream reach.

### 2.3.3.2 Rome Trout Brook

At 0 meters there we found a large sediment deposit on the right side of the stream which had significantly less gravel and silt (Scheffe, p<0.0001) than the stream average. There was no significant difference for sand.

The prominent feature at the 10 meter transect is the dam before the culvert. Along the dam on the left side there is a sediment deposit. The sample taken here was significantly lower in gravel (Scheffe, p<0.0001). There was no significant difference between sand and silt proportions and the stream average.

We noted a grassy, eroded slope on the right bank of transect 35. A sediment sample from here had no significant difference from the stream average for gravel. However, there was significantly more sand and silt (Scheffe, p<0.0001).

There was an undercut in the left bank near 80 meters. Compared to the stream average our sample here had significantly more gravel and less silt (Scheffe, p<0.0001). There was no significant difference in proportion of sand.

Transect 90 featured an approximately 3 meter undercut on the right side. We looked at the samples here and in the middle of the stream. The sediment sample from the right side was not significantly different for gravel but had significantly less sand and silt (Scheffe, p<0.0001). The sediment sample from the middle of transect 90 also had no significant difference for gravel but had significantly less sand and silt (Scheffe, p<0.0001). See figure 2.9 for noted points of erosion along stream reach.

### 2.3.4 Comparison Between Transects

The following section highlights only a subset of data to illustrate key trends. Transect numbers upstream to downstream starting with 0 meters. For all sediment proportions see Figures 2.8, 2.10 and 2.12.

### 2.3.4.1 Robbins Mill Stream

Silt proportion in Robbins Mill Stream differed significantly between all transects (Scheffe, p<0.05). For example, 20 meters was significantly higher than 0 meters and 10 meters (Scheffe, p=.00322; p=.018976). Silt at 80 meters was significantly greater than 20 meters (Scheffe, p=.009411). Sand proportion was significantly higher at transect 51 than transect 40 (Scheffe Test, p<0.0001). Gravel

proportion showed varying significance. Transect 40 had a significantly lower proportion of gravel than at 20 meters (Scheffe, $p < 0.0001$) and at 0 meters (Scheffe, $p < 0.0001$). See Figure 2.7 for map of stream reach.

**2.3.4.2 Rome Trout Brook**

All transects' silt proportions were significantly different from each other (Scheffe, $p < 0.05$), with the exceptions of 70 with 80 and 90, and 90 with 35 and 60.  Silt was highest at 0 meters, and 10 meters. There was significantly more silt at 10 meters than at 100 meters (Scheffe, $p < 0.0001$) likely due to the beaver dam at 10 meters. Transect 35 had a significantly lower proportion of silt than at 100 meters (Scheffe, $p < 0.0001$). Sand in all transects differed significantly from each other with the exceptions of 80 with 35, 60 and 70; 0 with 10 and 100; and 70 with 90.  The proportion at transect 10 was significantly larger than all downstream transects (Scheffe, $p < 0.0001$). The average gravel proportion was significantly higher at 0 than at 10 (Scheffe, $p < 0.0001$); at 70 than at 80 and 90 (Scheffe, $p < 0.0001$ both cases); and at 35 and 60 than at 80 (Scheffe, $p < 0.0001$ both cases).  See figure 2.9 for map of stream reach.

**2.3.4.3 Whittier Brook**

The average silt proportion in Whittier Brook varied significantly between each transect (Scheffe, $p < 0.05$). Silt was largest in the pool at 80, near the mouth of

58

the stream, with a proportion of 2.4%. Sand in Whittier Brook varied significantly

between transects -10, 0, 100 and 110 (Scheffe, p<0.0001) with the lower reach

transects being much higher (Figure 2.12). We also found significance between

transects 20 and 50, 70, 80, 85 and 90 where 20 had the largest proportion of sand

(Figure 12). Significant differences in gravel proportions in Whittier Brook were

found between roughly half of all transect to transect comparisons, with no clear

patterns along the length of the reach. See Figure 2.11 for map of stream reach.


### 2.3.5 Comparison Between Streams

Rome Trout Brook and Robbins Mill Stream have significantly greater

average proportion of silt than Whittier Brook (Scheffe, p<0.0001). Rome Trout

Brook has a significantly greater average proportion of sand than Robbins Mill

(Scheffe, p=0.031273), while Rome Trout Brook and Robbins Mill Stream both have a

significantly higher proportion of sand than Whittier Brook (Scheffe, p<0.0001).

Robbins Mill Stream has significantly larger average proportion of gravel than Rome

Trout Brook (Scheffe, p<0.0001).

# Robbins Mill Erosion
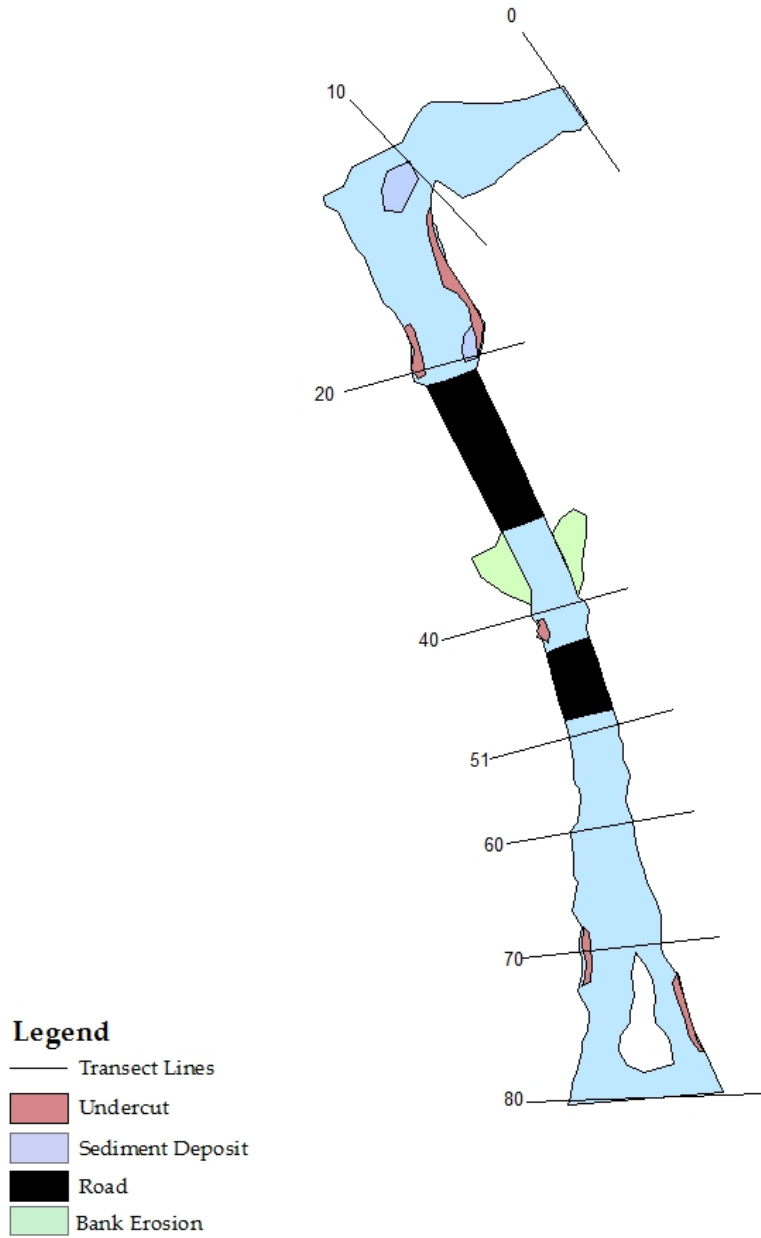


**Figure 2.7** *GIS map of study reach in Robbins Mill Stream.*
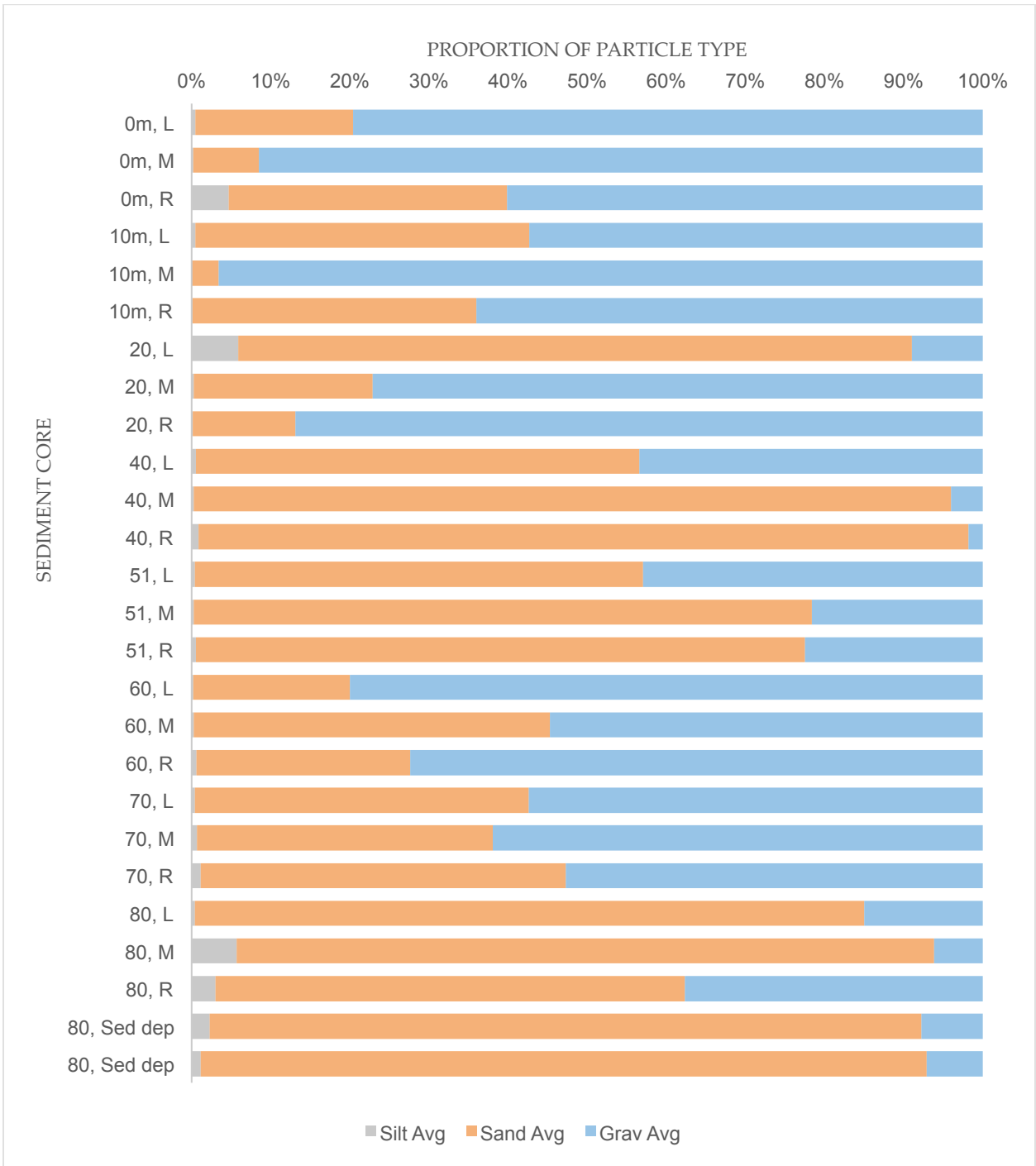
**Figure 2.8** *Stacked bar graph showing the proportion by mass of silt, sand and gravel in Robbins Mill Stream. Silt is defined as particles < 0.0625mm in diameter. Sand is defined as particles 0.0625mm – 1.41mm in diameter. Gravel is defines as particles >1.41mm in diameter. The y-axis specifies sediment core where the number refers to which transect it was taken at, and the letter refers to if it was taken from the left (L), middle (M), or right side (R) of the reach when facing upstream.*

# Rome Trout Erosion



**Legend**

—— Transect Lines
� Undercut
 Sediment Deposit
 Dam
 Road
 Scouring Pool
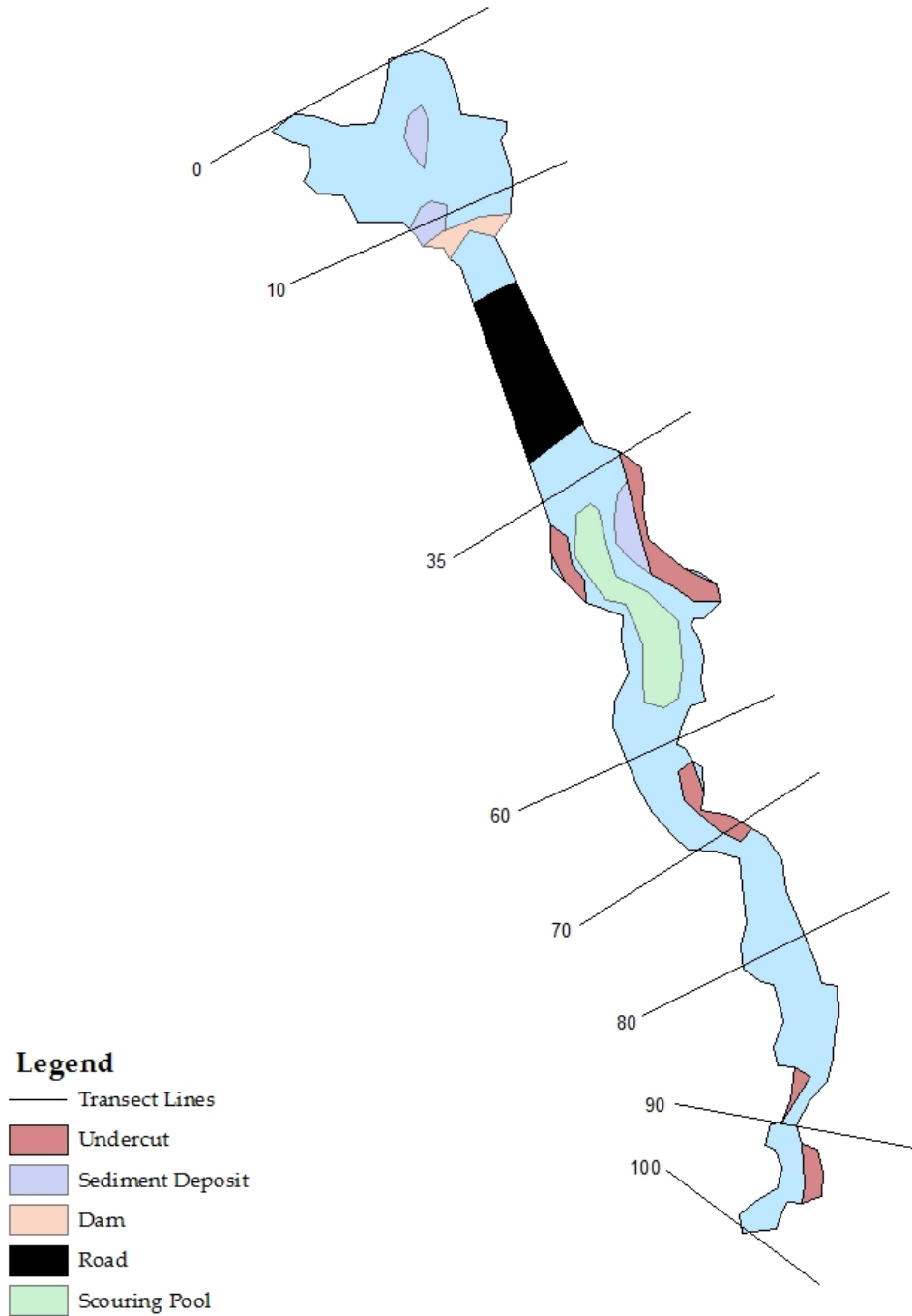
**Figure 2.9** *GIS map of study reach in Rome Trout Brook.*
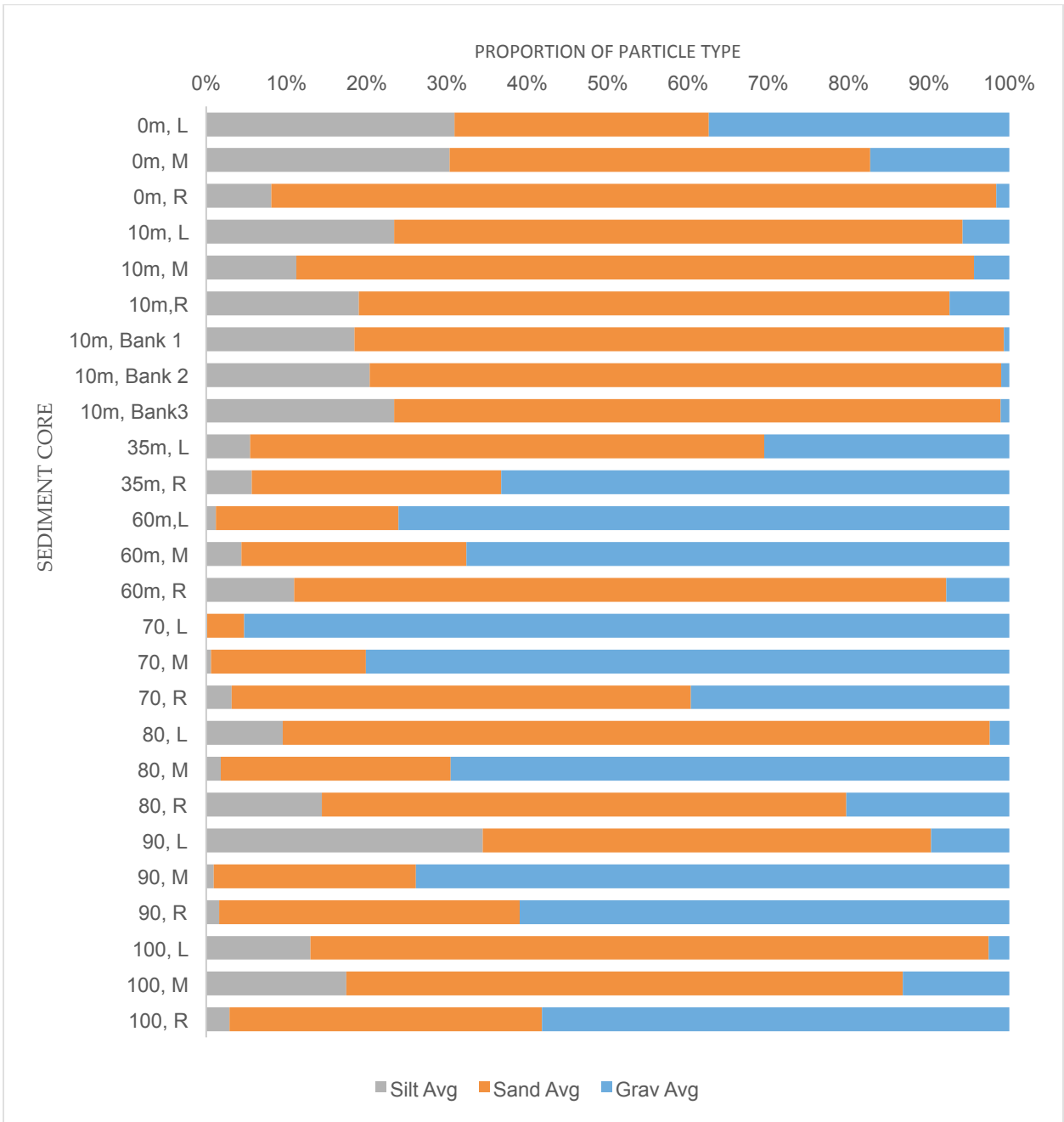
**Figure 2.10** *Stacked bar graph showing the proportion by mass of silt, sand and gravel in Rome Trout Brook.
Silt is defined as particles < 0.0625mm in diameter. Sand is defined as particles 0.0625mm – 1.41mm in
diameter. Gravel is defines as particles >1.41mm in diameter. The y-axis specifies sediment core where the
number refers to which transect it was taken at, and the letter refers to if it was taken from the left (L), middle
(M), or right side (R) of the reach when facing upstream.*
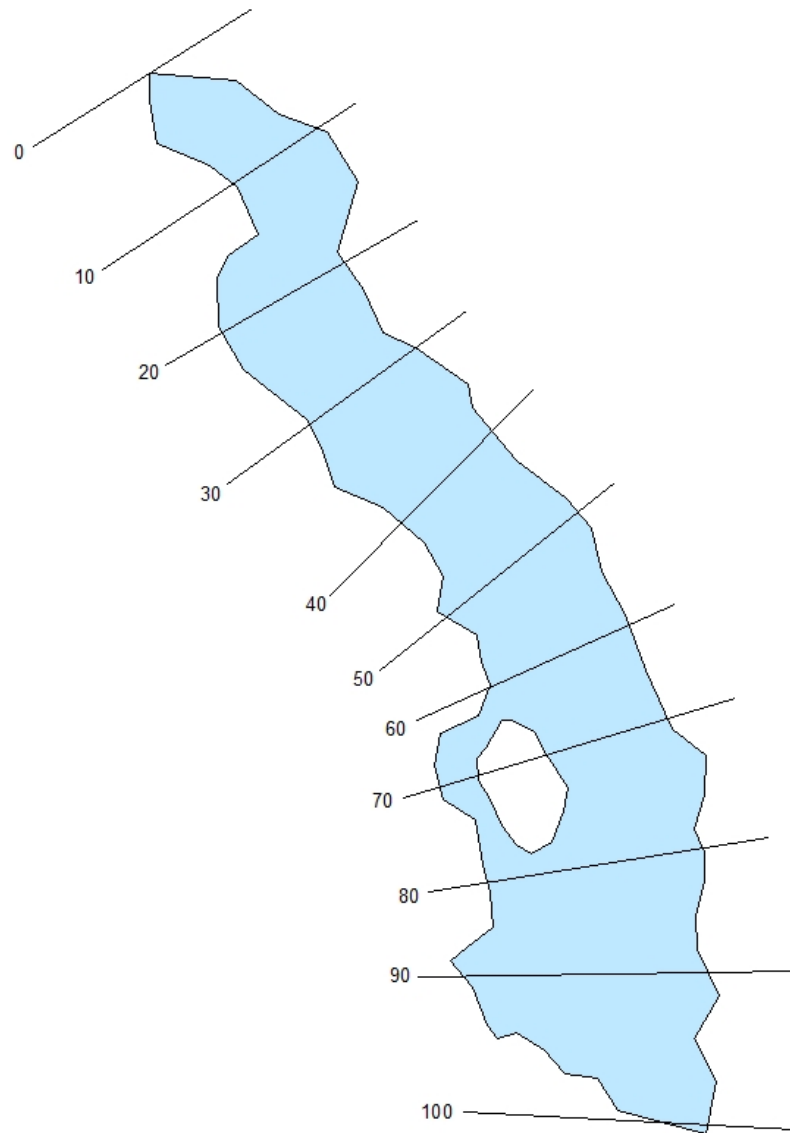
**Figure 2.11** *GIS map of study reach in Whittier Brook.*
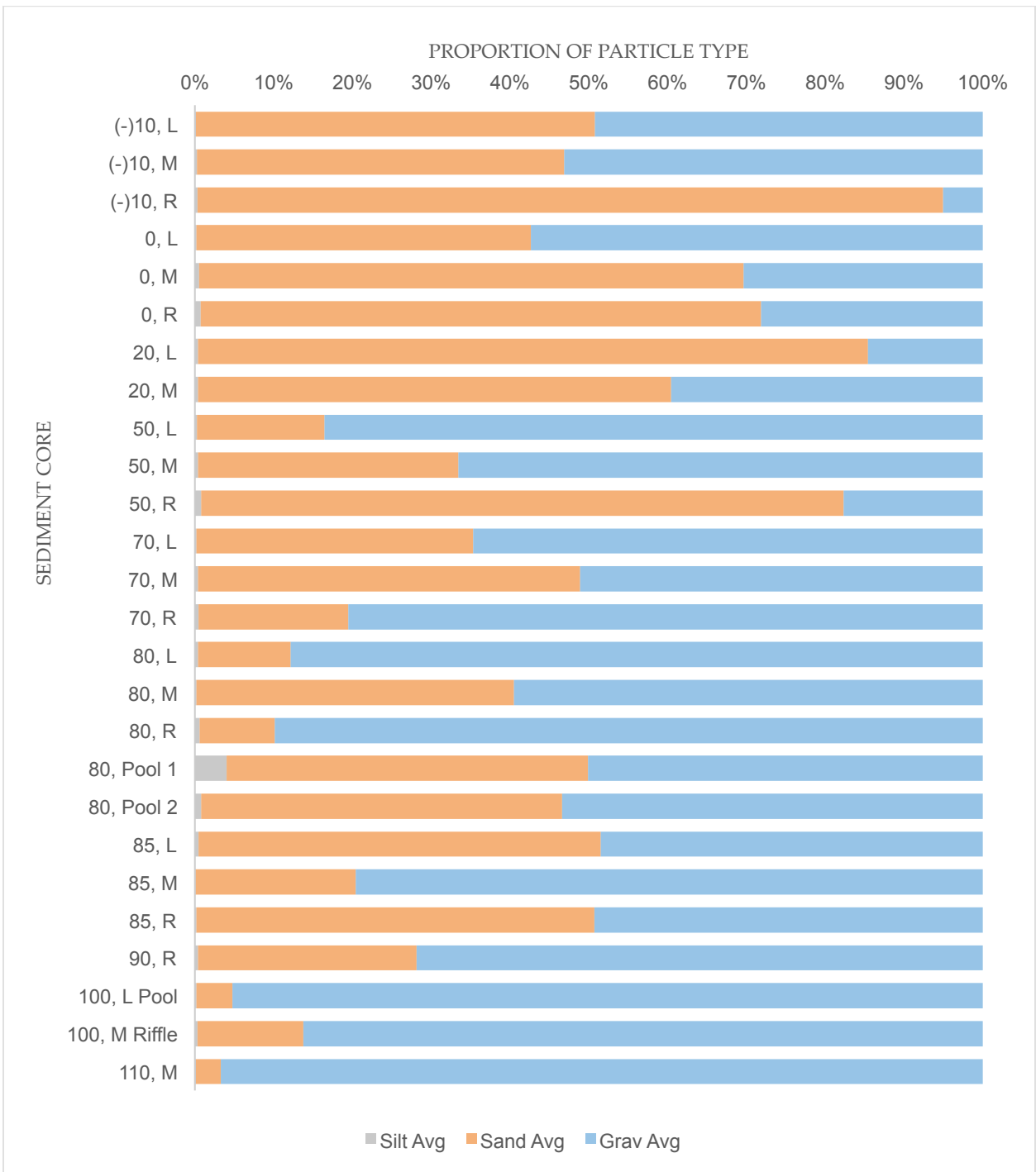
**Figure 2.12** *Stacked bar graph showing the proportion by mass of silt, sand and gravel in Whittier Stream. Silt is defined as particles < 0.0625mm in diameter. Sand is defined as particles 0.0625mm – 1.41mm in diameter. Gravel is defines as particles >1.41mm in diameter. The y-axis specifies sediment core where the number refers to which transect it was taken at, and the letter refers to if it was taken from the left (L), middle (M), or right side (R) of the reach when facing upstream.*

**2.4 Discussion**

**2.4.1 Interpretation of Significant Results**

**2.4.1.1 Hydrology**

Our three streams differ noticeably in hydraulic character. All three have different discharges (Table 2.2), different substrate material (Figure 2.8, 2.10 and 2.12) and different geologic contexts. Whittier Brook is a boulder-bed stream, which mostly flows over bedrock. Rome Trout Brook is a sand-bed stream, and has meander bends in the lower reach, a deep scouring pool immediately downstream of the culvert and the beaver dam immediately upstream of the culvert. Robbins Mill Stream is a gravel-bed stream, and is the simplest of them all, with a meander bend upstream of the culvert, a second unused crossing where an old bridge spans the stream, and a split in the channel around several boulders near the end of the reach. These differences represent compounding variable and may complicate our results regarding road impacts.

**2.4.1.2 Noted Points Of Erosion**

We hypothesized that sediment cores near to observed points of erosion in the streams would have higher relative proportions of silt and fine sediment. However, flow and sediment transport in the stream should be considered. In Robbins Mill Stream at 40 meters our left side core had less gravel, less silt, and

more sand than the stream average. On the right side there was more sand, less silt, but no significant difference in proportion of gravel from the rest of the stream. Since we define sand as anything >0.0625 and <1.41mm, the higher proportions of sand here support our hypothesis and provide evidence that the erosion points are a significant source of fine sediment material for the stream. The sediment deposit at transect 80 had more sand and silt than the stream average, but was not different from the stream average for gravel; further supporting our hypothesis. At the washout bank at transect 20 we found a higher proportion of gravel and a lower proportion of silt. We suspect that the lower proportion of fine sediments here is due to the high rate of flow measured at this transect. This fast current is responsible for the suspension rather than deposition of fine erosional inputs. This is further supported by the significantly higher concentration of suspended solids found downstream (Table 2.3).

In Rome Trout Brook the sediment cores from the sediment deposit on the right side of the 0 meter transect and the left side of the 10 meters transect both showed similar proportion of sand and silt to the stream average and a lower proportion of gravel. Here a beaver dam appears to be blocking fine sediment from being transported downstream, hence the low proportions of gravel. It is unclear whether this difference in proportions may be attributed to our observed erosion points from the road, or to the beaver dam retaining silt and sand (Pollock *et al.*

2007). At transect 35, just below a large washout from the road above, our right side core had higher proportions of sand and silt than the stream average. This is consistent with our hypothesis. Furthermore, this transect had a low flow of 10.7 L/s, supporting our flow-based argument concerning suspension versus deposition of fine particles in areas of fast flow and slow flow, respectively. We noted undercuts on the left bank of the 80 meters transect and the left side of the 90 meter transect, as well as a sediment deposit in the middle of transect 90. The left side 80 meter sediment core had a higher proportion of gravel and a lower proportion of silt. The right and middle samples examined at transect 90 had a lower proportion of sand and silt and a similar proportion of gravel to the stream average. Similar to transect 20 at Robbins Mill Stream we suspect the lower proportion of fine sediment near bank erosion is due to higher flow rate, which is the cause of bank erosion. This is supported by the relatively high (Table 2.2) discharges of 60.8 and 57.5 L/s measured at each transect.

### 2.4.1.3 Comparison Between Transects

Our original hypothesis was that the impacted streams would show higher proportions of finer sediments near the road crossings, and then decreasing proportions of these, and therefore increasing proportions of gravel, down the reach. Meanwhile, we predicted Whittier Brook to have no such trend.

In Robbins Mill Stream we found silt concentrations to be higher at 20 meters, immediately upstream of the road than at 0 meters and 10 meters, as predicted. But there was also greater silt proportion at the end of the reach than at 20 meters. These results indicate that there is an input of silt just above the road, and that this silt is then transported all the way down the reach. As we have already discussed, there is a relatively high (Table 2.2) discharge at 20m, and higher concentration of suspended particulate matter downstream (Table 2.3) where the stream accelerates into the culvert.  This argument can also be applied to our measures of sand and gravel proportions. Sand proportions increased from transect 40 to 51 where the stream emerges from below an old stone bridge and there is more erosional input, further supporting our hypothesis of increasing fine sediment downstream. Inversely, gravel proportions decreased at 40 meters from higher in the reach at transects 20 and 0, which also indicates the increasing proportion of fine sediment down the reach. Given the natural tendency of beaver dams to breach in large storms this could result in a large sediment flux downstream and onto the flow plains (Levine & Meyer 2014).

At Rome Trout we found the proportion of silt was highest at 0 meters and 10 meters and was lower at 35 meters and 100 meters. We would normally expect Rome Trout to have a similar silt pattern to Robbins Mill where much of it is transported downstream, but the dam at 10 meters appears to be retaining most fine

particles. This is common for beaver dams (Stefan & Klein 2004; Levine & Meyer 2014). The average proportion of gravel was higher upstream at 0 meters than 10 meters and higher at 70 meters than at 80 and 90 meters. These results suggest there is a trend of gravel decreasing down the stream reach. It appears that sediment from the road crossing is captured by the dam above the road, and is transported down the reach below the road.

After statistical analysis, in Whittier no clear trend in silt, sand, or gravel proportions was seen down the reach as expected. Therefore, variations are not due to any particular structure in the stream channel.


**2.4.1.4 Comparison Between Streams**

An important question of this study was whether impacted streams had a higher proportion of fine sediment. In comparing sediment between streams, we hypothesized there would be finer sediment in the impacted streams, Robbins Mill and Rome Trout, than in Whittier. Our hypothesis was supported by the average sand and silt data. There was a significantly higher average proportion of sand and silt in both impacted streams than the reference stream. Between our impacted study streams, Rome Trout has a greater proportion of sand than Robbins Mill Stream and Robbins Mill Stream had a larger average proportion of gravel than Rome Trout Brook. The results between impacted streams are consistent with the observed

geomorphology of the streams with Robbins Mill being a gravel-bed stream and Rome Trout as a sand-bed stream. Despite the complicating factor of differing stream types our erosion point data and trends along each reach show there are impacts associated with roads.

**2.4.2 Methods and Sources of Error**

Our methods, while they yielded much useful data, suffered from several potential systematic errors. The flow readings from the Marsh McBirney flow meter were variable and could have contributed to varying discharge between transects down each stream reach. This could be because the meter itself is faulty and needs to be recalibrated. In collecting our sediment samples we were unable to get a uniform amount of sediment at Whittier. Due to boulder-bed morphology it was difficult to get sediment samples. This morphology also contributed to the difficulty we faced in measuring flow and depth throughout the stream reach. We accounted for this error by calculating proportions for sediment grain sizes for each sample. In the process of sorting our sediment samples we consistently lost some sediment from spills and from particles getting caught in the filters. However, average percent error for all samples was 1.3%. Our suspended solids data was also influenced by random error in initial filter mass. This was a problem when we dried our filters, but then needed to subtract their initial mass to find the amount of particulate matter.

Unfortunately, many masses of used filters were less than the average filter mass -

indicating that the glass fiber filters we used vary much more than we expected.

However, we were able to get at least one replicate from each part of each reach with

usable data. A final source of error was the timing of our suspended solids and

hydrology data collection. Since our data collection taken over a period of three

weeks it is possible that small temporal variance could account for some of our

differences. Fortunately there were no large storms between sampling so this effect

should be minimal.


### 2.4.3 Context for Stream Biota

The sediment of stream influences the channel dynamics and the quality of

habitat for biotic organisms. Streambed conditions have important implications for

fish and other biota. Previous literature on the impacts of increased sedimentation

and input of fine particles found increased turbidity which interferes with fish gills

and decreases visibility along with oxygen levels (Burns 1970; Hartman *et al*. 1996).

This along with scouring from culverts alters or even destroys habitat for

macroinvertebrates. Our results show there was significantly more silt and sand at

the impacted streams, Robbins Mill Stream and Rome Trout Brook, than at the

unimpacted stream, Whittier Brook. There are increased finer sediment particles and

therefore it is only logical to look at the road crossings present at the impacted

streams as the source of this.


**2.4.4 Summary and Conclusions**

We initially hypothesized that road crossings would have a measurable and

negative impact on the health of headwater streams. We were able to reach several

significant conclusions in support of this. First, we discovered that our unimpacted

streams had significantly higher average proportions of fine grained sediment than

in our unimpacted control stream (see section 8.1 - comparison between streams).

We also found higher proportions of small sediment particles immediately upstream

of each road crossing than further upstream where there was less erosion from the

road. Complementing these, when looking at our measurements of suspended

solids in the stream, we saw that every sample collected in impacted streams was

significantly higher than every sample taken from Whittier Brook. With all of these

results in mind, we can conclude that erosion from road crossings increase the

sediment load of headwater streams and they do increase the proportion of finer

grain particles in streambeds.

A more detailed analysis of sediment cores of impacted and unimpacted

streams would give us a more complete picture of sediment erosion and deposition

in the stream reach. This research could include ashing cores to determine the

composition of organic material within each grain size, and chemical analysis to identify clays and other more complex soil types that are linked to nutrient transport. Other further areas of study include a more complete analysis of discharge including transient storage and groundwater connections, and increase the variety and number of streams.

As human population grows further into surrounding landscapes it is important to study their effects on natural ecological processes. Especially in environments similar to Maine where roads are salted in the winter, environmental impacts could be severe. Roads are becoming ubiquitous on the natural landscape and further research should be done on the impacts of road crossings and ways to mitigate these negative effects that our study infers.

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# CHAPTER 3
# ORGANIC MATTER

## 3.1 Introduction

### 3.1.1 Organic Matter and the Food Web

Organic matter is an important source of nutrients coming from living environments that enter a stream.  This includes logs, sticks, leaves, and other detritus that comes from non-aquatic based sources. (Allan & Castillo 2007). Organic matter in a forested stream is mostly from the riparian zone and is highest in the fall with trees shedding their leaves (Goldman *et al.* 2014). Leaves accumulate in the stream channel during the summer and then are exported during the fall flush, which can result in higher oxygen demand downstream, due to additional oxygen necessary for decompositional processes (Goldman *et al.* 2014).

Decaying particulate organic matter (POM) and the microbial biofilms that colonize POM serve as a major source of carbon for stream biota, especially developing insects (Kaushik & Hynes 1971). Since headwater streams are often shaded, causing photosynthesis rates to be low, the stream community's feeding and life cycle habits are based on the input, decomposition, transport and storage of allochthonous organic matter at different points in the stream (Wetzel 2001; Vannote *et al*. 1980). Organic material affects food webs far downstream of the point of entry. Organic matter is broken down as it moves downstream, and organisms take

advantage of inefficiencies upstream in organic matter use (Webster *et al.* 1999;

Dodds & Whiles 2010; Vannote *et al*. 1980).

Bacteria, fungus, and protists colonize organic material soon after it enters the

stream. Collectors, shredders, and detritivores rely on fungus, bacteria, and organic

matter for food (Cummins *et al.* 1973; Hall & Meyer 1998). Leaves are generally not

nutritious enough on their own to attract invertebrates, but once fungus and bacteria

have colonized them, various invertebrates are drawn to the leaf. Invertebrates,

specifically shredders, greatly increase the rate of decomposition in the streams

(Wallace *et al.* 1982). Therefore, primary microbial colonization of leaves is vital to

releasing nutrients and carbon from the organic matter to the stream ecosystem

biota (Dodds & Whiles 2010). Studies have shown that microbial organism and

macroinvertebrate populations in streams is controlled by a bottom-up relationship

(Gonçalves *et al.* 2014). This means that fungus and bacteria help to determine the

type of invertebrates that will colonize leaves. Therefore, factors affecting microbial

growth and health will affect invertebrate presence. (Ambrose *et al.* 2004; Flores *et al*.

2011). Macroinvertebrates that act as collectors are additionally affected by leaf

decomposition from physical abrasion from sediment and larger particles. Abrasion

can significantly break apart organic matter, making it easier for collectors to find

particles they are able to eat (Heard *et al.* 1999).

### 3.1.2 Shelter/Habitat

Organic matter provides habitat for invertebrates, bacteria, and fungi. Even during periods of drought and water stress, leaves can provide shelter and a source of organic matter (Yila *et al.* 2010; Straka *et al.* 2012). This is an example of how organic matter can contribute to ecosystem resiliency, serving as a buffer against the effects of climate change or land use disturbance. Large wood can also shape communities through providing habitat, altering stream niches, and changing the functional groups living in a particular stream segment through the creation of pools and falls. Biomass, number of insects and secondary production varied greatly between streams with logs to streams without (Wallace *et al.* 1995a).

### 3.1.3 Output of Nutrients

While streams are no longer thought of simply as "pipes" transporting water from inland catchments to the ocean, they still can carry large amounts of nutrients downstream. Organic matter from headwater streams breaks down from coarse to fine matter and dissolved carbon (DOC) and other nutrients. Fluxes of nutrient inputs affect the chemistry of estuaries, lakes and oceans as a whole (Holmes *et al.* 2012). Nutrient dynamics can also play a part in the export of organic matter downstream. Streams with higher nutrient levels result in faster breakdown of organic matter. This therefore increases amounts of fine particulate organic matter

(FPOM, <1 mm diameter) downstream rather than coarse particulate organic matter (CPOM, >1 mm diameter), important for stream cycling and macroinvertebrates, and can change communities accordingly (Benstead *et al.* 2009). If decomposition occurs too quickly in the streams, not all of the necessary limiting nutrients and carbon can be exported to the lakes (Bosch *et al.* 2014).

When anthropogenic activities impair stream dynamics and decomposition, it can result in excess export of nutrients downstream and subsequent hypoxia. Getting rid of wood debris in streams results in increased export of materials downstream (Eggert *et al.* 2012). Impacted land has been seen to still be contributing organic matter decades later after disturbance (Yamashita *et al.* 2011). When debris dams are removed, leaves have a shorter residence time upstream, prohibiting the coarse material from being fragmented by shredders, resulting in inefficiencies downstream where functional groups are less prepared to eat the larger food material (Bilby & Likens 1980). Leaves, too, are vital for proper stream functioning. POM, fine inorganics, and gravel were all found to be exported at higher rates without caches of leaves in the stream (Eggert *et al.* 2012). The type of leaves buried in sediments can also have an effect on stream nutrients as well. Leaves were found to increase nitrogen retention, suggesting that the quality of POC can have an effect on nitrogen levels, keeping downstream nitrogen amounts from reaching biologically problematic levels (Stelzer *et al*. 2014).

### 3.1.4 Input

Most organic matter comes from terrestrial sources in the form of plant material, especially from communities adjacent to the stream. Even with bank erosion or alteration of the riparian zone, terrestrial organic matter is often more important to organic matter loading in streams than internal carbon production via photosynthesis (Keith *et al*. 2014). Leaf input is minimized in the summer and is highest in the spring and fall (Wetzel & Otsuki 1974; Barlocher 1983). Hydrologic conditions can also affect the amount and quality of organic matter in a stream. During times of low flow and base flow, groundwater can provide a high proportion of the DOM, while leaves and terrestrial sources provide the dominant fraction during storm events (Inamdar *et al.* 2011). Source, amount, and quality of terrestrial organic carbon vary depending on hydrologic conditions (Dalzell *et al.* 2005). Reducing or eliminating organic matter input to streams has numerous effects on the food web of a stream; in one study, eliminating leaf detritus input resulted in higher consumption efficiency and more wood consumption. The stream that was denied litter inputs had an entire taxonomic group missing from the ecosystem compared to the reference stream (Hall *et al.* 2000), showing how limiting inputs of organic material to a stream affects consumers.

### 3.1.5 Retention

When organic matter enters streams, it cannot be used by biota unless it is held in the stream for a long enough time, for example, for microbes to effectively colonize leaves. Retention is a measure of the probability that an object in transport will get stuck on an obstacle in the stream reach, and the number of obstacles in a reach (Young, Koval & Del Signore 1978). Retention is affected by bed roughness and hydrological characteristics. Studies have shown that unimpacted reaches are more effective retainers than channelized ones at normal and low flows (Koljonin *et al.* 2012). Leaf bunches are important for keeping smaller Benthic Organic Matter (BOM), organic matter in the bed of a stream, and sediment in the waterway (Eggert *et al.* 2012). Sediment grain size of the stream bed can even have a large effect on retention; sandy streambeds hold more organic matter in the deeper interstitial zone than other sediment types (Cornut *et al.* 2012).

In regards to retention, large woody matter is the primary force behind stream structure shaping. Wood structures and their debris dams have a compounding effect; the more large wood dams that occur, the more total organic matter retained. Debris dams also increase the amount of invertebrates in a habitat by several times (Smock *et al.* 1989). Removing these dams takes away the energy base for the stream ecosystem by reducing the amount of leaves retained in the stream, and thus limiting the amount of organisms the stream can support (Bilby &

Likens 1980).  Organic matter retention is also affected by the discharge of a stream.

Stream order, which measures the size of streams based off of number of tributaries,

correlates negatively with retention.  As stream order increases, more CPOM is

suspended in the water column due to a lack of retentive structures (Wallace *et al.*

1982).  Large wood added to streams has a declining effect of retaining wood as

streams get larger (Flores *et al.* 2011).


### 3.1.6 Decomposition Rate

Microbial organisms are not only important for stability of the food chain, but

they are also some of the only creatures that can process the nutrients, cellulose and

lignin, that are prominent in organic matter. Therefore, they are highly important for

the beginning stages of decomposition of organic matter, and making it available to

the rest of the food web. Additionally, after the primary microbial colonizers,

macroinvertebrate feeding on organic material, especially leaves, increases and

furthers leaf decomposition (Ambrose *et al.* 2004; Flores *et al.* 2011). The

decomposition rate of organic material is also strongly influenced by the chemical

composition of the litter species; some species will decompose more quickly than

others based on their chemical make-up (Guendehou *et al.* 2014). Species that have

more labile nutrients, such as nitrogen, and fewer refractory compounds, such as

lignin, will decompose faster (Figure 3.1). Nitrogen content seems to be one of the

main determinants of species-specific decomposition rate because nitrogen is often a limiting nutrient (Alhamd *et al.* 2004; Abelho & Graça 2006; Jabiol & Chauvet 2012; Guendehou *et al.* 2014; König *et al.* 2014). Additionally, the decomposition of woody material is much slower than the decomposition of leaves. This is due to its structure and being less nutritious than leaves (Allan & Castillo 2007).
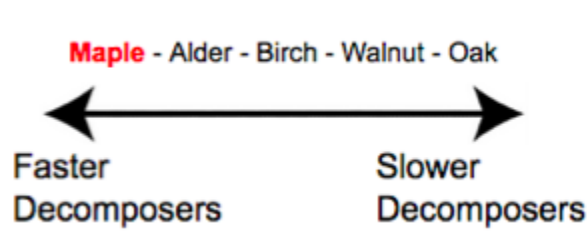


**Figure 3.1.** *Spectrum of speed of decomposition as function of species. We used maple leaves in our experiment. Figure adopted from Jabiol & Chauvet 2012.*

Abiotic factors also have a large influence on decomposition. Temperature increases can increase the rate of decomposition, especially in streams with lower nutrient levels (Fernandes *et al.* 2014). Areas with high sediment deposition rate have a slower decomposition rates while low deposition areas have faster decomposition (Meyer 2014). Sediment and larger particles such as stones can act as an abrasive that is significant in breaking apart organic matter (Heard *et al.* 1999). When all factors are considered together, the decomposition of organic matter in a stream over time is best represented by a negative exponential curve (Figure 3.2).
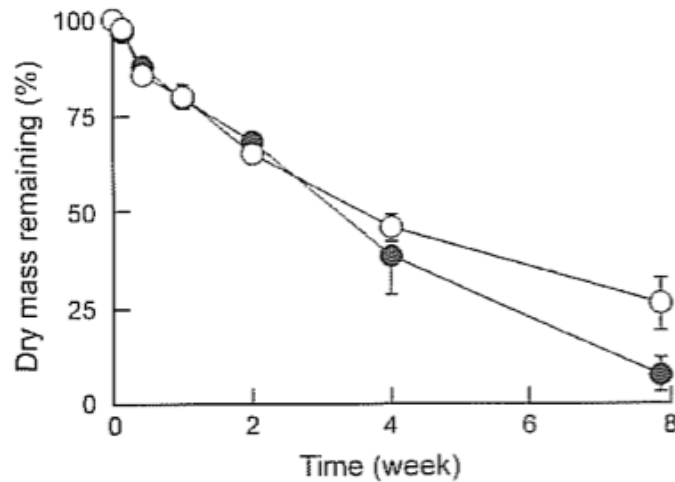
**Figure 3.2.** *Example of a graph measuring leaf decomposition in a stream using the percent of dry mass remaining over 8 weeks. Figure adopted from Allan & Castillo (2007).*

### 3.1.7 Floods

The amount of seston, the material in the water column, depends on hydrologic conditions. Floods increase the amount of DOM provided by terrestrial sources due to leaf leaching, running through soil, and other forms of contact with organic matter on the banks of streams (Inamdar *et al.* 2011). The fall flush is also important for leaf movement, as leaves accumulate until a heavy event in the fall season, which moves leaves downstream (Goldman *et al.* 2014). Concentration of seston during floods is highest when the stream water level is rising, and declines as the flood peaks; this displays how organic matter is flushed out primarily during the beginning of storms (Golladay *et al.* 1987). Backwaters of the creek, areas where flow is very slow, often hold a large portion of a stream's organic matter; floods deposit matter in backwater as they recede (Speaker *et al.* 1984). Floods also impact

anthropogenically-disturbed streams in a different way than unimpacted ones.

Because of the increased amount of non-permeable area in the catchment such as

roads and parking lots, floods tend to be more extreme and have less travel time

traveling from terrestrial areas to aquatic ones (Shuster *et al.* 2005). Concentrations

of seston in disturbed watersheds were also observed to have returned to normal

pre-disturbance baseflow conditions following a few years, but seston

concentrations of disturbed streams remain higher after several years during high

flows (Golladay *et al.* 1987). This displays a difference in human-impacted streams

that exists and persists only during high water.


**3.1.8 Research Questions**

We addressed two main topics in our organic matter research: retention and

decomposition. We developed two main research questions about stream retention:

1) Does the presence of roads affect organic matter retention of Belgrade region

    streams?

    and

2) Does the presence of large wood affect organic matter retention?

For decomposition, we asked the question:

1) Does the presence of roads in the Belgrade Lakes region affect decomposition

    rates in the stream?

We hypothesized that streams with higher amounts of large wood would be more retentive. Clearing of areas around the streams from urbanization will have fewer large wood structures to enter the stream. There will be fewer large wood structures to prevent organic matter from being flushed out of a reach. So, streams that are impacted by humans will have decreased retention. We also hypothesized that decomposition would be faster at streams with anthropogenic influence due to higher nutrient content, increased sunlight, and physical abrasion from fine sediments.

## 3.2 Methods

### 3.2.1 Large Wood Measurements

To measure the amount of large wood in the stream, we used a measuring tape and/or calipers to measure logs in the stream bed that were greater than 10 centimeters at their midpoint. We also recorded the length of the log. Using these measurements, we calculated the approximate volume of wood in the stream bed.

### 3.2.2 Leaf & Dowel Releases

We used pink fluorescent spray paint to coat three sets of 1000 Ginkgo (*Ginkgo biloba*) leaves and twenty five wooden dowels approximately twenty centimeters in length. Prior to deployment, we soaked the leaves and dowels in water to pre-saturate them to make our "plant matter" more realistic to conditions at our study site.

We went to one stream per week for three weeks. Each day, we began by setting up a net at the end of the reach. We released the leaves and then ten minutes later released the dowels. After twenty minutes, we began at the net and walked upstream, counting how many leaves and dowels we found in each ten meter section and recorded what stream structures they were caught on. These categories were, riffle or pool, rocks, roots, backwater, bank, wood, debris dam or floater. Using these data, we calculated how far an average leaf will travel, with an exponential decay model.

We repeated the dowel retention test at Rome Trout two weeks after the first test because a beaver dam blocked all of the dowels and leaves, not allowing any to move past the first ten meters of the reach. While this is valuable information about stream retention at a beaver dam, we also wanted to be able to gauge the retention of the whole stream length. We released the dowels for the second time downstream

of the culvert and pool, to observe retention in the lowest 50 meters of the stream.

To calculate average leaf movement downstream, see Equation 3.1.

$$P_d = P_0 e^{-k_r d}$$

**Equation 3.1** *Calculation of the average leaf movement downstream. $P_d$= number of leaves in transport at distance d, $P_0$=number of leaves initially released at distance=0, $k_r$=stream constant calculated from experiments, and d=distance downstream.*

### 3.2.3 Leaf Decomposition

To compare the decay of leaves in different streams, we prepared 72 mesh

bags each containing five grams of leaves.  We selected three sites in each stream,

and placed eight leaf bags at each one. For Robbins Mill and Rome Trout, our two

streams with culverts, we selected one site upstream of the culvert, one immediately

downstream of the culvert, and the last site was farther downstream of the culvert.

At Whittier, we tried to space our packs out at equal distance along our 100m reach.

However, due to all the boulders in Whittier, we selected sites based on accessibility

and ability to stake the bags in place. We removed one bag from every site in each

stream for eight weeks, so that n=3 per stream per week. For a control, we carried

five bags of leaves during each removal trip to account for breakage due to

handling.

After the leaf bags were removed from the streams, we emptied the bags and

rinsed them off.  Any insects residing in the leaves at the time of rinsing were

collected and placed in an alcohol solution. The rinsed leaves were placed in a paper bag and left to dry for 4-6 days at room temperature. After the drying period was over, we weighed the leaves and recorded their mass. The weight of the three sites was averaged together per stream to create one value for each week for each stream. However, for Whittier stream only site one and site two were used in the average for week 4 and after because the leaf packs at site three vanished after week three. This was likely due to meddling from passersby or stronger stream flow during rain. We graphed the relationship between weeks passed and the average weight of the leaf packs and used this to calculate a k-value ($k_d$) to act as a constant to compare decomposition rates in each stream. The higher the $k_d$, the faster the decomposition rate ($k^d$ is measured in $d^{-1}$).

We were unable to conduct any statistical analyses to compare the decomposition rates of the streams, because our data collection only supplies one $k_d$ value. However, more than one $k_d$ value would be necessary to conduct any form of statistical analysis. We can still observe differences in the calculated values and the $k_d$ values we obtain will be valuable to keep records of decomposition rates of Belgrade Region streams.

### 3.3 Results

The slopes of average weight vs. week were used to determine the rate of decay of each stream (Figure 3.3). The percent weight lost/day is the slope of each line and the $k_d$ is calculated by taking the Ln of percent weight lost/day. Rome Trout had the slowest rate of decay for the leaf pack experiment, and Robbins Mill had the fastest (Table 3.1). Whittier was between the other streams.
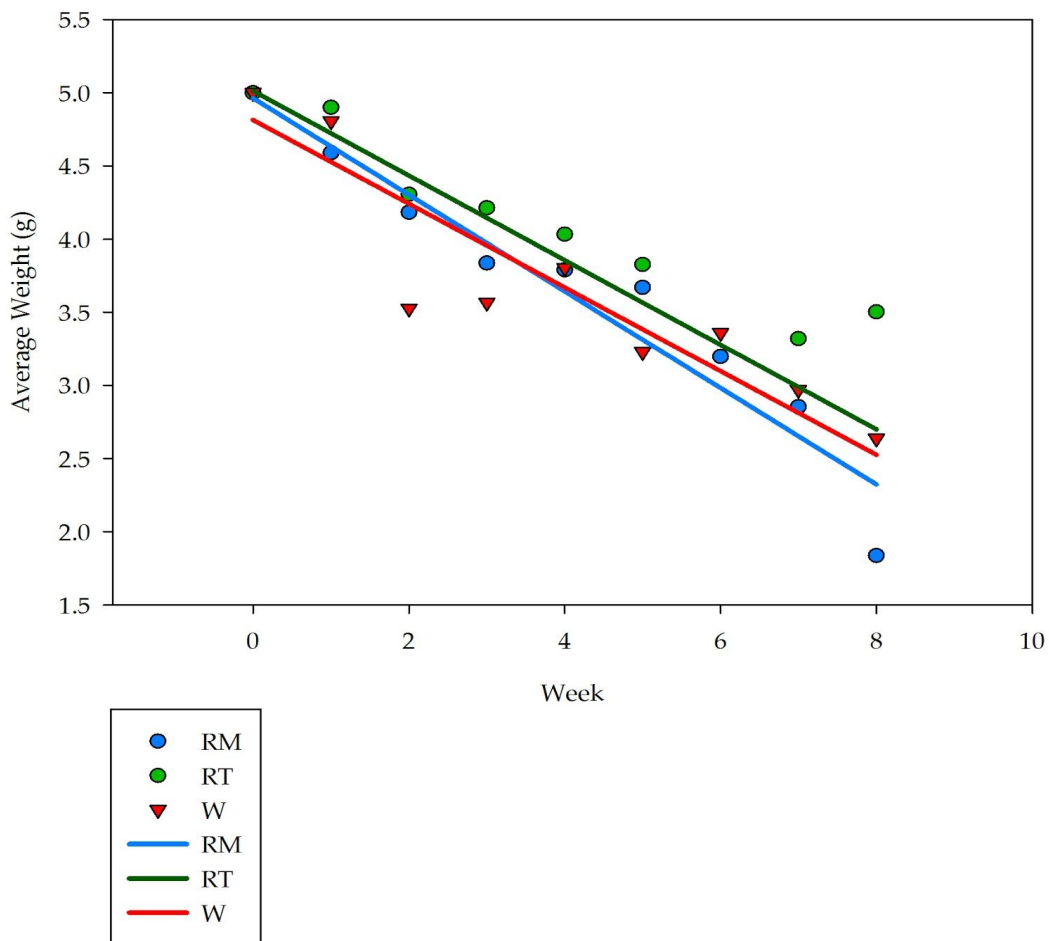


**Figure 3.3.** *Regression depicting mean change in leaf mass over eight weeks for Robbins Mill (RM), Rome Trout (RT), and Whittier (W). The points for Whittier week 4 and later used the average of two sites instead of three because of missing leaf packs.*

**Table 3.1.** $k_d$ *constants and percent of original leaf mass lost per day over eight weeks for Robbins Mill (RM), Rome Trout (RT), and Whittier (W).*

| Stream | $k_d$-value($d^{-1}$) | Percent weight lost/day |
|--------|----------|-------------------------|
| RM | 0.0151 | 0.95% |
| RT | 0.0074 | 0.59% |
| W | 0.0083 | 0.61% |

All three streams had large wood present in them. Whittier had the highest overall amount, and the highest per meter. Robbins Mill had the lowest total amount, and lowest per meter. Rome Trout had a moderate amount of wood (Table 3.2).

**Table 3.2.** *Amount of wood in stream reaches, both as a total amount and the average volume per meter for Robbins Mill (RM), Rome Trout (RT), and Whittier (W).*

| Stream | Total Wood ($m^3$) | $m^3/m$ |
|--------|-------------------|---------|
| RM | 0.77 | 0.001 |
| RT | 1.55 | 0.016 |
| W | 2.17 | 0.022 |

Out of the 1000 leaves released in each stream, only leaves in Whittier and Robbins Mill made it past the first ten meter reach. Twenty meters was the maximum distance traveled in Whittier, while sixty meters was the furthest that leaves in Robbins Mill traveled (Figure 3.4). Calculating the average distance traveled per leaf, Robbins Mill had the highest at 7.97 meters. Whittier was lower with an average distance traveled of 1.21. Rome Trout had an average of zero, because no leaves made it past the first ten meter section (Table 3.3).
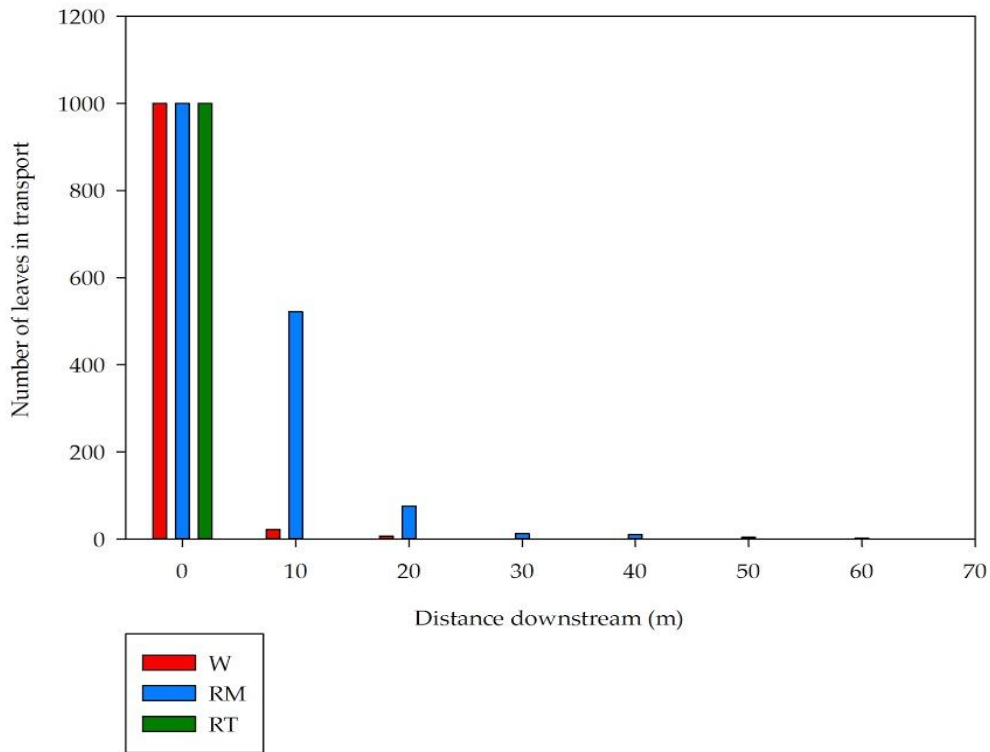
**Figure 3.4.** *Number of leaves remaining in transport as a function of distance downstream of release point for Robbins Mill (RM), Rome Trout (RT) and Whittier (W).*

For the dowel release in Robbins Mill, no dowel traveled further than the second ten meter reach. In the first reach, the primary retention mechanism was debris, while in the second reach, the primary retention mechanisms were riffles and pools (Figure 3.5). Many different retention structures were present.

**Table 3.3.** *Average downstream travel distance for released leaves for Robbins Mill (RM), Rome Trout (RT) and Whittier (W).*

| Stream | Average Distance Traveled per Leaf (m) |
|--------|----------------------------------------|
| RM | 7.97 |
| RT | 0 |
| W | 1.21 |
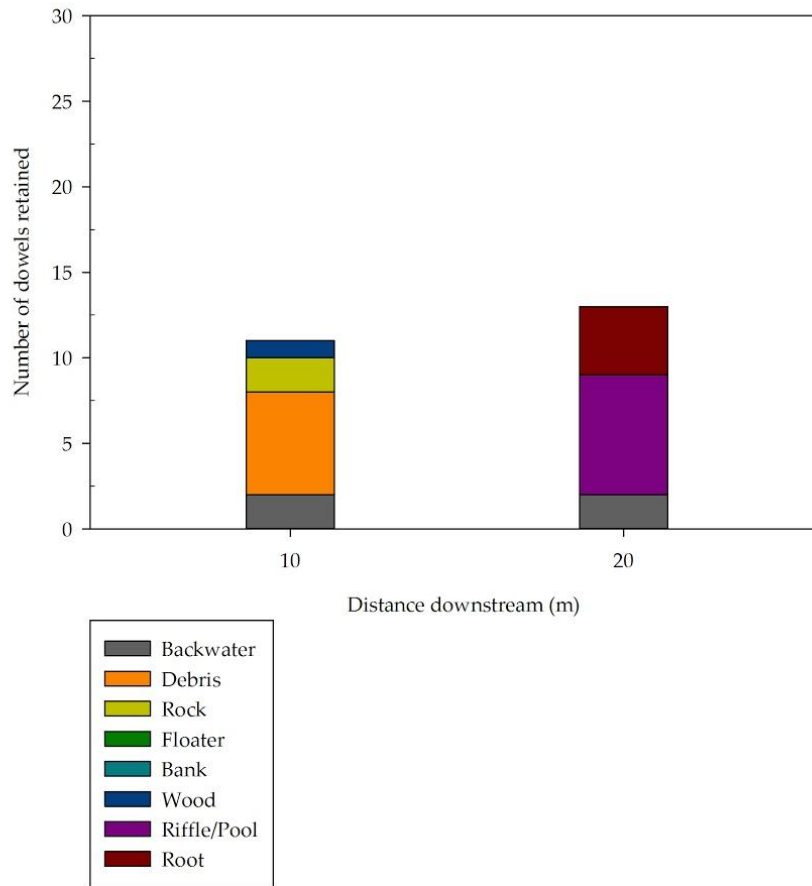
**Figure 3.5.** *Number of sticks retained as a function of distance downstream from release point and retention structure for Robbins Mill Brook (n=25).*

In our first of two dowel releases in Rome Trout, all dowels got stuck on a beaver debris dam present in the stream. In our subsequent release downstream, most dowels were stuck on debris, but this was randomly scattered and not an organized beaver dam. Sticks traveled up to 30 meters from the release point in our second launch (Figure 3.6).
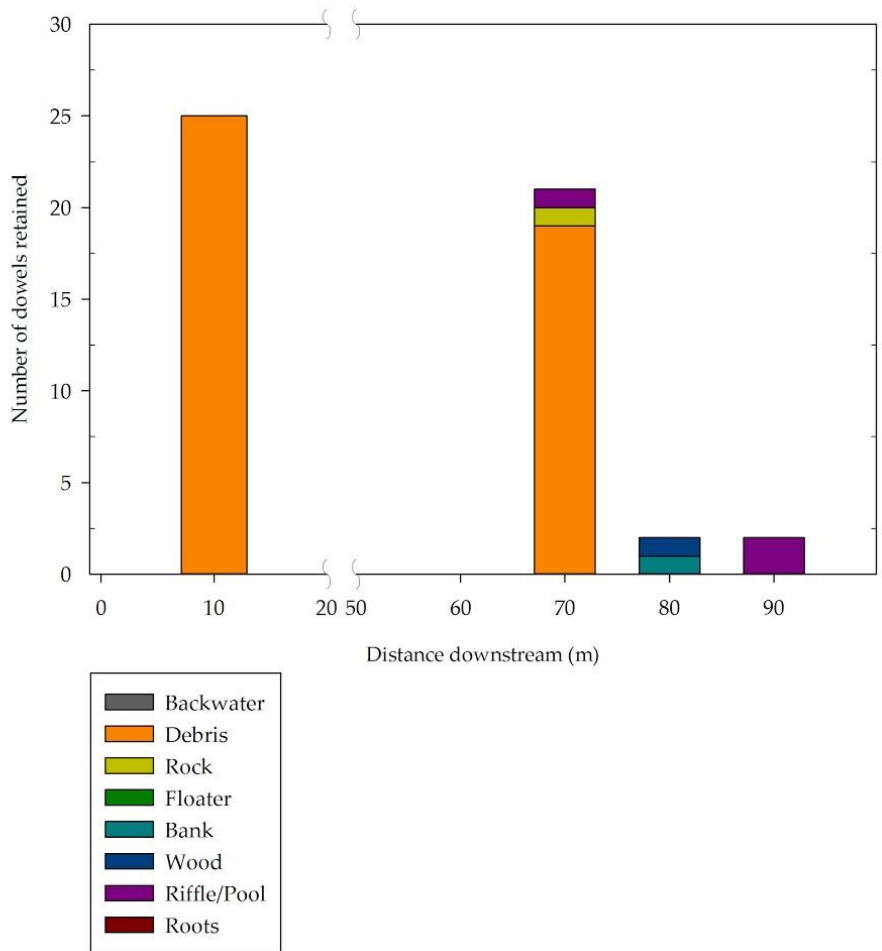
**Figure 3.6.** *Number of sticks retained as a function of distance downstream from release point and retention structure for Rome Trout Brook (n=25). This figure shows two separate releases; one beginning at 0m and one beginning at 60m. Two releases were conducted due to the presence of beaver dams at 10m and 40m.*

In Whittier Stream, no dowels traveled past the first ten meter reach.  Sticks were caught in backwater and debris, but both of these structures were due to the large boulders characteristic of the stream creating a pool and a debris eddy (Figure 3.7).
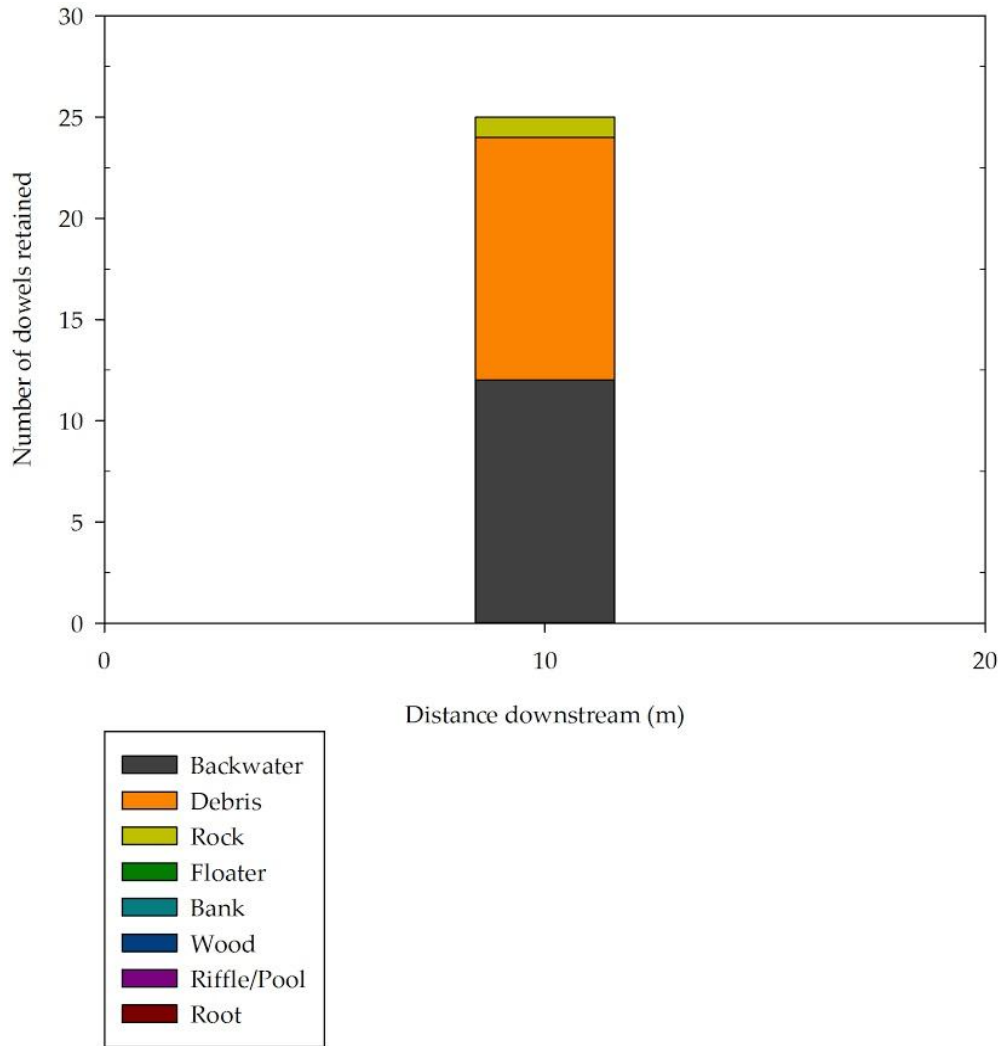
**Figure 3.7.** *Number of sticks retained as a function of distance downstream from release point and retention structure for Whittier Stream (n=25).*

## 3.4 Discussion

### 3.4.1 Retention

All three streams were very retentive, and were consistent with the literature (Jones & Smock 1991; Imberger *et al*. 2011). All of our average distances traveled for leaves fit within the range of two similar studies (Table 3.4), with the exception of Rome Trout, which was low because of its beaver dam (Table 3.3). The retention

mechanism differed between each stream.   Rome Trout was the most notable, with several beaver dams preventing movement of organic matter from traveling downstream. This notable structure is unusual as it is non-random and blocks the entire stream, rather than just a part.  This stands in comparison to other streams in our study, whose debris placement is due to the stream and its flow instead of an animal and its preferences.  Whittier was full of large boulders and had low flow during the day that we released the leaves.  Boulders and the pools they create stopped sticks and leaves from moving downstream, but few sticks were caught on the rocks themselves.  The primary retention mechanism is the pool created by the boulders, but the individual sticks were caught on structures or stream features created by the boulders (Figure 3.6).  A lot of large wood was present (Table 3.2), however, much of it sat above the stream level on the top of the boulders and would only touch water during high flows, such as those we observed during storms. Robbins Mill' lower retention rate is likely due to a lack of retention mechanisms. The volume of large wood in the stream was lower (Table 3.2), which can mean faster flow and fewer obstructions for organic matter to potentially be caught on. There were also not as many large rocks and boulders in the stream bed, which can halt transport of organic matter downstream both through creation of pools and through catching wood and branches to increase the retentiveness of the stream.

**Table 3.4.** *Literature values of average distance downstream traveled for released leaves in streams.*

| Study Site | Mean Distance Traveled (m) | Study |
|---|---|---|
| Colliers Creek (Virginia, U.S.A.) | 1.6 | Jones and Smock, 1991 |
| Buzzard Branch (Virginia, U.S.A.) | 5.2 | Jones and Smock, 1991 |
| Lyrebird Creek (Victoria, Australia) | 2.53 | Imberger, Thompson & Grace, 2011 |
| Monbulk Creek (Victoria, Australia) | 4.4 | Imberger, Thompson & Grace, 2011 |
| Cardinia (Victoria, Australia) | 3.9 | Imberger, Thompson & Grace, 2011 |
| Mullum (Victoria, Australia) | 7.3 | Imberger, Thompson & Grace, 2011 |
| Blind Creek (Victoria, Australia) | 2.15 | Imberger, Thompson & Grace, 2011 |

Large wood seems to be a correlating factor not only in our streams, but also in streams worldwide. In streams in northern Spain, adding wood resulted in a highly variable increase in organic matter retained. However, simply adding large wood doesn't guarantee that retention will increase. Instead, it seems that having both large and small wood is best for increasing organic matter retention, as the large logs catch smaller matter, which catch even smaller matter (Flores *et al*. 2011; Flores *et al*. 2013; Speaker *et al*. 1984). In particular, Flores *et al*. 2013 attributed increased organic matter retention to the complexity of structures introduced during the experiment of wood addition, which was more apt to replicate real life conditions. This study also suggested that if large wood is added to streams that are prone to extreme flooding and drought, and thus can carry a lot of organic material with them, organic matter buildup after experimental wood additions can happen

faster than previously thought.  Flores *et al.* 2013 added both large wood and smaller

branches, and these structures became more complex over time.

Large wood forms the base for which other sticks can be caught on. If this

wood is absent, sticks and consequently leaves will not be caught.  In Robbins Mill,

where large wood volume was significantly less, this may have the effect of

retaining less organic matter overall.  Studies of benthic organic matter and standing

leaf stocks in the stream could help confirm this hypothesis.  The root problem of

Robbins Mill, we believe, is that our study reach's riparian zone was much smaller

than that of the other streams', resulting in less input of organic matter from the

bankside vegetation.  If there are no sources of large wood or other structural debris

adjacent to the stream reach, then the stream may exhibit a deficiency in not only

organic matter input, but also in retention of matter that reaches the stream.

In order to increase organic matter retention, adding or preserving buffers on

stream banks can be a good step in either conservation or restoration of organic

matter dynamics.  If vegetation is adjacent to streams, it can prevent a slew of

negative consequences.  One of these is the contribution of woody debris to serve as

not only as a food source for stream taxa, but also as a barrier to downstream

movement of organic matter from large or medium size wood which can hold more

edible plant matter, like leaves and fruits.  These can possibly negate human

disturbance, such as the roads crossings at our streams.  Buffers can also

significantly help prevent excess nutrients from moving downstream.  Urban and agricultural streams with buffers with were found to retain nitrogen in similar concentrations to unimpaired forest streams, while streams with no buffers had exponentially higher nitrogen (Sobota *et al*. 2012).  Channelization and straightening of streams can also impair organic matter retention of streams as stream channels lose their complexity and have poor retention at all levels of flow.  The addition of wood is useful to restore streams that have been affected by riparian zone removal, as wood is retentive at all discharge rates (Koljonen *et al*. 2012).  However, the addition of wood should mimic natural positioning and avoid human-designed anchoring schemes, as these are less effective (Kail *et al*. 2007).

Non-anthropogenic biotic factors such as fauna may also contribute to stream retention.  The presence of beavers in the area will result in dams and an animal-created barrier to organic matter movement in streams.  Removing nuisance beavers has effects on organic matter, providing an unusual and unexamined example of an anthropogenic disturbance to headwater streams.

While all of our streams are very retentive, there is some evidence in Robbins Mill that humans might have disrupted a portion of the organic matter cycle due to removal of bankside vegetation.  As more of this region is developed, planners and citizens need to put thought into the state of these headwater streams, as anthropogenic disturbance has been shown in other areas to affect organic matter

and its role as the base of the food chain.  Perhaps Robbins Mill would benefit from wood additions, but a simpler way to help the stream is to allow buffers to return to the cleared portions of the streambanks adjacent to the roads.

**3.4.2 Decomposition**

**3.4.2.1 Temperature, Time, DO**

Our $k_d$ values are very different from $k_d$ values found for decomposing red maple leaves in a stream in a study in Kentucky, where the $k_d$ value for red maple leaf packs was $0.448d^{-1}$. Further, the $k_d$ values for all of their trials were significantly higher, even for packs with oak leaves which are known to decompose more slowly than maple leaves (Jabiol & Chauvet 2012). However, this study occurred over 60 months as opposed to two. If we had continued our study for a longer duration, perhaps we could have seen a higher $k_d$ value. As it stands, our two months represent only the beginning of an exponentially decaying curve of leaf decomposition (Allan & Castillo 2007). If we carried our study on longer, our $k_d$ values would be more representative and possibly more similar to values in the literature.

Additionally, Kentucky's mean annual temperature is 12°C while the mean annual temperature in the location of our researched streams is around -5°C (National Oceanic and Atmospheric Administration). The higher temperature could

have increased the rate of decomposition in Kentucky. Water at excessively high or low temperatures can kill some of the microbes and invertebrate that are vital to decomposition (Taylor & Chauvet 2014). Streams with higher temperatures that are not excessively high often have higher decomposition rates (Fernandes *et al.* 2014). From personal observations, we noted Rome Trout seemed to be the coldest stream towards the beginning of our study but not as cold relative to the other streams later in our research, suggesting it as a groundwater fed stream (Allan & Castillo 2007). Additionally, more E. coli bacteria was found in Rome Trout in comparison to our other streams, which could be due to septic tank contents seeping into groundwater and this groundwater entering the streams (Chapter 5). Groundwater entering causes a more constant temperature throughout the year (Allan & Castillo 2007). Perhaps Rome Trout water does not reach ideal temperature for some microbes to survive and so less colonization occurs on the leaves lowering decomposition rates.

Shaded areas have slower leaf litter decomposition primarily due to lower fungal colonization and lower shredder consumption rates (Lagrue *et al.* 2011). Qualitatively, we saw a trend that Robbins Mill had the least amount of riparian coverage over the stream while Whittier had the most. While this could mean greater organic matter input diversity, it also means more shade over the stream. Whittier also had many large boulders which blocked sunlight from reaching the water in many areas. Perhaps while riparian fauna diversity could increase

decomposition rates in Whittier, the shade inhibited decomposition at the same time. As urbanization reduces tree cover in favor of roads, more open spaces will increase sunlight reaching the stream and thus could increase decomposition rates.

### 3.4.2.2 Nutrients & Microbes

Litter that is less nutritious decomposes faster if small inputs of nutrients, like nitrogen, are added to the stream (Gulis *et al.* 2006). Nitrogen is a main limiting agent for stream life. Fungi and bacteria can obtain some of their nutrients out of the water, so water chemistry is important in their health. Being able to obtain necessary nutrients from the water column allows fungi and other microbes to consume less nutritious leaves for their carbon sources. (Suberkropp & Chauvet 1995). Whittier had the lowest amount of background nitrogen between the streams, and Rome Trout and Robbins Mill were similar in their nitrogen concentrations (Chapter 4). Perhaps Whittier's low amount of excess nutrients was a limiting agent for microbes and thus decomposition.

Robbins Mill and Rome Trout likely have small logging operations upstream. Logging can input nitrogen and other nutrients into the stream, thus adding limiting nutrients. This will stimulate microbial decomposition because many microbes are sensitive to nutrient changes in the water column (Benfield *et al*. 2001). Both streams

also likely have agricultural land in the catchment site. DOM in stream water from

agriculturally affected streams was found to support more microbial communities

than those from unaffected areas (Williams *et al*. 2010). So, perhaps background

nutrients from agriculture increased decomposition rates in Rome Trout. Whittier

has the lowest amount of background phosphorus content (Chapter 4). Although

microbes are sensitive to changes in nutrient content in general, they are not very

sensitive to phosphorus specifically. No groups responded to changes in

phosphorus in previous studies, suggesting nitrogen as the main limiting nutrient

stream ecosystems (Ferreira, Gulis & Graça 2006). As nitrogen and phosphorus are

often added to streams from logging and agricultural operations, high amounts of

urbanization could actually eliminate these operations from the area depending on

the sort of development taking place. If agriculture and logging wastes are

eliminated, decomposition could decrease. However, it would depend what

replaced logging and agriculture. More impervious surfaces like roads and

driveways can increase nutrient input from runoff (Chapter 4). This could

temporarily increase decomposition rates, but if nutrient level becomes too high or

toxic chemicals enter the stream, decomposition rates will slow down (Suberkropp

& Chauvet 1995). Intensive gardening for parks or lawns could have similar effects

to agriculture in terms of nutrient inputs. Input of nutrients from roads could have

very different effects on stream health. High inputs of salt from roads in the winter can decrease decomposition rates (Cañedo-Argüelles *et al*. 2014).

### 3.4.2.3 Invertebrates

Robbins Mill had the highest amount of macroinvertebrates between the three streams (see invert chapter). This could contribute to Robbins Mill' high decomposition rate because more macroinvertebrates need more food and would break down leaves faster. Whittier had the lowest percentage of shredders between the streams, 2.99% compared to 11.73% and 10.07% for Rome Trout and Robbins Mill respectively. This could account for Whittier having a slower $k_d$ value than Robbins Mill. Robbins Mill also had the lowest retention and amount of large wood present. If Robbins Mill has a lower amount of food available for the larger amount of macroinvertebrates, they will eat more of what is available even if it is not the most preferable food (Hall *et al*. 2000). Perhaps we would see a stronger effect of macroinvertebrates, specifically shredders, on leaf decomposition with a longer study period, as bugs become more important post microbial colonization (Allan & Castillo 2007).

**3.4.2.4 Riparian Zone Diversity**

The chemical makeup of different species influences decomposition rates. Also, combinations of different litter species can produce higher decomposition rates. This is largely due to the unique microbial community that will develop under different diversities of litter (Kominoski *et al.* 2010; Jabiol & Chauvet 2012; Chapman *et al.* 2013). Greater diversity in fungal species on the leaf can result in greater diversity of invertebrates colonizing the leaf because more fungal diversity offers greater range of nutrition to invertebrates. This makes the leaf possibly more appealing to macroinvertebrate consumption, so macroinvertebrates would consume more organic matter causing faster decomposition (Gonçalves *et al.* 2014). Streams that have greater diversity along the banks could have faster decomposition rates due to the diversity of microbial colonies. Robbins Mill and Whittier both had 12 tree species while Rome Trout had 6 tree species. Greater tree diversity could have helped to increase diversity in fungal communities in Robbins Mill and Whittier and thus increasing decomposition. If road construction and general urbanization reduces tree diversity, this could decrease decomposition rates.

**3.4.2.5 Sediment**

High sediment deposition (in our streams due to logging or roads) can bury leaves and therefore delay leaf colonization and decomposition from invertebrate

activity (Benfield *et al.* 2001; Sponseller & Benfield 2001). However, sediment and larger particles such as stones and sand can act as an abrasive that is significant in breaking apart organic matter (Heard *et al.* 1999). Robbins Mill had less sand than Rome Trout but more sand than Whittier. It was also more shallow than Rome Trout, so perhaps the sand had a greater chance of contact with the leaf packs leading to increased physical abrasion. Particularly at the third site at Robbins Mill, the sandy sediment seemed to be aiding in decomposition more than inhibiting it by not fully burying the leaf packs but flowing with sand particles in suspension over the packs rapidly. In Rome Trout, especially at our first site, our leaf packs were sometimes buried under the silt; this could have inhibited colonization and therefore slowing the decomposition process. Lastly, Robbins Mill had a higher proportion of gravel than Rome Trout which could explain higher decomposition rates in Robbins Mill. If urbanization of the area means more road crossings, there will be an increased rate of sediment deposition in the streams (Chapter 2). Increased sediment deposition rates generally lead to slower decomposition rates (Meyer 2014). The topography and composition of the stream floor will be a factor that determines if organic matter becomes buried by sediment.

### 3.5 Recommendations for Future Studies

Continuing the experiments we conducted this semester will be beneficial for documenting how the streams are changing over time. However, there are ways that our experiments can be improved. There should be a better way to secure the leaf packs to the stream bed that allows for easier removal of a single pack. For example, a small carabiner could be useful instead of using knots. Also, having one bobber attached to each group of leaf packs would be useful for locating the packs especially during high and/or clouded water resulting from storms.  A longer wait time for the leaf and dowel releases may be more realistic, perhaps this could even be extended over a week or two to study how leaves move on a longer time scale.

### 3.6 Conclusion

All three streams were very retentive, especially after comparisons with the literature values.  Robbins Mill is the most compromised, due to low bank vegetation and the least existing large wood in the stream channel.  Buffer restoration is recommended, and if this cannot be accomplished, large wood addition is another step to help ensure retention is adequate for stream organisms. A total inventory of current organic matter stocks is recommended for all three streams to establish baselines.

Robbins Mill, one of the streams with anthropogenic impacts, had the highest $k^d$. The other impacted stream, Rome Trout, had the lowest $k^d$ and Whittier, the reference stream, had a $k^d$ in between the two impacted streams. This partially supports our hypothesis because one of the impacted streams had the highest $k^d$. These measures of current decomposition rates are highly valuable in long-term studies and conservation planning. Changes in leaf litter composition, invertebrates populations, and water temperature are unavoidable due to climate change alone, and this could influence decomposition (Rouifed *et al.* 2010; Tank *et al.* 2010). We need to assess the current state of the streams in order to predict and plan for climate change effects. Using decomposition as a measurement can help assess stream health and predict changes in streams and in confluences downstream. Longer term studies and continued studies in future Capstone projects will be important in planning for the future.

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# CHAPTER 4
# NUTRIENT SPIRALING

## 4.1 Introduction

Nutrients are chemical elements that are necessary for life and include elements such as phosphorous (P) and nitrogen (N) (Allan & Castillo 2007; Hauer & Lamberti 2007). N and P commonly limit primary production in aquatic ecosystems, meaning that they are required for growth but are not widely available in the natural environment; if the supply of N or P is not high enough to meet biological demand, autotrophic production is limited (Elser *et al.* 2007; King *et al.* 2014; Reddy *et al.* 1999). In streams, nutrients that are in high demand will theoretically be taken up by biota more quickly and have a shorter uptake length than non-limiting nutrients (Allan & Castillo 2007). N and P cycle through various chemical forms as they move between biotic and abiotic parts of the environment (Lavelle *et al.* 2005; Vanni 2002). Ammonium ($NH_4^+$), nitrate ($NO_3^-$) and phosphate ($PO_4^{3-}$) are three of the most commonly studied forms of N and P found in freshwater ecosystems.

In lentic ecosystems like ponds, nutrients cycle in a circular pattern; molecules are are taken up by the benthos and remineralized. However, lotic ecosystems such as streams and rivers have an added dimension: flow. In streams, nutrient spiraling involves the cycling of nutrients through various chemical forms and environmental compartments—such as biotic assimilation and sorption to

sediment—while simultaneously being transported downstream (Figure 4.1; Webster & Patten 1979; Newbold *et al.* 1981). Nutrient uptake is a functional measure that, like metabolism and decomposition, evaluates how nutrients are used rather than just their concentration at a given time (Bunn *et al.* 1999). This spiraling process is used to assess ecosystem function as well as demand for N and P, and help compare the health of streams with catchment land use (Palmer & Febria 2012). This holistic view of the stream ecosystem is critical to the understanding of stream dynamics' influence on other processes in larger, downstream bodies of water. This is particularly important for headwater streams, which, despite their small size, play a disproportionately large role in watershed nutrient processing due to their relatively large total length and shallow depth, which allows for a higher proportion of benthic interactions than in larger streams.
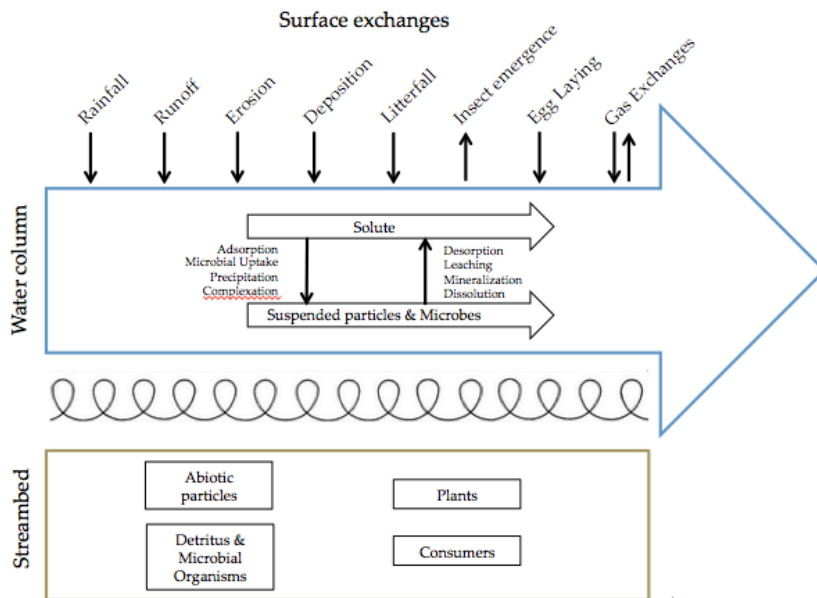


**Figure 4.1** *A conceptual diagram of nutrient spiraling in streams. The "spirals" represent how nutrients are transformed and exchanged between the water column and the streambed and interstitial water. Key processes highlighted here include adsorption and microbial and plant uptake (Stream Solute Workshop 1990).*

**4.2 The Chemistry of Nutrient Spiraling**

**4.2.1 Biotic Assimilation**

A dissolved nutrient molecule flowing downstream can be removed from the water column by biotic assimilation or by physical sorption to the sediment (Fisher *et al.* 2004). The uptake of inorganic nutrients from the water column is a major aspect of nutrient retention in streams (Fellows *et al.* 2006; Stream Solute Workshop 1990).

The biotic compartment of nutrient cycling begins when living organisms like algae and bacteria take up nutrients in biologically available inorganic forms (i.e. $NH_4^+$, $NO_3^-$, $PO_4^{3-}$) and transform them into organic forms (i.e. bound to carbon-based compounds). Nutrients are recycled back to their inorganic form through remineralization by decomposers, excretion, or sloppy feeding by heterotrophic consumers on biofilms (Lavelle *et al.* 2005; Hauer & Lamberti 2007). An average nutrient particle may go through this cycle of biotic assimilation and remineralization many times over the course of its journey downstream (McClain, Bilby & Triska 1998). As the particle moves down the reach, a higher frequency of cycles per unity of the stream length indicates higher demand for the nutrient.

**4.2.2 Influence of Abiotic Factors on Biotic Assimilation**

Biotic uptake is heavily influenced by abiotic factors including hydrology and substrate composition. For example, discharge dynamics—which include changes in flow rate and groundwater inputs—influence the length of time that stream organisms are exposed to nutrients, as well as those organisms' ability to assimilate nutrients (Haggard *et al.* 2001; Schneck *et al.* 2011). During periods of higher discharge such as storm events, nutrients have less interaction with the benthos. Streams typically experience periods of very long spiraling length during storm events because water flow rate is too high for organisms to take up nutrients (Newbold *et al.* 1982).

Substrate composition also plays an important role in nutrient spiraling. Microbial and invertebrate communities depend heavily on benthic conditions; their ability to process N and P is directly related to the composition and health of the stream bed substrate. Stream beds in unimpacted streams are characterized by lack of erosion as well as roughness and surface irregularity of sediments that enhances biofilm diversity (Schneck *et al*. 2011; Zaimes *et al*. 2011). Biofilms growing on rough, variable-substrate surfaces have greater exposure to water and therefore more access to nutrients (Lottig & Stanley 2007). Additionally, healthy stream beds have fewer small particles suspended in the water column and therefore provide greater access to light, allowing autotrophic organisms to undergo photosynthesis and utilize

nutrients in the process. Autotrophic activity by algae plays a significant role in nutrient processing, fixing both N and P to enable photosynthesis (Sabater *et al.* 2000).

### 4.2.3 Nitrogen and Phosphorous Cycling in Streams

N is a key nutrient in freshwater ecosystems that is used for reproduction and growth of most water-dwelling primary producers (Elser *et al.* 2007). Bioavailable nitrogen species include $NO_3^-$, $NO_2^-$, $NH_4^+$, and dissolved organic nitrogen (DON) (Durand *et al.* 2011). Sources of N include atmospheric deposition, N fixation, and inputs from runoff and groundwater (Allan & Castillo 2007; Boyer *et al.* 2002). Streams may be important sinks for bioavailable N, preventing downstream eutrophication (Sobota *et al.* 2012; Alexander *et al.* 2000; Mulholland *et al.* 2008).

$NO_3^-$ and $NH_4^+$ are two commonly studied forms of N in lotic ecosystems. Dissolved $NO_3^-$ can be removed via denitrification, storage in organic matter, or burial (Sobota *et al.* 2012); these processes may increase with $NO_3^-$ concentration in the stream (Mullholland *et al.* 2008). $NH_4^+$, on the other hand, is the most labile, or most easily assimilated form of N (Webster *et al.* 2003). It is rapidly immobilized and can be remineralized or converted into more mobile forms (Peterson *et al.* 2001). This conversion can occur through anaerobic ammonium oxidation (Annamox), a mechanism of the nitrogen cycle that converts nitrite ($NO_2$) and $NH_4^+$ to the more

mobile Nitrogen gas form. Because it is less labile, $NO_3^-$ typically has longer uptake length than $NH_4^+$ (Webster *et al.* 2003; Newbold *et al.* 2006).

Like N, P is another important driver of biological activity in lotic ecosystems (Withers & Jarvie 2008). P is a common limiting nutrient in freshwater ecosystems (Reddy *et al.* 1999). Unlike other nutrients, P is typically transported in particulate—as opposed to dissolved—form (Allan & Castillo 2007). The most biologically available form of P, the form which is assimilated most readily, is phosphate (Reynolds & Davies 2001). Measured as SRP, this is the most commonly studied form of P in fluvial research (Triska *et al.* 2006). Headwater streams, specifically, play a major role in P retention and regulating the amount of P transported downstream (Withers & Jarvie 2008).

**4.3 The Importance of Nutrient Spiraling in Headwater Streams**

Nutrient spiraling is particularly important for understanding headwater streams, because a stream's ability to take up nutrients directly affects the health of the ecosystem downstream. Streams control the amount of nutrients exported to lakes, rivers and estuaries, and stream nutrient dynamics influence the degree of eutrophication in the receiving body of water, which is directly related to the amount of nutrient use (uptake) or removal that occurs in streams (Peterson *et al.* 2001; Hall *et al.* 2002; Allan & Castillo 2007).

Although streams constitute only about one percent of the area of an average watershed, they process all runoff that does not enter directly into groundwater reserves, and play a major role in taking up, mobilizing and transforming nutrients (Webster & Swank 1985). A stream's ability to store and remineralize essential elements can be viewed as an ecosystem service (Meyer 1997). Increasingly, the importance of in-stream processes in headwater streams is shown to play a significant role in controlling N loads to downstream systems (Lowe & Likens 2005). Headwater streams are particularly active sites of N uptake and transformation and can influence catchment exports (e.g. Alexander *et al.* 2000; Peterson *et al.* 2001; Mulholland 2004; Bernhardt *et al.* 2005).

### 4.3.1 Nutrient Spiraling in Forested Streams

In nutrient spiraling studies, forested streams are generally used as reference sites for examining nutrient dynamics of anthropogenically-impacted streams. Seasonality of light, temperature, and organic matter input may affect biotic demand for inorganic nutrients in forested streams (Mulholland *et al.* 1985; Mulholland *et al.* 2000; Hill *et al.* 2001). Forested streams process, use, and remove N and P from the water column. The benthic surfaces of forested headwater streams represent heterogeneous habitats, including sand, rock, organic sediments and wood, which have varying degrees of influence on nutrient uptake (Cardinale *et al.* 2002; Hoellein

*et al.* 2007; Pringle *et al.* 1988). Long-term studies of nutrient uptake in forested

watersheds have shown distinct seasonal variation in $NO_3^-$ and SRP concentrations,

which fluctuate in response to seasonal variation in demand (Mulholland 2004). One

study of three temperate headwater streams observed the highest $NH_4^+$ and $NO_3^-$

uptake velocities during the spring (Hoellein *et al.* 2007). Using uptake length to

assess nutrient dynamics requires a knowledge of the range of expected values and

natural variability (Davis & Minshall 1999; Table 4.1).

**Table 4.1** *A selection of studies on nutrient uptake in forested headwater streams since 1999. Most of these studies only examined 1 or 2 nutrients; a "-" indicates the nutrient was not studied.*

| Location | Study | No. of Streams | Uptake length range (m) | | |
| --- | --- | --- | --- | --- | --- |
| | | | $NH_4^+$ | $NO_3^-$ | SRP |
| Hubbard Brook Forest (NH) | Hall et al., 2002 | 13 | 5 - 270 | - | 2 - 85 |
| Central Idaho | Davis & Minshall, 1999 | 2 | - | 549 - 1,839 | 370 |
| Mack Creek (OR) | Sobota et al., 2012 | 1 | 1,111 - 1,491 | 3,575 - 987 | - |
| North America | Webster et al., 2003 | 11 | 14 - 1350 | - | - |
| Michigan | Hoellein et al., 2007 | 3 | 112 - 648 | 144 - 1,625 | 98 - 532 |
| Mountains in NW and SE USA | Hill et al., 2012 | 36 | 42 - 143 | - | 106 - 353 |

## 4.5 Implications of Detrimental Anthropogenic Land Practice

Differing land use surrounding stream ecosystems can alter nutrient

processing procedures in several ways, the most prominent of which include:

change in substrate, sediment composition and hydrology, alteration of redox

conditions, increased nutrient influx and consequently effects on microbial,

consumer and producer populations and interactions (Zaimes *et al.* 2011; Niyogi *et al.* 2004; Sabater *et al.* 2000). Two major land uses that alter these systems include

urban development and agricultural land use (Teufl *et al.* 2013; Walsh *et al.* 2005; Merseburger *et al.* 2005).

**4.5.1 Urbanization**

Nutrient uptake is negatively correlated with indicators of urbanization, meaning that streams become less capable of processing nutrients effectively as urbanization increases (Meyer *et al.* 2005). Urbanization of surrounding land can alter stream bed composition, morphology and stability, consequently reducing biotic richness as indicated by increased dominance of nutrient tolerant species (Walsh *et al.* 2005). Studies show that both $NH_4^+$ and SRP uptake velocities decrease as urban development progresses, indicating that streams become saturated with nutrients and are therefore unable to fully process large amounts of N and P (Meyer *et al.* 2005). Urbanization can be characterized by two major indicators: increasing percentage of impervious cover and introduction of large scale storm drainage system. These two factors significantly amplify the flow rate and water level rise due to storm runoff and contribute to point source inputs of high amounts of dissolved nutrients and sediments (Miller *et al.* 2014). This limits the contact nutrients can have with the benthos, making them more difficult to process.

Impervious cover can be defined as material, such as asphalt, through which rainwater and other substances cannot flow and be absorbed into the soil. Increase

in impervious cover in proximity to freshwater streams is one of the most significant

sources of stream nutrient dynamic alteration (Schueler *et al.* 2009). Urban runoff

pollution frequently involves a 'flush' phenomenon, where nutrient influx occurs at

high rates and then sharply decreases (Wei *et al.* 2013).  As impervious cover

increases, percent of rainfall absorbed into the soil is reduced. Instead, rainwater is

turned into runoff, carrying excessive amounts of N, P and sediment into

surrounding bodies of water. These events represent non-point pollution, and enter

the stream in a singular pulse (Uyugun *et al.* 2014). With increasing urbanization of

surrounding land, freshwater streams are experiencing these 'quickflow' events

more frequently (Raney *et al.* 2014).

In addition to increased levels of dissolved nutrients, sediment from erosion

is carried into urban streams and deposited on the stream bed. As a result of the

influx of sediments from the surrounding areas, urban streams experience

sedimentation of stream beds, decreasing light availability to benthic algae and

sediment oxygen concentrations, which reduces the productivity of autotrophic

communities (Schneck *et al.* 2011; Lottig *et al.* 2007; Hoellin *et al.* 2012). This reduces

biodiversity of urban streams, eliminating species that are not tolerant of high

nutrient levels and further reducing the capacity of the stream to absorb and process

nutrients.

**4.5.2 Agriculture**

Increasing agricultural land use and evolution of agricultural practices can have significant lasting effects on nutrient processing in freshwater streams. Decades of agricultural land use has heavily impacted streambeds, resulting in excess sediment and nutrients moving downstream (Zaimes *et al.* 2011; Merseburger *et al.* 2005). Increased sedimentation affects streambed composition and suspended sediments, resulting in the reduction of viable habitat for biotic processors (Niyogi *et al.* 2003; Schneck *et al.* 2011). Streams with surrounding crop fields frequently experience deterioration in sediment composition from nutrient enriched soil (Teufl *et al.* 2013). These changes in streambed substrate have lasting effects on biotic functioning and limit biodiversity in the stream.

Development of land surrounding streams can change the composition of stream biotic communities, and such changes may have important consequences for critical ecosystem functions such as nutrient uptake (Wang *et al.* 2011). For example, if macrophytes dominate over benthic algae then they will drive ecosystem metabolism, but will have limited influence on water column nutrient concentrations because they become saturated with excess nutrients injected from surrounding agricultural practices (Tall *et al.* 2011; O'Brien *et al.* 2014). While agricultural streams can support high rates of nutrient processing via biotic uptake, the effects of these processes do not play as significant of a role as they would in

undisturbed streams, where nutrients are limited (Schaller *et al.* 2004). Streams with higher degree of development nearby ultimately send more nutrients downstream as the system becomes saturated with nutrients.

Over-fertilization of crops results in large amounts of excess nutrients entering the environment. Diffuse nutrient inputs from agricultural fields have significant effects on water chemistry, and frequently remain unprocessed as they travel (Merseburger *et al.* 2005). N and P occur in increasing levels in the water column and end up draining into larger bodies of water resulting in nutrient loading (Niyogi *et al.* 2003; Raney *et al.* 2014). In particular, excess P from soils leach into stream waters from catchments, a phenomenon frequently associated with dairy farming (Hooda *et. al.* 1997), and $NH_4^+$ from fertilizers can have a similar effect.

## 4.6 Quantifying Nutrient Dynamics

Nutrient transport and exchange can be modeled using equations that account for processes such as advection, dispersion, groundwater and tributary inputs, transient storage, and transformation by biotic and abiotic compartments (Stream Solute Workshop, 1990). Decline in nutrient concentration over the reach scale, incorporating processes of uptake and release, is proportional to the gross rate of uptake (O'Brien & Dodds 2008). There are several ways of quantifying this. One example is to use spiraling length (S), which is the average distance that a nutrient

molecule travels from its dissolved, available form in the water column to the streambed and back again (Webster & Patten 1979; Newbold *et al.* 1981; Mulholland *et al.* 2002; Newbold *et al.* 2006). Spiraling length is the sum of uptake length ($S_w$)—how far the nutrient is transported in the water column in its dissolved, inorganic form before it is taken up—and turnover length ($S_b$), which is the distance it travels in particulate form before being remineralized (Chaubey *et al.* 2007). Since dissolved nutrients move faster than benthic ones, $S_w$ is commonly used as a measure of spiraling length (Newbold *et al.* 1982; Hauer & Lamberti 2007). A shorter uptake length suggests the nutrient is more limited (Newbold *et al.* 1982; Davis & Minshall 1999).

The downside to using uptake length is that this metric is sensitive to discharge, making it difficult to compare uptake lengths between streams (Hall *et al.*, 2002). To account for this, we use uptake velocity, ($V_f$) or the mass transfer coefficient (Newbold *et al.* 2006). Unlike uptake length, uptake velocity accounts for differences in depth and velocity between streams and also emphasizes biological influence on nutrient concentrations (Niyogi *et al.* 2004; Stream Solute Workshop 1990; Hall *et al.* 2002; Davis & Minshall 1999; Fellows *et al.* 2006).

Several different methods can be used to obtain the measurements necessary to calculate uptake length and velocity, including short-term nutrient addition, isotopic tracers, and breakthrough curves (TASCC method). Short-term nutrient

additions are the most commonly used method (Ensign & Doyle 2006) for uptake

measurements.

**4.7 Research Questions and Hypotheses**

Our research employed nutrient addition experiments to evaluate each

stream to determine nutrient uptake lengths for $NH_4^+$, SRP and $NO_3^-$. Streams were

then compared to determine if uptake lengths differed, we were interested in

examining the influence of various surrounding environments on uptake. These

land uses ranged from undeveloped at our control stream and residential (urban) or

agricultural development at our two impacted streams. The independent variable

that we thought would affect nutrient uptake was occurrence of road crossings. We

hypothesized that the presence of road intersections would increase uptake lengths

in affected streams due to the presence of impervious cover, which increases input

of excess nutrients and sediments.

Secondly, our research aimed to determine the role of stream bed sediment

processing on nutrient uptake, and to determine if there are differences in nutrient

uptake rate in sediment samples taken from three different distances down the reach

of each stream with different proximities to the road (Figure 4.2). We hypothesized

that sediment samples taken above stream from the culvert (i.e. road crossing), just

below the culvert, and further down the reach from the culvert, would all have

different nutrient uptake capacities, with sediment samples taken closest to the

intersection having the slowest uptake rate due to sedimentation and increased

influx of nutrient originating from the road crossing.
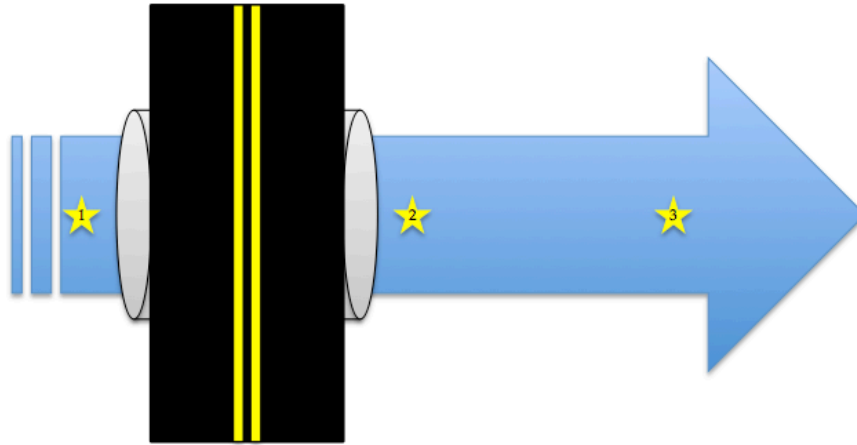


**Figure 4.2** *Conceptual diagram of sediment sampling locations for streams with road crossings (Rome Trout and Robbins Mill). At Whittier, samples were taken at the top of the reach, middle of the reach, and down the reach (to establish sediment uptake in streambed substrate uninfluenced by road crossings).*

## 4.8 Methods

### 4.8.1 Site Description

We studied three headwater streams in the Belgrade Lakes watershed in

central Maine. Two of the streams, Rome Trout and Robbins Mill, flow south from

North Pond into Great Pond. These two streams are crossed by Route 225, a paved

road with homes. The third stream, Whittier, flows into Long Pond and was

considered an unimpacted reference stream for this study because it has no road

crossings. A 80- or 100-meter reach was established at each stream using flagging

tape.

### 4.8.2 Short-Term Nutrient Addition Experiments

We conducted a short-term nutrient addition experiment for $NO_3^-$ (as $KNO_3$), $NH_4^+$ (as $NH_4Cl$), and SRP (as $KH_2PO_4$) at each of the three streams to determine uptake length and uptake velocity. These experiments involved adding a particular nutrient at a constant rate to a stream to increase background concentration by ~20 $gL^{-1}$. Subsequently we sampled at plateau concentrations, which were determined by using a conservative tracer (Stream Solute Workshop 1990). The nutrient addition was performed on each stream on a different day within a five week span during September and October of 2014.

For our nutrient addition experiments assumed a discharge ($Q$) of 115 L $s^{-1}$ based on a similar experiment conducted last year in the Belgrade Lakes watershed on streams of similar size (Poljak & Schell 2013); exact discharge could not be determined during this phase of the experiment due to a limited number of Marsh McBirney flow meters. Solutes were dissolved in 15 L of stream water, a value which we later increased to 20L to improve the dissolution of NaCl, and allow for a increased run time in order to be able to establish a plateau.

At each stream we took background conductivity measurements at five stations along the reach before performing the release. We collected three 60-mL water samples (one for $NO_3^-$, $NH_4^+$, and SRP) filtered through a 0.7 micron GF/F filter to determine background concentrations of each nutrient at each station.

We released our solutions into the stream using a battery-operated FMI Lab Pump (model RHB) with a drip rate of approximately 200 mL $s^{-1}$. We used a conservative tracer (NaCl) to determine plateau times, which we released simultaneously with $NH_4^+$ and SRP. We monitored conductivity at the farthest station downstream using an YSI EcoSense EC300 conductivity meter. The time from initial release to plateau conductivity is defined as the plateau time. We measured plateau time at each stream.

At plateau, we collected nutrient samples at each of the five stations. Three replicate samples were taken for each nutrient at each station. After a wait time (generally 24 hours), we repeated the nutrient release procedure for $NO_3^-$, sampling at the plateau time determined by the first release. We took the samples back to the lab and froze them until processing, which occurred about 5 weeks later.

### 4.8.3 Substrate-Specific Nutrient Injection Experiment

In addition to the field nutrient release experiments, we also performed a substrate-specific nutrient injection experiment in the laboratory to measure and compare uptake rates of sediments taken from locations above culverts, below culverts, and downstream (or, in the case of Whittier Stream where there was not culvert, samples were taken from upstream, midstream and downstream). We

obtained three sediment samples and 1L of stream water from each of these three sites along each stream on the same day.

We took the samples back to the lab and stored them in the refrigerator. We standardized sample volumes by taking 10 mL of sample from each of the three replicate samples from each site and transferring the sediment into a new 120-mL plastic cup. We filled each newly prepared cup with 80 mL of stream water. In total, we collected three replicates for each nutrient from each stream.

We added 1 mL of concentrated $NO_3^-$, SRP and $NH_4^+$ to each designated cup. We sampled from each cup at 0, 1 and 2 hours. Water samples were filtered through 0.7 micron GF/F filters, stored in 20-mL disposable plastic scintillation vials, and frozen until analysis.

### 4.8.4 Data Analysis

Nutrient concentrations were determined using a QuickChem 8500 series automated ion analyzer from Lachat Instruments. We mixed reagents and standards according to QuickChem Method 10-1115-01-1-A and followed the low-nutrient specifications to prepare working standards. Samples were thawed in hot water baths just prior to running the Lachat. SRP, $NH_4^+$ and $NO_3^-$ were analyzed on separate days.

We constructed standard curves for each nutrient. These equations were then used to calculate concentrations, which were derived from peak area for for SRP and $NH_4^+$. Air bubbles were present when analyzing the $NO_3^-$ samples, possibly because the reagents were not at room temperature; to minimize the effect that the air bubbles would have on concentration, we used peak height instead of peak area for $NO_3^-$. To determine uptake length ($S_w$), we found the inverse slope of a regression between distance downstream and the natural log of the net change in nutrient concentration divided by the net change in conductivity (Figure 4.4).

We calculated uptake velocity ($V_f$) by incorporating the width and discharge at each station to account for differences in discharge. Discharge measurements were taken by the Sediment and Hydrology team and incorporated in our calculations. We were only able to obtain one sample value for each of $S_w$ and $V_f$ per stream and were therefore unable to conduct any statistical analysis due to lack of sufficiently large sample sizes.

## 4.9 Results

### 4.9.1 Background Concentrations

Average background SRP concentrations were highest at the two impacted streams, Rome Trout and Robbins Mill at 0.195 gPL$^{-1}$ (Rome Trout) and 0.144 gPL$^{-1}$

(Robbins Mill) (Figure 4.3). $NO_3^-$ was highest at Rome Trout (.1346gPL$^{-1}$), and was below detection at Whittier. $NH_4^+$ was relatively low across all three streams, with an average of .0106 gPL$^{-1}$ at Rome Trout, .0177gPL$^{-1}$ at Robbins Mill, and .0163 gPL$^{-1}$ at Whittier.
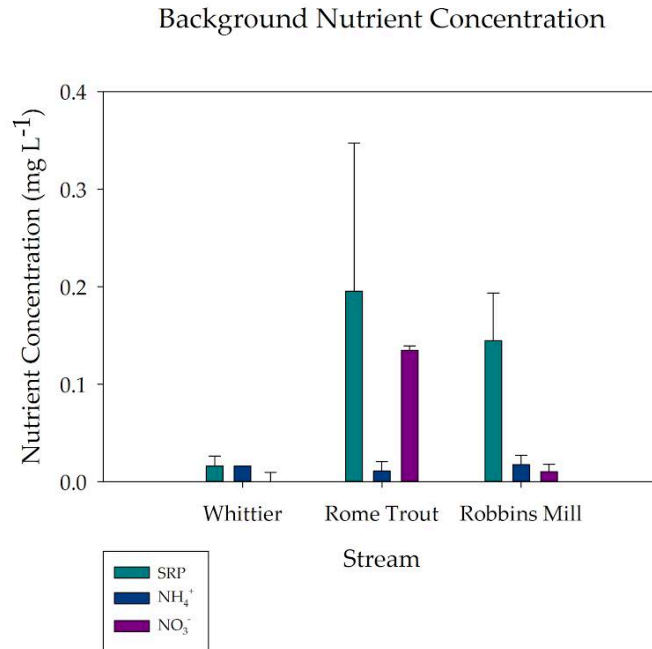


**Figure 4.3** *Background nutrient concentrations for each stream (±1 SE).*

### 4.9.2 Uptake Length

Uptake length for SRP was relatively similar across all three streams, ranging from 178 m at Whittier to 250 m at Robbins Mill (Table 4.1). $NO_3^-$ uptake length differed between Rome Trout (55 m) and Robbins Mill (208 m). All but one location had a positive uptake length; the only negative uptake length was $NO_3^-$ at Whittier, suggesting that there was virtually no uptake (Figure 4.5). $NH_4^+$ had both the

shortest observable uptake length (45 m at Whittier) and the longest uptake overall

in our study (833 m at Rome Trout) (Figure 4.5).

**Table 4.2** *Uptake length ($S_w$) and uptake velocity ($V_f$) for each nutrient. Values for Whittier $NO_3^-$ were negative, suggesting little to no $NO_3^-$ uptake.*

| | Uptake length (m) | | | Uptake velocity, Vf (mm min-1) | | |
|---|---|---|---|---|---|---|
| | $NH_4^+$ | $NO_3^-$ | SRP | $NH_4^+$ | $NO_3^-$ | SRP |
| Whittier | 45 | - | 178 | 13.6 | - | 3.43 |
| Rome Trout | 833 | 55 | 222 | 0.858 | 12.9 | 3.22 |
| Robbins Mill | 95 | 208 | 250 | 4.14 | 1.89 | 1.58 |
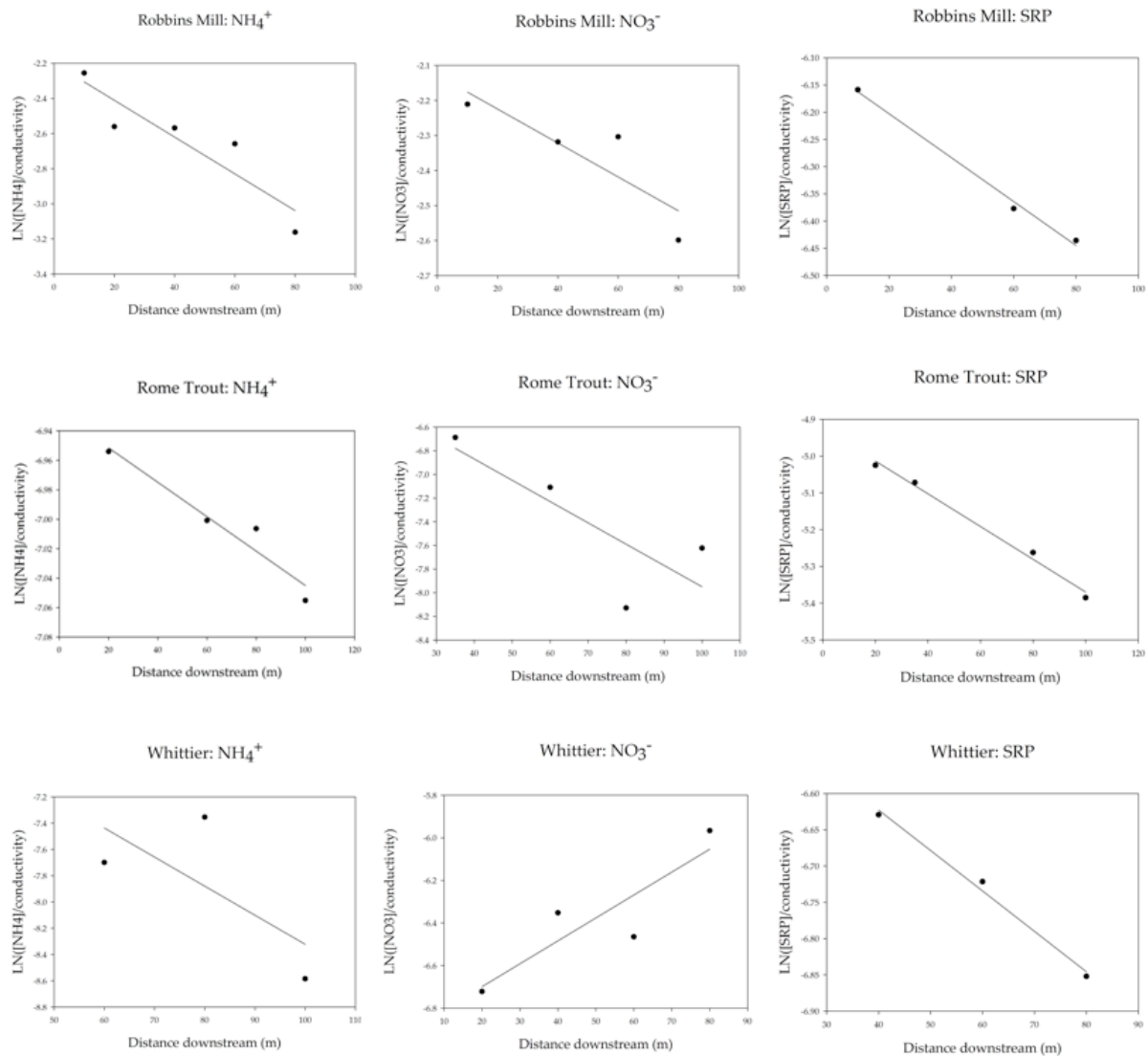


**Figure 4.4** *Regression lines of $NO_3^-$, SRP and $NH_4^+$ for each study area. Uptake length is the inverse slope of these regression lines. There was no observed presence of $NO_3^-$ or $NO_3^-$ uptake at Whittier Stream.*
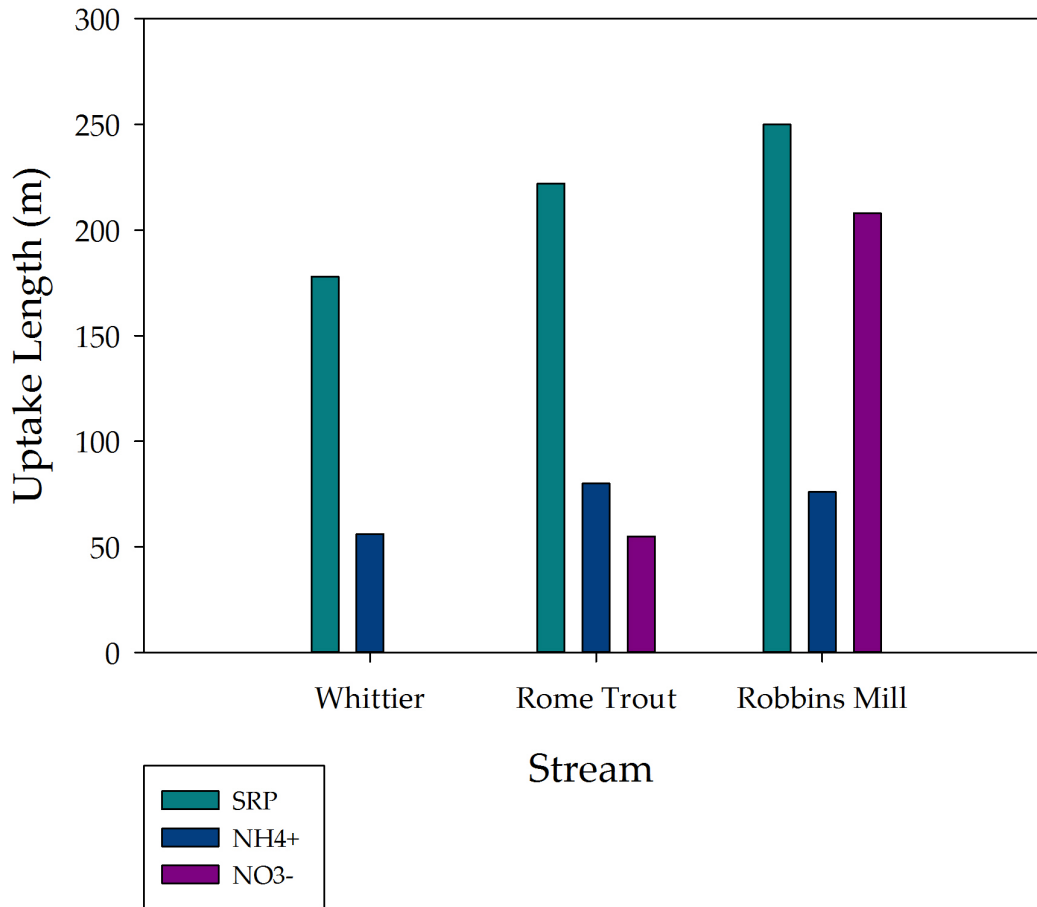
**Figure 4.5** *Comparison of uptake length for SRP, $NO_3^-$, and $NH_4^+$ at each stream.*

### 4.9.3 Sediment Exposure

The sediment exposure experiment showed various uptake rates for sediments taken from different locations at each stream (Figure 4.6). Lack of replicates prevented us from being able to test for statistical significance, but there was no observable pattern to suggest that sediment taken from above and below the culvert took up nutrients faster or slower than downstream sites (Table 2).

The fastest uptake rate was observed for $NH_4^+$ in the sediment from down the reach at Robbins Mill (-0.794 mg $NH_4^+$ $L^{-1}$ $h^{-1}$). The slowest was SRP down the reach at Rome Trout (0.15 mg SRP $L^{-1}$ $h^{-1}$). The stream with the fastest, most consistent uptake rates across all three sites was Whittier (0.43±0.019 mg $L^{-1}$ $h^{-1}$, $n=3$).

In some samples, nutrient concentration increased over time. This was the case for $NH_4^+$ at all three sites along Rome Trout. Two streams showed a combination of nutrient uptake and release at different sites along the reach. This was observed for $NH_4^+$ at Whittier (upper and lower reach) and for $NO_3^-$ at Rome Trout (above the culvert and down the reach). Robbins Mill showed uptake for all three nutrients at all three sites (Figure 4.6).

**Table 4.3** *Uptake rates for nutrients at each of three sites along the reach. Bold values are negative, suggesting nutrient uptake by the sediment (as opposed to nutrient release). Data are incomplete for $NO_3^-$ at Whittier due to outliers.*

|  |  | Uptake rate (mg $L^{-1}h^{-1}$) | | |
|  |  | $NH_4^+$ | $NO_3^-$ | SRP |
|---|---|---|---|---|
| Robbins Mill | Above the culvert | **-0.3791** | **-0.245** | **-0.335** |
|  | Below the culvert | **-0.2347** | **-0.2745** | **-0.27** |
|  | Down the reach | **-0.7943** | **-0.1862** | **-0.215** |
| Rome Trout | Above the culvert | 0.0632 | 0.0588 | **-0.37** |
|  | Below the culvert | 0.3141 | **-0.196** | **-0.475** |
|  | Down the reach | 0.3069 | 0.0392 | **-0.15** |
| Whittier | Upper reach | 0.0351 | - | **-0.405** |
|  | Middle reach | **-0.4314** | - | **-0.4314** |
|  | Down the reach | 0.0361 | - | **-0.44** |
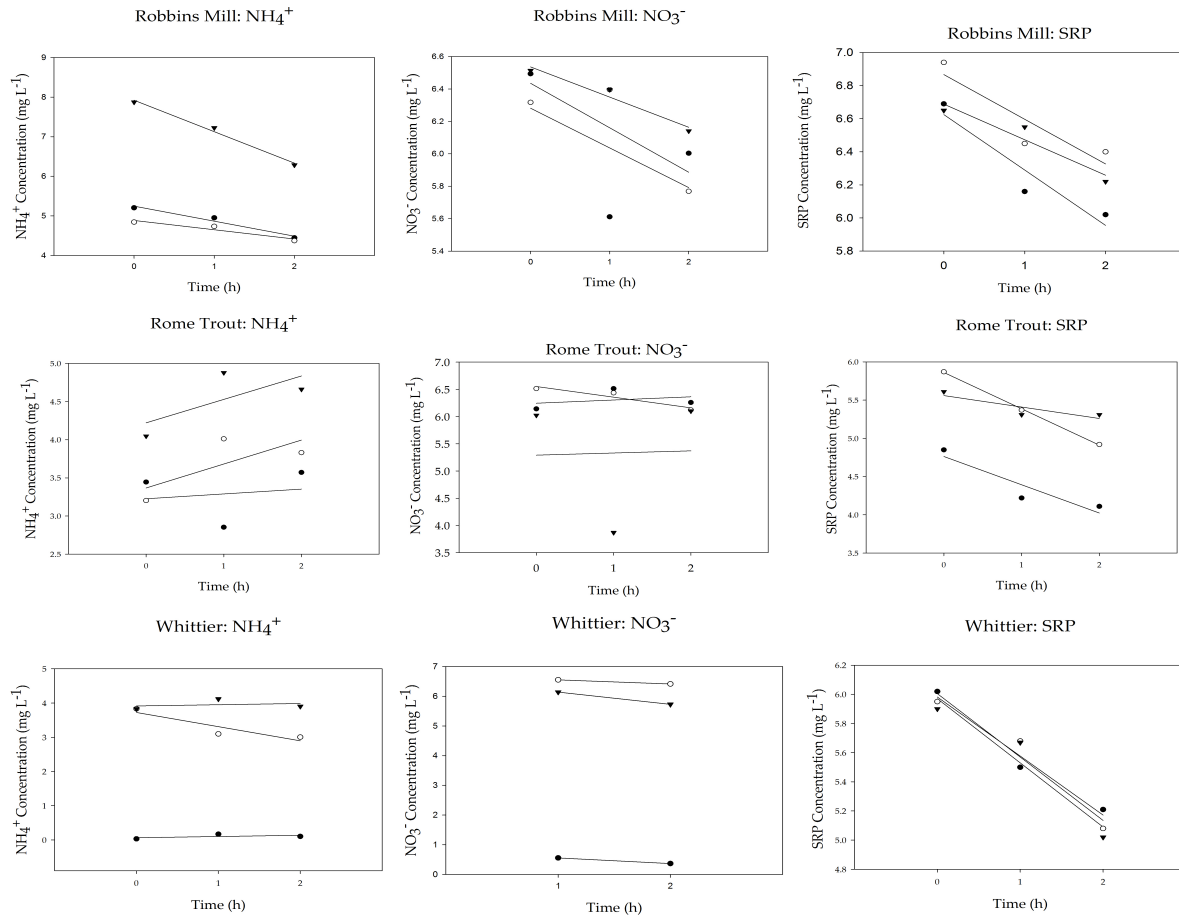
**Figure 4.6** *Regression showing the change in nutrient concentration (y axis) over time (x axis) for each nutrient across each of the three streams.*

## 4.10 Discussion

Extensive research has been done of the effects of anthropogenic land

development on nutrient processing in streams (Zaimes *et al.* 2011; Niyogi *et al.* 2004;

Sabater *et al.* 2000). Some of the most prominent ways in which land development

and road construction can influence nutrient spiraling in streams is by altering

substrate composition, hydrology, biotic communities and increasing the frequency

and concentration of nutrient influx events. Studies show that road intersections,

and consequently an increase in impervious cover in proximity to freshwater

streams, is one of the most significant sources of stream nutrient dynamic alteration

(Schueler *et al.* 2009). The health of lake ecosystems depend on that of the smaller

headwater streams and watersheds (Peterson *et al.* 2001; Hall *et al.* 2002; Allan &

Castillo 2007), making the assessment of nutrient dynamics in the Belgrade

Watershed important for the surrounding community, which depends largely on the

health of the watershed as well as the lakes that they influence. Results of our study

indicate that road intersections may have had significant effects on nutrient uptake

by decreasing instream nutrient processing. Calculated uptake lengths provide

evidence that the studied headwater streams are impacted by anthropogenic land

use, and these impacts are affecting the concentrations of nutrients exported to

larger bodies of water. Below, we examine several aspects of nutrient cycling

dynamics in these streams to determine what processes have been significantly

affected.


**4.10.1 Background Nutrients**

As expected, background concentrations of SRP and $NO_3^-$ were higher at the

two impacted streams, Rome Trout and Robbins Mill, than at Whittier. As well as

the two impacted streams having road intersections, both have anthropogenically-

developed catchments as shown in the satellite images of the two impacted streams

and their surrounding land usage (Figure 4.7). The image of Robbins Mill shows

housing developments directly on the intersecting road, and the upper right corner

of Figure 7b shows a fraction of the agricultural fields that surround the upper reach

of Rome Trout. In combination with observations of car traffic and human activity

that were seen during the research period, this strongly supports the hypothesis that

anthropogenic development and activity produces nutrient influx into the associated

stream as well as being positively correlated with uptake length. In contrast, the

image of Whittier stream (Figure 4.7c) shows limited surrounding land use, and no

other human activity was noted during the experimental period, two factors which

support our results showing that Whittier has the lowest background nutrient

concentrations.

While background concentrations of SRP and $NO_3^-$ were found in higher

concentration at impacted streams, $NH_4^+$ was consistently low across all three

streams. This could be explained by the fact that $NH_4^+$ may not be the form of N

being introduced into the environment by human activity. Instead, surrounding

anthropogenic land use is inputting $NO_3^-$, which explains higher background

concentrations in some cases. Additionally, $NH_4^+$ is generally the preferred source of

nitrogen for benthic organisms, being the most easily assimilated (Webster *et al.*,

2003), which could indicate why $NH_4^+$ levels are low. When the nutrient is limited, it

is required for growth but concentrations are not high enough to meet biological

demand (Esler *et al*. 2007; King *et al*. 2014; Reddy *et al*. 1999), benthic organisms take

up as much of the nutrient that is available. However, this does not explain why

$NH_4^+$ levels are consistently detected at the same levels. If it was a limiting factor, all

the $NH_4^+$ would be taken up and the background concentrations would be closer to

zero.



**Figure 4.7** *Google Maps images of three study streams: a. Robbins Mill  b. Rome Trout c. Whittier Stream*

### 4.10.2 Nutrient Uptake Experiments

The nutrient release experiment showed virtually no $NO_3^-$ uptake at Whittier.

Calculations showed that this uptake length value was negative, which could be

explained by an extremely long uptake length. When creating graphical

representations of uptake length, that of Whittier showed no decline as the nutrients

traveled down the reach. However, if we could have monitored the course of the

nutrients indefinitely, we would have most likely seen a decrease in nutrient

concentration. Therefore, it is possible that Whittier is, in fact, taking up $NO_3^-$, but so slowly that it takes much longer to actually begin cycling the nutrient.

This could have been expected if background $NO_3^-$ concentrations were high, signifying that the stream was not limited for $NO_3^-$; however, the average background concentration was below detection ($< \sim 10$ mg $L^{-1}$, *n=5*). The biota were not taking up $NO_3^-$ even though it was made available through the nutrient release. This discrepancy suggests that organisms were meeting their biological nitrogen requirement from other sources of nitrogen, possibly $NH_4^+$. This hypothesis is supported by our data, which show the uptake length for $NH_4^+$ at Whittier to be 45 m, the shortest uptake length in the entire study. This is a plausible scenario as $NH_4^+$ is the most easily assimilated form of N and typically has shorter uptake lengths than $NO_3^-$ (Webster *et al.* 2003; Newbold *et al.* 2006).

We did not have the statistical power to assess whether or not road crossings had a significant impact on uptake length due to lack of replication of the experiments, however Robbins Mill and Rome Trout tended towards higher uptake lengths than Whittier for each nutrient (Table 4.1). We expected to see this because the proximity of Rome Trout and Robbins Mill to impervious roads and human development may increase the possibility of sedimentation, nutrient loading, and consequently higher background nutrients. Sedimentation may increase uptake length by decreasing the amount of viable habitat for organisms that would

otherwise process nutrients (Niyogi *et al.* 2003; Schneck *et al.* 2011). We would need to conduct further studies to uncouple the effects of land use and road crossings on uptake length, but our data provide preliminary evidence that our hypothesis was correct.

**4.10.3 Nutrient Exposure**

Whittier took up SRP very quickly at all three sites, i.e. upper, middle and lower reach (0.43±0.019 mg L$^{-1}$ h$^{-1}$, *n=3*). These were three of the fastest, most similar uptake rates observed across all streams (Figure 4.6). We would expect uptake rates to be similar in an unimpacted stream where there is no major anthropogenic source of nutrient runoff or sedimentation to block nutrient uptake. We would also expect an unimpacted stream like Whittier to be nutrient-limited for similar reasons. The relatively fast SRP uptake rates at Whittier suggest that the stream was P-limited. However, this hypothesis is not strongly supported by our uptake length or velocity data, which show that Whittier had a much shorter uptake length for $NH_4^+$ than SRP, and that the SRP uptake velocity at Whittier (3.34 mm min$^{-1}$) was very similar to that at Rome Trout (3.22 mm min$^{-1}$). These discrepancies between the sediment-specific uptake rates and the reach-scale uptake length and velocity suggest that the sediment-specific SRP uptake rates at Whittier were not necessarily fast enough to

signify limitation. Overall, our data suggest that Whittier was more limited for $NH_4^+$ than SRP.

The relatively low rate of $NO_3^-$ uptake by sediment from Rome Trout and Whittier suggests that the samples were either already saturated with $NO_3^-$, or there was a lack of biota for which $NO_3^-$ was bioavailable. The faster uptake rate for SRP at all three streams suggests that either SRP was more limited than $NO_3^-$, or that there were more biota present to take it up, or more biota that could take it up efficiently.

The Sediment and Hydrology team found that Robbins Mill and Rome Trout had a significantly higher amount of sand and silt closer to the road then down reach compared to Whittier. Studies indicate that coarse grained, heterogeneous stream beds are indicative of efficient nutrient spiraling in unimpacted streams (Schneck *et al*. 2011; Zaimes *et al*. 2011). Higher percentage of fine grain sediment would therefore decrease uptake of nutrients from the water column. Furthermore, finer grain sediments such as sand and silt have been shown to contain higher concentrations of P (Kairserli, Voutsa & Samara 2012). This would indicate that fine sediments that are either saturated or blocking biotic uptake, and would not reduce nutrient concentrations in the water column. However, while our sediment uptake data indicated that some nutrients were taken up slower near the roads at certain streams (e.g. $NH_4^+$ at Robbins Mill, Figure 4.6), we would require more replicates to

show that there is a statistical correlation between road intersections, sedimentation and nutrient uptake.

**4.11 Directions for Future Study**

While results of our study indicated a significant relationship between road intersection and uptake length, further study must be done to fully evaluate the effects of human development on nutrient processing in the Belgrade watershed. The study period for our research did not allow investigation into the effects of seasonality and the implications of different seasons on nutrient spiralling. Studies have shown that uptake length varies seasonally, and side effects of temperature change and weather patterns could also significantly affect nutrient dynamics. This could include events such as snow melt, or application of road salts. Additionally, time constraints prevented us from conducting replicates, which limited our statistical analysis. With these factors in mind, it would be beneficial to conduct multiple nutrient releases and sediment exposure experiments to increase statistical power as well as performing releases over time to see seasonal changes in uptake. Further study could also include an in depth evaluation of sediment composition as well as stream biota, primary production and metabolism to get a more nuanced look at where, when and how nutrients are being used in the stream.

This study provides evidence that anthropogenic development, including road crossings and the presence of agriculture may influence background nutrient concentrations and uptake lengths at three streams in the Belgrade Lakes Watershed. The results of our study also provide a good jumping off point for further research in the area, being a good indication of the current nature of nutrient cycling dynamics in the Belgrade Lakes Watershed.

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# CHAPTER 5
# STORMS AND FLUXES

## 5.1 Introduction

One of the most unique factors of a low order stream is the dramatic capacity for change in flow on a daily, seasonal, and annual basis. Following storm events, low order streams can have discharge volume multiple orders of magnitude larger than during base (standard) flow, as seen in figure 5.1. These dramatic changes in stream discharge have large implications for ecosystem function, not only within the streambed, but also for the downstream body of water.

In low order streams, greatly increased discharge follows large rain events in the watershed. As rainwater saturates the ground, it begins to run downhill to the collecting stream. As this collecting stream volume of water increases, runoff picks up all manner of pollutants, nutrients, and particulate matter (in extreme cases even large animals!). Runoff (carrying pollutants, nutrients, and particulate matter) enters the streambed, can drastically change the concentrations of any nutrient, pollutant, or other material that is moved by water. These changes in concentrations, in conjunction with changes in discharge, are what we will refer to as flux, with units of mass of solute per unit time.

The relationship between flux patterns and storm intensity varies greatly by stream. In fact, developing a model for the flux of a single nutrient is extremely

difficult (Buck, Niyogi & Townsend 2004). This is due to the complexity of the delivery of nutrients into a stream. Certain types of ecosystems and land use tend result in predictable fluxes, however mapping an entire watershed from riparian zone to catchment edge is complex, and interactions (such as those related to groundwater) are difficult to determine. In the face of these complexities we have chosen to examine three metrics: flow and nutrient concentration to calculate nutrient flux, and concentration of pathogenic bacteria as an indicator of human impacts.

### 5.1.1 Storm Stages

A standard storm can be broken up into stages, with stream and watershed responses varying by stage during each storm event (Miller & Denver 1977). During the initial precipitation, the greatest influence on stream nutrient concentration comes from soluble salt solution within the soil. The transport of nitrate and chlorine occurs quickly after precipitation begins (Kennedy *et al.* 2012). Nutrients that are dissolved first are largely stored in the hyporheic zone, the area of sediment directly under the streamflow (Triska *et al.* 1990). Ferromagnesian minerals (such as olivine and pyroxene) and biomaterial potassium, contrastingly, are dissolved at a consistent rate during precipitation (Miller and Drever 1977). Additionally, other nutrients leach consistently for more than a week after a storm event (Triska *et al.*

1990).  Simply, the general pattern triggered by a precipitation event will trigger an initial rise in the concentration of nutrients through the transportation of dissolved nutrients into the stream, a decrease due to dilution, and ultimately a slow rise back to standard non-storm/precipitation event values (Miller and Drever 1977).  The timing of this rise to standard value depends on, among other factors, stream size, storm size, stream geomorphology, and land use patterns.
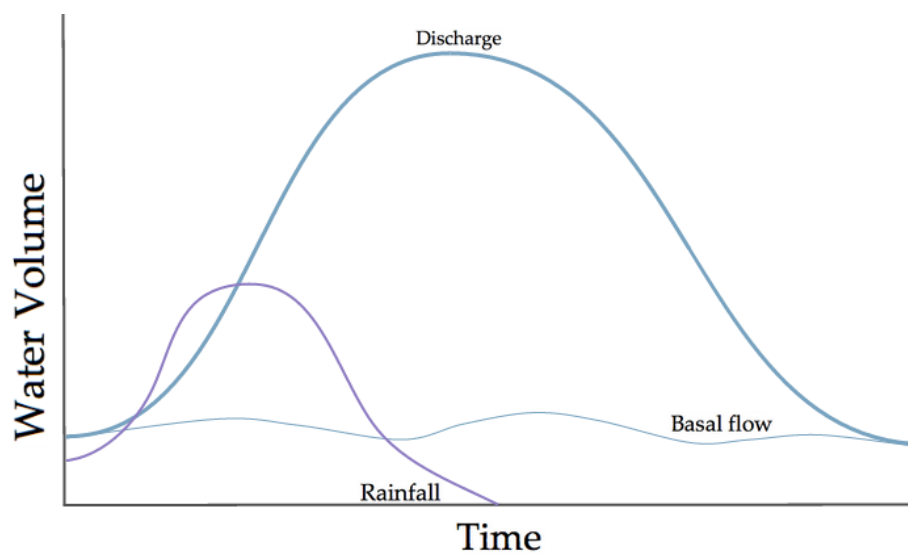


**Figure 5.1** *Conceptual diagram of water volume over time during a storm.  Note rainfall peak lags behind discharge peak, all relative to basal flow.*

As with many ecological examinations, we run into the issue of widely varying scales. Relative cycles and peaks of water discharge and nutrient concentrations vary and, subsequently lag from one another.  Flow lags behind precipitation peaks while water moves from ground to stream.  That lag varies from several hours to over a day given different watershed land characteristics (Chen *et al*. 2012).  The paths created by watershed type contribute to the length of the lag,

specifically, subsurface flow peaks (sub-surface channels) occur later and in a less

dramatic fashion than in surface flow (Albright 1991).  Additionally, nitrogen (N)

and dissolved organic carbon (DOC) lag behind flow peaks due to differences in

flow paths.  For example, $NO_3^-$ is more mobile than $NH_4^+$ which can slow its progress

through groundwater (Albright 1991).

Precipitation travels from landing point to stream channel in many ways. It is

important to consider whether the nutrient fluxes are a result of precipitation that

has picked up nutrients while flowing to the stream, or if water (and nutrients) were

simply displaced from within macropores (soil spaces).  This difference is referred to

as "new" (precipitation water picking up nutrients as it flows to the stream) versus

"old" (stored water and nutrients in macropores) water (Sklash, Stewart & Pearce

2010).  In storms of average magnitude, old water dominates: precipitation, or

"new" water, replaces "old" water that enters the water body.  We typically only see

"new" water as affecting stream composition in extremely severe storms with return

periods, or frequencies, of years to decades, likely with more than 100mm of flow.

During these extreme storm events, high levels of disturbance can lead to plant

removal within and near a stream (Fisher *et al*. 1982). Disturbance, such as large

storm events, can instigate succession, causing streams to stay as immature

communities (Fisher *et al*. 1982).

Flowpaths contribute to differences in discharge peaks, nutrient solution and nutrient fluxes. A major part of these differences is due to two major types of surface flow, sheet and channel flow.  Water moves more slowly in sheet flow as a result of the minimal gradient and high land resistance, and it is responsible for more nutrient transport as it erodes fine grained sediment (Guy 1964).  Sheet flow causes more erosion and has a greater influence on stream drainage areas than channel flow.  However, the tipping point from channel to sheet flow is a major point of emphasis in Guy, 1964 that is not fully understood (Verseveld, Mcdonnell & Lajtha 2009).  Channel, or "rill" flow, allows water to move much more quickly due to the depth of the channel, gradient, and lower drag resistance from water-bed interaction (Guy 1964). Watershed land use characteristics contribute greatly to the rate of erosion.  The various paths of water differ greatly given varying levels of precipitation intensity, from standard base flow to storm flow (Oda, Ohte & Suzuki 2011). In addition, deforested watersheds on steep slopes are identified as most susceptible to channelizing erosion (Yang *et al*. 2013).  On a much smaller level, the force of individual raindrops can affect the level of erosion, as the kinetic energy of raindrops causes splashing which leads both to suspended sediment transport and soil sealing, causing watersheds to be more susceptible to sheet flow (Guy 1964).

Due to the season and characteristics of our study area, we focused primarily on the effects of increased discharge, but drought-related effects on nutrient fluxes

are both complex and important.  Previous research has concluded that droughts are

responsible for the physical fragmentation of streams and water bodies, and

subsequently cause a larger relative role of deep groundwater inputs, which are the

opposite of "new" water (Dahm 2003).  In addition, droughts cause a heightened

representation of groundwater nutrient inputs (Dahm 2003).  The decreased flow

leads to decreased transport and export of organic carbon, DOC, nitrogen, and

phosphorus. Additionally, there is a related decrease in the availability of organic

nutrients relative to inorganic nutrients, which favors growth of autotrophs over

heterotrophs.

As discussed in the Conservation Lessons chapter, environmental policy fails

to protect streams in part, because influencing land is often located far from the

stream, as is the case with non-point sources.  The most important characteristics

that influence stream water movement are the type and amount of precipitation, the

shape and location of land slope, the soil types and underlying geology, the land use

and cover types, and the density and state of channel flow (Guy 1964). In addition,

temperature is a crucial variable of consideration, as colder water is more viscous,

relevant both to the movement of the water as surface tension varies, as well as to

the ability to hold suspended particles: warmer water can hold more suspended

particles  (Poole & Berman 2001).

**5.1.2 Chemical Cycling**

Nutrients in stream ecology affect not only the health of the stream ecosystem, but also the downstream body of (Vandenberg *et al.* 2005). Nitrogen and phosphorus have substantial impacts on stream organisms and can cause excess nutrient loading in downstream water systems (Berkowitz *et al.* 2014). Nitrogen exists as a part of several compounds in streams. The most likely inorganic forms to be delivered to streams are nitrate ($NO_3^-$) and ammonium ($NH_4^+$). Dissolved organic nitrogen (DON) is also present in streams. Nitrogen can be fixed from atmospheric $N_2$ to $NH_4$, a form that is readily available to organisms to utilized (Marcarelli, Baker & Wurtsbaugh 2008) . Freshwater nitrogen-fixing cyanobacteria assist in replacing $NH_4^+$ stores that are removed by $NH_4^+$ requiring algae and other primary producers that require fixed nitrogen (Vitousek 2002). However, the volume that these bacteria are able to fix has not been fully examined as artificially and anthropogenically-introduced nitrogen accounts for a far greater percentage of fixed nitrogen stores in stream systems (Vitousek 2002). This is a result of the introduction of the Haber Bosch process, and the pool of fixed nitrogen has more than doubled its natural level with significant negative ecological implications such as algal blooms and eutrophic bodies of water (Vitousek 2002).

A basic understanding of the varying nitrogen-based compounds and their fluxes in lower order streams is important in understanding nutrient cycling of the

entire waterway. In fact, riverine nitrogen is connected to responses of coastal systems hundreds of miles away (Chen *et al*. 2012). Storm systems can cause large pulses in nitrogen fluxes, which are integral in understanding the health of the stream and water system.  We examined three low order streams in this study. Northern Lakes tend to be phosphorus limited, i.e. the relatively lower concentration of phosphorus prevents exponential algal growth (Tank & Dodds 2003). Phosphorus concentration increases early in the storm cycle. Precipitation and initial erosion are the major source of this initial change in concentration (Edwards & Withers 2008). In addition, storm events following long dry periods or winter fertilizing on agricultural fields are known to create a "first flush" phenomena (Stutter, Langan & Cooper 2008). These "first flushes" are caused by eroding surface water which quickly picks up sediment and phosphorus and carries them into streams (Stutter, Langan & Cooper 2008).

### 5.1.3 Carbon

We were unable to examine particulate and dissolved organic carbon, but felt it important to include the current literature on this relevant topic, as it informed choices we made about where to focus our research.  Namely, that POC and DOC respond differently to storms.  Jeong *et al*. (2012) examined 42 storm events and found that POC was significantly less than DOC in base flow and small storms, but

that during periods of extreme flow, POC was greater than DOC.  Both POC and

DOC have a hysteretic relationship with discharge, but past a certain threshold of

water flow, POC is more affected by past periods of high flow.  The majority of DOC

flux occurs during standard flow conditions, on the other hand.  POC export as a

result of erosion will only continue to increase with climate change induced

increases in extreme storm events.  Both POC and DOC will transport organic forms

of N and P, particulate and dissolved.


### 5.1.4 Storms and Humans

Rivers and streams across the country can no longer support healthy

ecosystems (Poff *et al*. 1997).  This is due to failed management approaches as well as

a failure to acknowledge the need to maintain the natural state of our land.

Protection is consistently too narrow in scope and fails to protect the land beyond

the area of base flow.  The effects of anthropogenic development on our waters are

clear. As we increase impervious surfaces (such as paved areas) and pollution, we

increase the degradation of our waters (Schoonover & Lockaby 2006).

Urban streams worldwide have the highest biological oxygen demand (BOD)

the highest levels of suspended sediment, and the lowest amount of organic carbon

(Mallin, Johnson & Ensign 2009). Watershed development has been directly tied to

biological oxygen demand, which can lead to fish kills.  Terrestrial sinks are

becoming saturated through fertilizers and concentration of waste.  We are polluting

aquatic sinks, and increasing bioavailable nitrogen (Mulholland *et al.* 2008, Vitousek

*et al.* 1997).  In the short term, increased $CO_2$ concentrations lead to increased plant

growth, but this will drop off due to a decrease in available nitrogen due to the

amount in biomass and decomposing plant matter (Vitousek *et al*. 2002).   Inland

waters form a large portion of these sinks due to the transport, sequestering, and

mineralizing of anthropogenic $CO_2$ emissions (Jeong 2012).

Water quality has direct implications on human use of water resources.

Several recent studies have shown a decline in water quality with increased

development in the metrics of percent impervious cover and housing density. In

addition, pathogen concentration tends to increase with watershed development

(Young & Thackston 1999).  Further negative effects will continue to become visible

and damaging with continued pollution.


**5.1.5 Contamination**

In addition to nutrient loading, streams are susceptible to the flush of toxins

and other contaminants, especially during times of high flow such as storm events.

Toxins, such as pesticides have been shown to surge with increased discharge, and

account for contamination of downstream water bodies (Neumann et al. 2002).

Pesticides are not the only contaminant that has significant ecological and human

health implications; coliform and *E.coli* bacteria are known indicator bacteria of fecal

contamination (Ottoson & Stenstorm 2003, Sorensen *et al.* 1989). In addition to fecal

contamination, these bacteria indicate pathogenic bacteria and viruses (Calderon *et al.* 1991). High concentration of these bacteria can severely limit the recreational,

agricultural, and industrial use of a stream or downstream lake due to human health

risks such as severe illness and death, as well as risks to pets and livestock (Schiff,

Weisberg & Colford 2009).

Storms and large fluxes in discharge impact the levels of pathogenic bacteria,

particularly indicator bacteria (Muirhead *et al.* 2004). The increase in flow caused by

storms is associated with a significant increase in turbidity and in the concentration

of pathogenic bacteria (Hunter *et al.* 1999). The major sources of these pathogenic

bacteria are point sources, such as wastewater treatment plants, and non-point

sources such as fertilizer and waste runoff from fields, farm systems, and septic

systems (George, Anzil & Servais 2004). In our three streams we have no major point

sources of pollution but there is a significant farm system in the Rome Trout

watershed, and all homes rely on septic tanks for their wastewater treatment. In

addition to these traditional sources of pathogenic bacteria, literature has shown the

potential storage of *E.coli* specifically in

stream beds (Bai & Lung 2005, McDonald et al. 1982). Stored *E. coli* is then released

back into the water column during storm events and transported downstream (Bai

& Lung 2005). Turbidity and concentration of coliform and *E.coli* concentrations are also closely related (Bai & Lung 2004). Pathogenic bacteria are able to bind to suspended solids, and thus, as turbidity increases the amount of bacteria transported by these suspended solid increases as well (Bai & Lung 2004; Guber *et al.* 2007).

### 5.1.6 Our Research

Maine has the highest percent of forested land of any state, with roughly 90% forest cover, which remarkably hasn't changed since at least 1986 (Dennis 1986). Our hypotheses are based on the work of Guy (1964) and Wagner *et al.*'s (2008) conclusions that the majority of suspended sediment export in streams is triggered by storm events, and we examined how storm events related to different fluxes in forested stream systems. Fisher *et al.* (1982) suggest flooding is the most common form of stream disturbance, but that this disturbance is a fundamental aspect of the ecosystem. However, because of the brevity and stochastic nature of storms we developed a flexible sampling plan that allowed us to capture a greater portion of the storm's activity and resulting stream changes (Neumann *et al.*, 2001). In addition our sampling allowed us to determine the basal flow of all three steams as we had several weeks of basal flow conditions.

Our major research questions are threefold. First, how does change in discharge affect change in nutrient flux? Second, how does this relationship vary by level of anthropogenic influence? Finally, what is the role of pathogens *(E.coli* and indicator bacteria, coliform) in these relationships? Our hypotheses are as follows. First, increased flow will be correlated with significantly increased phosphorus of our anthropogenically impacted streams of Robbins Mill and Rome Trout. However, we predict that our less impacted stream, Whittier will show a lower increase in phosphorus with elevated discharge.  Second, discharge and nitrogen; increased flow will be correlated with increased $NH_4^+$ at our anthropogenically impacted streams of Robbins Mill and Rome Trout. However we predict that our less impacted stream, Whittier, will show a significantly lower increase in $NH_4^+$ with increased discharge. Finally we predict increased levels of *E.coli* and coliform at impacted streams, the highest at Rome Trout due to the farming operation just upstream to our sample site, and the lowest at minimally impacted Whittier stream.

### 5.2 Methods

Our research for this chapter involved water sampling, flow measuring, and *E.coli* and coliform sampling. We took water samples prior to all other metrics to minimize additional nutrients and sediment disturbed during the process of taking flow measurements. In all three streams, we looked for sampling locations with fast

moving (riffle) water in the central channel of the stream flow to control for localized

variation within the stream water column.

After finding a suitable spot, we filtered 60mL of water through a triple-

rinsed syringe and filter system. We used a 60 mL plastic BD Luer-Lok syringe, filter

attachment and 0.45 µm glass fiber filtration paper. We filtered the 60 mL samples

through the paper and directly into pre-labeled 125 mL Nalgene bottles.  In addition

to these filtered samples, we collected water directly (unfiltered) into triple-washed

pre-labeled 250 mL Nalgene screw top plastic bottles.  We placed these samples into

a dark Igloo cooler with two Nordic-ice chemical freezer packs for refrigeration

during transportation from the stream sites to the laboratory.  We placed each set of

filtered and unfiltered water (one of each sample from each stream) in a single

gallon bag which we then frozen and stored until the end of our sampling period

(9/11/14-10/28/14).

After sampling across a variety of flows over two months, we prepared and

digested water samples for nutrient testing. Our testing consisted of two main

workflows for our filtered and unfiltered water samples. For our unfiltered water

samples we examined total nitrogen (TN) and total phosphorus (TP). To do this we

first digested our samples using procedures outlined in QuickChem Method 10-107-

04-6-A protocol using an autoclave to ensure all phosphorus was converted to total

reactive phosphorus (SRP), and all nitrogen was converted to $NO_3^-$. After digesting

all unfiltered water samples, we stored them in a refrigerator prior to testing for

nutrient concentration.  We tested our digested water samples for total reactive

phosphorus using the Lachat QuikChem 8500 and using a Lachat Autosampler XYZ

ASX 520 to determine the concentration of TRP in each sample following the

QuickChem Method 10-107-04-6-A protocol.  After completing all TRP samples, we

moved to reactive $NO_3^-$ concentration testing using the same equipment and

following QuickChem Method 10-107-04-6-A protocol.

For filtered water samples we did not digest samples, and moved directly to

SRP and NO3- concentration testing.  After first thawing our filtered water samples

in a warm water bath, we followed an identical concentration testing procedure and

used identical equipment as we used with our unfiltered water samples after

digestion.

In conjunction with our water sampling, we also measured stream discharge.

We accomplished this using a Marsh McBirney Flow Meter 1000. To find the ideal

location for flow discharge measurement, we attempted to find moderately moving

water, and ensured that there were no side channels diverting water around our

sampling site and the stream bottom had minimal rocks.  By selecting main channel

sites with minimal large rocks we were able to produce a precise depth profile in

conjunction with flow speed rates.  When sampling conditions allowed, we

measured flow at two separate locations at each stream during each sampling date

to minimize bottom contour error and confirm the validity of our measured flow. Finally, we measured at the same section on each sampling day to decrease variability. To measure discharge, we first measured the width of the stream using a meter-marked tape measure. We then simultaneously measured the depth using a wooden meter stick (in meters) and flow using the Marsh McBirney Flowmeter 1000 with probe at 0.6 of the total depth attached to a Flow-meter probe in Ms$^{-1}$ at intervals equivalent to either ⅕ or ⅙ of the total width (as seen in Figure 5.2). By using the segment width, depth, and water flow speed we were able to calculate the total flow and discharge of the stream using Equation 5.1.

$$\sum_{n-i} \left( width_m \cdot \overline{depth}_m \cdot \overline{velocity}_{m^3/s} \right)$$

**Equation 5.1** *Equation used to calculate discharge flux.*

 We calculated flow for each measurement using Excel and averaged the two readings for each sampling day. (All graphics show averaged flow values when more than one flow discharge reading was taken.)

In addition to our nutrient concentration data and discharge measurements, we took two rounds of coliform and pathogenic *E.coli* concentration measurements. Initially we used Colliert *E.coli* qualitative testing kits (one replicate per stream) to determine qualitative presence or absence. We followed identical stream location selection to that of our filtered and unfiltered water sampling (mid-channel riffle of

the main channel) and filled triple rinsed 100ml plastic Nalgene bottles. We transported each sample in an igloo cooler with chemical ice packs until we compiled all samples and returned to the lab. We tested the samples in an incubator using presence/absence protocol described in Colliert testing manual (included with testing kit) directly after we returned to the lab to ensure all bacteria were present. We used unassisted visual analysis to determine the results in comparison to descriptions in Colliert manual.

Our second round of coliform and *E.coli* testing consisted of a quantitative analysis and 5 replicates per stream. We took all samples on the same testing day and within our 100m reach of analysis. We collected two samples, (A and B) from above any major anthropogenic influence within our study reach. This includes road crossings for both Robbins Mill stream and Rome Trout stream. Sample A was collected from slower moving water, while sample B was collected in fast moving riffle water from the main channel. We collected three additional samples downstream of the major anthropogenic influence at approximately 20-meter intervals when stream flow allowed. All samples were collected in 200mL sterile containers provided by Northeast Labs in Winslow, Maine and were transported in an Igloo cooler with two large chemical ice packs. We transported all samples directly to Northeast Labs following sampling where the samples were processed by standard protocols.

We conducted our research with the aim of sampling each week at each site in order to give a profile of stream characteristics under variable conditions of precipitation, but as such our replicates were insufficient for statistical analysis. Additionally, many of the assumptions for statistical tests were not met by our data.

## 5.3  Results

Through much of our study period we recorded similar basal flow discharge rates at each stream (Figure 5.2).  Prior to the storm event on October 21st, discharge for all streams remained relatively stable with no notable changes in discharge.  The most drastic change in discharge followed the storm event leading up to October 21st. Whittier shows the highest peak of 1.29 $m^3s^{-1}$.  The discharge peaked after the storm from October 21st through 26th. The peak at Rome Trout was nearly as high as the peak at Whittier on the 21st, but flow had fallen much more by the following sampling day. The discharge peak at Robbins Mill was lower than that of both Whittier and Rome Trout, but exhibited a slower return to basal flow.
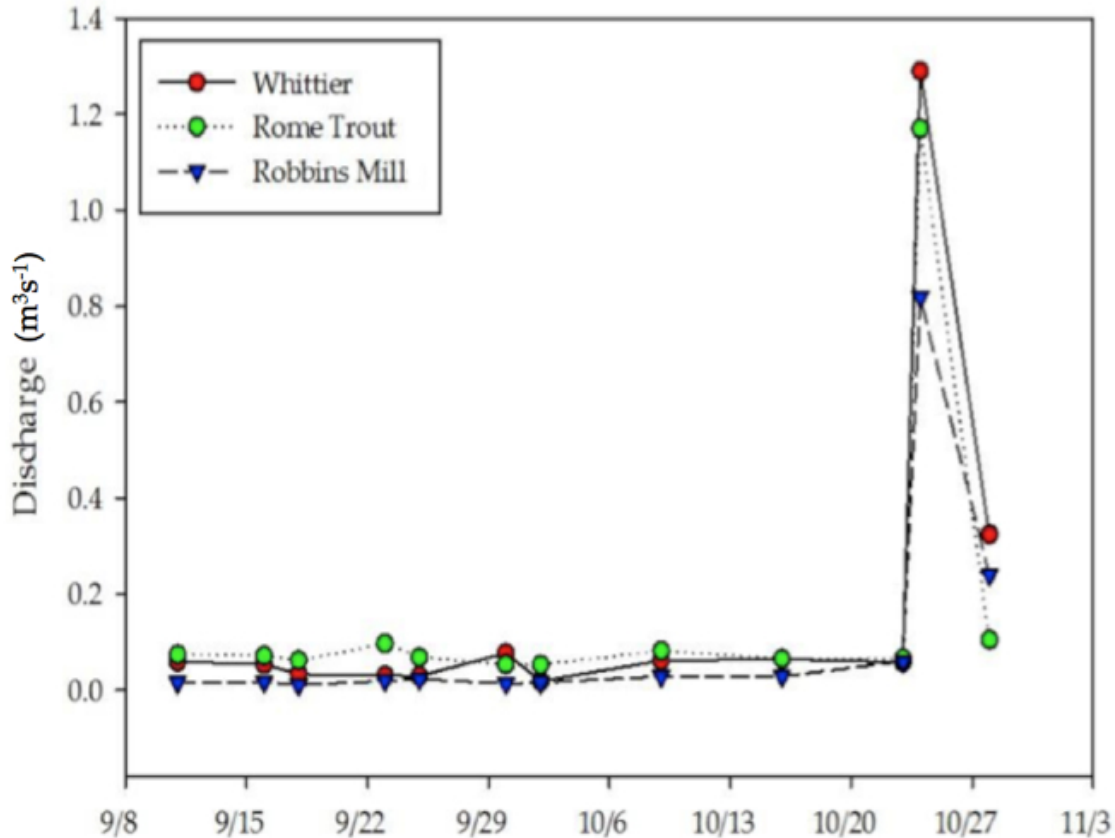
**Figure 5.2** *Discharge across the three streams at each sampling date. Storm occurred on 10/24.*

### 5.3.1 Nutrient Flux Assessment

Robbins Mill SRP flux remains relatively stable throughout the sampling period prior to the storm event, resulting in a large spike in SRP concentrations (Figure 5.3). SRP concentrations remain elevated longer after the storm event than was observed in Whittier and Rome Trout. Whittier shows two major spikes in phosphorous concentrations. We recorded the highest peak flux of SRP in Whittier on October 16th, with a peak flux of 24.3 mg SRP s$^{-1}$. Following the storm event Whittier shows a second bump in phosphorus discharge of 21.0 mg P s$^{-1}$. In contrast, Rome Trout shows two major increased SRP fluxes on September 23rd and October

25th of 26.0 and 26.5 mg/s respectively, although they are of similar magnitude. The

first peak is not connected to a change in discharge, while the second is connected

directly to an increase in discharge due to the storm event.
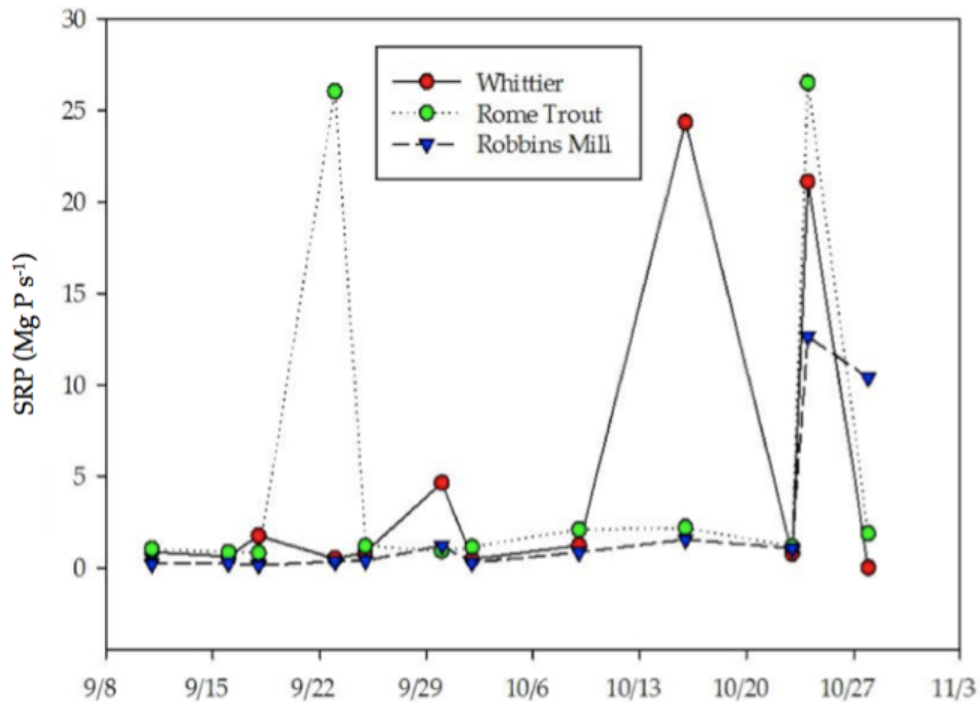


**Figure 5.3** *Soluble Reactive Phosphorus (SRP) flux over sampling time at three streams.*

We also assessed the total phosphorus (TP) concentrations. This includes all

particulate phosphorus compounds and all dissolved phosphorus compounds. All

three streams show a stable baseline flow of very similar phosphorus discharge over

the entire sampling period prior to the storm event (Figure 5.4). Following the storm

we found the highest change in TP flux in Whittier stream, followed by Rome Trout

and Robbins Mill. All three streams show similar return to basal phosphorus

discharge, although we did record Whittier to have more elevated phosphorus

concentrations on the sampling day that was three days after the peak of the storm event.
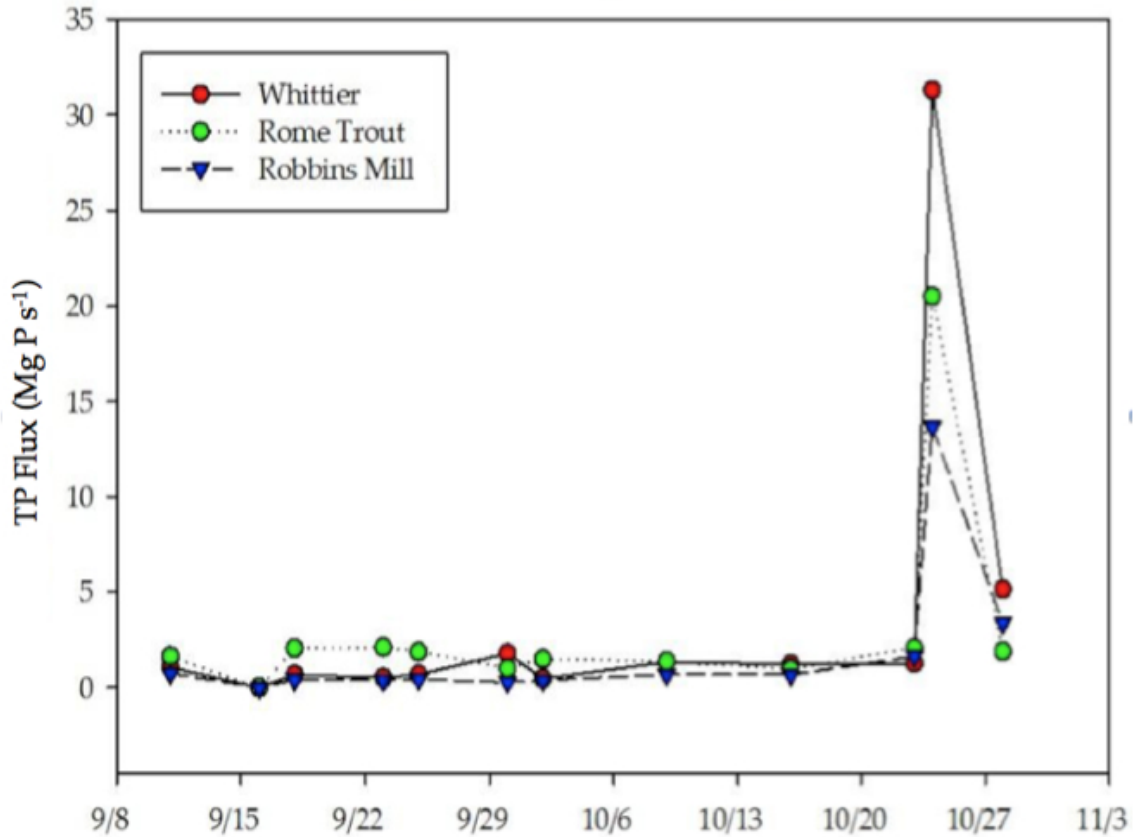


**Figure 5.4** *TP fluxes over sampling period for three streams.*

In addition to phosphorus flux we examined several nitrogen compounds. Including $NH_4$ and $NO_3^-$ as well as TN. We found a relatively stable $NH_4^+$ flux for both Whittier and Robbins Mill prior to the storm event. Rome Trout shows one large bump in $NH_4^+$ on September 23, which corresponds with the P flux increase, but remains relatively constant besides this singular change in $NH_4^+$. We found that

the storm event caused similar changes in $NH_4^+$ as in SRP. Whittier had the highest

peak in $NH_4^+$ flux, followed by Robbins Mill and Rome Trout respectively.
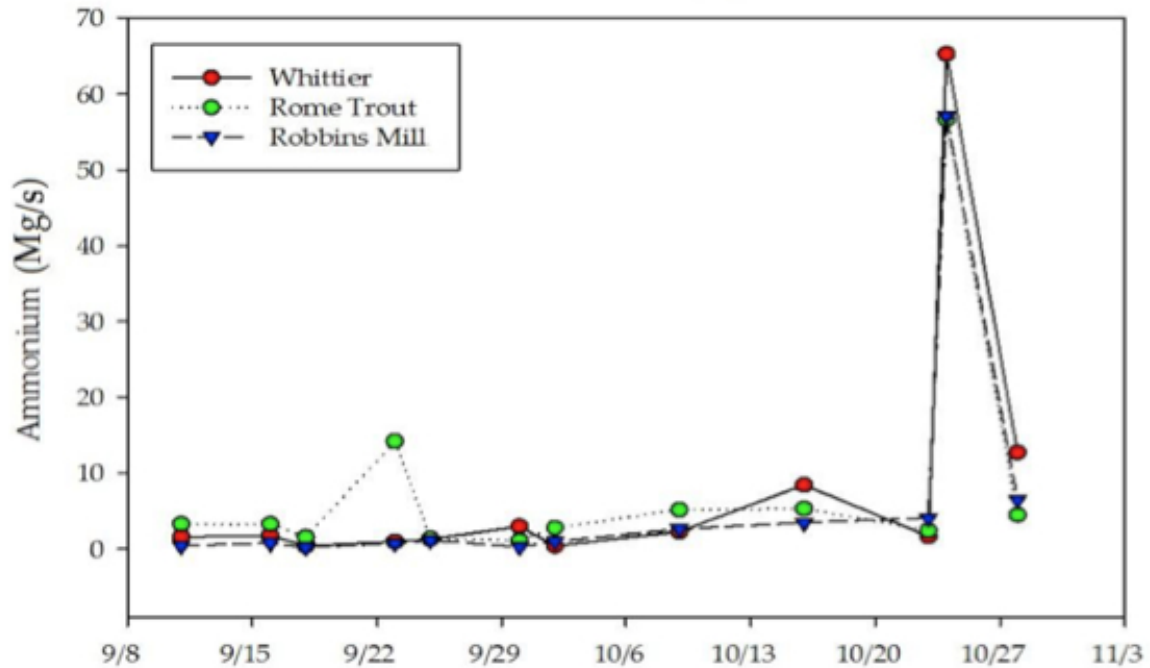


**Figure 5.5.** *$NH_4^+$ flux over sampling period.*

Second we examined $NO_3^-$ flux (figure 5.6). We found a very similar pattern to that

of our TN data (figure 5.4). All three streams displayed similar basal discharge, and

a large peak in $NO_3^-$ flux following the storm event. Whittier shows the highest $NO_3^-$

flux (653.3 mg s$^{-1}$), followed by Rome Trout (317.7 mg s$^{-1}$) and Robbins Mill (303.9

mg s$^{-1}$).
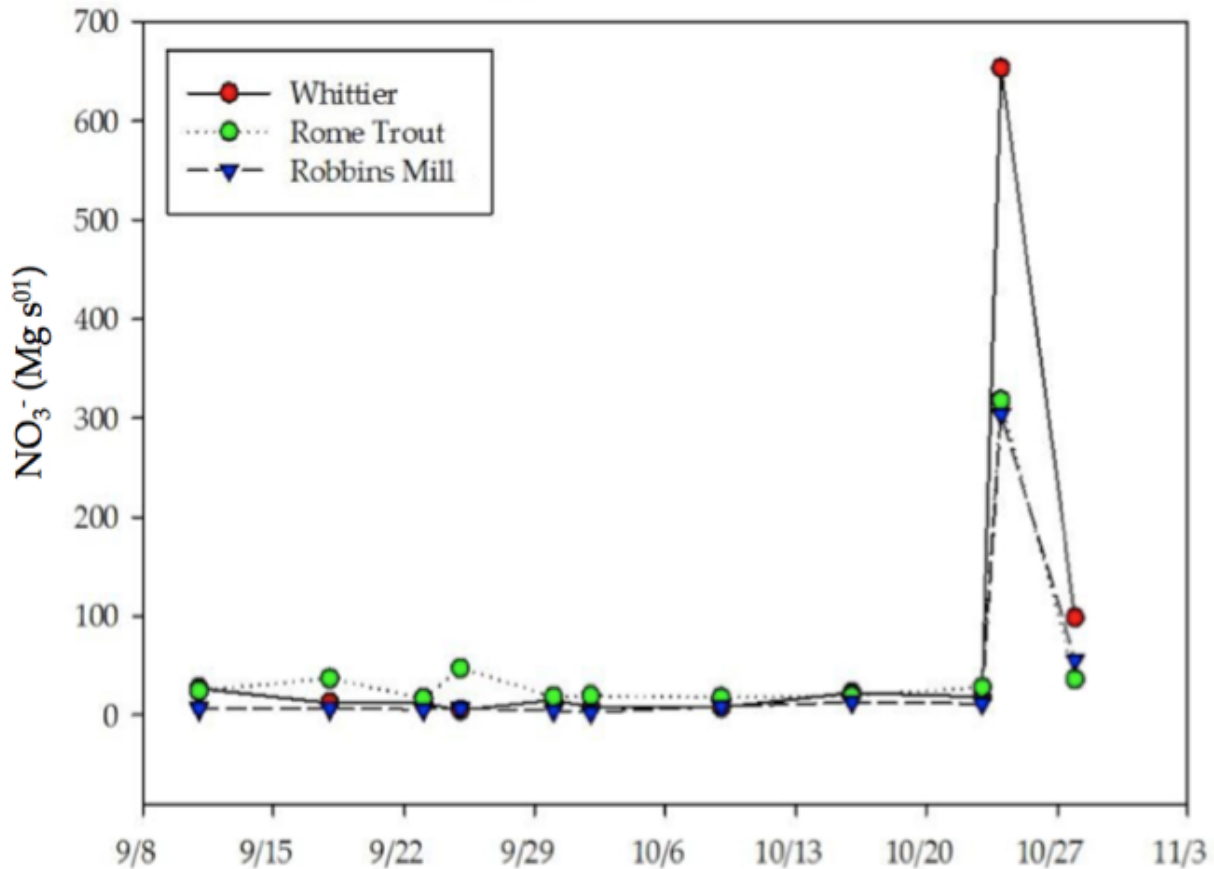
**Figure 5.6.** *NO$_3^+$ flux over sampling period.*

We examined *E.coli* and coliform bacteria both qualitatively and
quantitatively. Our first (qualitative) assessment of *E. Coli* presence in our streams
determined presence of *E. Coli* in all of our water samples. The Colliert tests showed
clearly that there was a significant level of *E. Coli* in each stream sample, but we
sought more quantitative results. We took the samples at five locations across the
stream, trying to take at least one to capture every variable part of the stream (Figure
5.7). An asterisk in figure 5.7 represents a road crossing or culvert, to indicate where
anthropogenic inputs may be concentrated. Unfortunately, due to the low number

of replicates and high variability we were unable to make any statistical conclusions about differences between streams. In addition, we noted large variation in *E.coli* counts within all streams but found the average *E.coli* counts of , 23, 79.8 and 73.4 MPN 100[1]mL for Whittier, Robbins Mill and Rome Trout respectively.

| | Whittier | Robbins Mills | Rome Trout |
|---|---|---|---|
| E. Coli | 41 | 200 | 74 |
| MPN/100 mL | 34 | 38 | 74 |
| | 19 | 59 | 62 |
| | 10 | 59 | 83 |
| | 11 | 43 | 74 |
| Average | 23 | 79.8 | 73.4 |
| Total | 1100 | 600 | 500 |
| Coliforms | 500 | 500 | 600 |
| CFU/100 mL | 500 | 700 | 1000 |
| | 600 | 600 | 600 |
| | 800 | 900 | 700 |
| Average | 700 | 660 | 680 |

**Figure 5.7.** *Measures of E. Coli and total coliforms within the three streams. Samples are in order of collection, from upstream to downstream. Road crossings between samples are represented by asterisks.*

## 5.4 Discussion

The highly variable nature of watershed characteristics have a great impact on the stream's response to a rain event (Bernhardt *et al.* 2003). We found evidence of this in the results of our study with three different watersheds responding

differently to a singular storm. While the storm did not precipitate evenly at all sites, to the best of our knowledge the storm was wide reaching and released similar amounts of rain over the three distinct watersheds. For our discussion we will assume that all three watersheds received approximately equal amounts of precipitation per square meter, but we acknowledge that there may have been some variation in rainfall by site. We measured similar increases in Q, and visually noted similar precipitation across all watersheds.

### 5.4.1 Flow Discharge

We must examine the differences in discharge between the three streams before we can make conclusions about the results of our nutrient data. We were able to establish basal flows for all three streams (figure 5.2). Robbins Mill had, throughout our basal flow period, (Sept 11th - Oct 23rd) on average the lowest flow. This is important as it points to this watershed holding less water during periods of drought, which is important when considering a storm-related discharge peak. We observed similar basal flows in Rome Trout and Whittier, but found Rome Trout to have slightly higher average basal flows (.148 m³s⁻¹) through the period than Whittier average basal flow was (.146 cm/s). We found that Rome Trout exhibits more variation in flow discharge than Whittier or Robbins Mill. This may indicate that Rome Trout is more susceptible to smaller rain events, i.e. less water is absorbed

into the soil during smaller rain events than is absorbed by the less impacted

Robbins Mill and Whittier watersheds.

Following the storm event of October 24th we measured a drastic change in

flow discharge in all streams.  This allowed us to examine this change in flow as a

post storm event flood. As we expected, several cm of rain (in this case 2.64),

translated to observed flows several orders of magnitude larger than our basal flow.

More specifically, Whittier provided the highest flow peak, followed by Rome Trout,

and ultimately Robbins Mill.  While we tried to sample streams at approximately the

same time, we always sampled Robbins Mill, Rome Trout and then Whittier, in that

order. This is in reverse order of the peak discharge which may indicate that

additional time (approximately 45 minutes from Robbins Mill to Whittier) that

Whittier was able to collect rain, and as such were potentially able to reach a higher

discharge. However, we did not note any extraordinary amount of rain, other than

the consistent rain of the previous 48 hours, and as such we believe that the slight

variation in sample time is insufficient to create discharge differences.  However, we

were unable to measure a storm profile and discharge response of all three streams.

Ideally we would have hourly measurements of flow for each stream throughout the

entire storm event to create a discharge time series for each watershed. However, we

were limited by time and scheduling limitations, and as such we do not know with

certainty where our measurements fall within each stream's storm discharge curve (Figure 5.1).

This makes it difficult to compare our measurements but we will attempt to do so while acknowledging the potential that each stream may have reached peaks in discharge at different times. During our measurements we recorded Whittier to have the highest overall discharge, followed closely by Rome Trout. These are the two larger streams with similar basal flows, so the increase in flow to a higher level than our smaller stream, Robbins Mill, following a storm event is consistent with our understanding that a larger stream will reach a higher peak discharge than a smaller stream (Guy 1964). We found an interesting difference in discharge the sampling day that was two days after the peak of the storm event between Whittier and Rome Trout. While they peaked at similar discharge, Rome Trout returned to nearly its basal flow levels, while Whittier remained elevated above its base flow levels. This leads us to believe that Whittier's more forested and less impacted watershed retains more water and reduces the "flashiness" or the tendency of flow to peak quickly and return to basal flows more rapidly. Rome Trout's more developed watershed seems to be more susceptible to rapid changes in discharge, due to the increased anthropogenic impact in its watershed. This difference in flashiness between watersheds with varying levels of anthropogenic influence is consistent with previous research on watershed land use (Mallin, Johnson & Ensign 2009).

**5.4.2 Phosphorus**

Phosphorus concentrations are of special interest as phosphorus is usually the limiting nutrient in downstream lakes (Schindler *et al.* 2008). TP fluxes follows very similar trends to that of discharge for all three streams during basal flows. Robbins Mill, with consistently the lowest discharge, has the lowest TP. The lower discharge of the stream means that even if Robbins Mill had similar concentration of TRP to our larger streams its net flux of TRP would be lower due to the difference in discharge volume. We found Rome Trout stream to have slightly elevated SRP flux over Whittier during the basal sampling period. Rome Trout's watershed has a farming operation upstream of our reach that may be responsible for the elevated basal TP flux over the less impacted Whittier watershed.

Following the storm event, our sampling shows Whittier to have the highest SRP flux of the three streams. This is not what we predicted: we expected that Rome Trout would have the highest SRP flux due to its upstream farming operation, followed by Robbins Mill and Whittier respectively. Phosphorus concentrations tend to peak early in the storm cycle as the eroding rainwater transports terrestrial sources of phosphorus into the stream system (Edwards & Withers 2008). After this initial peak, however, phosphorus concentrations tend to slowly decrease over the storm cycle as all easily transported terrestrial phosphorus has reached the stream

(McDiffet, Beidler & Domminic 1989). This phenomenon may explain why Rome Trout has lower SRP flux than Whittier does during our sampling period following the storm. Whittier has a more forested and less developed watershed, meaning that phosphorous discharge will likely take longer to reach peaks during the storm cycle than the less forested more developed Rome Trout watershed. We can account for this examining the watershed flashiness. Rome Trout may have already reached peak SRP flux due to its more flashy nature prior to our sampling, while Whittier was closer to its peak SRP flux because of its more elongated storm profile. This would explain why SRP flux was higher in the more impacted watershed (Rome Trout) than in our less impacted watershed (Whittier). In addition, Whittier has a higher overall discharge. With similar concentrations of SRP, Whittier will discharge more SRP than Rome Trout due to the difference in water volume discharge. Robbins Mill has the lowest peak in SRP likely due to lower overall flow discharge following the storm event.

SRP, which is dissolved phosphate concentrations are more difficult to explain. TP flux should be lower than SRP flux, as it does not contain particulate phosphorus compounds. However, we did not find this. Rome Trout and Whittier both have large peaks in undigested phosphorus during the basal flow period. These peaks are not correlated with any variation in flow discharge, but are only the result of undigested phosphorus concentrations variation. These variations are several

orders of magnitude larger than our other basal SRP concentrations and do not show up on TRP, leading us to believe these variations are likely due to sampling error or contamination. With additional time we would re-test these water samples to determine if our analysis was faulty or we had an incomplete digestion process.

### 5.4.3 Nitrogen

In addition to phosphorous, nitrogen is another important commonly limiting nutrient in stream ecosystems, as discussed in the Nutrient Cycling chapter. Our examination of nitrogen starts with nitrate ($NO_3^-$). $NO_3^-$ flux follows very similar trends to that of STP, with several notable differences. Relatively constant nitrate levels in all three streams during the basal flow period are similar to that of TP. However, Rome Trout does show slightly elevated nitrate flux compared to that of Robbins Mill and Whittier. This may be due to the close proximity of farming, and some level of background nitrate addition to the stream system at basal levels from surrounding land use.

We found nitrate discharge levels to peaks following the storm event for all three streams, and we found Whittier to have the highest peak. This is not what we expected to find, however our explanation for TRP may also explain why Whittier's nitrate discharge is higher than our more anthropogenically affected streams. The more anthropogenically affected watersheds reach peak nitrate concentrations more

rapidly, then begin to decrease in nitrate discharge more rapidly than the less flashy and less impacted Whittier stream.  Our single sampling method did not allow us to see these differences, but this phenomenon would explain the drastic differences in our single sample method.

Ammonium is a form of nitrogen that is more readily accessible for primary producers to utilize (Vitousek 2002).  Measuring $NH_4^+$ levels is important as it can show anthropogenic inputs of nitrogen more clearly than nitrate levels (Marcarelli, Baker & Wurtsbaugh 2008). We found that Rome trout had a singular peak in $NH_4^+$ flux levels during the basal period flow. This can be explained by upstream farming operation that likely uses $NH_4^+$ based fertilizers.  If this farm had recently applied an $NH_4^+$-based fertilizer, a small amount of precipitation could cause the bump in $NH_4^+$ flux as fertilizer was transported via run-off into the stream system.

Our measurements of $NH_4^+$ flux following the storm event diverge from TRP and Nitrate discharge trends. While we measured Whittier with the highest, both Rome trout and Robbins Mill had similar discharge levels, closer to that of Whittier's levels than in nitrate, or either phosphorus flux.  While Whittier's discharge levels remain the highest due to slower discharge peaks, and less dilution of $NH_4^+$, similar Rome Trout and Robbins Mill levels may point to much higher $NH_4^+$ runoff in these watersheds. Although we did not measure this, if $NH_4^+$ flux in Rome Trout and Robbins Mill follow similar trends to that of TP, nitrate, and phosphate, these

discharges should have dropped to lower levels. We did not find this, and found

Rome Trout and Robbins Mill $NH_4^+$ levels much closer to Whittier's. This can be

explained by high $NH_4^+$ input into Robbins Mill and Rome Trout and by larger

stores of $NH_4^+$ for rain water to transport into streams of the impacted watersheds.

With much larger stores of terrestrial $NH_4^+$, as would be found in fertilized lawns or

fields, deforested areas, and more impacted watersheds, these stores are depleted

less quickly and the peak of $NH_4^+$ is delayed. This explains why $NH_4^+$ levels of Rome

Trout and Robbins Mill are relatively closer to that of our unimpacted stream in

comparison to TRP, nitrate and phosphate.


### 5.4.4 Pathogens and Bacteria

Our *E.coli* and coliform qualitative results follow our hypothesis and understanding

of *E.coli* and coliform bacteria. We found all streams to have *E.coli* and coliform

bacteria, constant with our understanding that nearly all streams have low levels of

coliform and *E.coli* regardless of anthropogenic impact (Schoonover & Lockaby

2006). Fecal matter from wild warm-blooded organisms such as deer, or moose, can

make its way into water systems without anthropogenic activity (Whitlock, Jones &

Harwood 2002). For all three streams we likely observed a mixture of both

anthropogenic and naturally occurring *E.coli* and coliform bacteria in this qualitative

first testing.

Our quantitative results show a greater level of detail, but a with a important pitfall. We found *E.Coli* concentrations to be generally lower at Whittier than Robbins Mill and Rome. This is of interest as all these measurements were taken during basal flows, and not following the storm event. Thus we are able to ignore all the differences in flashiness of the three streams and examine the base flow data. The lower *E.Coli* in Whittier stream in comparison to our impacted streams of Robbins Mill and Rome Trout points to a lower input of basal anthropogenic fecal bacteria in Whittier than in Rome Trout and Robbins Mill. However all streams were below the EPA limit for recreational use of 235 MPN/100mL for *E.coli* concentrations, and do not have drastic variation in *E.coli* concentration. Coliform bacteria was similar across all streams which limits our ability to make wide statements about the difference in basal anthropogenic inputs or how the difference in land use affect coliform bacteria levels in our three streams. A longer running study with more replicates may have allowed us to asses *E.coli* and Coliform bacteria more thoroughly.

### 5.5 Conclusions

Our original hypothesis that our impacted streams with road crossings and increased development of watersheds will have higher concentrations of nitrogen and phosphorus did not entirely match our data. However, we do believe that that

our more impacted streams do in fact have higher fluxes of these nutrients, but our

sampling technique was unable to adequately quantify this. Looking at discharge,

along with all nutrient data backs up our explanation that the flashier more

impacted streams were likely further along in the storm response profile: i.e. they

reached peak discharge prior to sampling due to less forested cover. To adequately

address this concern we must conduct time-series sampling, of measurements of

discharge and flux of nutrients throughout the course of the storm on an hourly

scale. Unfortunately, this was outside the scope of this study and is an area potential

future research. However, we found our impacted streams to be flashier, have

higher relatively higher $NH_4^+$ fluxes, and higher *E.coli* and coliform bacteria. These

conclusions back up our hypothesis that that Rome Trout and Robbins Mill, the

more impacted watersheds, were more affected by anthropogenic use following a

storm.

In conclusion, our research shows that storm related increases in flow results

in large increase in discharge of nutrients for all three streams. In addition, we found

that $NH_4^+$ does not follow the similar patterns of TRP, phosphate and nitrate. We

believe that this is likely due to large stores of $NH_4^+$ in our anthropogenically-

impacted watersheds. Fertilized lawns and fields, which are present in both

anthropogenically-impacted watersheds could provide a source of this $NH_4^+$ that is

absent in Whittier's watershed. Although we found some variation in *E.coli*

concentrations that trended towards higher in impacted streams, all of these levels

were below the EPA recreational use levels. This leads us to believe that the

variation in *E.coli* is likely based on local conditions and has limited impact on

downstream, or in-stream communities.

Storms provide us with a unique opportunity to examine the anthropogenic

impact on these watersheds that may not be visible during basal flows. The added

stress of large rain events expose anthropogenic influence and allowed us to se a

huge increase in nutrient flux in all three streams. We were able to show similar

levels of Nitrogen and Phosphorus during basal flow periods, but notable variation

in nutrient flux following a storm event between these three streams. This is

undoubtedly the first step in assessing these three watersheds' reaction to storms

and will allow future research to document these fluxes in greater detail.

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**CHAPTER 6**
**RIPARIAN ZONE**

## 6.1 Introduction

### 6.1.1 Significance of Riparian Zones

One of the most important aspects of a lotic freshwater ecosystem is the

riparian zone, the dynamic interface between terrestrial and aquatic ecosystems

(Gregory *et al.* 1991; Auble *et al.* 1994). These zones, or ecotones, are known to be

some of the most diverse and complex biophysical, terrestrial habitats due to their

high exposure to both fluvial and non-fluvial disturbances (Naiman *et al.* 1993;

Pollock *et al.* 1998; Merritt & Cooper 2000). Riparian zones have many connections to

stream and river health as hotspots for nutrient cycling and sediment deposition.

The high diversity of riparian vegetation, soil composition and invertebrates not

only plays a major role in stream ecosystem function, but it also provides migration

corridors for numerous plant and animal species, consequently increasing

biodiversity (Jones *et al.* 2009; Lavelle *et al.* 2006; Daily 1997). Unfortunately, due to

the encroachment of human activity, the health of riparian habitats has been

declining (Jones *et al.* 2009), which could have many implications regarding the

overall quality of natural water sources across the globe.

Much research has been done regarding the importance of riparian vegetation

and its potential ecosystem services. A healthy riparian ecosystem, with wide

ranging species diversity and abundance, has the capacity to reduce water flow during floods, manage sediment storage, and intercept anthropogenic pollution and nutrients that otherwise would enter the stream (Jones *et al.* 2009; Lowrance *et al.* 1983).  Riparian zones can also serve as useful watershed management tools by providing natural flood-storage capacity as well as groundwater recharge (Warner *et al.* 1984). Perhaps the most notable service provided by riparian zones is their ability to maintain stream water quality. Areas with riparian zones that have been cleared for development have much more turbid waters and higher nutrients (Rajbhandari 2003). This is due to the riparian vegetation's abilities to filter surface runoff before it can enter the stream (Schultz *et al.* 2004). Higher plant diversity and density provide dynamic root and canopy structures, which are necessary to ensure the continuation of these services.

### 6.1.2 Fluvial Geomorphology

Fluvial landforms and processes that affect soil composition are limited by the success of riparian vegetation growth, and are directly related to plant species distribution (Hupp & Osterkamp 1996; Merritt & Cooper 2000). Floodplains are of particular interest when studying plant communities of riparian ecosystems due to the constant geomorphic change that results from flooding, erosion as well as sediment transport and deposition (Naiman & Decamps 1977). Constant changes in

substrata facilitate morphologically diverse species, with abilities to adjust to more or less saturated soils, as well as changing water levels. Some plants, such as trees, are able to help in establishing sequences of fluvial landform creation and maintenance as ecosystem engineers. This is carried out largely by the plants' roots, which have the ability to interfere with water and sediment movements during floods. Flood interference by plant roots results in bio-stabilization and bio-construction (Gurnell *et al.* 2005). In this way, the importance of riparian vegetation to stream geomorphology is very apparent due to its unique contributions.

### 6.1.3 Ecosystem Impacts

The stream ecosystem benefits at all scales from a healthy riparian zone, ranging from localized microhabitats found in soil and rocks, to entire riparian forest habitats. It has been found that the diversity of riparian plant populations has the ability to regulate light and stream shading, temperature and water flow. Erosion, bank stabilization, nutrient cycling and water quality are also managed by factors in the riparian zone (Naiman *et al.* 1993). Stable populations of riparian vegetation provide woody debris and organic matter to both aquatic and terrestrial biota (Naiman *et al.* 1993; Steiger *et al*. 2005). The aforementioned micro and macro habitats serve as a food source, shelter and reproductive sites for a wide range of terrestrial and amphibious animals, leaving the riparian zone as a good potential

corridor for migrating and breeding populations. These areas are key systems in the regulation of aquatic and terrestrial linkages. Riparian zones can also serve as early indicators for changes in the environment, which could be useful for future management and conservation efforts (Naiman & Decamps 1977).

Riparian zones are a major component in the cycling of nutrients in streams (Mulholland 1992). Riparian forests have many biogeochemical processes in their upper soil horizons that will retain groundwater nitrogen and phosphorus, while the nutrients that successfully enter the stream are rapidly taken up by algae and microbes (Mulholland 1992; Burt *et al.* 1999). Previous studies show that the riparian zone can either be a source or sink for inorganic nutrients from agricultural runoff in individual watersheds (Peterjohn & Correll 1984; Mulholland 1992; Hill 1996). As the soil moisture passes through the riparian rooting-zone, the vegetative demand for nutrients decreases the load present in groundwater. This process reduces the inorganic substances that enter the stream (Gregory *et al.* 1991). Due to these properties, it is clear that the more densely vegetated riparian zones will be the most successful at regulating stream nutrient inputs.

### 6.1.4 Organic Matter

Organic matter from plants and insects provides a significant input of energy for stream inhabitants, especially in mature, fully canopied, densely vegetated

riparian ecosystems (Nakano *et al*. 1999). The various types of riparian vegetation

control the quantity and type of organic matter inputs through differences in leaf

structure and chemical composition (Gregory *et al.* 1991). The size and type of

organic matter from plants entering the stream is a determining factor in stream

invertebrate feeding strategies. Stream food web dynamics are further influenced by

the entry of terrestrial arthropods, which serve as a major food source for fish

(Cummins & Klug 1979). While inorganic nutrient inputs facilitate or inhibit primary

production, organic matter inputs affect higher trophic levels. Due to these factors,

stream food-web dynamics are influenced by the status of riparian habitats in both

bottom-up and top-down processes.


**6.1.5 Changes in Land Use**

Anthropogenic land alteration, such as cultivation, urbanization and

domestic livestock, often result in changes in the vegetation patterns. Seed

transportation processes and habitat alterations are also common by-products of

land-use changes (Kauffman *et al*. 1983; Knopf *et al*. 1988; Mathooko 2000; Snyder *et

al*. 2003; McTammany *et al*. 2007). Agricultural practices and urbanization contribute

to the increased erosion of stream banks, which results in higher volumes of

suspended sediment (Zaimes *et al*. 2004). Land use for road construction results in

the removal of riparian vegetation, allowing more sunlight and runoff to reach the

stream ecosystem. Increased solar energy and inorganic nutrients from runoff facilitate the growth of benthic algal populations. Higher levels of algae have many negative implications for the health of the stream as a whole (MacKenzie 2008).

The three streams that we will be looking at, Whittier Brook, Robbins Mill Stream and Rome Trout Brook are currently lacking in research regarding riparian zone status and vegetation. Two of the streams examined are currently threatened by the encroachment of human development, water-flow redirection and riparian deforestation, which are known factors in the alteration of riparian habitats and stream health (Resh *et al.* 1988; Sweeney *et al.* 2004; Meyer *et al.* 2005; Patten 1998). Although it is known that riparian forests in the United States have been declining, there is still a need for more in-depth data and detailed classification in order to implement successful riparian land-use management practices (Jones *et al.* 2009; Quinn *et al.* 2007). Information collected from a baseline survey of vegetation diversity, density and species richness in the Belgrade Lakes region, as well as characterizations of soil and light conditions, will contribute greatly to future local research and watershed management. A baseline characterization will provide a foundation on which to draw comparisons across both temporal and spatial scales.

## 6.2 Materials and Methods

### 6.2.1 Vegetation Coverage

Stream reaches of 100 meters were designated at each location, with 10 meter intervals demarcated. At each 10-meter interval we laid a 1 x 0.5 meter quadrat on the bank perpendicular to the stream. Then we identified all plant species within the quadrat rectangle and assessed their approximate percentages of ground coverage. Small leafy plants were categorized as 'forbs', and larger individuals were distinguished more specifically. After the initial quadrat was evaluated, we flipped the rectangle length-wise, to extend the study area a second meter perpendicular to the stream bank. Identification and coverage procedures were repeated ten times along both the right and left sides of each stream. To compare the vegetation coverage across the three streams we utilized the Simpson's Diversity index (Equation 6.1).

$$D = \sum \frac{n(n-1)}{N(N-1)}$$

**Equation 6.1** *Simpson's Diversity Index, where D measures the probability that two individuals randomly selected from a sample are from the same species we compared vegetation coverage across the three streams, n represents the total number of organisms of a species, and N represents the total number of organisms for all the species.*

### 6.2.2 Tree Density and Canopy Coverage

A 10 x 10 meter quadrat was measured at the 30, 60 and 90 meter intervals along the reach, perpendicular to the right-side bank. Within these areas, we

identified tree species and measured their diameters (dbh, in centimeters). We used

a spherical densiometer to estimate percent canopy coverage and stream shading,

while facing north, east, south and west. We measured any trees wider than 2

centimeters and taller than 1.5 meters high at breast-height, using a diameter tape.

We also recorded species information for these trees. Dead trees were not included

in the study. This procedure was repeated on the left side bank.


### 6.2.3 Soil Density

At each stream, we collected three soil samples at the top of the reach, and

three at the bottom of the reach on the right side of each stream. We then brought

the samples back to the lab and weighed them for their initial masses. We re-

weighed dry samples. The difference in weight between wet and dry was calculated

to determine percent moisture content. We measured percent organic matter by

ashing samples in a ceramic oven, burning off organic matter. Finally, we weighed

samples a third time to determine the weight of matter that was lost. Percent organic

matter was calculated using the following equation: (mass of ash – mass of soil /

mass of soil) x 100.

### 6.2.4 Light Energy Inputs

To measure the amount of light energy reaching the riparian zone, we placed two PAR sensors at each stream on the right side. At Robbins Mill and Rome Trout, one sensor was placed closer to the open-canopy culvert area, and one was placed towards the middle of the reach where vegetation was more consistent. At Whittier, one PAR sensor was placed near the road approximately 200 meters away from the reach, while the second was placed at random within the vegetated riparian zone.

### 6.3 Results

Average vegetation coverage is given in percentages, determined by the approximate density within each 2 x 0.5 meter quadrat. Tree densities are also given in percentages, determined by average density within each 10 x 10 meter quadrat. The averages presented were calculated using the total coverage in each quadrat combined, depending on stream as well as bank side.

### 6.3.1 Robbins Mill

Black Cherry, Red Pine, and Speckled Alder trees dominated the right side of Robbins Mill, while the ground cover was predominately ferns, forbs, grasses, and raspberry. The left side was mostly ash, black cherry, dogwood, and speckled alder

trees, and the ground cover was primarily ferns, forbs, grasses and raspberry (Figure 6.1, Table 6.1, Table 6.2. Appendix). On the right side, we measured a total of 23 trees and 10 different species, with average diameters ranging from 2.5cm to 39.8cm and on the left side five species composed the 27 trees measured, with average diameters ranging from 2.68cm to 9.17cm (Table 6.3, Appendix). Using the Simpson's Diversity Index, it was determined that Robbins Mill had the highest vegetation diversity of all three streams, with a value of 0.9967.

| | Left | | Right |
|---|---|---|---|
| **Species** | **Percent Coverage (%)** | **Species** | **Percent Coverage (%)** |
| Grass | 14.21 | Forbs | 16.22 |
| Forbs | 13.19 | Grass | 12.16 |
| Dogwood | 11.39 | Fern | 11.15 |
| Fern | 10.15 | Speckled Alder | 8.34 |
| Speckled Alder | 7.25 | Red Pine | 4.17 |

**Figure 6.1** *Most abundant species at Robbins Mill measured in September, 2014. All plant species and percent coverage within ten 0.5m x 2m quadrats on each side of the stream were recorded, and all trees within three 10m x 10m quadrats on each bank were recorded.*

Average light density measured on the right side of Robbins Mill was 86%, and 47% on the left (Table 6.4, Appendix). The maximum amount of light near the road was 10,718 $\mu Em^{-2}s^{-1}$ with an average of 1,933 $\mu Em^{-2}s^{-1}$. Near the stream, we recorded a maximum of 16,026 $\mu Em^{-2}s^{-1}$ with an average of 2,038 $\mu Em^{-2}s^{-1}$ (Figure

6.5, Appendix). The differences between roadside and forested light intensity was

33.12%.

The three upstream soil samples had percent moistures of 21.41%, 21.12% and

22.24%. The three downstream samples had percent moistures of 19.47%, 20.63%

and 20.78%.  Overall, the upstream and downstream average soil moisture contents

displayed a significant difference (p-value = 0.03). Organic matter percentages for

upstream samples were 30.71%, 31.75%, 28.94%, while the three downstream

samples had 57.77%, 66.82% and 59.13%. The average upstream and downstream

organic matter also had a significant difference (p-value = 0.0002) (Figure 6.4, Table

6.5, Appendix).


### 6.3.2 Whittier Brook

Beech trees, hemlock trees and forbs dominated the right side of the stream,

while the left side of was dominated by hemlock, red maple, and witch hazel, along

with forbs and moss (Figure 6.2, Table 6.6, Table 6.7, Appendix). We observed a total

of 58 trees and 8 species with average diameters between 2.43cm and 18.37cm on the

right side, and a total of 55 trees composed of 10 different species, with average

diameters ranging from 2.77cm to 20.68cm on the left (Table 6.5, Appendix). The

Simpson's Diversity Index for Whittier yielded a measurement of 0.9934, making

this the intermediate of the three streams in terms of species diversity.

|  | Left |  | Right |
| --- | --- | --- | --- |
| **Species** | **Percent Coverage (%)** | **Species** | **Percent Coverage (%)** |
| Hemlock | 23.42 | Hemlock | 19.33 |
| Moss | 22.49 | Beech | 16.28 |
| Red Maple | 10.18 | Forbs | 11.19 |
| Forbs | 7.16 | Grass | 3.5 |
| Witch Hazel | 7.13 | Red Maple | 3.5 |

**Figure 6.2** *Most abundant species at Whittier Brook, measured in September, 2014. All plant species and percent coverage within ten 0.5m x 2m quadrats on each side of the stream were recorded, and all trees within three 10m x 10m quadrats on each bank were recorded.*

Average light density on the right side was 86%, and on the left there was an average of 75% (Table 6.4, Appendix). The maximum amount of light near the road was 4,822 $\mu Em^{-2}s^{-1}$ with an average of 646 $\mu Em^{-2}s^{-1}$. Near the stream, 2,715 $\mu Em^{-2}s^{-1}$ with an average of 545 $\mu Em^{-2}s^{-1}$ was recorded (Figure 6.6, Appendix). The differences between roadside and forested light intensity was 43.70%.

The three upstream soil samples had percent moisture of 21.16%, 18.25% and 21.46%, while three downstream samples had 16.31%, 17.50% and 19.61%. Upstream samples had 35.16%, 29.48% and 28.08% organic matter, and the three downstream samples had 25.61%, 28.77% and 23.69%. The average upstream and downstream soil moisture (p-value = 0.07) and organic matter (p-value = 0.07) were not significantly different from each other (Figure 6.4, Table 6.5, Appendix).

### 6.3.3 Rome Trout Brook

Speckled alder trees dominated the right side of Rome Trout, while the vegetation cover was mostly ferns, grasses, and raspberry. The left side of Rome Trout was predominately composed of speckled alders and red maples, and the ground cover was primarily ferns, forbs, goldenrod, grass and raspberry (Table 6.9, Table 6.10, Appendix). On the right side, five species made up the 32 observed trees, with average diameters ranging between 11.0cm and 16.5cm. On the left side, we measured a total of 63 trees, composed of 3 species, with average diameters between 3.8cm and 18.84cm (Table 6.11, Appendix). Rome Trout had the lowest Diversity Index, with a value of 0.9927.

| | Left | | Right |
| --- | --- | --- | --- |
| **Species** | **Percent Coverage (%)** | **Species** | **Percent Coverage (%)** |
| Speckled Alder | 54.84 | Speckled Alder | 26.79 |
| Grass | 15.19 | Forbs | 15.2 |
| Raspberry | 14.18 | Grass | 14.19 |
| Forbs | 11.14 | Raspberry | 10.14 |
| Fern | 10.12 | Fern | 8.11 |

**Figure 6.3** *Most abundant species at Rome Trout, measured in October, 2014. All plant species and percent coverage within ten 0.5m x 2m quadrats on each side of the stream were recorded, and all trees within three 10m x 10m quadrats on each bank were recorded.*

On the right side of Rome Trout, average light density was 83%, while on the left it was 70% (Table 6.4, Appendix). The maximum light near the road was 62 $\mu Em^{-2}s^{-1}$ with an average of 11 $\mu Em^{-2}s^{-1}$. Maximum light near the stream was 46

μEm$^{-2}$s$^{-1}$ with an average of 7 μEm$^{-2}$s$^{-1}$ (Figure 6.7, Appendix). The differences between roadside and forested light intensity was 25.81%.

At Rome Trout, the three upstream soil samples had percent moistures of 17.44%, 20.90% and 20.38%. The three downstream samples had percent moistures of 29.25%, 22.07% and 28. 16%. The average percent soil moisture upstream was significantly different than the average percent soil moisture downstream (p-value = 0.02). Upstream samples had 49.85%, 36.62% and 31.17% organic matter, and the downstream samples had a percent organic matter of 44.29%, 48.97% and 44.36%. Percent organic matter was not significantly different between upstream and downstream samples (p-value = 0.15) (Figure 6.4, Table 6.5, Appendix).
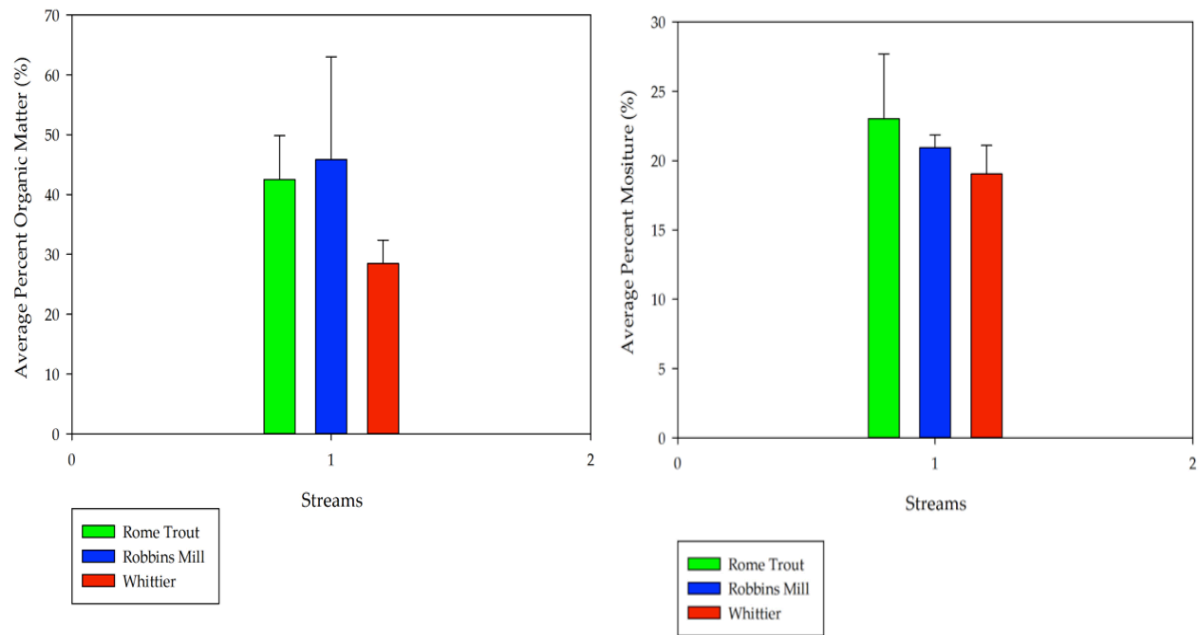


**Figure 6.4** *Average percent organic matter (left) and average percent soil moisture (right) from soil samples collected in two locations at each stream. Three samples were collected at an upstream location, and three more were collected downstream. All samples were collected in October, 2014.*

## 6.4 Discussion

### 6.4.1 Robbins Mill

It is important to note that Robbins Mill is a heavily impacted stream. A road runs along the left bank and cuts through the reach at approximately 20-35 meters, where there is a large, deep culvert. The right side of the stream is bordered by lawn and a small pine tree stand. Homes with front and back lawns surround the reach. The canopy cover at Robbins Mill was different from the other streams, where coverage was highest in the northern and southern directions. The difference in canopy cover on the west bank was likely due to the large, anthropogenically planted red pine trees along the north-west side. The highest light intensity near the road was greater than 30% in the vegetated riparian zone. This has many implications for light availability in regards to photosynthesizing organisms along stream banks that have roads or other similar structures nearby. Soil percent moisture and organic matter were significantly different across upstream and downstream locations. Upstream soil had both higher percent moisture and organic matter compared to downstream. This could be due to the presence of a road crossing and culvert upstream, with lawns and houses downstream. There was also a high abundance of pine needles collected in downstream samples.

### 6.4.2 Whittier Brook

As our control stream, Whittier was the least affected by anthropogenic activities. A single road leads to the stream, though it is separated from the reach by approximately 200 meters or more of a moderately wooded, sloping terrain. The light intensity near the stream was receiving approximately 45% less compared to the sensor placed near the road. Such significant differences can be attributed to the dense canopy coverage within the riparian zone. Light coverage was highest coming from the north and south directions. Soil moisture content and percent organic matter showed no significant differences between upstream and downstream samples, likely due to high volumes of rock particles collected within the samples.

### 6.4.3 Rome Trout Brook

Rome Trout was moderately impacted by anthropogenic development. A single road passes over the stream between the 30-50 meter intervals along the reach, and a culvert has been constructed as well. The immediate stream banks are not affected by residential or commercial properties, though some small homes are located nearby.  Light intensity was consistently high near the roadside, compared to the forested riparian zone, which was receiving almost 26% less light due to canopy coverage. North and south compass directions were found to have the highest canopy coverage. Similarly to Whittier and Robbins Mill, such low sample

sizes have resulted in large variations in tree trunk diameters and large standard

deviations. Percent soil moisture was significantly different between the upstream

and downstream samples, but there were no significant differences in organic

matter. This is likely due to the large culvert and road crossing located midway

through the upstream section, causing higher percent moisture downstream.


### 6.4.4 Watershed Implications

Our findings have many implications for other key aspects of stream

ecosystems in the Belgrade Lakes region. The variations in canopy coverage and

light intensity not only facilitate plant primary production, but also the growth of

algae within stream systems. Both of these factors provide significant energy inputs

for in-stream inhabitants, specifically the macro-invertebrates (Nakano S. *et al*. 1999;

Invertebrate Chapter). Studies have found that increased sunlight exposure in

vegetated riparian zones will lead to a higher energy input, therefore increasing the

abundance of macro-invertebrates and a more complex benthic community (Tait *et

al.* 1994; Quinn *et al.* 1997). The relationship between our stream invertebrate

communities and light inputs could potentially display similar correlations

(Invertebrate chapter). Further, higher diversity in plant species and size may have

effects on the feeding strategies of stream invertebrates (Cummins & Klug 1979),

resulting in a wide range of species.

Based on our data, it appears as though anthropogenic impacts did not have a significant effect on species diversity measures across the three streams, though the species compositions did seem to vary based on level of impact. Similarly to previous studies (Mathooko & Kariuki 2000), we found that riparian vegetation at impacted streams was primarily pioneer species or early successional trees. This can be attributed to the decreased time allowed for growth and restoration following land-use developments. We observed that Whittier was primarily composed of older succession species, such as eastern hemlock, which indicates a lack of disturbance allowing long-term growth. In contrast, early succession plants and trees, such as speckled alder, dominated Rome Trout and Robbins Mill, which is reflective of impacted growth. Due to their bio-stabilization attributes, more developed riparian plant roots and older plant communities may have long-term effects on the sediment and geomorphologic processes of streams (Gurnell *et al*. 2005; Sediment Chapter).

The results of our diversity measurements could have direct links to the organic matter inputs and nutrient cycling of each study site (Organic Matter and Nutrient Cycling Chapters). The healthier and more abundant riparian vegetation is, the higher the likelihood that excess nutrients in groundwater and runoff will be absorbed by plant roots as opposed to entering the stream (Gregory *et al* 1991). High variation in plant communities may also contribute to differences in organic matter inputs, specifically in terms of leaf litter composition. The presence of anthropogenic

development and its influence on riparian vegetation growth, specifically species composition, could result in differences in both nutrient cycling and organic matter processes dependent on the level of impact.

These essential components of watershed environments are directly influenced by the health and composition of riparian vegetation. It is clear that impacts to the riparian zone could disrupt numerous processes within the stream ecosystem, therefore affecting watershed conditions and overall water quality. Though our findings did not demonstrate a significant difference in diversity across the three study sites, there are still many potential consequences of having either a younger or older plant community. Future studies regarding the effects of early versus late successional forests on stream processes would be incredibly beneficial in generating a more conclusive analysis on the relationship.


### 6.4 Conclusion

Our findings present a general characterization of the riparian ecosystems of three streams within the Belgrade Lakes watershed. Tree size and coverage, as well as approximate ground vegetation coverage allow future, long-term studies of changes in species composition and light coverage. Variations in light intensity across locations, specifically the observed increases in intensity near roads and culverts, have numerous implications for primary productivity of riparian and

aquatic photosynthesizing organisms. Should anthropogenic development continue in these regions, major increases in light intensity have the potential to disrupt current trophic processes, resulting in potentially unbalanced ecosystems. Fluctuations in soil moisture and organic matter in riparian sediment also have various implications for primary productivity, as this directly impacts the growth and sustained health of the plant community. Evidence from this study suggests that anthropogenic activity, specifically in the form of roads, culverts, homes and artificially planted trees, may have profound effects on the productivity levels of a stream riparian zone. In the future, it is increasingly pertinent that any changes in land use and development must consider these impacts, so as to mitigate any potential harm to stream ecosystems or the causation of trophic disruptions.

## 6.6 Appendix

**Table 6.1** *Percent ground coverage of riparian plant species at Robbins Mill measured in September, 2014 based on ten 0.5m x 2m quadrats per stream bank side.*

| Plant Species Robbins Mill Left | Percent Coverage (%) | Plant Species Robbins Mill Right | Percent Coverage (%) |
|---|---|---|---|
| Aster | 3.40 | Ash Seedling | 2.30 |
| Bed Straw | 2.30 | Aster | 4.60 |
| Black Cherry | 5.70 | Bed Straw | 1.10 |
| Fern | 10.15 | Black Cherry | 4.50 |
| Forbs | 13.19 | Fern | 11.15 |
| Goldenrod | 6.90 | Forbs | 16.22 |
| Grass | 14.21 | Goldenrod | 3.40 |
| Moss | 2.30 | Grass | 12.16 |
| Raspberry | 8.12 | Moss | 4.50 |
| Red Maple Seedling | 1.20 | Raspberry | 7.10 |
| **Sugar Maple** | **1.10** | Red Maple Seedling | 2.30 |
| | | **Speckled Alder** | **2.30** |
| | | **Viburnum** | **2.30** |

**Table 6.2** *Percent coverage of tree species in the riparian zone of Robbins Mill measured in September, 2014 based on three 10m x 10m quadrats per stream bank side.*

| Tree Species Robbins Mill Left | Percent Coverage (%) | Tree Species Robbins Mill Right | Percent Coverage (%) |
|---|---|---|---|
| Ash | 3.11 | Ash | 2.80 |
| Black Cherry | 4.14 | **Balsam Fir** | **1.40** |
| **Dogwood** | **11.39** | **Beech** | **1.40** |
| **Red Maple** | **2.70** | Black Cherry | 3.13 |
| Speckled Alder | 7.25 | **Red Pine** | **4.17** |
| | | Speckled Alder | 8.34 |
| | | **Sugar Maple** | **1.40** |
| | | **White Birch** | **1.40** |
| | | **White Pine** | **1.40** |
| | | **Winterberry** | **1.40** |

**Table 6.3** *Average tree diameters at Robbins Mill.*

| Robbins Mill Left Side | | | Robbins Mill Right Side | | |
| --- | --- | --- | --- | --- | --- |
| **Plant Species** | **Total Trees** | **Average Diameter (cm)** | **Plant Species** | **Total Trees** | **Average Diameter (cm)** |
| Ash | 3 | 9.17 | Ash | 2 | 3.70 |
| Black Cherry | 4 | 7.00 | **Balsam Fir** | **1** | **3.60** |
| **Dogwood** | **11** | **2.68** | Beech | 1 | 3.20 |
| **Red Maple** | **2** | **5.40** | Black Cherry | 3 | 2.97 |
| Speckled Alder | 7 | 7.50 | **Red Pine** | **4** | **36.63** |
| TOTAL | 27 | 6.35 | Speckled Alder | 8 | 3.70 |
| | | | **Sugar Maple** | **1** | **2.50** |
| | | | **White Birch** | **1** | **39.80** |
| | | | **White Pine** | **1** | **2.80** |
| | | | **Winterberry** | **1** | **2.90** |
| | | | TOTAL | 23 | 10.18 |

**Table 6.4** *Average densiometer readings from each stream.*

| | **Left** | **Right** |
| --- | --- | --- |
| Robbins Mill | 0.47 | 0.86 |
| Whittier | 0.75 | 0.86 |
| Rome Trout | 0.70 | 0.83 |

**Table 6.5** *Percent moisture determined from soil samples collected from upstream and downstream locations at all three streams (top) and percent organic matter measured from these same samples (bottom). Asterisks note statistical significance.*

| | Percent Moisture Upstream | Percent Moisture Downstream | P Value |
|---|---|---|---|
| **Rome Trout** | 17.441 | 29.250 | |
| | 20.899 | 22.074 | 0.02* |
| | 20.379 | 28.164 | |
| **Robbins Mill** | 21.410 | 19.473 | |
| | 21.115 | 20.628 | 0.03* |
| | 22.235 | 20.782 | |
| **Whittier** | 21.158 | 16.309 | |
| | 18.251 | 17.503 | 0.07 |
| | 21.457 | 19.605 | |

| | Percent Organic Matter Upstream | Percent Organic Matter Downstream | P Value |
|---|---|---|---|
| **Rome Trout** | -49.84768039 | -44.28918001 | |
| | -36.61984044 | -48.97079521 | 0.15 |
| | -31.17059451 | -44.35558862 | |
| **Robbins Mill** | -30.71132857 | -57.76861127 | |
| | -31.74729314 | -66.8226577 | 0.0002* |
| | -28.93612983 | -59.13218006 | |
| **Whittier** | -35.15541265 | -25.61991204 | |
| | -29.481725 | -28.768295 | 0.07 |
| | -28.08312294 | -23.69424323 | |

**Table 6.6** *Percent ground coverage of riparian plant species at Whittier Brook measured in September, 2014 based on ten 0.5m x 2m quadrats per stream bank side.*

| Plant Species Whittier Left | Percent Coverage (%) | Plant Species Whittier Right | Percent Coverage (%) |
| --- | --- | --- | --- |
| **Black Cherry** | **1.20** | **Beech** | **15.26** |
| Forbs | 7.16 | Forbs | 11.19 |
| Grass | 2.50 | **Goldenrod** | **2.30** |
| Hemlock | 6.13 | Grass | 3.50 |
| **Hemlock Seedling** | **3.70** | Hemlock | 19.33 |
| **Ironwood** | **1.20** | Moss | 3.50 |
| Moss | 22.49 | Red Maple Seedling | 1.20 |
| **Mushroom** | **1.20** | Witch Hazel | 2.40 |
| Red Maple Seedling | 1.20 | | |
| Witch Hazel | 1.20 | | |

**Table 6.7** *Percent coverage of tree species in the riparian zone of Whittier Brook measured in September, 2014 based on three 10m x 10m quadrats per stream bank side.*

| Tree Species Whittier Left | Percent Coverage (%) | Tree Species Whittier Right | Percent Coverage (%) |
| --- | --- | --- | --- |
| Beech | 4.70 | Beech | 16.28 |
| **Big Tooth Aspen** | **1.20** | **Grey Birch** | **3.50** |
| Hemlock | 23.42 | Hemlock | 12.21 |
| Ironwood | 3.50 | Ironwood | 3.50 |
| **Maple Leaf Viburnum** | **3.50** | Red Maple | 3.50 |
| **White Pine** | **2.40** | Striped Maple | 2.30 |
| Red Maple | 10.18 | Sugar Maple | 3.50 |
| **Red Oak** | **1.20** | | |
| Sugar Maple | 1.20 | | |
| **Witch Hazel** | **7.13** | | |

**Table 6.8** *Average tree diameters measured at Whittier Brook.*

| | Whittier Right Side | | |
|---|---|---|---|
| **Species** | **Total Trees** | **Total cm** | **Average Diameter (cm)** |
| Beech | 16 | 86.10 | 5.38 |
| **Grey Birch** | **3** | **55.10** | **18.37** |
| Hemlock | 12 | 191.70 | 15.98 |
| Ironwood | 3 | 34.70 | 11.57 |
| Red Maple | 3 | 36.50 | 12.17 |
| **Striped Maple** | **2** | **5.50** | **2.75** |
| Sugar Maple | 3 | 40.60 | 13.53 |
| **Witch Hazel** | **16** | **38.80** | **2.43** |
| Total | 58 | | 10.27 |

| | Whittier Left Side | | |
|---|---|---|---|
| **Species** | **Total Trees** | **Total cm** | **Average Diameter (cm)** |
| Beech | 4 | 82.70 | 20.68 |
| **Big Tooth Aspen** | **1** | **31.50** | **31.50** |
| Hemlock | 23 | 331.90 | 14.43 |
| Ironwood | 3 | 42.90 | 14.30 |
| **Maple Leaf Viburnum** | **3** | **8.30** | **2.77** |
| **White Pine** | **2** | **95.20** | **47.60** |
| Red Maple | 10 | 71.70 | 7.17 |
| **Red Oak** | **1** | **19.50** | **19.50** |
| Sugar Maple | 1 | 7.60 | 7.60 |
| TOTAL | 48 | | 18.39 |

**Table 6.9** *Percent ground coverage of riparian plant species at Rome Trout measured in October, 2014 based on ten 0.5m x 2m quadrats per stream bank side.*

| Plant Species Rome Trout Left | Percent Coverage (%) | Plant Species Rome Trout Right | Percent Coverage (%) |
|---|---|---|---|
| Aster | 2.20 | Aster | 4.50 |
| Black Cherry | 1.10 | Bed Straw | 1.10 |
| Fern | 10.12 | Black Cherry | 1.10 |
| Forbs | 11.14 | Culvert | 2.30 |
| Goldenrod | 9.11 | Fern | 8.11 |
| Grass | 15.19 | Forbs | 15.20 |
| Moss | 5.60 | Goldenrod | 2.30 |
| **White Pine** | **2.30** | Grass | 14.19 |
| Raspberry | 14.18 | **Jewel Weed** | **1.10** |
| **Red Maple** | **3.40** | Moss | 8.11 |
| Speckled Alder | 5.60 | Raspberry | 10.14 |
| **Winterberry** | **1.10** | Speckled Alder | 3.40 |
| | | **Viburnum** | **2.30** |

**Table 6.10** *Percent coverage of tree species in the riparian zone of Rome Trout measured in Ocrtober, 2014 based on three 10m x 10m quadrats per stream bank side.*

| Tree Species Rome Trout Left | Percent Coverage (%) | Tree Species Rome Trout Right | Percent Coverage (%) |
|---|---|---|---|
| **Elm** | **1.20** | **Balsam Fir** | **1.30** |
| Red Maple | 8.12 | **Black Cherry** | **1.30** |
| Speckled Alder | 54.84 | Red Maple | 3.90 |
| | | Speckled Alder | 26.79 |
| | | **Sugar Maple** | **1.30** |

**Table 6.11** *Average tree diameters at Rome Trout.*

**Rome Trout Right Side**

| Species | Total Trees | Total cm | Average Diameter (cm) |
|---|---|---|---|
| **Balsam Fir** | **1** | **12.40** | **12.40** |
| **Black Cherry** | **1** | **11.00** | **11.00** |
| Red Maple | 3 | 52.20 | 17.40 |
| Speckled Alder | 26 | 117.10 | 4.50 |
| **Sugar Maple** | **1** | **16.50** | **16.50** |
| TOTAL | 32 | | 12.36 |

| Rome Trout Left Side | | |
|---|---|---|
| Species | Total Trees | Average Diameter (cm) |
| **Elm** | **1** | **7.70** |
| Red Maple | 8 | 18.96 |
| Speckled Alder | 54 | 3.84 |
| TOTAL | 63 | 10.17 |
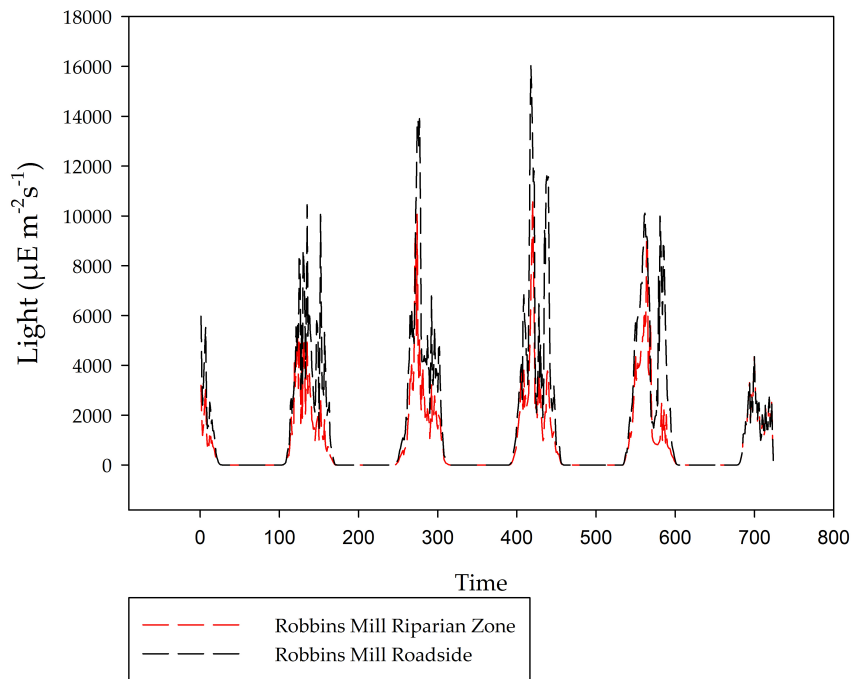


**Figure 6.5** *Light intensity measured by two separate PAR sensors at Robbins Mill. One sensor was located along the side of the road, while the other was placed in the middle of the reach, within riparian vegetation. Sensors were placed on October 16, 2014 at 1:55pm and retrieved on October 21, 2014 at 2:25pm.*

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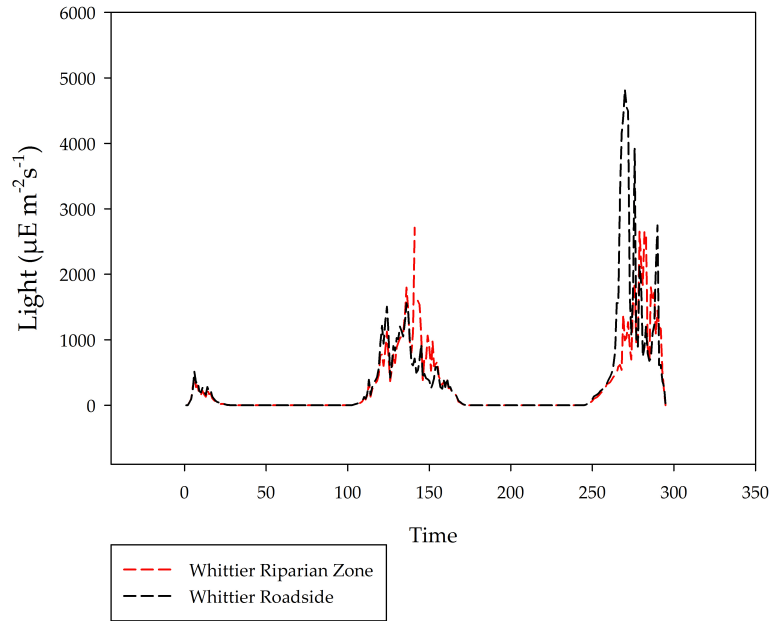
**Figure 6.6** *Light intensity measured by two separate PAR sensors at Whittier Brook. One sensor was located along the side of the road, while the other was placed in the middle of the reach, within riparian vegetation. Sensors were deployed at 1:15pm on October 7, 2014 and retrieved at 1:55pm on October 9, 2014.*
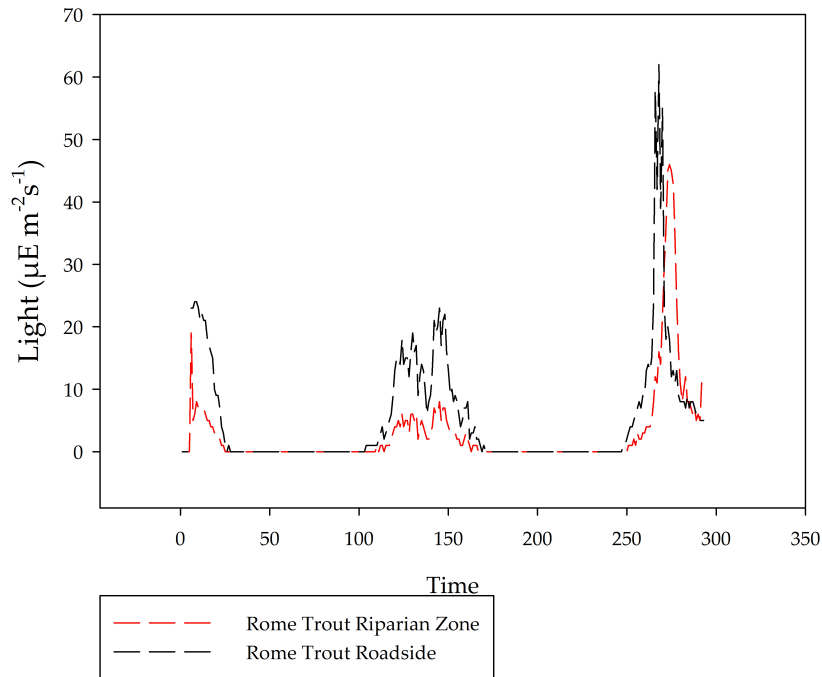


**Figure 6.7** *Light intensity measured by two separate PAR sensors at Rome Trout. One sensor was located along the side of the road in the middle of the culvert, while the other was placed in the middle of the reach, within riparian vegetation. Sensors were deployed at 1:55pm on September 30, 2014, and retrieved on October 2, 2014 at 2:25pm.*

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**CHAPTER 7**
**MACROINVERTEBRATES**


## 7.1  Introduction

### 7.1.1  Importance of Macroinvertebrates to Headwater Streams

Maintaining the overall health of headwater streams is critical for

downstream water quality and habitat diversity in the catchment.  Typically,

conservation efforts are prioritized for large river systems and lakes through projects

like dam removal or lake shore protection.  However, streams provide essential

water, nutrients and biota to downstream communities and have the ability to

process nutrients and organic matter.  Macroinvertebrate communities comprise a

diverse and integral component of riparian and stream ecosystems.  A

macroinvertebrate is defined as an organism without a backbone that is greater than

500 mm in size (Hauer & Lamberti 1996).  Occupying multiple lower trophic levels,

they are essential for nutrient cycling via decomposition of organic matter and serve

as food for predators at higher trophic levels (Wallace & Webster 1996). In addition,

different species exhibit a variety of sensitivities to environmental stressors; as a

result, macroinvertebrate species diversity, composition, and even behavior can

indicate the overall health of a given stream ecosystem (Clarke *et al*. 2008).

Ecological indicators serve to provide a measure of response to anthropogenic

disturbance, such as landscape change, eutrophication or alteration of flow, and the

presence of invasive species (Dolédec & Statzner 2010). Macroinvertebrate

composition in streams also serves as an indicator of long-term environmental

change, such as the changes in frequency of droughts and floods due to global

climate change (Jones 1988; Boulton 2003; Gibbins *et al*. 2007). Apart from just their

presence, macroinvertebrate behavior can point to both natural and anthropogenic

disturbances (Figure 7.1). The presence of pesticides in the water due to agricultural

runoff has been shown to prompt invertebrates to drift, which can disrupt food

chains and nutrient cycling in an affected stream (Schulz & Liess 1999). The behavior

known as invertebrate drift occurs when environmentally stressed aquatic

macroinvertebrates drift downstream until they encounter a more favorable habitat.

(Waters 1965). Studies determining the patterns of species richness along headwater

streams play an important role in informing policy decisions on conservation

planning and reserve design to improve aquatic biodiversity (Clarke *et al*. 2008).
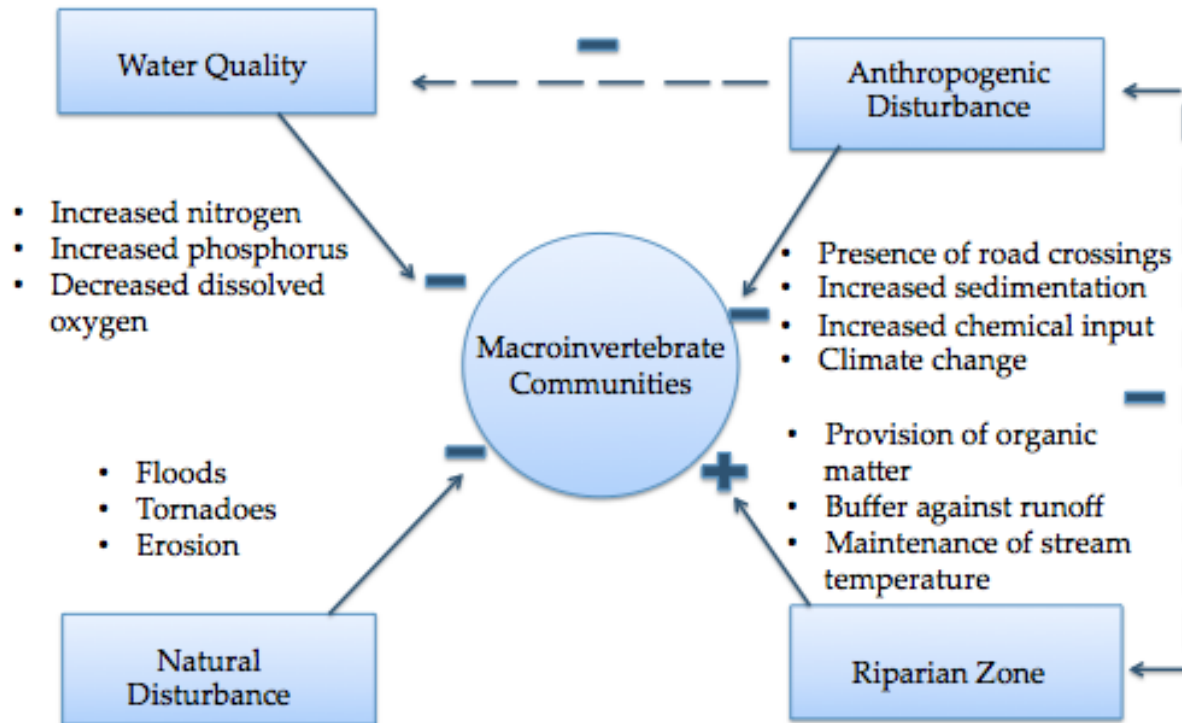
**Figure 7.1** *Conceptual diagram representing some of the positive and negative biological, physical and chemical impacts on macroinvertebrate communities.*

### 7.1.2 Classification of Macroinvertebrates

In temperate stream ecosystems, the taxonomic richness of stream macroinvertebrates can be high. Common temperate stream macroinvertebrates usually belong to one of four phyla: Annelida (segmented worms), Mollusca (molluscs), Platyhelminthes (flatworms), and the most numerous, Arthropoda (arthropods, which includes the insects; Voshell 2002). Diversity is usually assessed at the family level due to difficulties in distinguishing between many species. Macroinvertebrates can also be classified by their functional feeding diversity, or feeding habits, which include: shredders, scrapers, piercers, collectors, and engulfer-

predators (Cummins 1974).  Shredders, such as giant stoneflies, have large

mandibles and chew on large or intact pieces of plant material.  Collectors gather

and feed on fine particulate organic matter (FPOM); some spin webs or use hairs to

collect and filter their food (e.g., netspinning caddisflies and brush-footed mayflies),

and others generate water currents to collect suspended FPOM (e.g., mussels).

Scrapers, such as water pennies, have jaws adapted to scrape thin layers of algae

from rock surfaces.  Piercers, such as predaceous diving beetle larvae, have

mouthparts which stab prey.  The last category, engulfer-predators, consists of

insectivore such as dragonfly larvae, which often have large chewing mouthparts

(Voshell 2002).  These differences shape the ways macroinvertebrates interact with

algal biofilms, other invertebrates and organic matter in the stream system.

Macroinvertebrate diversity can also be assessed through differing tolerances

to natural and anthropogenic environmental stressors.  The primary stressors for

aquatic macroinvertebrates are attributed to changes in water quality, changes in

pH, dissolved oxygen, turbidity, suspended particles, and concentration of various

ions and toxins (Richards *et al*. 1993; Schulz & Liess 1999; Roy *et al*. 2003).  Taxa

known to be sensitive to such changes include the EPT orders—Ephemeroptera

(mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies); in contrast, those

that are tolerant to environmental change include the subclass Oligochaeta

(earthworms) and family Chironomidae (midges; Lenat 1993; Roy *et al.* 2003; Wang

*et al.* 2007).

Biotic measures are used to assess these macroinvertebrate assemblages in

streams. For stream ecosystems it is important to measure richness rather than

abundance when determining community structure. A biotic index is an extremely

useful tool for the bioassessment of stream ecosystems because it describes the

quality of the habitat through taxonomic observations. The Hilsenhoff Biotic Index

was introduced in 1977 and uses weighted pollution tolerance values assigned to

various aquatic macroinvertebrate taxa to evaluate the water quality of the streams

they inhabit (Hilsenhoff 1998; Table 7.1). Another commonly used metric is percent

EPT, a measure of the percent abundance of Ephemeroptera, Plecoptera and

Trichoptera (mayflies, stoneflies and caddisflies, respectively) in a stream. Due to

their sensitivity to perturbation, EPT taxa can be widely used to monitor a number

of abiotic, biotic, and human impacts to streams in environmental monitoring

programs across the globe (Wallace *et al.* 2014).


### 7.1.3 Anthropogenic Impacts and Land Use

The influence of logging, mining, deforestation, urbanization and agriculture

on stream habitat quality has been widely studied over the past few decades

(Lammert & Allan 1999; Lussier *et al*. 2008). There are very few streams left with

catchments unaltered by human activity.  For example, a land use gradient starting

from forested headwaters, to agriculture, to pasture, then to urban land showed

organismal density increased along this gradient, but richness, diversity and

evenness decreased (Hepp & Santos 2009).   The conversion of land for agricultural

use has been widely studied in terms of the impact on macroinvertebrate

community structure.  Although agricultural input has not been shown to alter the

diversity of invertebrates, it does however modify the distribution of organisms

among different habitats such as pools and riffles (Schulz & Liess 1999).  The shift in

taxonomic composition can be attributed to a change in food sources entering

forested versus impacted streams, which can be caused by activities such as logging

that diminish organic matter inputs to streams (Carlson *et al*. 2012).  Specific to the

New England region, streams that had been near recent logging operations

documented higher macroinvertebrate abundance due to increased light penetration

and nutrient availability for grazers (Nislow & Lowe 2006). The direct impacts of

urban activity, pollution and roadways can be seen through the community of

benthic organisms that inhabit these stream ecosystems. Overall, in areas where

urban development and pressure are the highest, richness and population densities

of macroinvertebrates are low and only tolerant organisms are supported (Garie &

McIntosh 1986).  Therefore, maintaining an intact and robust riparian zone is vital to

mitigating the effects of agricultural and urban runoff on both stream and lake

ecosystems (Virbackas *et al*. 2011).

### 7.1.4  Pertinence of Macroinvertebrates to the Belgrade Lake Community

Algal blooms are a concern in parts of the Belgrade Lakes region. An

inventory of macroinvertebrate fauna in catchments can point to possible sources of

pollution due to anthropogenic runoff that may contribute to this eutrophication.

Disruptions in macroinvertebrate community structure caused by pollution, human

activity, or environmental changes may affect organisms at other trophic levels, such

as insectivorous fish, which are vital to Belgrade's small-scale recreational fisheries.

While macroinvertebrates perform many roles in streams, their use as indicators of

stream health is valuable to humans both ecologically and economically (Wallace &

Webster 1996). Aquatic invertebrates play a significant role in engaging and rallying

communities around stream conservation because they provide people with a

tangible connection to their ecosystem.

### 7.1.5  Research Objectives

The purpose of this study is to characterize the structure and diversity of

macroinvertebrate communities in headwater streams of the Belgrade watershed.

By using biotic indices, we also aim to characterize the health of the tributary

streams that feed into the Belgrade Lakes.  Using our results, we seek to correlate

measures of macroinvertebrate diversity with other biotic and abiotic stream

measures, as well as compare our data to previous studies.  We assessed taxonomic

diversity using Shannon-Weiner diversity (*H'*) and water quality using Hilsenhoff's

biotic index (H.B.I.; Hilsenhoff 1987).  We also used a third measure, %EPT, which

assesses the relative proportion of pollution-sensitive taxa—Ephemeroptera

(mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)—to help

characterize stream health and macroinvertebrate diversity.  Our research questions

are:  1) What is the macroinvertebrate diversity and composition in the Belgrade

Lakes watershed, and 2) how does human activity affect macroinvertebrate

composition and/or behavior?  Based on previous macroinvertebrate assessments in

the Belgrade Lakes region and in other ecosystems, we hypothesize that healthier

streams will be further away from human activity, have better water quality, and

have a higher biodiversity of macroinvertebrates, especially pollution-intolerant taxa

(Roy *et al.* 2003; Wang *et al.* 2007; ES494 2013).


## 7.2  Methods

### 7.2.1  Study Sites

We sampled macroinvertebrate diversity at three streams in the Belgrade

Lakes watershed. The first of the sites was Whittier Brook, a well-preserved stream

set far back from any roads. Whittier Brook has a dense canopy cover and many large boulders throughout the reach. The other two sites, Rome Trout Brook and Robbins Mill Stream, are impacted by logging upstream and both flow through culverts at road crossings near the start of the reach. Both Robbins Mill and Rome Trout are in Rome, Maine off of Route 225. Whittier Brook is located in Belgrade, Maine off of Route 27 (Figure 7.2).
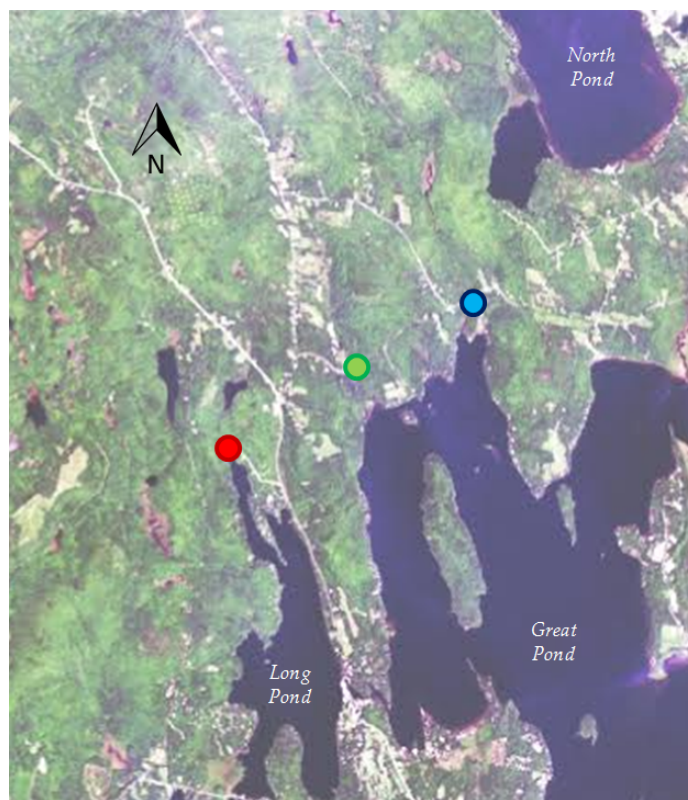


**Figure 7.2** *Map of the three macroinvertebrate sampling locations in the Belgrade Lakes watershed. Key: red = Whittier, green = Rome Trout, blue = Robbins Mill.*

## 7.2.2 Sampling

We performed our field sampling at each of the aforementioned three streams over the course of three weeks between September 16-30, 2014. Both Rome Trout

and Whittier Brook featured 100-meter study sites while Robbins Mill was 80 meters, due to geomorphological variation.  Along each reach, we sampled using two different techniques every 20 meters.  We collected kick net samples in areas with loose, sandy substrate, and sampled by kicking up substrate into a net downstream for 30 seconds. We then put the contents of the net through a 500-micron mesh sieve and, using a squirt bottle, transferred the contents trapped by the sieve a wide-mouthed sampling jar.  We stored these samples in the refrigerator at 4° C to be sorted and preserved within 48 hours.

We performed hand collections by selecting cobbles and boulders (64 mm in diameter and larger) lying within a 1 m$^2$ area, placing them into a bucket, and scraping off macroinvertebrate-rich detritus.  We then placed scraped rocks from the bucket back to the stream, and sieved and transferred the detritus-rich sample in the bucket to a wide-mouthed sampling jar for cold storage in the same way as for the kick net samples.

### 7.2.3  Macroinvertebrate Behavior

To study the dispersal of macroinvertebrates in their habitats, we deployed three 100 mm drift nets for 20 minutes near the end of each stream reach; two nets were placed in riffle areas and one in a pool area.  We performed a total of two three-net replicates at each stream, for a total of 18 drift trials.

### 7.2.4 Processing and Analysis

After the macroinvertebrates were separated out from the organic matter and the sediment in the laboratory, we placed them in 70% ethanol for preservation. We identified macroinvertebrates to the family level under a microscope (Olympus SZ61), using Voshell (2002) and Merritt *et al.* (2008) as references. To assess water quality in the three study streams, we used the H.B.I. and percent Ephemeroptera, Trichoptera and Plecoptera (%EPT; Equation 7.1). We also used the Shannon-Weiner diversity index to help characterize family-level diversity in the communities of the three study sites (Equation 7.2).

$$\%\text{EPT} = \frac{\text{number of macroinvertebrates belonging to EPT taxa}}{\text{total number of macroinvertebrates}}$$

**Equation 7.1** *Calculation of percent EPT, which indicates the proportion of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) relative to the total number of macroinvertebrates sampled.*

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

**Equation 7.2** *Shannon-Weiner diversity index (H'), where $p_i$ is the proportion of species i in the sample and R is the number of species in the sample.*

## 7.3  Results

### 7.3.1  Taxonomic Richness of Samples

Based on Shannon-Weiner diversity, percent EPT, and H.B.I. calculations, we found that all three streams included in our study were diverse both taxonomically and functionally.  Across the three streams, we sampled a total of 879 individuals, which were identified to at least the family level (with the exception of the flatworms and annelids, which were identified at the phylum level).  Due to sampling difficulties across the three study streams, our sample sizes and taxonomic richness varied widely:  134 individuals in 16 taxa from Whittier, 189 individuals in 24 taxa from Rome Trout, and 566 individuals in 34 taxa from Robbins Mill.  Together, our samples comprise 39 taxa.

The majority of macroinvertebrates collected from all three streams were arthropods, of which nearly all were insects.  The Trichoptera (caddisflies) were the most numerous order across all three streams (318 individuals), followed by the Ephemeroptera (mayflies; 145 individuals), Diptera (105 individuals), and Plecoptera (stoneflies; 76 individuals); Figure 7.3).  The most abundant families across the three streams were the Hydropsychidae (common netspinner caddisfly; 242 individuals), Heptageniidae (flatheaded mayfly; 138 individuals) and Perlidae (common stonefly; 53 individuals).

### 7.3.2 Diversity and Biotic Indices

Through calculation of Shannon-Weiner diversity indices, we determined that Whittier Brook had the lowest family-level diversity ($H'$ = 1.98), while Robbins Mill had the highest family-level diversity ($H'$ = 2.66).

Calculation of the Hilsenhoff Biotic Index (HBI) indicated that Whittier had the best water quality (HBI = 3.10) while Rome Trout had the poorest water quality (HBI = 4.55; Table 7.2). We were unable to perform statistical analyses due to the small number of replicates (n = 1) per stream.

### 7.3.3 Percent EPT

More than half of all macroinvertebrates collected from the three study streams belonged to the pollution-sensitive EPT taxa. Whittier had the greatest percent EPT at 85%, while Rome Trout had the lowest at 54%, although these values varied between sampling points within each stream. Robbins Mill Stream, for example, had a culvert between 20 and 40 m along the reach, and percent EPT decreased from 78% immediately upstream of the culvert to 55% immediately downstream of the culvert. Rome Trout, with a culvert at 40 m along the reach, had an even more stark decrease in %EPT; the upstream samples, at 0 m and 20 m, had %EPT at 82%, while the samples downstream had half the %EPT at 43% (Figure 7.4).

As with diversity and biotic indices, the sample sizes at each sampling point along

the three reaches were too small to perform statistical tests (n = 2).

**Table 7.1** *Hilsenhoff Biotic Index values with corresponding water quality grade and degree of organic pollution (Hilsenhoff, 1987)*

| HBI 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 significant 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 7.2** *A summary of HBI index values, %EPT and diversity measures at each of the three streams.*

| Stream | Total macroinvertebrates | Number of taxa | Shannon-Weiner Index | % EPT | HBI |
|---|---|---|---|---|---|
| Whittier | 134 | 16 | 1.98 | 85 | 3.10 |
| Rome Trout | 189 | 23 | 2.41 | 54 | 4.11 |
| Robbins Mill | 566 | 34 | 2.66 | 67 | 3.54 |
| TOTAL | 879 | 39 | 2.73 | 69 | |

## Whittier Brook



- Trichoptera 51
- Ephemeroptera 34
- Plecoptera 29
- Odonata 11
- Coleoptera 5
- Other insects 3
- Oligochaeta 1

## Rome Trout Brook



- Trichoptera 54
- Ephemeroptera 41
- Annelida 37
- Coleoptera 24
- Diptera 22
- Plecoptera 3
- Other macroinvertebrates 7

## Robbins Mill Stream



- Trichoptera 213
- Ephemeroptera 145
- Diptera 82
- Coleoptera 45
- Plecoptera 44
- Pulmonata 13
- Other macroinvertebrates 24

## All Streams



- Trichoptera 318
- Ephemeroptera 220
- Diptera 105
- Plecoptera 76
- Coleoptera 67
- Annelida 42
- Other macroinvertebrates 51

**Figure 7.3**  *Taxonomic composition of macroninvertebrates collected at Whittier Brook, Rome Trout Stream, and Robbins Mill Stream.*
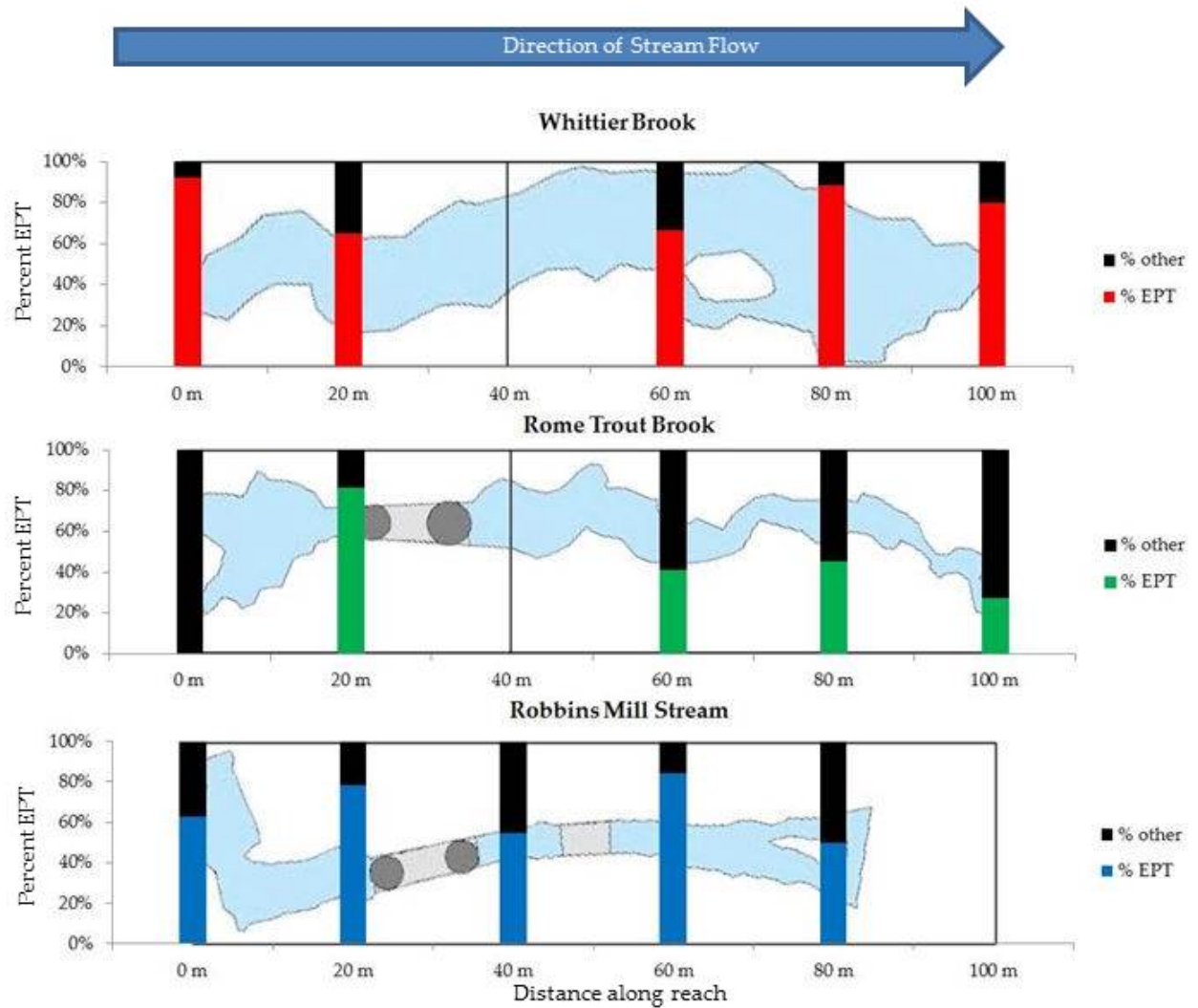
**Figure 7.4** *Percent EPT composition of collected macroinvertebrates as a function of distance along the reaches of Whittier Brook, Rome Trout Brook, and Robbins Mill Stream.*

### 7.3.4  Functional Feeding Group Diversity

Since different species within the same family may belong to different

functional feeding groups (e.g., Baetidae, the small minnow mayflies, which can be

collectors or scrapers), we found it generally more difficult to accurately assign

functional feeding groups.  Hence, we followed Voshell (2002) and Bouchard (2004)

and thus assigned our insects to six categories:  collector-filterers, collector-gatherers, scrapers, shredders, piercer-predators, and engulfer-predators (examples presented below).  We found collector-filterers to be most abundant overall, but there were some significant differences between the three streams (Figure 7.5).  At Whittier, the most numerous macroinvertebrates were the collector-filterers, followed by engulfer-predators and scrapers.  By contrast, at Rome Trout, scrapers were the most abundant feeding group, followed by the engulfer-predators and collector-filterers; this may be partially due to increased algal growth due to ample light and possible agricultural runoff into the brook.  Robbins Mill was dominated by collector-filterers, scrapers, and collector-gatherers (Table 7.3).  The most numerous collector-filterers and scrapers in all three streams were the Hydropsychidae and Heptageniidae, respectively.  In Robbins Mill and Rome Trout, the dominant shredders were the Tipulidae (crane flies), while in Whittier, the only shredders present were the Nemouridae (nemourid stoneflies) and Haliplidae (crawling water beetles).  Piercer-predators were absent in Whittier and insignificant in Rome Trout, but present in Robbins Mill as Athericidae (snipe flies).  Engulfer-predators were present in all three streams as Aeshnidae (darner dragonflies) and Perlidae (common stoneflies), as well as Rhyacophilidae (freeliving caddisflies) in Robbins Mill alone.

## Whittier Brook

Legend:
- Collector-filterer 47
- Collector-gatherer 11
- Engulfer-predator 42
- Scraper 30
- Shredder 4

## Rome Trout Brook

Legend:
- Collector-filterer 40
- Collector-gatherer 52
- Engulfer-predator 21
- Piercer-predator 2
- Scraper 43
- Shredder 21

## Robbins Mill Stream

Legend:
- Collector-filterer 195
- Collector-gatherer 73
- Engulfer-predator 61
- Piercer-predator 20
- Scraper 160
- Shredder 57

## All streams

Legend:
- Collector-filterer 282
- Collector-gatherer 136
- Engulfer-predator 124
- Piercer-predator 22
- Scraper 233
- Shredder 82

**Figure 7.5** *Functional feeding group classification of macroninvertebrates collected at Whittier Brook, Rome Trout Stream, and Robbins Mill Stream.*

**Table 7.3** *Breakdown of number of organisms in each functional feeding groups by stream.*

| Feeding group | Whittier | Rome Trout | Robbins Mill | TOTAL |
|---|---|---|---|---|
| Collector-filterer | 47 | 40 | 195 | 282 |
| Collector-gatherer | 11 | 52 | 73 | 136 |
| Engulfer-predator | 42 | 21 | 61 | 124 |
| Piercer-predator | 0 | 2 | 20 | 22 |
| Scraper | 30 | 43 | 160 | 233 |
| Shredder | 4 | 21 | 57 | 82 |
| TOTAL | 134 | 179 | 566 | 879 |

### 7.3.5 Invertebrate Drift

Finally, we found drift to be insignificant across all three streams. From all 18 trials, one pronggilled mayfly was collected each from Rome Trout and Robbins Mill, and one small minnow mayfly was collected from Robbins Mill. No drifting insects were collected from Whittier.

### 7.3.6 Correlations Between Macroinvertebrate Measures and Other Stream Measures

For each of the three streams, we correlated taxonomic richness and percent EPT at each sampling point along the reach with other stream measures, including

sediment composition (e.g. percent sand, percent gravel), and found no significant

correlations between any of the measures (n = 3).


## 7.4  Discussion

### 7.4.1  Diversity and Biotic indices

Through Shannon-Weiner diversity indices and biotic indices, we determined

that all three streams were diverse and in good health.  Although we expected the

stream without a road crossing along the reach (Whittier) to have greater

macroinvertebrate diversity, greater percent EPT, and lower HBI than streams with

road crossings (Rome Trout and Robbins Mill), our data does not consistently

support our hypothesis across all three measures.

For the Shannon-Weiner diversity index, we observed moderate-to-high

family-level richness when compared to other macroinvertebrate studies in

temperate riparian ecosystems (Flecker 1984; Barbour *et al*. 1996; Lammert 1999;

Boulton 2003; Roy *et al*. 2003).  However, our diversity results did not support our

hypothesis:  both Rome Trout and Robbins Mill had higher diversity indices than

Whittier; this may be due to the much larger sample sizes in the former two streams

(Table 7.2).

More than half of all individuals sampled across the three streams belonged to the pollution-sensitive Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly) taxa, indicating that all three streams must have relatively low pollution levels. In addition, although Whittier had lower family-level diversity, it had proportionally fewer chironomids and annelids (pollution-tolerant taxa) than either Robbins Mill or Rome Trout, suggesting that pollution may have an effect on the macroinvertebrate community structure of these latter two streams.

Using the diversity indices, we also calculated biotic indices and determined that all three streams had high water quality overall; following Hilsenhoff (1987), we found that Whittier and Robbins Mill had "Excellent" water quality, while Rome Trout had "Very Good" water quality (Table 7.1).

### 7.4.2  Functional Feeding Groups

From a functional feeding group standpoint, since both collector-filterers and collector-gatherers were numerous in all three streams, we can infer that fine particulate organic matter (FPOM), the main food source for both kinds of collectors, must be an abundant source of food for many taxa in all three streams. Closer examination of the less abundant functional groups indicate that Whittier has far fewer shredders than Rome Trout or Robbins Mill, suggesting that the amount of coarse detritus falling from the canopy into Whittier or retained in the reach is

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comparatively smaller than that of the other two streams. This is potentially due to

Whittier's large number of boulders that limit the amount of leaf litter that can fall

into the stream and reach the benthos. Rome Trout had a high abundance of

scrapers, indicating a higher degree of algal growth; this could be due to lower

canopy cover along the reach and runoff from nearby farms, which would provide

ample light and nutrients for algae to grow in the stream. In addition, Whittier had

a proportionally greater number of engulfer-predators than either Rome Trout or

Robbins Mill, suggesting that predation of and by macroinvertebrates plays a

comparatively greater role than herbivory in energy and nutrient cycling in Whittier

than in the other two streams (Table 7.3; Figure 7.5).


### 7.4.3 Invertebrate Drift

The primary trigger of invertebrate drift across aquatic ecosystems is a

change in light intensity; as a result, drift tends to exhibit diel periodicity, with peak

drift rates around the hours of dawn and dusk (Flecker 1992; Hauer & Lamberti

1996). However, multiple other abiotic, biotic, and anthropogenic factors can trigger

invertebrate drift, including storms (Gibbins *et al*. 2007), competition with other

organisms (Flecker 1992), presence of culverts and road crossings (Hay *et al*. 2008),

and pollution (Schulz & Liess, 1999). As a result, explanations for why drift occurs

can be complex and difficult to identify.

Experimental design may have significantly limited the amount of drifting

macroinvertebrates we collected (n = 3). Due to temporal limitations, we were only

able to sample between the hours of 2 and 4 PM, when light intensity is still fairly

strong. In addition, our constraint of 20 minutes per replicate may also have limited

the amount of drifting macroinvertebrates that could be collected.

Several factors may explain the differences in drift magnitude across all three

study streams. First, storms may have increased the magnitude of invertebrate drift,

as all three drifting macroinvertebrates were collected from Rome Trout and

Robbins Mill during heavy rain, and rain did not occur during the drift experiments

conducted at Whittier. Secondly, anthropogenic disturbances may have also

increased drift; all three drifting macroinvertebrates were collected downstream of

the culverts at Rome Trout and Robbins Mill. Consequently, our failure to collect

any drifting macroinvertebrates from Whittier could be attributed to its sheltered

location away from human activity, absence of culverts or road crossings, lower

flow rate due to the absence of stormy weather, and perhaps even stochasticity due

to small sample size. Despite the difficulty in identifying the cause of drift, we

nevertheless observed a low magnitude of invertebrate drift despite significant

natural and anthropogenic stressors such as inclement weather and road crossings.

As a result, we hypothesize that background drift in the three study streams is likely

very low, but more extensive drift studies should continue to be conducted to more

fully investigate their causes within the headwater streams of the Belgrade Lakes

watershed.

### 7.4.4  Human Impact

Though sample sizes and replications were small, we determined that percent

EPT appeared to be lower from sampling sites immediately downstream of culverts,

suggesting that anthropogenic activities, such as the construction of road crossings,

may have localized impacts on the community structure and health of streams in the

Belgrade Lakes watershed.  These results were consistent with studies in the

Belgrade Lakes watershed in previous years (ES494 2013).

Human activity must have at least a localized effect on stream diversity, but

whether due to point-source pollution or changes in sediment structure and

permeability is difficult to determine using these methods. Possibilities of

disturbance include decreased substrate stability, increased chemical inputs,

increased flow rates and increased suspended solids.  Culverts are characterized by

shallow water habitat with a relatively homogenous and flat substrate.  Dams,

levees and culverts decrease water quality and dissolved oxygen availability in

stream environments (Genkai-Kato *et al*. 2005).  Combined with a lack of diverse

substrate types, dissolved oxygen can limit the number of invertebrate taxa present

near these alterations in the stream's channel. The presence of stream

impoundments and the subsequent decline in water quality and flow rates impact

biota composition throughout the culvert at its outlet, but have not been shown to

affect downstream water quality (Ogbieubu & Oribhabor 2002). EPT taxa may be

virtually absent at culverts or impoundments in streams, but tend to reappear

further down the reach.  This observation indicates that pollution from road

crossings may be point-source, and therefore more easily managed and mitigated

than diffuse pollution.

### 7.4.5  Methodology and Logistics

In an effort to reduce sampling error, we used two different sampling

methods (brushing and kicking) at each sampling point along the three reaches.

However, each stream presented logistical difficulties that made sampling more

challenging in some areas due to inaccessibility. For example, Whittier was covered

in large boulders that made sampling nearly impossible along the entire reach,

which may account for the relatively low number of individuals collected.  At Rome

Trout, the sampling point at 40 m along the reach was located inside a culvert, and

so no samples could be collected.  At Robbins Mill, the culvert was located between

20 and 40 m, and samples were collected both upstream and downstream of the

culvert. However, at 100 m, the depth of the stream increased dramatically due to

the presence of a beaver dam downstream, and no samples could be collected at this

point either.  As a result, total number of individuals and taxa collected varied

significantly between sampling points, and future studies should incorporate more

sophisticated and balanced sampling techniques to obtain more equal sample sizes

between sampling points in order to try and bypass these difficulties.

### 7.4.6  Correlations

Due to small replicate sizes for our stream measures, we were unable to

identify any significant correlations between taxonomic diversity, percent EPT, and

HBI with other stream measures such as sediment composition, nitrogen

concentration, or pH.  However, many other studies have evaluated possible

correlations in a variety of temperate and tropical stream ecosystems, and these can

be used as models to guide the conservation and preservation of the Belgrade Lakes

region as a whole.  Richards *et al.* (1993), for example, correlated changes in

macroinvertebrate composition with changes in catchment morphology.  Fourteen

years later, Wang *et al*. (2007) identified a decrease in variance of family-level

diversity as the concentrations of both soluble nitrogen and phosphorus in the water

column increase.  Land cover can also exert a similar effect, as an increase in

urbanization decreases the variance in HBI scores for affected streams; following

Roy *et al* (2003), streams in Georgia that had little surrounding urbanization could

have water quality ranging from excellent to poor, whereas streams running

through highly urbanized areas always had poor water quality.

Since the Belgrade Lakes watershed is a largely agricultural, rural region in Maine located between the larger towns of Farmington, Augusta, and Bangor, point-source pollution due to automobiles and agricultural runoff are likely the major stressors to macroinvertebrate composition.  Construction of culverts and road crossings to improve transportation across central Maine can elicit changes in catchment morphology, and careless farming practices can increase the amount of nitrogen-, phosphorus-, and pesticide-rich runoff entering these streams.  Consequently, future studies in the Belgrade Lakes watershed should monitor these changes over time using larger sample sizes to identify any significant statistical relationships between macroinvertebrate diversity and other biotic, abiotic, and anthropogenic factors.

### 7.5  Conclusion

Overall, our results present a simple but conclusive evaluation of the health and macroinvertebrate diversity in Belgrade Lakes streams.  We found that Whittier, Rome Trout, and Robbins Mill streams were all healthy and exhibited few signs of pollution.  Given the importance of macroinvertebrates in lotic food webs, through analysis of functional feeding group diversity, we were also able to hypothesize ecological trends regarding nutrient cycling and organic matter decomposition in all three streams.

Apart from being biological indicators of stream health, macroinvertebrates allow for important trophic linkages and cycling of organic matter in aquatic ecosystems. To maintain these crucial ecosystem functions, it is essential to protect the diversity of macroinvertebrate taxa present in streams. This can be facilitated through the maintenance of robust and intact riparian zones, as well as sustaining unaltered stream channels and substrates. If not managed or monitored, polluted streams have the potential to feed into the Belgrade Lakes and cause algal blooms. These unsightly blooms can have detrimental effects to Belgrade's tourism and recreational fisheries.

We also discovered that logistical difficulties in sampling can impede the identification of any correlative trends with other stream measures such as sediment composition. With increased sampling capacity at headwater streams that feed into Belgrade's lakes, macroinvertebrate communities can be monitored and compulsory water quality improvements can be made on a localized scale. Macroinvertebrate sampling and analyses remain easily calculable and highly versatile and indicative tools to evaluate the conservation status of temperate stream ecosystems. Targeting streams for conservation efforts provides potential early warning assessments and valuable insights as to how anthropogenic impacts to our watersheds can be mitigated.

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# CHAPTER 8
# PHARMACEUTICALS IN STREAMS

## 8.1. Introduction

### 8.1.1 Pharmaceuticals in the Environment

Pharmaceuticals can be defined as any chemical substance that contains one or more active ingredients that cause therapeutic effects intended for human or veterinary disease treatment and prevention. As the world's growing population ages and new medical discoveries are made, the demand for prescription medication is expected to rise; the decade spanning 1993 to 2003 in the U.S. saw an increase in the number of purchased prescriptions by 70% while the U.S. population increased by only 13% (Ruhoy & Daughton 2007).

The growing population will place additional pressure both on the pharmaceutical industry as well as on the demand for fresh water. It is, therefore, prudent to better understand how increasingly present concentrations of pharmaceuticals are making their way into our waterways in order to determine how to best stymie these biologically active organic wastes. Reducing this source pollution will aid in protecting our limited water sources and the biodiversity and ecosystem functions they provide. The concept of toxic substances entering the environment was not a tangible concept to the general population until the 1960's due to the publication of *Silent Spring* by Rachel Carson. Within the last two

decades, attention to fresh water contamination by pharmaceutical substances has increased. In 2002, the U.S. Geological Survey measured that over 80% of United States waterways contained trace amounts of at least one of 95 commonly used medications and 75% of the samples tested positive for more than one substance (Kolpin *et al.* 2002). Pharmaceuticals have been detected in surface waters such as rivers (Ashton *et al.* 2004; Batt *et al.* 2006; Glassmeyer *et al.* 2005), in ground water (Rodriguez-Mozaz *et al.* 2004; Verstraeten *et al.* 2005), and even in treated drinking water (Daughton 2008; Heberer 2002b; Stackelberg *et al.* 2007). This issue is now globally recognized (Andersen *et al.* 2003; Al-Odaini *et al.* 2013; Wang *et al.* 2010; Yang *et al.* 2010; Schallenberg & Armstrong 2004; Wang *et al.* 2014; Sim *et al.* 2010; Calamari *et al.* 2003).

### 8.1.2 Mechanisms of Entry into Freshwater Systems

By design, pharmaceuticals are intended to alter biological processes within the human body. Consequently, it is no surprise that these substances have the ability to initiate profound effects on the biota of freshwater ecosystems. These effects, such as the suppression of bacterial communities, also have the potential to impact human health. However, these possible impacts are presently understudied. Pharmaceutical substances enter the environment in both biologically active and

non-active forms, with those of major concern being active pharmaceutical

ingredients (APIs). The four major categories of contamination of freshwater

ecosystems by APIs are: human ingestion and excretion, manufacturing and hospital

effluent, inappropriate disposal, and the veterinary and agricultural industries

(Figure 8.1).
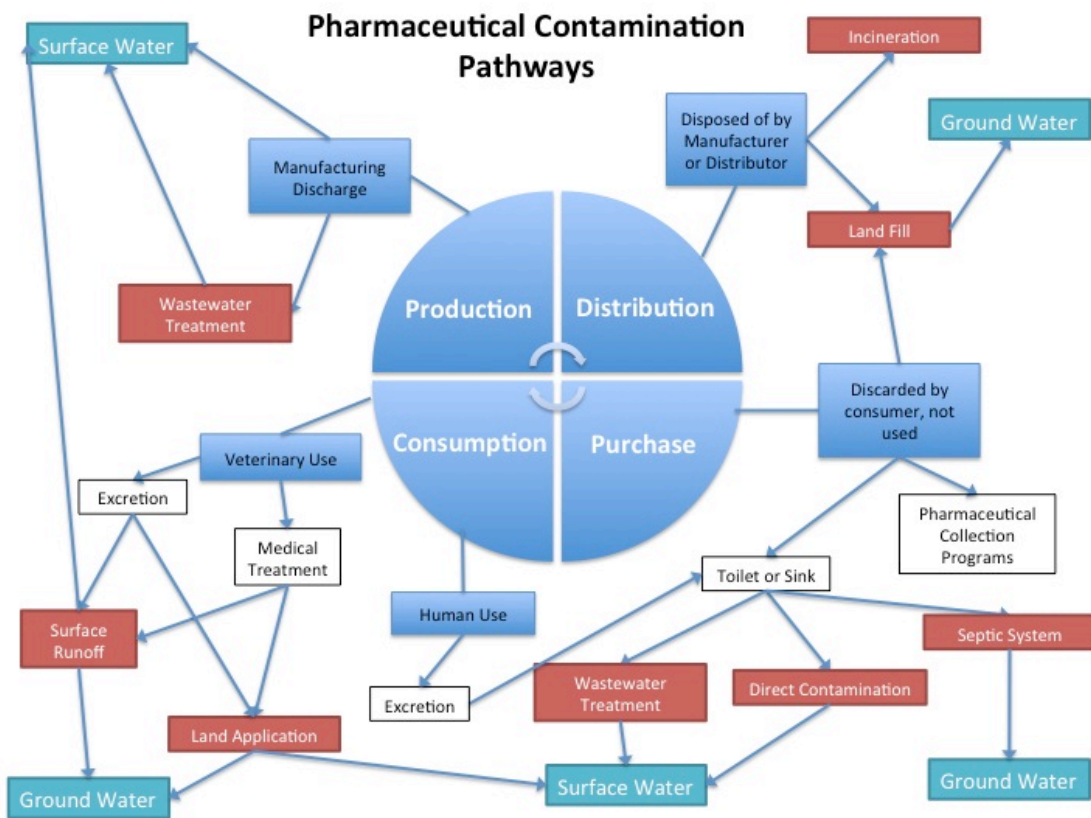


**Figure 8.1** *Pharmaceutical contamination pathways for freshwater ecosystems. Beginning with the four major sources, (production, distribution, purchase, and consumption) this schematic diagram displays how group and surface water can be contaminated by pharmaceutical waste. Blue: origin of use; white: disposal method; red: where the disposed pharmaceutical ends up; teal: final contamination.*

## 8.1.3 Incomplete Human Metabolism

The majority of human pharmaceutical compounds make their way into

aquatic ecosystems after human ingestion and excretion in the form of an active

pharmaceutical or as a metabolite of the active compound (Ashton *et al.* 2004). After

a patient consumes a medication, a percentage of that chemical passes through the

body unmetabolized, or unchanged from its original form. The degree to which this

occurs depends upon the structure of the chemical compound, the mechanism of

action within the body, as well as a patient's unique body chemistry (Bound &

Voulvoulis 2005). In some cases, greater than 90% of a drug is excreted in its original

and active form into the environment (Halling-Sørensen *et al.* 1998). The chemical

can also undergo chemical change inside the body into a biologically active or non-

active metabolite (Kumar *et al.* 2010).

This input of APIs and their derivatives into municipal sewage has led to

their reported detection at sewage treatment plants (STPs) as well as in tertiary

septic systems (Carrara *et al.* 2008; Fick *et al.* 2009; Godfrey *et al.* 2007; Heberer 2002b;

Kolpin *et al.* 2002; Swartz *et al.* 2006; Ternes 1998). Furthermore, many of these APIs

are not fully removed by way of conventional STP processes or onsite septic

systems. As a result, many occur in freshwater ecosystems and potable water

supplies (Stackelberg *et al.* 2007). Given the modernity in this understanding, STPs

are not yet designed to remove these pharmaceuticals from effluent (Halling-

Sørensen *et al.* 1998; Kolpin *et al.* 2002; Stackelberg *et al.* 2004). Pharmaceutical

concentrations are usually reduced by ~90% in treated effluent released into surface

waters when tertiary treatment is used (Hedgespeth *et al.* 2012). However, many

studies have shown that excreted metabolites formed by conjugation in the body

often cleave back into the original, active pharmaceuticals in natural environments

(Heberer 2002a; Ternes 1998; Hedgespeth *et al.* 2012). Within the Environmental

Protection Agency's Region 1 (CT, ME, MA, NH, RI, VT) 71% of all homeowners

rely on septic tanks for sewage disposal rather than municipal sewage treatment;

this statistic is even higher in very rural reaches such as those in our study area (U.S.

EPA 2012). Septic systems often go unchecked for years, leading to ineffective

treatment due to improper installment, tank failure, and inadvertent anaerobic

conditions (Carrara *et al.* 2008; Kolpin *et al.* 2002; Swartz *et al.* 2006). Since waste

moves through the septic system to groundwater aquifers, septic tank effluent is a

source of contamination for ground water (Godfrey & Woessner 2004).

### 8.1.4 Point Sources: Manufacturing and Hospital Waste

Wastewater from drug manufacturing regions has shown extremely high

levels of APIs in the surface, ground, and drinking water in the surrounding areas

(Fick *et al.* 2009; Larsson *et al.* 2007). Ordinarily, sewage effluent pharmaceutical

concentrations are ≤1 µg/L, but near one production site in India, ciprofloxacin, a

common antibiotic, was found at concentrations of 28,000-31,000 μg/L. This unprecedented level is over 1000 times beyond bacterial toxicity level (Larsson *et al.* 2007). While industrial effluent of this magnitude is rare, smaller epicenters of highly concentrated effluent, such as hospitals, exist in almost every town across the globe (Coutu *et al.* 2013; Hartmann *et al.* 1998; Kümmerer 2001; Lindberg *et al.* 2005; Ruhoy & Daughton 2007). These familiar point sources of pollution provide typical municipal STPs with a much higher load of pharmaceutical waste than that associated with excretion.

### 8.1.5 Inappropriate Disposal Methods

The consumer also influences the amount of pharmaceuticals reaching freshwater ecosystems. Oftentimes, prescription medications are over-prescribed or patients are noncompliant with a physician's directed consumption (Daughton 2002). At least $1 billion in prescription drugs prescribed to patients are discarded each year due to non-compliance, expiration, or death, leading to an excess of unconsumed pharmaceuticals in the home (Morgan 2001). Many of these wasted medications are disposed of improperly and find their way into water systems.

The two most common modes of pharmaceutical disposal are flushing down the toilet or washing down the sink. Flushing APIs that would otherwise undergo

metabolism before excretion is a significant watershed input source of APIs. For example, flushing one dose of carbamazepine, a commonly prescribed seizure medication, causes an impact roughly equal to 29–87 metabolized doses (Ruhoy & Daughton 2007). Despite these consequences, audits on household pharmaceutical disposal practices have shown that the general public is thoroughly unaware of proper disposal methods (Bound & Vouvoulis 2005; Kuspis & Krenzelok 1996; Seehusen & Edwards 2006). A 1996 study found that 35.4% of study participants disposed of unused medications via municipal sewage (toilet or sink) (Kuspis & Krenzelok 1996). Ten years following, another American study found that 89% of respondents disposed of their unused pharmaceuticals via municipal sewage (toilet or sink) and 56% of these respondents were unaware that this is an inappropriate practice (Seehusen & Edwards 2006). These results point to a lack of education within the general public about the ecological dangers and potential human health effects that result from these practices.

### 8.1.6 Agriculture and Veterinary Medicine

Finally, the agriculture and veterinary industries both contribute a large amount of APIs directly into ground and surface water. A 2010 estimation identified that approximately 70% of all antibiotics used in the Unites States are used for nontherapeutic purposes in animal agriculture, typically to compensate for

inadequate diet. Antibiotics and growth hormones are the pharmaceuticals most commonly added in animal feed (Martin *et al*. 2010). In 2000, antibiotics and hormones were used in over 88% of the US swine industry for growth as well as illness prevention (Mackie *et al*. 2006). Similar to human metabolic processes, an estimated 25% of these antibiotics are absorbed by animal metabolic processes while the remaining 75% are excreted into the environment via urine and feces (Mackie *et al*. 2006). Similar effects have been documented in STP effluent downstream from industrial feedlots due to runoff into surface water. This surface water has shown to contain sufficient levels of hormonally active agents to cause both ecological and public health concern (Lange *et al*. 2002; Soto *et al*. 2004). Not only is this environmental contamination caused by veterinary excretion, but also by the extensive use of manure in the agricultural industry. The utilization of manure for fertilization practices is a direct application of the pharmaceuticals in livestock waste to the environment via surface runoff as well as subsequent groundwater infiltration (Bartelt-Hunt *et al*. 2011; Zhao *et al*. 2010).

### 8.1.7 Documented Effects on Freshwater Ecosystems

Recent literature has shown severe effects on a plethora of freshwater biota. While pharmaceuticals are often not found at toxic levels in the environment, they are present in levels high enough to cause negative impacts on aquatic organisms

(Crane *et al.* 2006). Most detection studies reveal that approximately 80-100

pharmaceuticals and their subsequent metabolites are present in sewage, seawater,

groundwater, and drinking water with the highest concentrations of

pharmaceuticals reported in sewage treatment plant (STP) effluent. Most

pharmaceuticals that significantly impact the environment share similar properties,

including high production volume, long-term environmental persistence, and strong

biological activity (Fent 2006).

**Table 8.1.** *Results of various studies observing impacts on aquatic organisms due to pharmaceutical exposure*

| Therapeutic Class | Substance | Organism impacted | Effects | Exposure | Concentration | Reference |
|---|---|---|---|---|---|---|
| Antiandrogen | Flutamide | Guppy (*Poecilia reticulata*) | Decreased sperm count | Chronic (30-day) | 10 μg/mg | Baatrup and Junge (2001) |
| Antibiotic | Fluoroquinolones (group) | Duckweed (*Lemna gibba*) | Frond bleaching | Acute (7-day) | 300 μg/L | Brain et al. (2004) |
| Antibacterial | Triclosan | Daphnia (*Daphnia magna*) | Increased sex ratio | Chronic (30-day) | 36 μg/L | Flaherty and Dodson (2005) |
| Anti-cancer/aromotase inhibitor | Fadrazole | Fathead Minnow (*Pimephales promelas*) | Inhibition of brain aromatase activity, decrease in mature oocytes (females), increased androgen levels (male) | Chronic (21-day) | 2-50 μg/L | Ankley et al. (2002) |
| Antidepressant | Fluoxetine | Daphnia (*Daphnia magna*) | Increased fecundity | Acute (6-day) | 36 μg/L | Flaherty and Dodson (2005) |
| Antiinflammatory | Diclofenac | Rainbow Trout (*Oncorhynchus mykiss*) | Renal lesions and gill alterations | Chronic (28-day) | 5 μg/L | Schwaiger (2004) |
| Beta-blockers | Propranolol | Japanese Medaka (*Oryzias latipes*) | Changes in plasma steroid levels, reduced egg count | Chronic | 0.5 μg/L | Huggett et al. (2002) |
| Benzodiazepines | Diazepam, digoxin, amlodipine | Fresh-water Polyp (*Hydra vulgaris*) | Inhibited ability to regenerate a hypostome, tentacles and foot | Chronic (17-days) | 10 mg/L | Pascoe et al. (2003) |
| Cholesterol-lowering agent | Clofibric Acid | Daphnia (*Daphnia magna*) | Skewed sex ratio (toward male) | Acute (6-day) | 10 μg/L | Flaherty and Dodson (2005) |
| Estrogenic compounds | EE2 | Fathead Minnow (*Pimephales promelas*) | Lack of secondary sexual characteristics in males, skewed sex ratio (toward female) | Chronic (84-day) | 4 ng/L | Länge et al. (2001) |
| Estrogenic compounds | EE2 | Zebrafish (*Danio rerio*) | 56% reduction in fecundity, infertility (males) | Chronic (40-day) | 5 ng/L | Nash et al. (2004) |
| Estrogenic Compounds | EE2 | Fathead Minnow (*Pimephales promelas*) | Decrease in gonadosomatic index, egg count and egg fertilization rate | Chronic (21-day) | 10-100 ng/L | Pawlowski et al. (2004) |
| Estrogenic Compounds | EE2 | Fathead Minnow (*Pimephales promelas*) | Decreased secondary sexual characteristics, decreased egg fertilization | Chronic | <1 ng/L | Parrot and Blunt (2005) |

**8.1.8 Impact on Humans**

Due to the observed impacts of pharmaceuticals in ecological systems, many researchers believe that waterborne pharmaceuticals could have unintended effects in humans (Kumar *et al.* 2010). Though there are drinking water standards for organic compounds in the U.S. set by the EPA, these standards do not apply to pharmaceutical concentrations (Webb 2003). Water testing studies worldwide illuminate the prevalence of pharmaceuticals in drinking water. Some drugs are detected even in cities that utilize advanced, tertiary sewage treatment (Jones *et al.* 2005). Studies indicate that over half of America's drinking water is contaminated with approximately 11 different pharmaceuticals, including estrone, naproxen, and trimethoprim (Benotti *et al.* 2009). To date, many studies conclude that the concentrations of waterborne pharmaceuticals, particularly in drinking water, may be too low to impact humans (Christensen 1998; Schwab *et al.* 2005; Webb 2003), however, other research has found that waterborne pharmaceuticals can reduce human cell proliferation and alter protein expression and cell structure (Pomati *et al.* 2006).

Contaminated STP effluent and septic discharge entering freshwater ecosystems is directly related to human health. This discharge is of particular concern given its association with the contamination of freshwater with antibiotics

(Hirsch *et al.* 1999, Kümmerer 2009). These emissions, present at different

concentrations, cause environmental risk in terms of promoting bacterial antibiotic

resistance (Guardabassi *et al.* 2000; Halling-Sorensen *et al.* 1998; Mackie *et al.* 2006). In

recent years, antibiotic resistance has become a significant challenge to the global

medical community with potentially drastic epidemiological consequences. This is

due to over prescription as well the use and misuse of antimicrobial medications

and substances (World Health Organization 2014). Anthropogenically impacted

aquatic ecosystems have the potential to serve as a breeding ground for antibiotic

resistant genes in microbial communities; horizontal transference to pathogenic

bacteria in human populations through water systems and food webs is a true

public health concern (Negreanu *et al.* 2012). This is a natural process that takes

place when microorganisms replicate inaccurately; however, the anthropogenic

introduction of antimicrobial drugs into aquatic ecosystems can accelerate the

emergence of these potentially catastrophic drug-resistant bacterial strains (World

Health Organization 2014).

### 8.1.9 Current Research

For our research, we investigated how pharmaceuticals affect bacterial

respiration in microbial biofilm communities in the streams in the Belgrade Lakes

watershed. To date, the majority of literature has focused on the basic detection of pharmaceutical concentrations in freshwater ecosystems as well as the subjection of pharmaceuticals to biota in controlled toxicology laboratory environments. Because of the availability of existing information on this topic, and the importance of the development of this body of research in the future, we did not actively test for the presence or absence of pharmaceuticals in these streams. Rather, we sought to understand the impacts of pharmaceuticals on an important ecosystem function within an authentic stream ecosystem. We chose bacteria as the study organism because of their important role in energy flow and in the movement of organic matter throughout the food web. Bacteria are an important constituent in stream biofilm, the basis in the stream food web; therefore, if bacterial communities are disrupted in freshwater ecosystems, the composition of the stream biofilm is inherently altered. Biofilm composition can, in turn, influence the stream populations and the composition of upper levels within the food web (Baesmer *et al.* 2009).

The experiment was run in three different streams varying in anthropogenic impact. Whittier Stream has no road crossings and little direct anthropogenic influence. Additionally, Robbins Mill Stream has two road crossings within three miles upstream of our study site, while Rome Trout Brook, which has multiple road

crossings upstream of our study site. Furthermore, Rome Trout Brook is located

downstream of logging and quarrying ventures.

**8.1.10 Pharmaceuticals Investigated**

Our experiment involved four common pharmaceuticals: Estradiol,

Erythromycin, Loratadine, and Metformin. Estradiol is an estrogenic compound

found in various forms of birth control around the United States

(www.nlm.nih.gov). Its presence in wastewater has been monitored because it is

considered to be an ideal representation of estrogen-based compounds in the

environment (Chawla *et al.* 2014; Andersen *et al.* 2003 & Table 2).  Erythromycin is a

frequently prescribed antibiotic for common respiratory infections

(www.nlm.nih.gov). Loratadine and Metformin are inexpensive and widely used for

common allergies and type II diabetes respectively (www.nlm.nih.gov; Wang *et al.*

2014; Table 2).  Metformin is one of the most highly prescribed drugs to treat type II

diabetes and lower the risk of atrial fibrillation (Chang *et al.* 2014; Sheurer *et al.*

2012). However, it can be removed from drinking water sources using tertiary

treatment passages, such as oxidation (Sheurer *et al.* 2012).

**Table 8.2.** *Summary of the compounds used in this study and their initial major environmental concerns (treatment information retrieved from www.nlm.nih.gov).*

| Pharmaceutical | Target Treatment | Major Environmental Concern |
| --- | --- | --- |
| Estradiol | Contraceptive/ Endocrine Disrupter | Reduced endocrine-based effects of different organisms (Andersen et al. 2003). Human health risks. |
| Erythromycin | Respiratory infections | Resistant bacteria |
| Loratadine | Hay-fever and allergies | Unknown |
| Metformin | Type II Diabetes | Unknown |
| Control | N/A | None (meant to simulate freshwater microbial communities) |
| Cumulative mix | N/A | The effects of a conglomerate mixture of pharmaceuticals being additive in the environment. Used in Rosi-Marshall et al. 2013 |

Since these compounds are all widely consumed and accessible, we selected them to investigate the following research questions:

1.) Is the impact of each pharmaceutical on stream microbial communities a function of the stream's proximity to anthropogenic influence? Our hypothesis was that as a stream's proximity to human influence increases, the impact of pharmaceuticals on bacterial respiration rate would increase because of the stresses these systems already face prior to pharmaceutical exposure.

2.) How will bacterial respiration be observed to change as a function of each pharmaceutical in isolation? Our hypothesis was that antibiotic compounds will have the largest negative impact on the bacterial communities in these streams.

3.) Are the effects of multiple pharmaceuticals on stream microbial communities additive? We hypothesized that an aggregated mixture of

pharmaceuticals will have a larger impact on the microbial communities of the streams, and the effects of which will be additive. This effect, called the combination effect, is supported by previous research (Cleuvers 2004; Flaherty & Dodson 2005; Brian 2005).

### 8.2 Materials and Methods

The following procedures were adapted from the work of Tank and Dodds (2003).

### 8.2.1 The Preparation of Pharmaceutical Diffusing Substrata (PhaDS):

We generated gel-based substrates for each pharmaceutical replicate to serve as controlled means of exposing bacterial communities to these pharmaceuticals without directly releasing them into the environment (Rosi-Marshall *et al.* 2013). We labeled each of the 90 individual 35 mL polycon cups prior to the creation of the gel substrates. We generated five replicates for each of the four pharmaceuticals, control, and mix to make a total of 30 PhaDS per stream.

### 8.2.2 Preparation of Agar Gels

To prepare each of these PhaDS, we boiled 200mL of water while constantly stirring with a 2.5cm stir bar and keeping the level of the water below half the volume of the Erlenmeyer flask. Once the water began to boil, we added the

designated amount of the appropriate pharmaceutical to the solution and waited for

it to completely dissolve into solution (Table 8.2). Once dissolved, we added the

appropriate 2% percent by weight of agar powder (3% by weight for the mixed

solution) and waited for the solution to boil. The solution was ready to pour when it

became clear and began to bubble.

**Table 8.3**. *Pharmaceutical and agar masses used in the creation of PhaDs.*

| Target Pharmaceutical | Molecular Weight (g/mol) | Target Molarity of pharmaceutical in each stream (mol/L) | Molarity of pharmaceutical in each stream (mol/L) | Mass of agar/ stream (g) |
|---|---|---|---|---|
| Estradiol | 272.38 | 0.015 | 0.01499 | 4 |
| Erythromycin | 733.94 | 0.015 | 0.01499 | 4 |
| Loratadine | 382.88 | 0.015 | 0.01501 | 4 |
| Metformin | 129.16 | 0.015 | 0.01503 | 4 |
| Control | N/A | 0 | 0 | 4 |
| Mix | N/A | 0.06 | 0.06004 | 6 |

**Figure 8.2** *Method of pouring hot agar solution into 35mL poly-con cups.*
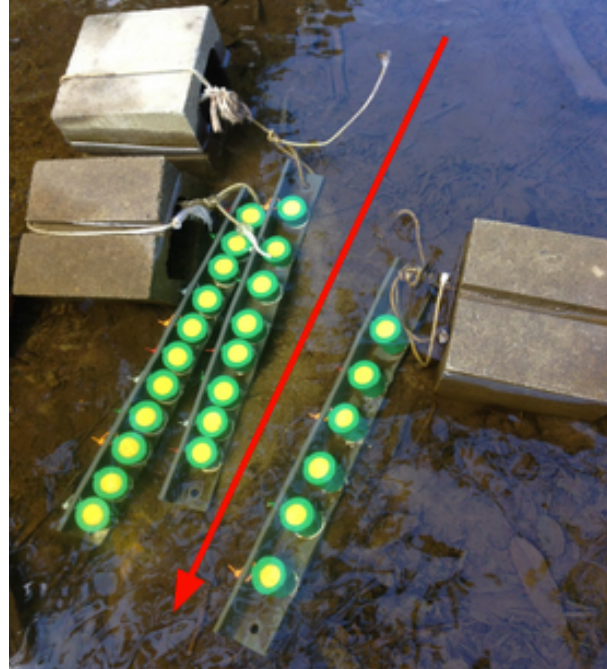


**Figure 8.3** *Deployment of PhaDS into Robbins Mill Brook. Arrow indicates direction of stream flow.*

Next, we used heat-resistant gloves to pour the newly created hot agar solution into each of the appropriate treatment cups until they were nearly full. A rounded meniscus formed once the gel cooled for about fifteen minutes. Once the gels had cooled, we placed a cellulose sponge disc on the surface of the agar to encourage bacterial colonization and ensured that the lids of each cup were securely fastened. If they were loose, or if the lid was unable to be closed initially, we either replaced the disc or carved out a small groove in the agar gel for the disc to settle into.

Once each replicate cooled we covered and attached them to L-bars in random order using zip ties color coded for each particular treatment type. For additional security, we applied silicone glue to the bottom of each cup and the L-bar

to better prevent against the cup flipping over in the stream. We then covered each

L-bar with plastic wrap and refrigerated them, until field deployment.


**8.2.3 Deployment of PhaDS in the Environment**

Once in the field, we securely fastened each L-bar to a cinder block or other

weighted apparatus using twine or rope and left them in their respective stream for

approximately 18 (± 1) days to allow for bacterial colonization to take place. We

periodically monitored the PhaDS once to twice a week to ensure that no replicates

were either loosened or lost in the stream. This was to ensure that all replicates

remained in the stream for the duration of the experiment in order to maximally

power our statistical analysis.


**8.2.4 Retrieval of PhaDS from the Field**

Prior to retrieving the PhaDS, we labeled ninety 50 ml centrifuge tubes with

the site, treatment, and replicate number corresponding to each PhaDS.

Additionally, we labeled three 1L bottles to fill with unfiltered stream water from

each site. While in the field, we removed the PhaDS and L-bars from the stream, and

placed them on the stream bank. While using latex gloves, we filled each centrifuge

tube with stream water, and used forceps to fully immerse each cellulose disk into

its corresponding tube. We then transported the tubes and cellulose discs back to the lab and placed them and the three 1L collection bottles in the refrigerator. We kept them refrigerated for 24 hours before beginning to measure the respiration rate of each disc.

### 8.2.5 Measuring Respiration Rates

We conducted the following experiment at room temperature. Before starting, we used a dissolved oxygen probe to measure the DO concentration (mg $L^{-1}$ DO) for the stream water in each 1L bottle. We then replaced the water inside of the centrifuge tube with some of the additional stream water, capped it, and stored it in the incubator for four hours at twenty-two degrees centigrade. We recorded the starting time for each tube as the time it was capped, while ensuring that there were no bubbles inside the tube when it was capped. We repeated this process for each replicate and placed the 1L bottles into the incubator with the tubes.

After four hours, we removed the tubes and 1L bottles from the incubator and measured the final dissolved oxygen for the 1L bottles, or "blanks", as well as each of the tubes. We repeated this process for each stream's respective data before placing the materials back into the incubator for another forty-four hours. After approximately two days, we removed the materials and measured the dissolved oxygen content for each tube and blank one last time before discarding the water

and discs in each tube. Therefore, we found the change in DO over 4 hours and then

44 hours. We then used the following equation to calculate respiration rate for each

cellulose disc:

$$\text{Respiration Rate} = (DO_f - DO_i)/cm^2/hr$$

**Equation 8.1** *Calculation for the respiration rate of bacterial communities on each cellulose disk.*

### 8.2.6 Statistical Analysis

To determine any relationships between bacterial respiration rate and the

variables in question, we performed a two-way analysis of variance (ANOVA) that

explored the potential impact of the following: proximity to anthropogenic

influence, the presence of pharmaceuticals, and any interaction between the two that

might exist. This served as a means for us to assess whether or not the respiration

rate was influenced by each of these variables, and whether or not any detected

influence differed significantly depending on which stream or specific

pharmaceutical was in question. We further explored all statistically significant

influences suggested by this ANOVA by ensuring that the data were balanced, and

performing a Tukey-Kramer pairwise comparison of the results to determine which

specific streams or pharmaceuticals generated the most significant results.  In each

of these analyses, we used a significance threshold of 0.05.

## 8.3. Results

**8.3.1 What is the Pharmaceutical Impact on Stream Bacteria Relative to its Proximity to Anthropogenic Influence?**

We analyzed all 88 replicates (two were lost in the field) that were retrieved from the three streams for the respiration rate of the bacterial communities colonizing the cellulose disc. We focused our first research question on determining whether or not anthropogenic influence would impact bacterial respiration in comparison to the controls and determined that the respiration rate varied by stream ($F_2$=154.71, p<0.001). In addition, we found that respiration rate was influenced by the presence of pharmaceuticals ($F_5$= 11.36, p < 0.001). Furthermore, Whittier Stream had a significantly lower respiration rate of 0.017 mgDO/$m^2$/hr compared to Rome Trout (0.028 mgDO/$m^2$/hr) and Robbins Mill (0.027 mgDO/$m^2$/hr) (p<0.001). The respiration rates of Rome Trout and Robbins Mill did not significantly differ from one another (Figure 8.4).

In all three streams, Erythromycin yielded a lower respiration rate (W= 0.014 mgDO/$m^2$/hr, RT= 0.025 mgDO/$m^2$/hr, RM= 0.021 mgDO/$m^2$/hr) in comparison to the control values (see above). Estradiol yielded a higher respiration rate in Robbins Mill Stream (0.035 mgDO/$m^2$/hr), but had no appreciable impact in both Whittier Stream and Rome Trout Brook. Loratadine had a higher respiration rate in Whittier Stream

(0.019 mgDO/m²/hr), but there was no appreciable change in both Robbins Mill Stream and Rome Trout Brook. Metformin had a lower respiration rate in Robbins Mill Stream samples (0.025 mgDO/m²/hr), but there was no appreciable change in Whittier Stream and Rome Trout Brook (Table 8.3).
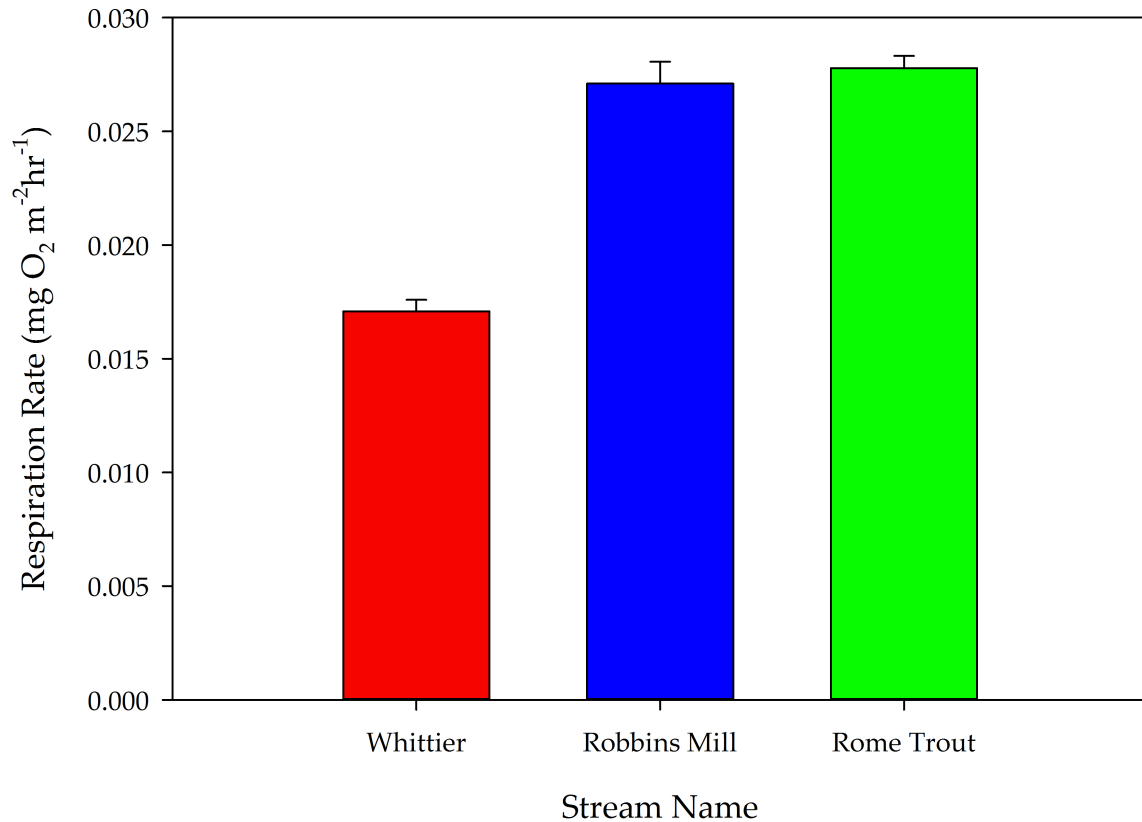


**Figure 8.4** *Average respiration rate of pharmaceuticals in each of the three target streams in this experiment (W=30, RM=29, RT=29).*

Table 8.4 *Summary of all impacts of pharmaceuticals on respiration rate after 4 hours of incubation as compared to that of the control replicates.*

| Pharmaceutical Respiration Rate Compared to Control | Whittier | | | Robbins Mill | | | Rome Trout | | |
|---|---|---|---|---|---|---|---|---|---|
| | Increase | Decrease | No Appreciable Change | Increase | Decrease | No Appreciable Change | Increase | Decrease | No Appreciable Change |
| Erythromycin | | ◆ | | | ◆ | | | ◆ | |
| Estradiol | | | ◆ | ◆ | | | | | ◆ |
| Metformin | | | ◆ | | ◆ | | | | ◆ |
| Loratadine | ◆ | | | | | ◆ | | | ◆ |
| Cumulative Mix | | | ◆ | | | ◆ | | | ◆ |

## 8.3.2 What is the Impact of Various Dissolved Pharmaceuticals on Microbial Stream Communities?

In the aforementioned analysis of variance, we sought to determine whether or not the respiration rate of stream bacterial communities was influenced by the presence of pharmaceuticals. We determined both that the presence of pharmaceuticals and the stream site did significantly impact the bacterial respiration rates in this experiment ($F_5$=11.36, p<0.001), and that the level of impact varied depending on what type of pharmaceutical was present ($F_{10}$=5.66, p<0.001). A qualitative summary of each pharmaceutical's impact on the bacterial respiration rate is provided in Table 8.5. In addition, a Tukey-Kramer test comparing each of the fifteen possible drug combinations suggests that the difference in bacterial

respiration, after 4 hours of incubation, between control communities and those

exposed to Erythromycin is statistically significant (p=0.003). This significant

difference is consistent across all three streams (Figure 8.5).
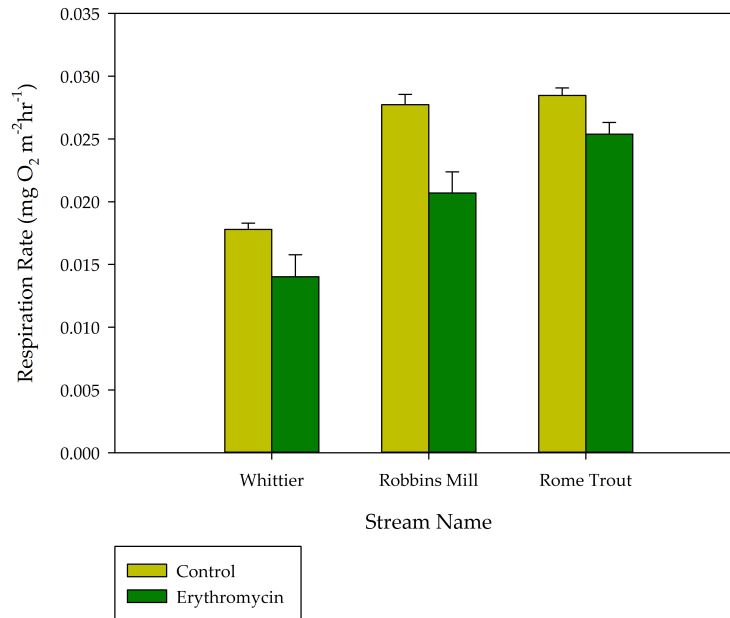


**Figure 8.5** *Respiration rates of bacterial communities for all Erythromycin and control replicates in each of the three study streams of this experiment (n=10 per stream).*

### 8.3.3 How are Microbial Stream Communities Impacted by Multiple Dissolved Pharmaceuticals? Are the Effects Additive?

In an effort to assess whether or not the presence of multiple pharmaceuticals

in the environment would yield additive effects, we referenced the result of the

Tukey-Kramer pairwise comparison of the impacts of individual pharmaceuticals.

Through this analysis we determined that there was no significant differences in the

respiration rates of the mixed pharmaceutical replicates compared to the controls

(Figure 8.6). We verified this with visual examination of the relationship between the

respiration rates of the control replicates and the average respiration rates of all the

pharmaceutical replicates in a given stream (Figure 8.7).



**Figure 8.6** *Respiration rates for the pharmaceutical mixture replicates compared to the control replicates for each stream after four hours of incubation (n=10 per stream)*
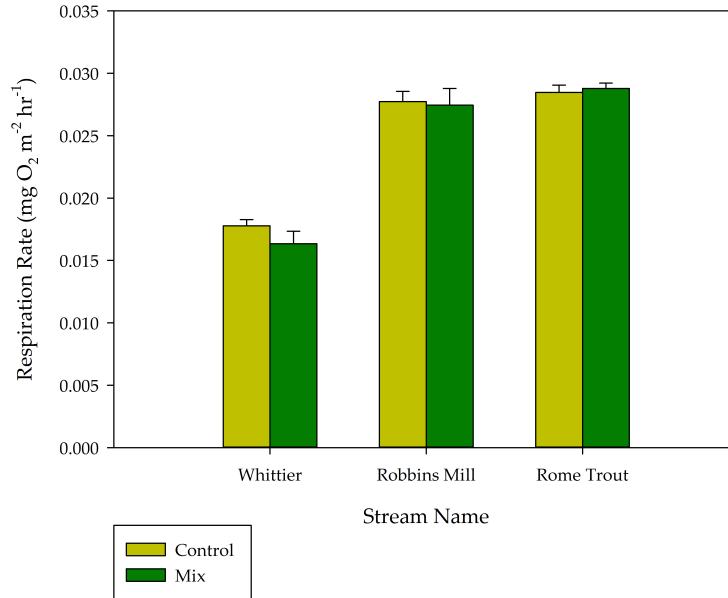
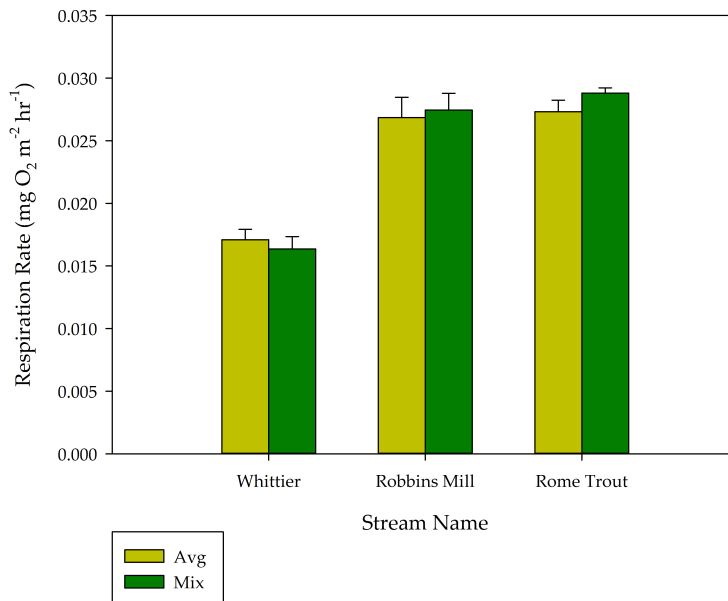

**Figure 8.7**  *Respiration rates for the pharmaceutical mixture replicates compared to the average of all individual pharmaceutical replicates for each stream after four hours of incubation (n=19, 20, 19 respectively)*

## 8.4. Discussion

### 8.4.1 What is the pharmaceutical impact on stream bacteria as a function of stream development proximity?

Our cumulative data clearly illustrates that anthropogenically influenced streams have higher populations of bacteria and/or activity as measured by respiration. This is contrary to our hypothesis that the control stream would provide a better environment for microbial communities to flourish. The respiration rates for Rome Trout Brook and Robbins Mill Stream after incubation were each over 2.5 times greater than that in Whittier Stream. This effect can be explained by excess nutrients, particularly nitrogen and phosphorus, in anthropogenically impacted streams. Previous chapters in this study found larger concentrations of both phosphorous and nitrogen in Rome Trout and Robbins Mill; however this data was not subjected to statistical analyses (Chapter 4). The source of these excess nutrients is most attributable to runoff from agricultural or other developed land applied with fertilizer or manure and to sewage or septic effluent. These nutrients can cause an overgrowth of stream biofilms including algae and heterotrophic bacterial species via the breakdown of the excess organic matter. The respiration from these bacterial communities depletes dissolved oxygen in the aquatic environment (Mallin *et al.* 2006). This can have devastating effects on the ecosystem biota, such as causing

hypoxic conditions. Studies show that excess nutrients in aquatic environments are directly related to reductions in harvestable fisheries (Rabalais 2002). Targeting the source of excess nutrients, particularly phosphorus and nitrogen, can effectively reduce hypoxia in stream environments caused by bacterial respiration (Mallin *et al.* 2006).

The observed high bacterial levels in Rome Trout Brook and Robbins Mill Stream could be attributed to higher nutrient levels as well as bacterial influx via septic tank leaching in and around the Belgrade lakes watershed. Both *E.Coli* and other fecal coliforms were measured at high levels in both Rome Trout Brook and Robbins Mill Stream (Chapter 5). Homes and businesses throughout the Belgrade region primarily rely on septic tanks for waste processing. This reliance is problematic because the presence of *E.coli* bacteria and other fecal coliform contamination in the Belgrade lakes watershed suggests that septic tanks in this region might not be efficiently removing excreted waste, let alone pharmaceutical waste.

Another cause for amplification in stream bacterial communities in Rome Trout Brook and Robbins Mill Stream could be the reduced canopy cover that increases light reaching the stream waters (Chapter 6). Sunlight, particularly UV-B radiation, induces macromolecule cleavage. This increases dissolved organic matter concentrations and thus, bacterial growth in aquatic environments along with tight

coupling of primary production and respiration (Lindell *et al.* 1995; Hoellein *et al.*

2013). Increased temperature, a result of reduced canopy cover, can caused an

increase in bacterial growth as well (Felip *et al.* 1996).  Excess nutrients as well as

reduced canopy cover induce bacterial growth in streams.


**8.4.2 What is the Impact of Various Dissolved Pharmaceuticals on Microbial**

**Stream Communities?**

The four individual pharmaceuticals generated varying effects on the bacteria

that colonized the cellulose disc. As hypothesized, Erythromycin, an antibiotic, had

the most negative effect on the bacteria. However, the effects of Estradiol,

Loratadine, and Metformin were variable, as predicted.

Estradiol, a sex hormone, increased bacterial respiration rates in Robbins Mill

Stream, but had no effect on bacterial respiration rates in Whittier Stream and Rome

Trout Brook when compared to the control samples. Recent studies have shown that

Estradiol and other sex hormones can cause increased bacterial growth in humans

and other mammals (Amirshahi *et al.* 2011; García-Goméz *et al.* 2012). One such

study showed a direct effect on bacteria; both Estradiol and Progesterone

metabolically replace an essential growth factor in the bacterial species *B.*

*melaninogenicus* and promote bacterial growth (Kornman & Loesche 1982).

Researchers presume that this replacement has the potential to impact ecological

and human health (Kornman & Loesche 1982). Another study by Kidd *et al.* (2007) found no change in bacterial growth due to exposure to estrogenic compounds.  Our data displayed both increased and negligible changes in respiration rate, depending on the stream, showing that different effects are possible.

Loratadine, an antagonist of the histamine receptor H1, also caused an increase in bacterial respiration in only Whittier Stream. Other H1 antagonists have been shown to exhibit antimicrobial properties (Dastidar *et al.* 1976). However, another antihistamine, diphenhydramine, displayed antibacterial activity in *Flavobacterium*, but increased bacterial activity in *Pseudomonas* (Rosi-Marshall *et al.* 2010). *Pseudomonads*, common in freshwater ecosystems, is a bacteria that display high levels of antibiotic resistance when in close proximity to multidrug wastewater effluent (Heydorn *et al.* 2000; Palleroni 2010; Poole *et al.* 1993). Our results vary by stream, but other studies indicate the possibility of multiple effects.

Metformin displayed a decrease in bacterial respiration rate in only Robbins Mill Brook, but no appreciable change for the other two study sites. An unpleasant side effect of this anti-diabetic is bacterial overgrowth in the human small intestine (Caspary *et al.* 1977; Diamanti-Kandarakis *et al.* 2010). However, a more recent study on the gut bacteria of *C. elegans,* a species of roundworm, showed that Metformin causes changes in metabolic pathways of gut bacteria, inhibiting bacterial growth (Maratos-Flier 2013). Our data supports a decrease in bacterial respiration rate in

stream bacterial communities in the presence of Metformin. The only

pharmaceutical that consistently changed respiration rate in all three streams was

Erythromycin; the other three pharmaceuticals did not consistently affect bacterial

respiration.

The results that we generated were created using 0.015mol/L

concentrations of pharmaceuticals in a manner adopted from a previous similar

study (Rosi-Marshall *et al.* 2013). Based on our results, and the results of this

previous research, we found these concentrations to be reasonably

environmentally safe, but acknowledge that their impacts could be more severe at

higher concentrations.

While significant effects may not be present in all stream microbial

communities, there is still risk for other biota to be severely affected. The

statistically significant effects of Erythromycin display convincing evidence for

the formation of antibiotic resistance. When bacteria are exposed to antibiotics,

some organisms survive through natural selection and replicate, creating a new

population that contains a higher proportion of antibiotic resistant genes (Figure
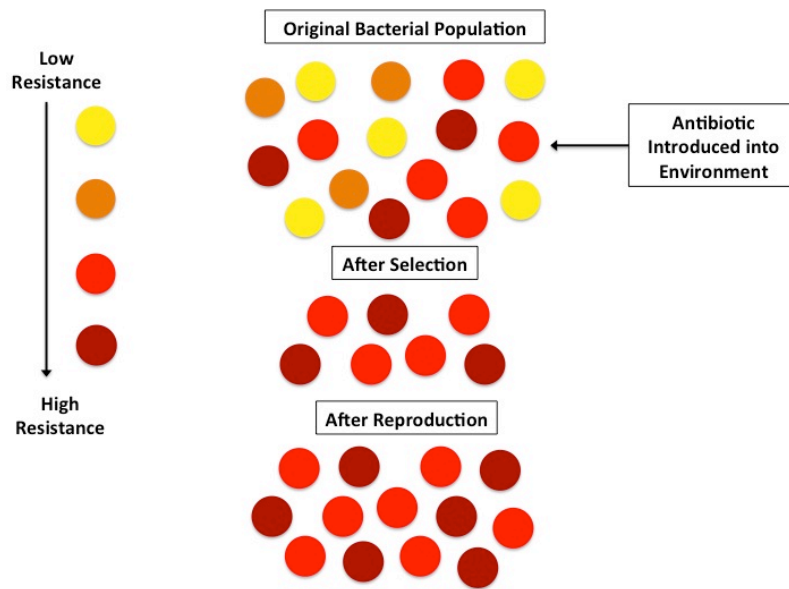
8.8).

**Figure 8.8** *Conceptual diagram displaying the mechanism behind the formation of antibacterial resistance in an aquatic environment.*

Horizontal transference from stream bacteria to human disease causing bacteria through water systems and food webs is a true public health concern (Negreanu *et al.* 2012). Thus, anthropogenically impacted aquatic ecosystems potentially serve as a breeding ground for antibiotic resistant genes in microbial communities. While this may seem like a trivial matter, developing resistances across the globe inhibit our ability to treat very common infectious diseases such as pneumonia, pertussis, or tetanus. This often results in the death and disability of individuals who could easily be saved with the use of simple antibiotics (World Health Organization 2014). Without this effective treatment, many routine medical procedures will fail or be medically classified as high risk.

Ecologically, antibiotics in freshwater ecosystems suppress microbial respiration, however, their effect on the ecosystem community respiration and

other ecosystem properties as a whole is understudied and not well understood.

Effects on anthropogenically influenced streams can be hypothesized: a

continued input of nutrients through runoff combined with a reduction in

nutrient consuming bacterial communities in streams, the health of these streams

could degrade quickly, particularly with respect to water quality and

biodiversity.

**8.4.3 How are Microbial Stream Communities Impacted by Multiple Dissolved**

**Pharmaceuticals, a Better Representation of Actual Environmental Scenarios?**

We hypothesized that the mixture containing all four pharmaceuticals would

have an additive effect on bacterial respiration rate. We found an averaged effect.

The respiration rates after four hours of incubation for the mix treatment were

statistically similar to both the control and the average, displaying that the

pharmaceuticals that caused an increase in respiration were coupled with those that

decreased respiration in the mix, causing them to cancel each other out. Other

researchers have also experienced this phenomenon (Rosi-Marshall *et al.* 2010).

However, other studies support the impacts of additive effects. In a study exploring

the additive effects of pharmaceuticals on *Daphnia magna* in isolation, the effects of a

mixture of three antibiotics was more impactful than any antibiotic individually

(Cleuvers 2004). These results stand in contrast to our results and are likely due to

complex stream conditions and the mixture being comprised of three

pharmaceuticals of the same class.

Various pharmaceutical mixtures of low-level concentrations are more

indicative of actual conditions in streams (Crane *et al.* 2006). While this study

focused specifically on impacts pertaining to bacterial respiration, the impacts on

other fundamental processes such as primary and secondary production, leaf

composition, or behavior of animal species could be the focus of future studies.

Therefore, more research is necessary to fully illuminate the impact of mixed

pharmaceuticals at realistic concentrations in aquatic ecosystems.

### 8.4.4 Sources of Error

One source of experimental error in this experiment include the PhaDS

becoming inverted in the stream during periods of increased water flow.  Inversion

of the cups may have impacted bacterial growth on the cellulose discs.  The loss of

PhaDS over the course of the stream may have altered our data; however, we

anticipated this complication and created five replicates of each type of PhaDS for

this reason. We ultimately lost only two PhaDS therefore mitigating any error due to

this problem.  Another source of error was the difference in the time PhaDS were

kept in the streams.  We removed the PhaDS in Whittier Stream approximately three

days earlier than advised due to a significant loss of cellulose that we attributed to

bacterial activity or macroinvertebrate activity. Since we needed intact cellulose

discs to efficiently test for DO loss, we chose to remove the discs early, risking

experimental error, in order to preserve our data. An additional source of error from

this complication was that some cellulose discs had non-uniform surface areas. We

suspected that this might have the potential to slightly impact our respiration rate

calculations for those replicates. This variability in surface area should be corrected

for in future studies.


## 8.5 Conclusions

Our study provides insight into the varying impacts of pharmaceuticals, both

individual and mixed, on stream microbial communities. Our results are variable;

the impacts on microbial communities at one stream often do not match that of other

streams to the same extent. Consistently, we found results supporting the inhibiting

effect of Erythromycin on bacterial growth. Our results also suggest that Estradiol

promotes bacterial growth, a finding that agrees with numerous other studies, and

that bacterial respiration increases with human land use (Amirshahi *et al.* 2011;

García-Goméz *et al.* 2012).

This topic requires further research in order to solidify our knowledge on the

impacts of pharmaceuticals on stream microbial communities. As previously

mentioned, our study, in addition to other studies, supports contradicting

conclusions, particularly regarding the impact of mixed pharmaceuticals. Currently, researchers at Ball State University in Muncie, Indiana are working on a compelling study, entitled RiverPACE, exploring the concentrations of numerous pharmaceuticals in stream water nationwide. Their study sites include the Messalonskee Stream in Waterville, ME, a stream that all of our study eventually streams flow into. The results of this study are expected to be extremely informative towards the magnitude of pharmaceutical contamination in U.S. waters.

The management implications that this study provides inform a need for the acknowledgement of inefficiencies and the need for improvement of wastewater treatment systems. In addition to the need for infrastructural improvement, there are significant actions that individuals can take that can have a major impact on their local communities. By ensuring that the prescribed course of a medication is taken in full, consumers are able to reduce the probability that a second course of the medication will be needed. By doing so, consumers can reduce their excretion of unmetabolized pharmaceuticals into the waste stream as well as prevent the need for the disposal of unused medications. Consumers should utilize pharmaceutical take-back programs to ensure that all unused medications are incinerated rather than being sent to a landfill or introduced to the waste stream. Homeowners can also ensure that home septic systems are properly maintained to guarantee the highest possible functionality. Therefore, while expulsion of all pharmaceuticals

from freshwater ecosystems is currently implausible, improvements can be made on

many scales by a variety of organizations to reduce pharmaceutical impact. In

particular, the steps that can be taken by individuals are a good starting point

towards minimizing pharmaceutical impact on the environment, specifically in

communities such as Belgrade that are intimately intertwined with freshwater

ecosystems.

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