

SEASONAL MACROINVERTEBRATE INHABITANTS OF NATURAL LEAF PACKS IN  
TWO WESTERN PENNSYLVANIA HEADWATER STREAMS

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Title: Seasonal Macroinvertebrate Inhabitants of Natural Leaf Packs in Two Western Pennsylvania Headwater Streams

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The importance of macroinvertebrates in stream ecosystems is well known as these animals serve a variety of functions. Many studies have shown that they occupy leaf packs (collection of detritus in streams). Most studies use artificial leaf packs or detritus traps. This study examines total density, generic richness and feeding group densities of macroinvertebrate occupancy in leaf packs seasonally. Samples were collected from naturally occurring leaf packs found in 2 Western Pennsylvanian headwater streams. Total density of macroinvertebrate occupants of the leaf packs were shown to vary seasonally. The shredder feeding group was shown to have higher density in the summer season. Trends of higher density and generic richness were evident as the temperature difference between stream and leaf pack increased. This study shows the viability of sampling natural leaf packs. It also shows that numbers of macroinvertebrates in leaf packs change seasonally and points to temperature difference between stream and leaf pack being an important factor in macroinvertebrate occupancy of leaf packs.

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## Chapter 1

### INTRODUCTION

The importance of macroinvertebrates in stream ecosystems is well known (Anderson and Sedell 1979; Berg 1995; Casas 1997; Cuffney et al 1990; Cummins et al 1989; Wallace and Webster 1996). These animals serve as a food source for a variety of fish (Wallace and Webster 1996; Markle and Grant 1970) and other animals that inhabit riparian ecosystems (Sealander 1943). Macroinvertebrates also play a role as decomposers by breaking down plant material (Anderson and Sedell 1979; Cummings et al 1989; Richardson 1992; Gessner 1991; Wallace and Webster 1996; Parkyn et al 1997; Wallace et al 1982). This function is vital for stream health and the ecology of its inhabitants, as much organic material that enters low order streams are in the form of coarse particulate organic matter (CPOM; >1mm diameter) and many organisms rely on fine particulate organic matter (FPOM; <1mm diameter) as a food source (Anderson and Sedell 1979). Additionally, presence of certain aquatic macroinvertebrates can be used as an indicator of water quality (Bohmer et al 2004; Lenat 1988; Roy et al 2003), because many are intolerant of even small amounts of pollution (Thorne and Williams 1997). Macroinvertebrate occupancy of leaf packs have been shown to also be a possible indicator of stream quality (Nelson 2000).

Given the variety of ecosystem functions performed by macroinvertebrates in stream systems, knowledge relating to their life cycles is important in understanding stream ecology (Sylvestre and Baily 2005; Tokeshi 1995). An improved knowledge of season specific habits of benthic macroinvertebrates may lead to being more informed on what to expect while examining water quality and lead to a more accurate interpretation of the results and seasonal comparisons.



These data may also aid in development of more effective stream management plans that consider multiple trophic level interactions.

Previous studies have demonstrated the propensity of a variety of macroinvertebrates to inhabit leaf packs (Dobson and Hildrew 1992; Dobson 1994; Hieber and Gessner 2002; Richardson 1992; Murphy and Giller 2000; Petersen and Cummins 1974), a concentration of detritus leaf litter in a stream (Dobson 1994). Examining the makeup of leaf packs has been an accepted method of studying macroinvertebrates for several reasons (Dobson 1994; Dobson 1991; King et al 1987). Leaf litter in streams has been shown to be a factor that determines composition of benthic macroinvertebrate communities (Wallace et al 1997). Many aquatic organisms use leaf packs as refugia in greater numbers during high flow events in the stream due to either a short term or seasonal response (Lancaster and Hildrew 1993). Even given the volatile constantly changing environment of stream bottoms, leaf packs have been shown to be important habitat for benthic macroinvertebrates (Palmer et al 2000). Removal of leaves from streams can be detrimental to macroinvertebrate populations (Wallace et al 1997). In fact, detritus dams have been shown to hold most organic matter present in first order streams (Bilby and Likens 1980) Removal of macroinvertebrates from streams can have deleterious effects on stream ecosystems (Cuuney et al 1990; Wallace et al 1982). By lowering the number of insects present in stream, detritus processing rates are lowered and transport, both physical and between trophic levels, of particulate material is lowered as well (Ruetz 2000; Cuffney et al 1990; Wallace et al 1989).

Although much work has been done regarding use of leaf packs by macroinvertebrates, most studies have focused on artificial leaf packs which may or may not be analogous to leaf packs formed naturally in streams (Hieber and Gessner 2002; Dobson 1991; Dobson 1994;

Dobson and Hildrew 1992; King 1987; Murphy et al 1998; Gessner 1991; Benefield et al 1977; Richardson 1992; Petersen and Cummins 1974). This study examined macroinvertebrate colonization of natural leaf packs found in two headwater streams in Pennsylvania. The goals of my research were: 1) identify which macroinvertebrate use leaf packs; 2) determine seasonal differences in species and feeding group richness and abundance between seasons; and 3) discern what conclusions, if any, can be found from interpreting data regarding importance of leaf packs in life cycles of certain macroinvertebrates.

These questions have led me to form two hypotheses. 1.) I hypothesize that generic richness and abundance of macroinvertebrate inhabiting leaf packs will vary according to the season in which they are collected. 2.) I theorize that numbers of leaf pack residents will be greater in winter than summer. The lack of emerging adults and possibility that macroinvertebrates use leaf packs as refugia to avoid colder stream temperatures leads me to think that densities of macroinvertebrates in leaf packs will be greatest in winter.

## Chapter 2

### REVIEW OF RELATED LITERATURE

#### *Importance of Allochthonous Input to Low Order Stream*

Low order streams are found in headwaters river systems and account for as much as 85% of all running waters (Anderson and Sedell 1979). By definition lower order streams have a greater streamside to stream bottom ratio than higher order streams (Anderson and Sedell 1979). As such, the influence of streamside deposits into streams will have a greater effect on lower order streams than on higher order streams (Bilby and Likens 1980).

Streams receive energy, as carbon, from two sources allochthonous, or input from outside the stream, and autochthonous, or energy input from within the stream itself. Autochthonous input is derived in large part from algae growing in streams and fine particles of organic material filtering down from lower order streams (Anderson and Sedell 1979). Allochthonous input into streams is generally in the form of leaves, grasses or woody material falling into the stream, some of which is retained in debris dams (Bilby and Likens 1980). Leaves can make up 40% of carbon input into some streams (Bilby and Likens 1980). Most allochthonous input is in the form of larger particles which cannot be used by many organisms smaller than macroinvertebrates (Jonsson and Malmqvist 2000; Lepori et al 2005). In short, allochthonous input is of utmost importance for headwater streams and benthic macroinvertebrates (Casas 1997). Another study has shown the importance of retaining large particles of detritus when restoring streams for overall health of the stream ecosystems (Lepori et al 2005). Distinction in carbon sources of low order and high order streams make it reasonable to expect differences in faunas found in these streams.

### *Macroinvertebrate Use of Leaf Packs*

Many studies have shown that macroinvertebrate use leaf packs as a food source (Casas 1997; Cuffney et al 1990; Cummins et al 1989; Dobson 1994; Hieber and Gessner 2002 Rowe and Richardson 2001). Breakdown of detritus from coarse particulate organic material takes place by a combination of processes which include physical leaching creating dissolved organic material, and detritivore breakdown creating fine particulate organic material (Hieber and Gessner 2002). Greater species richness of macroinvertebrate detritivores positively affects the rate of leaf litter break down (Jonsson and Malmqvist 2000). Furthermore, removal of macroinvertebrates has been shown to limit processing of detritus material (Ruetz 2000; Cuffney et al 1990).

Leaf packs have also been shown to be important sources of refugia in cooler seasons or high flow events (Lancaster and Hildrew 1993; King et al 1987). Refugia have been defined as "...places not subject to raised hydraulic stress during spates..." (Lancaster and Hildrew 1993).

Macroinvertebrates have been shown to use refugia on a seasonal basis to deal with high flow events (Lancaster and Hildrew 1993). Detritus material has also been shown to be a limiting factor of benthic macroinvertebrates' presence in streams (Dobson and Hildrew 1992). However, not all studies recognize the importance of leaf packs as macroinvertebrate refugia. Studies have compared polyester or paper leaves against naturally occurring leaf packs and found that few individuals colonized imitation leaves compared to leaf packs made with real leaves (Dobson 1994; Richardson 1992). These results were interpreted to mean that macroinvertebrates' use leaf packs primarily as a food source (Dobson 1994; Richardson 1992). These studies did not account for seasonal temperature differences which may lead to occupancy of leaf packs by macroinvertebrate for reasons other than food.

### *Natural Versus Constructed Leaf Packs*

There are many studies that have used artificial leaf packs to examine life cycles, of macroinvertebrate detritivores (Hieber and Gessner 2002; Dobson 1991; Dobson 1994; Dobson and Hildrew 1992; Murphy et al 1998; Gessner 1991; Benefield et al 1977; Richardson 1992; Petersen and Cummins 1974). However, use of artificial leaf packs has led to equivocal results or interpretations. First, leaf packs created using mesh bags have been shown to cause leaves to degrade at a different rate than those naturally existing in streams (Cummins et al 1980; Gessner 1991). Also, natural leaf packs can continuously receive new input from riparian zones while mesh bag leaf packs remain static (Braioni et al 2001; Lepori et al 2005; Petersen and Cummins 1974). Secondly, mesh bags may prevent access to leaves by larger detritivores such as tipulidae (Anderson and Sedell 1979). Additionally, determining which animals associate with leaves as opposed to those which associate with the bag itself is also an inherent problem with artificial leaf packs (Winterbourn 1978). While contents of artificial leaf packs may resemble the contents of natural leaf packs, they could vary based on how long the leaf pack has been present in stream and the frequency of disturbances (Dobson 1991). Finally, no studies could be found in which construction of artificial leaf packs contained detritus material other than leaves, such as grasses or twigs. Yet leaf packs in streams often are found to include these other materials (Anderson and Sedell 1979). The lack of these other materials in constructed leaf packs may skew results as some chironomids, tipulidae along with some families of ephemeroptera macroinvertebrates have exhibited feeding preferences for woody material (Anderson and Sedell 1979; Hoffman and Hering 2000). Other studies have tried to create 'natural' leaf packs in streams using detritus traps (Murphy and Giller 2000). However, in each of these instances, data must be interpreted to relate to naturally occurring leaf packs (King et al

1997). Some studies specifically addressed the question of how natural leaf packs compare to mesh bag or detritus trap leaf packs, and found that although they can be analogous in some situations, they varied significantly depending on stream conditions (Dobson 1991; King et al 1987). Another study revealed a much higher taxon richness in natural leaf pack than detritus dams (Casas 1997).

It is also important to note the role conditioning plays in leaf pack use by macroinvertebrates. Conditioning is colonization of detritus material which makes for easier feeding and digestion by macroinvertebrates (Gessner 1991, Anderson and Sedell 1979). Leaf material has higher protein content after some conditioning (Anderson and Sedell 1979) but can lose much of its nutritional value from being conditioned for an extended period which affects its desirability to macroinvertebrates (Anderson and Sedell 1979). Conditioning is a continuous process that can take place over a matter of weeks or months, (Gessner 1991) and possibly vary seasonally which can make it difficult to match conditioning state of artificial leaf packs to that of those found naturally in stream. Finally one study has shown that a combination of artificial leaf packs and natural leaf packs is necessary to get a full picture of a stream's functioning (Braioni et al 2001).

#### *Classification of Macroinvertebrates by Feeding Habits*

In addition to traditional taxonomic identification of macroinvertebrates, it has been common practice to identify macroinvertebrates into feeding groups by diet and foraging behavior, e.g., as shredders or detritivores, grazers or collectors, and predators (Dobson 1992, 1994; Richardson 1992; Cummins et al. 1989; Cummins 1973). Knowledge relating to feeding activity of aquatic macroinvertebrates is important when implementing any stream management strategies (Lepori et al 2005; Cummins 1973). Shredders generally feed on leaf litter in streams

and include species of letophlebiid mayflies, filipalpid plecoptera, case-bearing trichoptera, chironomid, culicid, ptychopterid, simuliid, tipulid, ephydrid, stratiomyid, and syrphid diptera, along with some species of amphipods and isopods (Cummins 1973; Cummins et al 1989; Friberg and Jacobson 1994). Non-shredders, such as grazers or collectors, normally feed on algae that are found on stones (Dobson and Hildrew 1992) and include animals such as species of gastropods, some crayfish species and others (Evans-White and Lamberti 2005). Predators are those macroinvertebrates which feed on other animals and include some species Megaloptera, Tricopetera, Zygoptera, and Anisoptera among others (Lancaster and Robertson 1995; Jefferies 1990; Wissinger 1988).

Numerous studies have shown that many leaf pack inhabitants are shredders, accounting for approximately 20% of total biomass (Cummins et al 1989; Dobson 1994; Richardson 1992; Hieber and Gessner 2002). Some shredders may eat algae or macrophytes but in the absence of this food type, they will consume leaf litter (Friberg and Jacobsen 1994). In fact, diversity of shredders found in streams has been shown to directly affect the rate of leaf breakdown in streams (Jonsson et al 2001; Simon and Benefield 2001; Cuffney et al 1990). However, grazers have been shown to be present in large numbers in leaf packs before June (Dobson 1994) which could be due to more food found on leaves at this time or other life cycle related reasons (Cummins et al 1980; Evans-White and Lamberti 2005). Predators have also been found in leaf packs though in smaller numbers (Dobson 1994). This could point to use of leaf packs as refugia because it has been shown that the average ratio of invertebrate predators to prey is 0.36 (Jefferies and Lawton 1985). In addition to identifying inhabitants of leaf packs to the most definitive taxon possible, aquatic invertebrates can be grouped into classes of shredders, grazers/collectors/scrapers (labeled collectors for this study), predators, and miscellaneous, for

purposes of comparison. While many benthic invertebrates may occasionally feed in different manners, classifying them in functional groups is helpful when looking at questions dealing with process.

### *Results of Studies Using Artificial Leaf Packs*

Studies have used artificial leaf packs to examine a variety of questions. These studies often focused on a comparison of sampling techniques or rate of detritus breakdown. However, information regarding macroinvertebrate use of leaf packs was available in a variety of forms, including feeding guild number, family or genera richness. A study examining a pastureland stream showed an increase in macroinvertebrate colonization of leaf packs through the first 141 days, beginning in October, which corresponded with conditioning periods of the leaves used (Benefield et al 1977). Macroinvertebrates found during peak period of colonization included mostly mayflies and midges (Benefield et al 1977). Another study showed peak colonization of leaf packs by macroinvertebrates after only 4 weeks, beginning in October (Hieber and Gessner 2002). While comparing macroinvertebrate colonization of leaf packs made of polyester leaves to those made with real leaves, they found that densities of macroinvertebrates increased only until day 30 (Richardson 1992). A 2 month study using mesh bags filled with leaves of different ages noted nine common genera in the first month with an increase to fourteen common genera in the second month (Dobson 1994). Shredders were more common in the second month while numbers of grazers decreased (Dobson 1994). A study that compared mesh bags and plastic leaf traps revealed higher numbers of collectors and shredders with a low amount of predators and grazers (Dobson 1991). Chironomids dominated the number of invertebrates found in mesh bag leaf packs (Dobson 1991). One study examined leaf pack breakdown rates over several different stream orders and found 26 different shredder species in



autumn (Jonsson et al 2001). While another study beginning in November found 76 taxa over the period of the study (Murphy et al 1998); in a study running from August through June in Africa, King et al. (1987) found densities of macroinvertebrates were consistently higher in leaf packs than adjacent benthos except during peak leaf fall. Finally, a study conducted from March through July noted highest densities of macroinvertebrates in summer with maximum richness found in autumn (Murphy and Giller 2000). While many studies had similar results, it is apparent that differences existed and may have been due to different stream types, weather or water conditions. Macroinvertebrate feeding habits have been shown to alter with changes in stream temperature (Buzby and Perry 2000). If macroinvertebrates are using leaf packs for benefit of a more temperate habitat, abundance and/or richness could be expected to be higher in cooler seasons if leaf packs are warmer than stream temperature. It follows then that higher density or richness results would be expected in leaf packs cooler than stream temperature in warmer seasons. In short, greater temperature differences between leaf pack and stream should mean higher genera richness and/or total abundance.

## Chapter 3

### METHODS

In this study, I examined naturally occurring leaf packs at two representative headwater streams in Indiana County Pennsylvania. Samples were collected once per season at two different locales within each stream, two samples from each locale.

#### *Study Sites*

Ramsey Run (659271, 4497574), and McGee Run (657717, 4503974), two headwater streams in Indiana County Pa were used for this study. Both areas of streams where samples were collected would be classified as first order using the system design by Horton (Wetzel 2001) and modified by Strahler (Wetzel 2001). Ramsey Run runs through a pasture used by cows and then into a wooded area. Riparian trees are dominated by willows. McGee Run travels along a semi-residential area. The grass is mowed to the stream bank edge. Trees found along the stream consist mostly of mature oaks and maples. Two 15m lengths of each stream were chosen and delineated, on the basis of leaf pack presence prior to beginning the study.

#### *Stream and Leaf Pack Chemistry*

Prior to each sample collection, water temperature, pH, conductivity (umhos/ dissolved oxygen (mg/l), and redox potential(+/- ORP) were recorded at each site using a YSI Model No. 650 MDS instrument. Temperature and ORP were recorded inside the leaf pack as well. The physical arrangement of probes on the subsurface sonde prevented accurate measurements of pH, conductivity and dissolved oxygen at any levels within leaf pack.

### *Leaf Pack Core Sampler Construction*

The sampler used in this study was an original design and construction. The body consisted of a 15.24cm diameter PVC pipe 60.96cm long, and one end was sharpened to aid in penetration of leaf packs. Six 10cm holes were drilled at selected intervals into the pipe and covered with 0.5mm mesh to allow for water to drain while keeping the sample intact. A metal handle was bolted to the top of the pipe to allow pressure to be applied to force the sampler into the leaf pack. A small shovel was used to wedge under the bottom end of the device and bring samples out of the water. The sampler functioned by being forced into a leaf pack, the shovel was slid under it and then both lifted simultaneously to keep the sample intact. The entire sample was then deposited into an 18.9271l (5 gallon) bucket with its bottom cut out and replaced with 0.5mm mesh; the sample was then rinsed repeatedly and the remaining material was placed into a labeled glass mason jar (0.946 liter capacity). All samples were fixed in 10% buffered formalin until they could be sorted.



Figure 1. Leaf Pack Core Sampler

### *Leaf Pack Core Sampler Efficiency*

Due to the original design and construction of the sampler, its efficiency was unknown. In order to determine its effectiveness, one sample of a leaf pack was taken with the leaf pack core sampler, (test sample), and remaining portions of the leaf pack was collected using mesh nets (control sample). Family richness (number of families of organisms present) and overall macroinvertebrate abundance were used in density calculations for the test and control samples using the wet weight of collected detritus. Efficiency of the sampler was calculated by comparing densities of the test and control samples.

### *Sampling*

Two samples from leaf packs were collected with the leaf pack core sampler from both study sites at both streams, during fall, spring, winter and summer. Each season was defined as a group of three months. For example, summer contained the months June, July and August. Flexibility in sampling date allowed weather events, such as floods that would disrupt leaf packs. Winter samples in Ramsey Run were collected December 5, 2008. Spring samples were taken March 13, 2009, for both streams. On June 13, 2009, summer samples from both streams were collected. All fall samples were collected on October 1, 2009. Finally, as McGee Run was added midway into the study its winter sample was collected on December 15, 2009. This translates to a total of 16 samples taken during the course of the study (n=16). Data recorded were: location, depth of sample, water type, and general weather conditions. After fixing samples with 10% buffered formalin they were returned to the laboratory for further processing.

### *Sorting*

Each sample was thoroughly rinsed in a 0.5mm mesh strainer. Samples were then placed into a sorting tray where detritus material was separated from the sample and then left to air dry. Any macroinvertebrates found were preserved in 75% ethanol. This process was repeated with each sample until no remaining macroinvertebrates could be found. After the detritus material had been air dried to it was placed into a zippered plastic bag to minimize further decomposition, re-hydrated and weighed.

### *Identification of Macroinvertebrates*

A variety of dichotomous keys were used to identify macroinvertebrates to the most definitive taxon possible (Burch 1975; Byers 1996; Cogman and Ferrington 1996; Edmunds and Waltz 1996; Hilsenhoff 2001; Kathman and Brinkhurst 1998; Klem 1982; Walker and Brodersen 1997; Welsch 1959; Wiggins 1978). Most identifications could be accomplished with keys and use of a dissecting microscope; however, some identifications required more extensive work. Oligocheates were mounted on slides in Hoyer's medium (Anderson 1954) and allowed to clear. A light microscope was then used to locate penis sheaths and identify differences in setae type and number. Chironomids also required slide mounting. Each specimen was decapitated, then body and head were mounted on the same slide. The head was mounted ventral side up in order to expose its labial plate which was critical for identification using a light microscope.

### *Data Analysis*

Due to variation in depth and composition of natural leaf packs, samples had to be standardized in order to make comparisons between them. Using genera richness (numbers of genera; Table 1) and macroinvertebrate abundance (Table 1) compared to grams of wet detritus (Table 2) to calculate densities allowed comparisons between samples. Detritus material was

rehydrated by adding water to the bags and allowing the material to soak up water for 2 nights. Excess water was then siphoned off, the sample weighed and densities were calculated. Standardizing data in this way made it possible for distribution of genera and abundance comparisons to be drawn between seasons.

These densities were used to create a Shannon-Weiner diversity index ( ) to compare diversity between streams and when averaged could be used to compare overall seasonal densities standardized using (Peet 1974). Evenness was then examined using (Larsen and Herlihy 1998). Temperature differences between leaf pack and stream were averaged for the two samples taken from each site. In order to determine if temperature differences between stream and leaf pack affected densities, absolute values of temperature differences were used for statistical testing.

### *Statistical Analysis*

Densities of generic richness and total macroinvertebrate abundance were compared with a Two-way ANOVA using Minitab statistics program (Minitab Inc. State College Pa) to determine if any differences existed due to season or streams. The same test was used to examine total abundance in streams. Kruskal-Wallis tests were used to examine genera richness and total abundance regarding sample depth due to unequal variances of samples. Effects of temperature differences between leaf packs and stream temperatures on generic richness and total abundance were examined using the absolute value of the difference between leaf pack and stream temperature at each site using the same test. This test was also used to examine feeding group densities in comparison to stream and season separately due to one or more feeding group densities having unequal variances compared to each factor.



Figure 2. Sampling in Action

## Chapter 4

### RESULTS

#### *Efficiency Test Results*

The control leaf pack contained 238.64g of detritus material compared to 98.31g in the test sample. One hundred and sixteen macroinvertebrates were collected in the test sample representing 4 families. The control sample had 404 individuals representing 10 families. Densities were calculated for total abundance and family richness for both control and test samples (Table 1). These densities were then compared by dividing the test result by the corresponding control result in order to determine the efficiency of the leaf pack core sampler (Table 1).

Table 1. Densities and Efficiencies Calculated for the Leaf Pack Core Sampler

Efficiency Test Densities	Test	Control	Efficiencies
Family Richness	0.0407	0.0419	0.9714
Total Abundance	1.1799	1.6929	0.697

#### *General Results*

Throughout the course of this study, 4155 individual macroinvertebrates were collected from the two streams (Table 2). All were identified to genus if possible. Some specimens could not be identified to genus because they were immature, gender of the individual prohibited identification, poor condition of specimens, or shifting in position during slide preparation and drying.

Of the total individuals collected, 3288 or approximately 80% were identified at least to genus and represented 51 distinct genera (Table 2). Twenty-seven families were identified representing fifteen orders (Table 2).



Table 2. Number of Macroinvertebrates collected per stream each season (M=McGee Run R=Ramsey Run)

Individuals Collected			M	R	M	R	M	R	M	R
Order	Family	Genus	Fall	Fall	Winter	Winter	Spring	Spring	Summer	Summer
Basommatophora										
	Lymnaeidae									
		<i>Pseudosuccinea</i>	0	0	1	3	0	2	0	0
	Physidae									
		<i>Aplexa</i>	0	0	0	1	0	1	0	0
Coleoptera										
	Elmidae									
		<i>Dubiraphia</i>	0	1	0	1	0	3	2	4
	Psephenidae									
		<i>Ectopria</i>	0	0	0	0	0	1	0	0
		<i>Psephenus</i>	0	0	0	1	0	0	0	0
Decapoda										
	Astacidae									
		Unknown	1	2	0	0	0	0	12	5
Diptera										
	Chironomidae									
		<i>Ablabesmyia</i>	8	7	8	2	1	6	0	1
		<i>Brillia</i>	0	0	0	0	0	1	0	2
		<i>Chironomus</i>	1	1	7	62	2	12	15	138
		<i>Cryptochironomus</i>	1	0	0	2	7	0	0	2
		<i>Dicrotendipes</i>	1	6	9	0	2	5	4	15
		<i>Diplocladius</i>	0	0	11	0	0	1	0	0
		<i>Microspectra</i>	0	0	2	0	0	0	1	0
		<i>Microtendipes</i>	4	37	19	0	0	10	0	2
		<i>Orthocladius</i>	0	0	2	0	18	1	1	0
		<i>Parachironomus</i>	0	1	0	0	0	0	0	0
		<i>Paralauterborniella</i>	0	1	0	0	2	0	0	0
		<i>Penteneura</i>	0	0	0	1	0	0	0	0
		<i>Phaenospectra</i>	0	0	0	0	0	0	0	1
		<i>Polypedilum</i>	0	1	0	2	0	0	1	2
		<i>Procladius</i>	4	7	3	19	3	2	0	9
		<i>Psectrocladius</i>	0	0	0	0	0	1	0	0
		<i>Rheotanytarsus</i>	0	2	0	0	0	1	0	0
		<i>Stictochironomus</i>	37	15	30	74	31	179	331	1626
		<i>Tanytarsus</i>	0	0	0	0	0	0	0	1
		<i>Tribelos</i>	1	3	0	1	1	0	4	27
		<i>Trichotanypus</i>	0	0	0	0	0	5	0	0
		Unknown	7	25	12	51	52	49	48	221

Table 2. Continued

Individuals Collected			M	R	M	R	M	R	M	R
Order	Family	Genus	Fall	Fall	Winter	Winter	Spring	Spring	Summer	Summer
	Ceratopogonidae									
		Unknown	0	0	1	0	0	0	0	0
	Empididae									
		<i>Hemeordromia</i>	0	0	2	0	1	0	0	0
	Tabanidae									
		<i>Haematopota</i>	4	0	0	0	0	0	1	0
		<i>Crysops</i>	0	0	5	3	0	6	0	0
	Tipulidae									
		<i>Leptotarsus</i>	0	0	1	0	0	3	0	0
		<i>Tipula</i>	0	0	0	0	1	18	0	0
Emphemeroptera										
	Ephemerellidae									
		<i>Serratella</i>	0	1	0	0	0	0	0	1
	Leptophlebiidae									
		<i>Habrophlebia</i>	0	0	2	0	0	0	0	0
		<i>Paraleptophlebia</i>	4	1	2	0	0	0	0	0
Hemiptera										
	Corixidae									
		Unknown	0	0	0	1	0	0	0	0
Lepidoptera										
	Unknown									
		Unknown	0	0	0	0	0	1	1	0
Megaloptera										
	Sialidae									
		<i>Sialis</i>	0	2	1	1	0	0	0	0
Odonata										
	Gomphidae									
		<i>Dromogomphus</i>	0	2	1	0	0	1	1	1
	Libellulidae									
		<i>Libellula</i>	0	0	0	2	0	0	0	0
	Zygoptera									
		<i>Lestes</i>	3	4	0	0	0	0	0	0

Table 2. Continued

Individuals Collected			M	R	M	R	M	R	M	R
Order	Family	Genus	Fall	Fall	Winter	Winter	Spring	Spring	Summer	Summer
Oligochaeta										
	Lumbricidae									
		<i>Eiseniella</i>	0	0	0	0	0	3	0	0
	Naididae									
		<i>Stephensonia</i>	0	3	2	10	0	0	0	0
		Unknown	0	0	0	1	0	0	0	0
	Tubificidae									
		<i>Limnodrilus</i>	5	0	3	163	3	17	5	0
		Unknown	23	6	4	290	2	29	4	19
Plecoptera										
	Leuctridae									
		<i>Perlomyia</i>	0	0	16	2	0	1	0	0
Rhynchobdellida										
	Glossiphoniidae									
		<i>Helobdella</i>	0	0	0	0	0	0	0	1
Tichoptera										
	Hydropsychidae									
		<i>Smicridea</i>	0	0	1	11	0	6	1	1
	Leptoxyphidae									
		<i>Tricorythodes</i>	0	0	0	0	0	1	0	0
	Linephilidae									
		<i>Grensia</i>	0	0	7	0	0	0	0	0
		<i>Nemotaulius</i>	1	0	2	3	0	1	1	0
	Philopotamidae									
		<i>Chimarra</i>	0	0	0	4	0	0	0	0
Veneroida										
	Sphaeriidae									
		<i>Pisidium</i>	0	4	0	23	8	5	3	8
		<i>Sphaerium</i>	0	1	0	0	0	0	0	0
<b>Subtotals</b>			105	133	154	734	134	370	436	
<b>Total</b>										4155

Table 3. Average Weight (in Grams) of Rehydrated Detritus Collected at Each Site per Season

Detritus Weights	McGee Run		Ramsey Run	
	Site 1	Site2	Site 1	Site 2
Season				
Fall	259.97	93.01	248.11	337.92
Winter	56.62	201.79	354.94	133.97
Spring	254.19	335.07	329.14	369.91
Summer	340.64	267.92	228.78	298.41

*Diversity*

Results of the Shannon Weiner calculations showed similarities and differences between the two streams (Table 4). Both streams had low diversity in the summer season, at

Table 4. Shannon-Weiner Diversity Results Standardized using

Diversity	Ramsey Run	McGee Run	Average
Fall	11.1117	8.0631	9.5874
Winter	6.4051	14.479	10.4656
Spring	7.0104	6.4592	6.7348
Summer	2.5262	2.7668	2.6465

least twice as small as the next lowest season (Table 4). Ramsey Run had the highest diversity results in the fall, while McGee Run’s highest diversity results were found in winter. When the two streams were averaged, summer had the lowest result, and winter diversity was highest (Table 4).

### *Evenness*

Fall samples collected from McGee Run had the lowest evenness at 0.3386688 (Table 5).

Evenness for all other samples fell between 0.5 and 0.6 (Table 5).

Table 5. Evenness results by stream and season

Evenness	McGee Run	Ramsey Run
Fall	0.52378	0.523496
Winter	0.594164	0.555394
Spring	0.554412	0.502691
Summer	0.338688	0.538295

### *Total Density*

When examined together, using a two-way ANOVA, both stream ( $p= 0.034$ ,  $df= 1$ ) and season ( $p= 0.02$ ,  $df= 3$ ) were shown to affect total density. Each stream showed its highest total density result in summer samples (Figure 3). Differences in densities were evident between the seasons ( $p=0.012$   $df=3$ ).

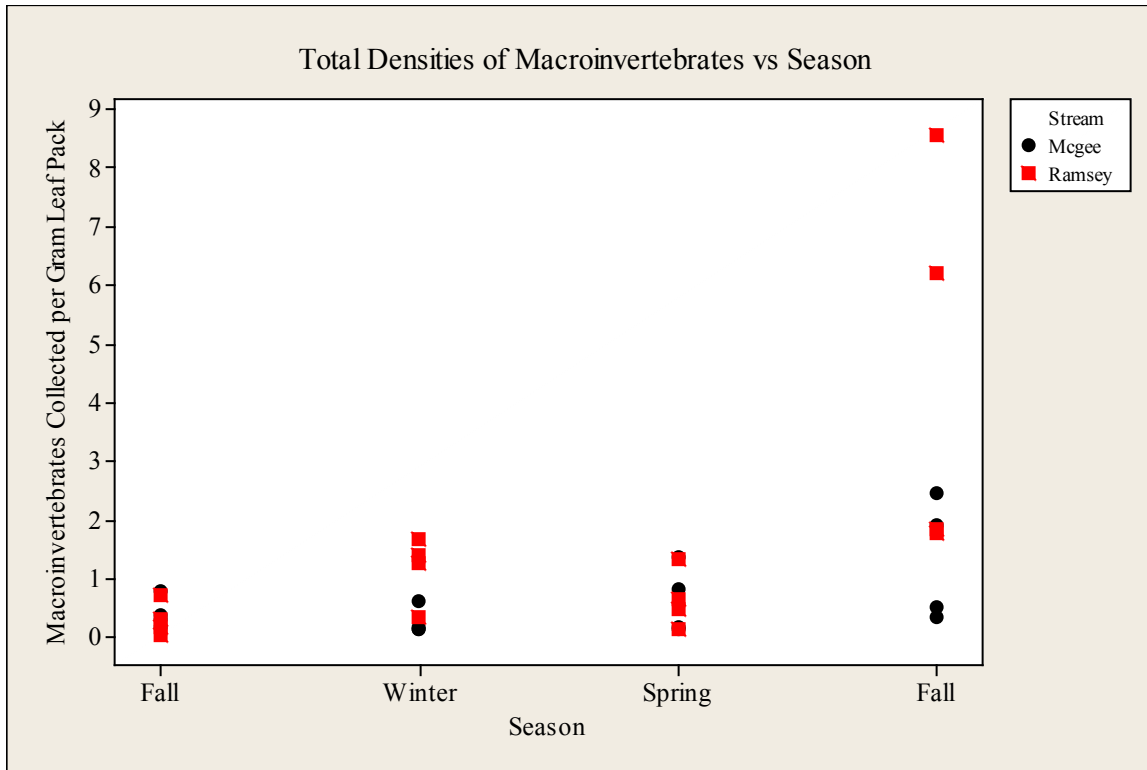


Figure 3. Densities of Macroinvertebrates Collected in each Sample by Season

Although differences in temperature between stream and leaf pack were not evident ( $p= 0.205$ ,  $df=18$ ), a trend of higher densities recorded with greater differences in temperature was present (Figure 4).

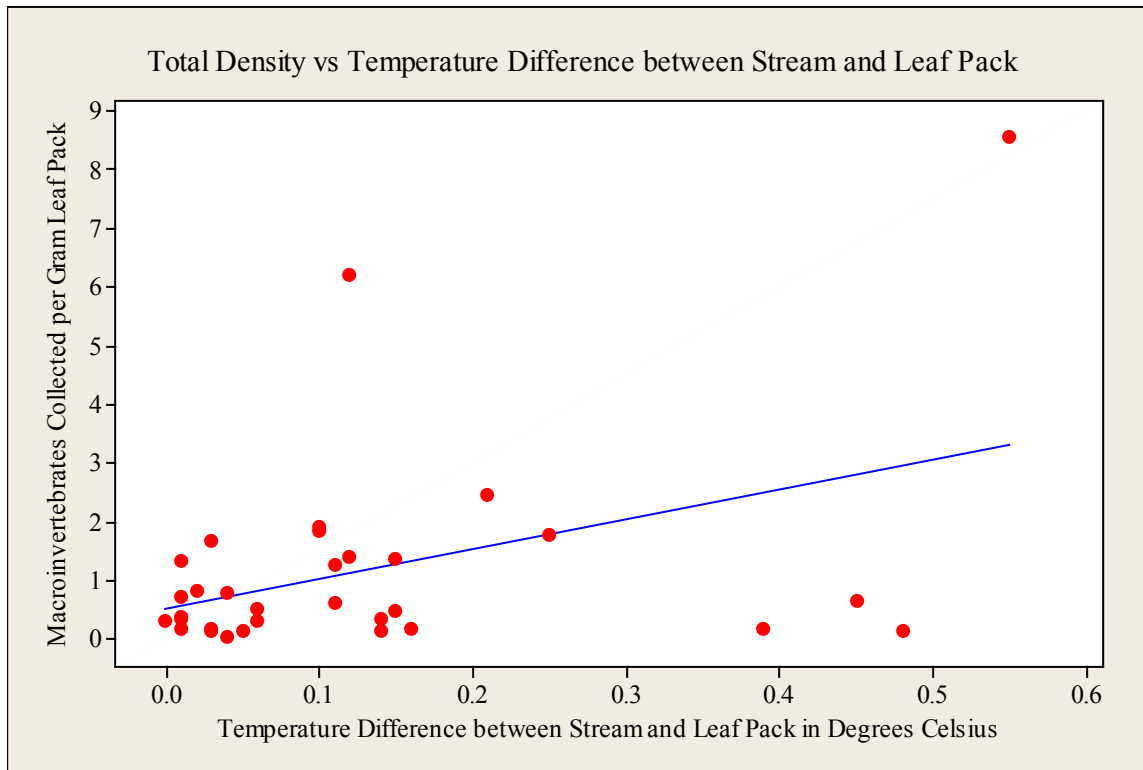


Figure 4. Total Densities of Macroinvertebrates from each Sample and Temperature Change between Stream and Leaf Packs

Water depth at which the sample was collected from had no affect on total density of macroinvertebrates collected ( $p= 0.430$ ,  $df= 24$ ; Figure 5).

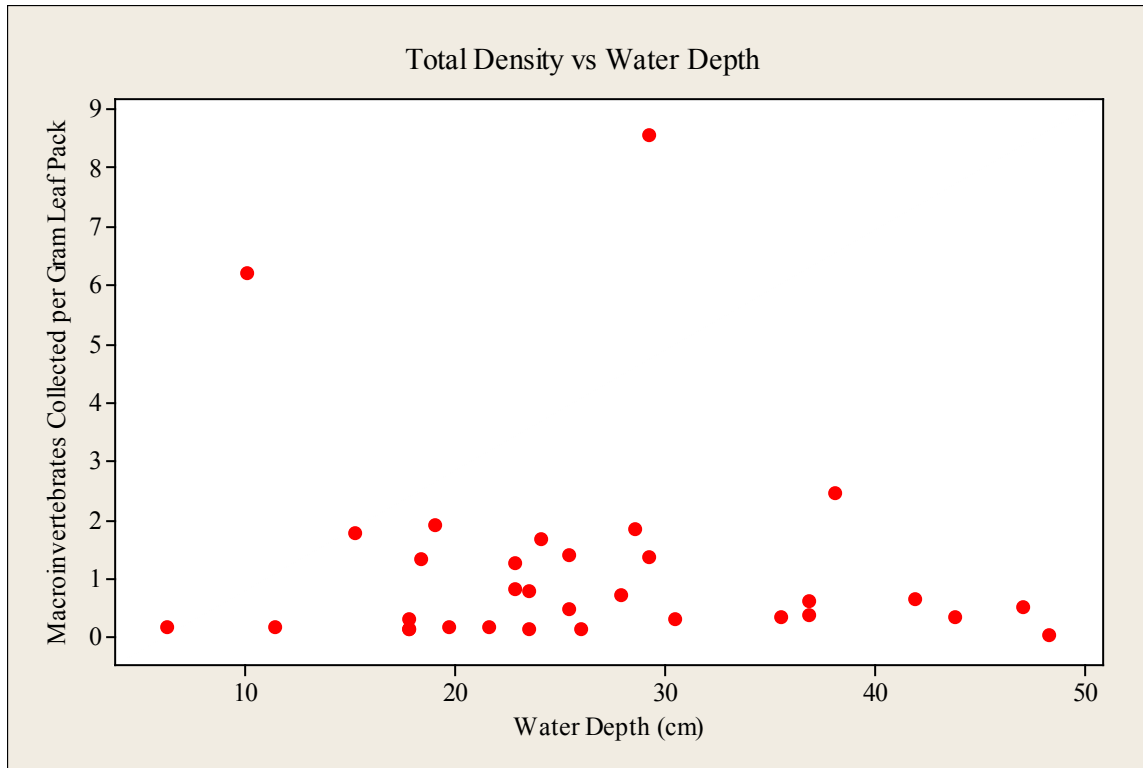


Figure 5. Density and Water Depth in cm of Macroinvertebrates Collected at each Site

*Generic Density*

Generic density was defined as the number of genera collected divided by the weight of re-hydrated detritus material, in grams, from that sample. Ramsey Run had its highest generic richness in the summer, while McGee Run’s highest result was in the spring (Figure 6).

However, season had no statistically relevant effect on generic density ( $p= 0.647$ ,  $df= 3$ ). The stream depth at which the sample was taken also had no affect on generic richness ( $p= 0.290$ ,  $df= 24$ ).



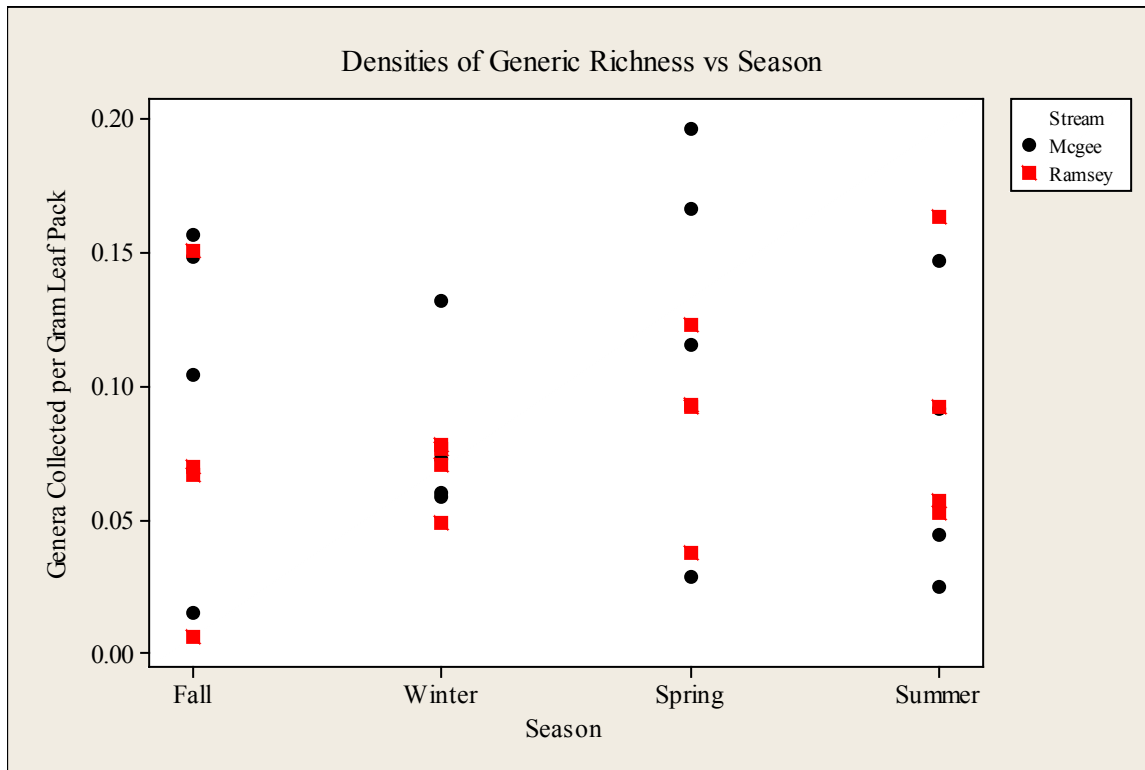
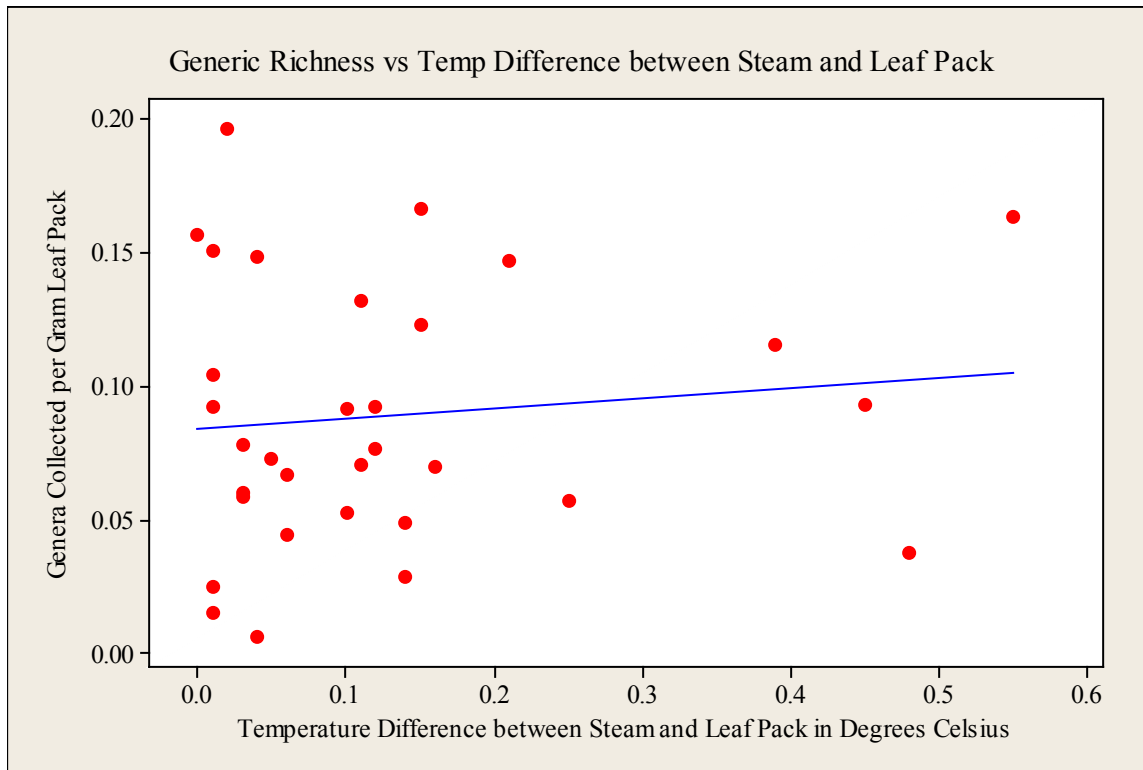


Figure 6. Densities of Number of Genera at each Site per Gram of Leaf Pack by Stream and Season

The absolute value of difference in temperature between leaf pack and stream was not statistically relevant either, ( $p= 0.408$ ;  $df= 18$ ) regarding generic density. However, as with total density both stream tended to have higher generic density results with greater differences in temperature between leaf pack and stream (Figure 7).



**Figure 7.** Generic Richness Densities Compared to Temperature Difference Between Stream and Leaf Pack with Trend Line

### *Feeding Group Densities*

Of the three feeding groups recognized by this study, only shredders and predators had any statistically significant results. Season had a significant effect on shredder density ( $p=0.001$ ,  $df=3$ ) and predators ( $p=0.05$ ,  $df=3$ ). Density of collectors was not affected by season ( $p=0.844$ ,  $df=3$ ). Summer had a much higher shredder density than other seasons (Figure 6). In fact, Ramsey Run’s summer density of shredders was more than 6 times as high as any other season (Figure 8).

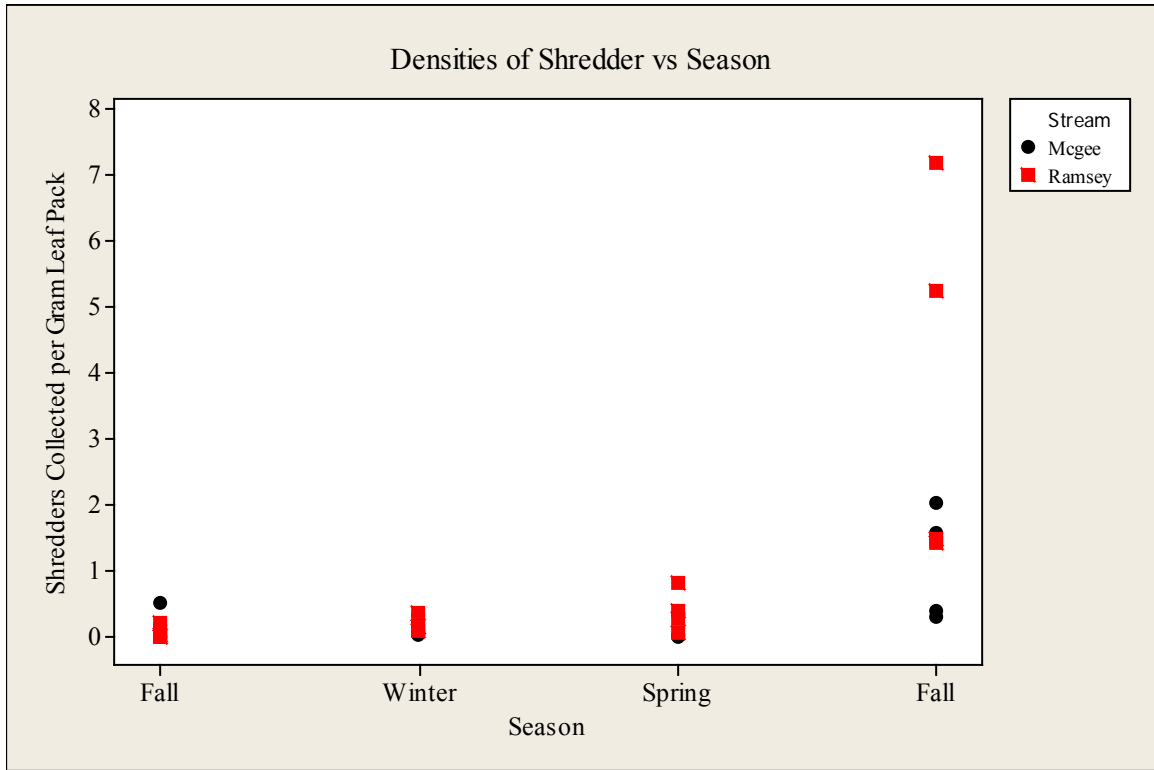


Figure 8. Densities of Shredders Collected by Stream per Season

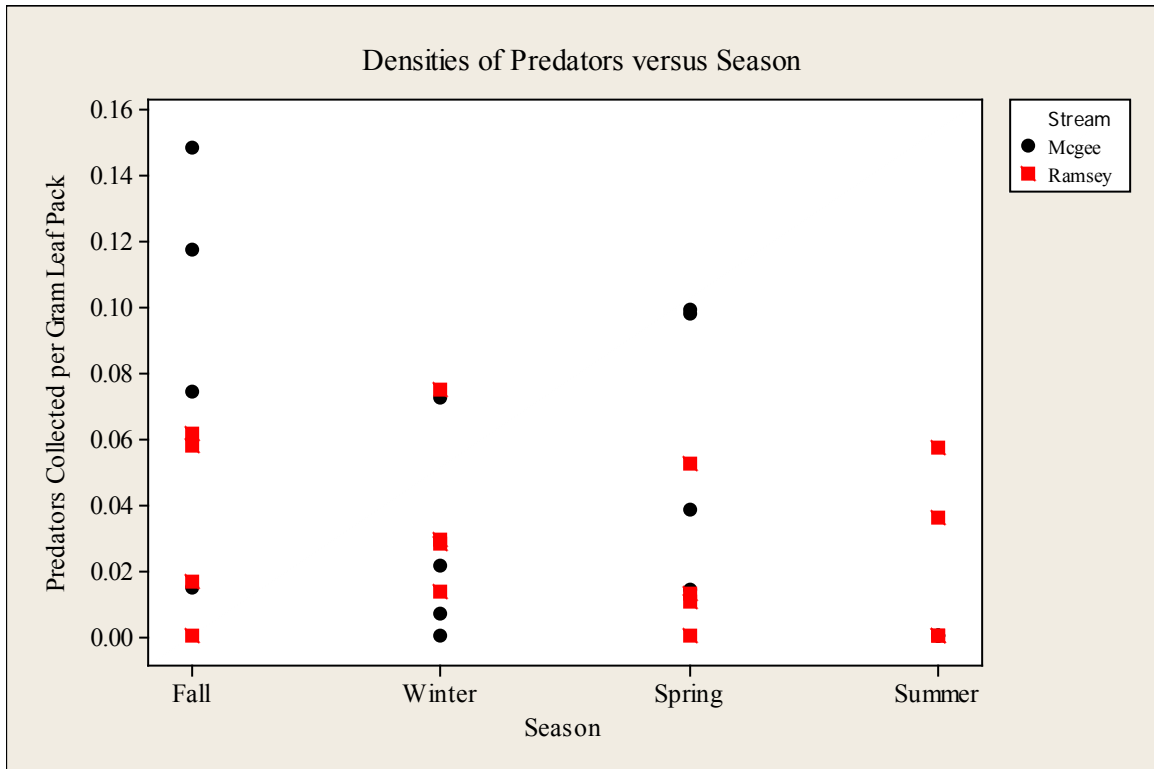


Figure 9. Densities of Predators Collected by Stream per Season

Predator densities were highest in McGee Run in fall samples while winter samples had the highest densities for Ramsey Run (Figure 9).

Differences in temperature between leaf packs and streams had no effect on feeding group densities (Table 6). Sample depth also showed no affect on any of the three feeding groups examined in this study.

Table 6. p Values Obtained from Kruskal-Wallis Testing

P values for Feeding Groups	$\Delta$ Temperature	Sample Depth
Shredder Density	0.286	0.432
Collector Density	0.928	0.490
Predator Density	0.346	0.535

The only feeding group that exhibited a similar trend to total density or generic richness regarding the temperature difference between leaf pack and stream was the shredder group (Figure 10).

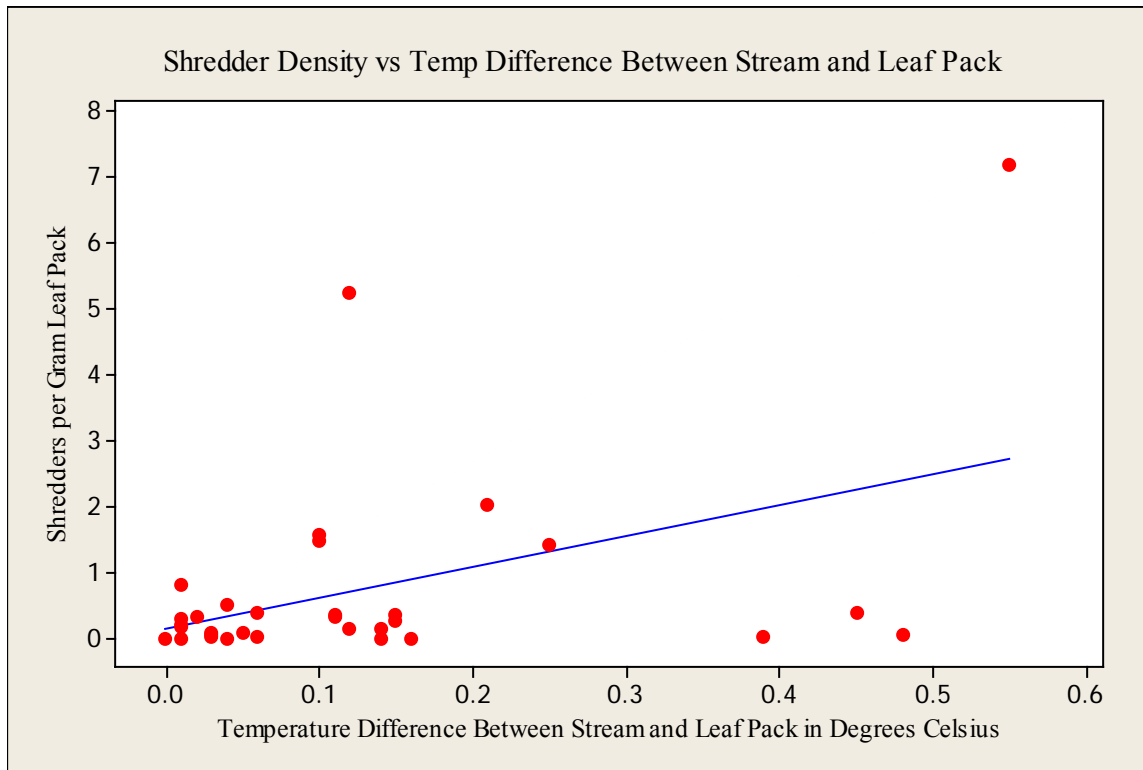


Figure 10. Shredder Density versus Absolute Temperature Change between Stream and Leaf Pack with Trend Line.

### *Vertebrates Collected*

Several vertebrates were collected over the course of this study. Although they were not the focus of this study, I felt it was important to note their appearance in leaf packs. A yearling white sucker (*Catostomus commersonii*) was collected from Ramsey Run in winter. Additionally two other white sucker larvae were collected during summer sampling, one from each stream. A creek chub (*Semolitus atromaculatus*) was also collected from Ramsey's Run in summer. Only 2 other vertebrates were collected during spring sampling. An unidentifiable ammocoete was collected from McGee Run along with a juvenile black nose dace (*Rhinichthys atratulus*) from Ramsey Run.

## Chapter 5

### DISCUSSION

Results for the efficiency test were mostly positive. When sampling at the family level, the leaf pack core sampler was shown to be 97% effective in showing densities of family richness compared to collecting the entire leaf pack (Table 1). When examining total abundance densities, the sampler was shown to be nearly 70% as effective relative to collecting the entire leaf pack. Overall, I believe that these results are acceptable. Potentially they could even out with repeated testing: family richness efficiency decreasing and total abundance effectiveness increasing. While the sampler was very efficient at capturing family richness, the fact that it was less effective at capturing total individuals was not surprising due to the large amount of heterogeneity generally known to be inherent in benthic sampling.

Overall results showed total densities tended to be higher in summer. This was due in large part to high numbers of family Chironomidae, specifically genus *Stictochironomus*. This genus is generally univoltine emerging in May or June (Tokeshi 1995), and non-biting midges' are short lived after emergence, primarily focused on mating after which oviposition often occurs within hours (Armitage 1995). Eggs of many genera can hatch after a few days in stream (Pindar 1995). Many eggs would have hatched only days before collection. This would limit time for predators to consume the new shredders before sampling. Therefore, high numbers of early instar *Stictochironomus* and other members of Chironomidae in summer would be expected.

Diversity results in the summer samples were nearly 3 times lower than diversities found in any other season (Table 4). Ramsey Run had the highest diversity in winter while McGee Run highest diversity in fall (Table 4). This is probably due to the difference in conditioning levels of leaves over the year (Andersen and Sedell 1979; Cummins et al 1980; Graca 2001;

Nelson 2000; Wallace et al 1997). Spending time in streams leeches nutrients out of leaves (Andersen and Sedell 1979). As the remaining nutrients are depleted from leaves, by stream leaching and macroinvertebrate feeding, there will be fewer macroinvertebrates able to use the leaves as food. As most of the riparian trees at the study were deciduous, the greatest input of leaves to the stream would take place in the fall. Therefore it makes sense that highest diversity results would be in within months after the new leaf input (Gessner 1991). Both highest diversity readings for the streams were recorded in the same season that yielded their highest predator densities (Table 4; Figure 9). While this contributes to the diversity results, it could also be an artifact of the higher diversity of macroinvertebrates attracting predators to the leaf packs (Jefferies and Lawton 1985). It could also explain the low diversity in McGee Run summer samples (Table 4). Members of the Tubificidae family, either of the *Limnodrilus* genus or unidentified, were collected in higher numbers in winter than any other season (Table 2). Unidentified Tubificidae were easily the second largest group collected from any season, with *Stictochironomus* being the largest for each season (Table 2). This would explain the higher diversity of winter (Table 3). One explanation for the appearance of tubificids in such large numbers is that some macroinvertebrates may be attracted to leaf packs for their relative warmth compared to streams; as winter was the only season that average temperature of leaf packs were warmer than stream temperatures.

The tendency of macroinvertebrates to avoid extreme temperatures is supported by the trend of higher generic richness being related to greater temperature differences between stream and leaf pack. However, in spring temperature differences between stream and leaf pack were more positive than those in fall and yet fall samples showed higher diversity. This may be explained by greater generic richness of macroinvertebrates collected affecting diversity results.

Richness may be higher in spring for several reasons. Many macroinvertebrates would be preparing to undergo metamorphosis. They may have gathered at leaf packs for the abundance of food in order to store energy for their upcoming transformation. This is supported by studies suggesting that the function as a food source is the primary reason that macroinvertebrates associate with leaf packs (Dobson 1994, Richardson 1992). Another reason for higher density in spring could be the hydrology of streams in that season. In areas where snow is common, the snow melt is one of the main contributors to the spring flooding events (Jost et al 2007). Snow melt coupled with precipitation high flow events could be more common in spring than other seasons (Jost et al 2007). One study in particular suggests that association of macroinvertebrates with leaf packs could be a response to seasonal changes in flow (Lancaster and Hildrew 1993). A third possible explanation for higher density could be temperature difference between stream and leaf pack previously discussed in this study. Some macroinvertebrates may be temperature sensitive and need slightly warmer temperatures to continue active feeding. A combination of these factors would explain why a large variety of macroinvertebrates are using leaf packs during this season. While other seasons may exhibit one or two of these factors, spring seems to be the only season where all three factors are evident.

Generic richness exhibited the same trend as total density regarding temperature differences between stream and leaf pack. The fact that both generic richness and total density respond in similar ways to differences in temperature leads one to conclude that this factor is an important determinate of macroinvertebrate association with leaf packs. While some studies have found food to be the primary use of leaf packs by macroinvertebrates (Dobson 1994; Richardson 1992), and another has noted their importance as refugia from high flow events (Lancaster and Hildrew 1993), few, if any, studies have shown leaf packs to be important refugia



for avoiding extreme stream water temperatures allowing continuation of normal feeding behavior. While results obtained from this study regarding this factor were not conclusive or statistically relevant, alterations to the sampling schedule may have corrected this problem. Given more time, it would have been possible to take multiple samples per season which could have further illuminated the results. Also, using a sampler with a smaller diameter would have made it more feasible to collect and process multiple samples each season. I believe that either of these methods would have led to more obvious correlations between temperature differences between stream and leaf pack regarding both total density and generic richness.

Another result that was statistically significant was the relationship between the shredder feeding group density and season. Summer was shown to have a statistically significant higher density of shredders than other seasons ( $p=0.001$   $df=3$ ). As previously discussed, the genus *Stictochironomus* of the family chironomidae was found in much greater number and higher density in summer samples. *Stictochironomus* are generally placed in shredder feeding group (Berg 1995; Coffman and Ferrington 1996). This would explain the higher density of shredders in summer samples. The results from the 3 feeding groups show differences from total density. Not every macroinvertebrate collected would fit neatly into a feeding group; feeding groups are used in most cases only with insects. Therefore, a feeding group classification was not applicable to all individuals collected (e.g., crayfish or worms). These individuals were ignored in calculations of feeding group statistics. Another reason for differences in results is the fact that differences in number of shredders collected in non-summer seasons compared to summer was greater than differences between total individuals collected in summer when compared to other seasons. These reasons may address the discrepancy in p values between effect of season on total density and its effect on shredder density. Predator density was also shown to change

over the seasons ( $p= 0.05$   $df=3$ ). However, predator densities were extremely low throughout the study. The collector feeding group was much more evenly spread over seasons. Interestingly, temperature difference between stream and leaf pack had no statistically significant effect on densities of any individual feeding group density (Table 3). In fact, shredder density was the only feeding group density to be positively related to temperature difference between stream and leaf pack (Figure 10). Again affects of temperature differences may be more defined with further sampling.

Water depth at which samples were collected from had no statistically significant impact on any response examined. This is most likely due to the nature of low-order streams. These streams are usually located in headwaters of the river continuum. As such they generally will not be as deep as higher order streams. Since the depth gradient is minimal, it seems unlikely that any macroinvertebrates will be attracted to an area of stream due to its depth. Also, the leaf pack core sampler was of adequate length to reach bottom in all stream areas sampled.

Appearance of the variety of vertebrates found over the course of this study could point to the importance of leaf packs as refugia for more than just macroinvertebrates. While the sucker yearling and larvae are probably using leaf packs as refugia for either temperature or high flow reasons, reasons for association of other vertebrates is not as clear. The creek chub and black nose dace, in particular, could be attracted to leaf packs for the high occupancy of their food sources. High concentrations of macroinvertebrates found in leaf packs could attract a variety of predator vertebrates. In fact, one study suggests that fish feeding on shredders may affect the rate of leaf breakdown (Ruetz et al 2002).

I believe that vertebrates may use leaf packs more extensively as either refugia or hunting ground for food sources than was evident from this study. However, mobility of these animals

probably allows them to escape capture from the leaf pack core sampler used in this study. A quicker capture technique could yield a higher density of a variety of vertebrates in leaf packs.

Overall, results of this study were fairly compatible with those from a variety of studies using artificial leaf packs. High total density (of Chironomidae in particular), of summer samples meshes well with other studies conducted during the summer (Murphy and Giller 2000; King et al. 1987). Also, shredder and collector feeding groups were more common in this study than the predator group which correlates with other studies (Dobson 1991). Similarities of results between this study and those performed with artificial leaf packs shows that sampling naturally occurring leaf packs is a viable option.



Figure 11. A Leaf Pack Sample in a Sorting Tray

## Chapter 6

### CONCLUSIONS

In general, I believe that this study has shown the viability of sampling natural leaf packs to determine presence and abundance of macroinvertebrates. While other studies have shown little difference between sampling natural leaf packs and artificial leaf packs created using mesh bags, detritus traps or debris dams (Casas 1997; Dobson 1991), it is important to not overlook possible confounding effects mentioned earlier. While testing with artificial may be effective, sampling naturally occurring leaf packs would eliminate these potential complications.

This study has also shown that densities of macroinvertebrates found in leaf packs changes seasonally. While the differences in densities were apparent, more study with multiple samples would help to confirm this result.

Another inference one could take from this study is the importance of temperature difference between stream water and leaf packs. More detailed studies of this kind may reveal more statistically significant data that would support the import of temperature difference as a factor influencing macroinvertebrate use of leaf packs in headwater streams.

In conclusion, this study has shown both the viability of sampling natural leaf packs and the possible importance of temperature difference between stream water and leaf pack as a determinate of macroinvertebrate use of leaf packs. When one is examining macroinvertebrate populations of streams, these may be important factors to keep in mind.

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