Colonization of Leaf Litter by Benthic Macroinvertebrates in Redwood Creek of Mt.Tamalpais State Park, California

Vega Shah

ABSTRACT

The importance of riparian zones to aquatic ecosystems is well recognized. Benthic macroinvertebrates (BMI) are an important aspect of freshwater stream systems due to the ecosystem roles they play. BMI preference for leaf letter can be examined using colonization in leaf packs and also breakdown of leaf litter. In this study, I examined differences in colonization and leaf breakdown among four different riparian species: big leaf maple (*Acer macrophyllum*), California bay (*Umbellularia californica*), coast redwood (*Sequoia sempervirens)* and white alder *(Alnus rhombifolia)* were examined. I used artificial leaf bags at various sites in Redwood Creek, Marin County, California to study colonization over a period of 2 weeks and 4 weeks. The variables used to study leaf specific BMI colonization and breakdown were abundance, family richness, genus richness, Shannon diversity index, functional feeding group distribution and leaf litter mass loss. It was found that Maple supports highest abundance and diversity of BMI while Redwood supports lowest abundance as well as diversity. Functional feeding group richness and leaf litter mass loss was found to be uniform across all leaf types. This research supports and re-affirms need for conservation of riparian vegetation in order to facilitate healthy aquatic fauna.

KEYWORDS

Diversity, EPT, leaf bag, decomposition, functional feeding groups (FFG)

INTRODUCTION

The importance of riparian zones to aquatic ecosystems is well recognized (Welcomme 1979).Terrestrial primary production derived from the riparian zone and floodplains is known to be the vital source of energy for limnic food webs (Junk et al 1989). Riparian zones act as a source of carbon by dropping leaf litter and woody debris into streams. This input not only acts as nutrition and habitat for macro-invertebrates, it also forms habitat for fish and substrate for growth of microalgae (Pusey and Arthington 2003). The riparian zone is defined as the bank side vegetative region encompassing the stream channel between the low and high water marks and that portion of the terrestrial landscape from the high water mark toward the uplands where vegetation may be influenced by elevated water tables or flooding (Naiman and Decamps 1997). Some of the important influences of riparian zones include thermal buffering (Lynch et al 1984), the provision of shade and its influence on in-stream primary production (Bunn et al,1999), storage interception and release of nutrients (Smith 1992), enhancement of bank stability (Prosser et al 2001), the provision of leaf litter and course woody debris as habitat and substrate for fish, invertebrates and algae (Sheldon and Walker 1998) and mediation of changes in channel morphology as well as habitat diversity (Nakamura and Swanson 1993).Thus it is important to consider the riparian zone to better understand benthic macroinvertebrate colonization. It has been shown that change in vegetation type in the riparian zone (e.g.: replacement of forest with grass or cropland) has a direct impact on composition and abundance of benthic macroinvertebrates (Cummins et al 1989). Consequently, changes in the macro-benthic zone are thought to be a result of altered plant litter quantity, quality and seasonality (Laćan et al 2009). Thus quality and quantity of leaf litter has a direct impact on freshwater benthic macro-invertebrate (BMI) health and diversity. Benthic macroinvertebrates are essentially defined as small stream inhabiting creatures that are large enough to be seen with the naked eye and spend all or part of their life cycle in or on the stream bottom (Fore et al.1996)

BMIs are an important aspect of freshwater stream systems due to the ecosystem roles they play. Several studies have dealt with the "goods" produced by benthic species, such as the quantity of prey items consumed by fish (Covich et al 1999). They are equally significant due to their ability to process leaf litter into smaller components. Cumulatively their ecosystem functions include sediment mixing, nutrient cycling, and energy flow through food webs. (Covich et al 1999). BMI respond to a variety of environmental variables like water quality,

sediment quality, shading, hydrological conditions as well as biological changes and due to this characteristic they are often used as an effective measure of stream health (Rosenberg and Resh 1993). As result, BMIs have become the most commonly used biological indicators in freshwater systems and are often used for conservation purposes. A common method to rapidly assess the water quality stream is EPT analysis which studies three orders of aquatic insects that are easily sorted and identified, namely Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (Fore et al.1996).EPT analysis is based on the premise that healthy streams have higher abundance and richness in EPT since these orders are sensitive to pollution (Fore et al.1996).

Figure 1. Photographs of Orders Ephemeroptera, Plecoptera and Trichoptera L to R

Another measure of BMI diversity is richness and abundance in functional feeding groups which are a classification approach that is based on the morphotype as well as behavioral mechanisms that certain taxa use to acquire and ingest food.(Merritt and Cummins 1996) .The benefit of this method is that a large number of taxonomic groups can be easily studied by grouping them in categories based on feeding habits ,examples of commonly found functional feeding groups are shredders, predators and collectors.(Short and Maslin 1977)

A reliable method to obtain information about the relationship between leaf litter and BMI is the study of colonization of invertebrates in leaf packs and also the breakdown of leaf litter by invertebrates. Leaf colonization and breakdown in limnic systems may be an especially useful assessment tool because, along with being an indicator of microbial and leaf feeding invertebrate functioning, leaf breakdown links invertebrate trophic levels. (Nelson 1999). Processing by leaf-feeding invertebrates (shredders) produces fine particles consumed by other invertebrate populations, such as collectors (Cummins and Klug 1979). Thus the transfer of nutrients is mainly facilitated by BMIs that breakdown leaf litters into smaller organic matter. The relationship between BMI and leaf litter can be studied by comparing invertebrate

colonization and breakdown rates between different species of leaves commonly found in riparian areas. This procedure provides information on three relevant aspects namely the preference of BMIs to specific type of litter, the diversity of BMI that colonize different types of leaf litter and the decomposition of leaf litter in streams (Cortes et al 1997). Several past studies have compared leaf litter colonization and breakdown in different leaf species (Bunn 1988; Basaguren and Pozo 1994; Cortes et al 1997) but these have been primarily conducted in tropical freshwater stream systems. This study specifically looks at colonization and breakdown in leaf litter derived from commonly found riparian species in the Mediterranean climate of Northern California. The above site was chosen based on knowledge that there is a strong influence of climate and catchment vegetation on stream structure and function and the Mediterranean climate is unique because it is characterized by distinct cool and wet season followed by a warm and dry season, they are influenced by a sequence of regular and often extreme flooding and drying periods. (Gasith and Resh 1999)

The objective of this research was to examine three general questions about colonization of benthic macroinvertebrates in riparian leaf litter. The first question examines BMI preference for leaf type and whether this preference is based on nutritional quality and or substrate quality of leaf species. I predicted that different leaf species would colonize different combinations and numbers of BMI based on differences in leaf litter quality as well as physical characteristics of each leaf species. The second question I want to address is whether a mixture of leaf litter supports higher diversity in colonization of BMI than any single species of leaf litter. I examined diversity using the Shannon diversity index and also by examining differences in functional feeding group for each leaf litter type. For the above question I predicted that leaf bags with higher diversity would colonize BMI that would have a higher Shannon diversity index and visible differences in functional feeding group proportions. The third question examines the change in mass of leaf litter after two different time periods of colonization and whether there were variations in the rates of decomposition across different types of leaf species. Since the leaf litter decomposition was a difficult variable to calculate, I predicted that different leaf species would have different amounts of biomass loss due to differences in their physical attributes. This experiment does not take into account the microbial breakdown of leaf litter in streams. It is assumed that the degree of microbial breakdown of leaf litter is ubiquitous through the same

stream and thus safe to compare bags for just breakdown caused by BMI related or leaf taxa related factors.

METHODS

The system under study is the lotic ecosystem in Mediterranean climate of Northern California. The study site was situated at Redwood creek in Mt.Tamalpais state park of northern California. The above mentioned creek has been classified as a class 1, 2nd degree stream. I chose this study site because of its location in a Mediterranean climate, accessibility and ideal topography for installation of research materials. Redwood creek, like most Mediterranean streams is characterized by distinct cool and wet season followed by a warm and dry season; they are influenced by a sequence of regular and often extreme flooding and drying periods. (Gasith and Resh 1999).Figure 2 depicts a map of Mt.Tamalpais state park and each red dot marks each of the sites where leaf bags
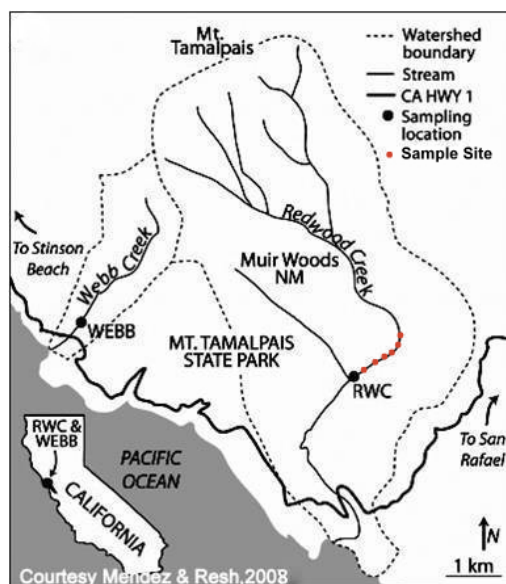

Figure.2 Map of Mt.Tamalpais State Park

The individuals of interest were benthic macro-invertebrates that inhabit the creek. This study was conducted over a period of 5 weeks in Fall 2009 (October 31st – December 2nd) and another period of 5 weeks in Spring 2009 (March 30 – April 30) .This time scale was chosen to compare the variations in colonization during wet season and dry season and also to avoid any loss of experimental data as a result of high flow rates during rainy seasons in December and January. The research was conducted under the supervision of Patina Mendez and with help from the Resh Lab at U.C. Berkeley.

Preparation of Leaf Bags

I collected leaves from Maple, Redwood, Alder and Bay trees from banks of Webb Creek and Redwood Creek both located in Mt.Tamalpais state park. The leaves collected were specifically those found on the forest bed and were brown or yellowish brown in color and usually whole. (Fig.4) .The leaves were cleaned as much as possible during collection and were sufficiently free of soil and other detritus. Later I air dried the leaves indoors and then weighed them into packages of 10 g each. Every replicate had 5 treatments each in the form of a leaf litter bag. The five treatments consisted of four single species bags and one mixed bag. The mixed bag was made using 2.5 g of each leaf type. The artificial leaf bags were constructed using two layers 12x15 in.es rectangular pieces of deer block mesh. Two layers were used to avoid loss of leaves from stream turbulence and mechanical manipulations. The top of each bag was secured with two cable ties 1 inch from each other on the bag. The bags were also tagged using color coded cable ties and labels. (Fig.3)
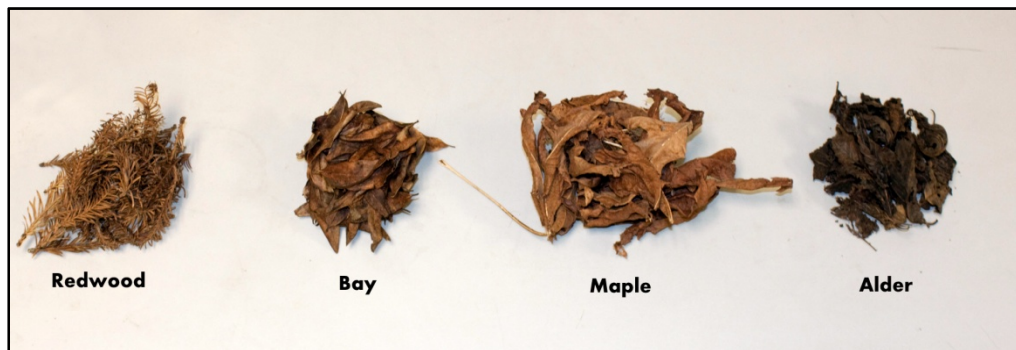

Figure 3. Artificial Leaf Bag

Figure 4.Types of Leaf Litter

In-stream Installation of Leaf Bags

The second part consisted of installations of leaf bags at each site. In order to do so, I first installed 3 re-enforcement bars at a distance of 20 inches from each other. I then put a chain; 40 inches long held down taut by the re-enforcement bars and fully submerged. Then each bag was installed on the chain using cable ties approximately 9 inches apart from each other (Fig.5). The bags were submerged at a depth of about 0.33 meters at each site. The order of bags was decided randomly and each bag was labeled with colored cable ties based on type.
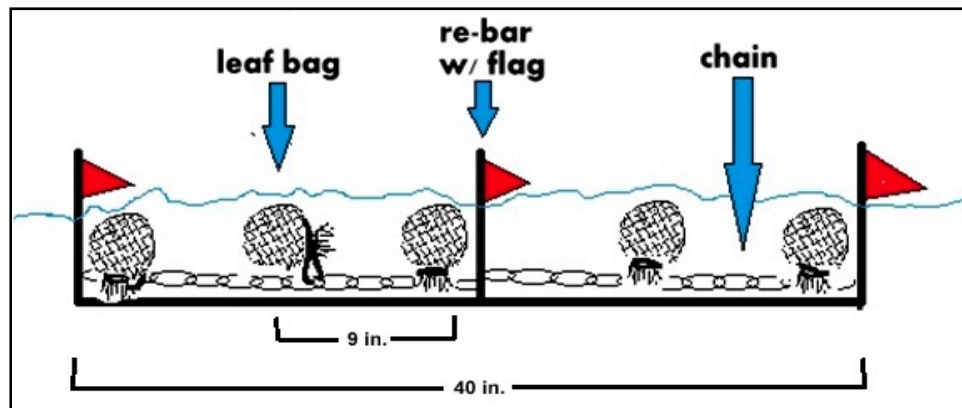

Figure 5.Diagram of In-stream Setup of Artificial Leaf Bags

Figure 6. Photograph of In-stream Setup

Collection, Sorting and Statistical Analysis

All the bags at sites 2, 4 and 6 were collected after a period of 2 weeks and were replaced by new leaf bags .After another 2 weeks (a total of 4 weeks from start of the experiment) , all bags from all six sites were collected. As a result, I had 15 leaf bags from sites 1, 3 and 5 which had been exposed for a time period of four weeks and I had 30 leaf bags from site 2, 4 and 6 which had been exposed for a time period of 2 weeks. The collection was conducted using a 10 micron sieve and a kick net. The sieve was used to hold the bottom part of the bag while the cable ties were severed. The kick net was held on the downstream side of the bag to prevent any BMI from escaping. Both the sieve and the bag were lifted out and the sieve was then washed with 75% ethanol in a white collecting pan .The entire contents of the bag were emptied into a 1 U.S. gallon Ziploc bag and pre-made labels indicating site, leaf type and date were put inside the bags. The leaf bags were cut open and the organisms were washed out of leaf litter using 75% ethanol. The leaf litter was air dried and weighed after which the change in biomass was measured. The change in biomass for the two different sets from sites 2, 4 and 6 were averaged out and the mean was used in analysis.

The organisms obtained from sites 1,3 and 5 were sorted ,counted and identified down to genus level using a Nikon 8140 dissecting microscope at a magnification range of 10X to 20X . These were the only organisms studied due to time and budget constraints of this project. After this the organisms were further sorted into functional feeding groups. The obtained data was first analyzed using Microsoft Excel 2007 and then using software R version 2.10. BMI abundance ,family richness ,genus richness and Shannon diversity index distribution box plots were made using R and package R Commander (Rcdmr).The statistical significance of distributions were tested using Kruskal-Wallis one way non-parametric analysis.

RESULTS

Abundance

I measured the BMI abundance across sites 1, 3 and 5 and analyzed them across each leaf type. I found that BMI abundance was highest in Alder and lowest in Redwood (Fig.7a) I also measured the abundance of EPT across each leaf bag type and found that it was highest in Maple and lowest in Redwood. (Fig.7b). I analyzed the statistical significance of both these distributions using the Kruskal-Wallis nonparametric one way analysis and found that the p-value for distribution of BMI abundance across different leaf types was 0.615 and the p-value for distribution of EPT abundance across different leaf types was 0.6687.
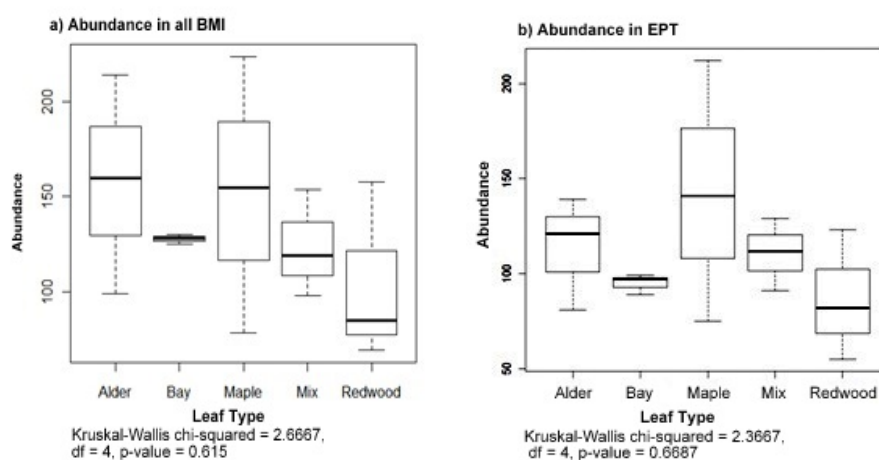

Figure 7.Abundance across leaf types

Richness

I measured family level richness and genus level richness across each leaf type for all BMI (Fig 8a,8b) and also for EPT (Fig.8c,8d).I found that among BMI, Alder, Mix and Redwood had highest family and genus richness while Bay had lowest. I also found that among EPT, family level as well as genus level richness was uniformly distributed across each leaf bag type. I analyzed each of these distributions using the Kruskal-Wallis one way non-parametric analysis. I found that distribution of family and genus richness of BMI across leaf types had a p-value of 0.481. I also found that family and genus distribution of EPT across different leaf types had a p-value of 0.9875
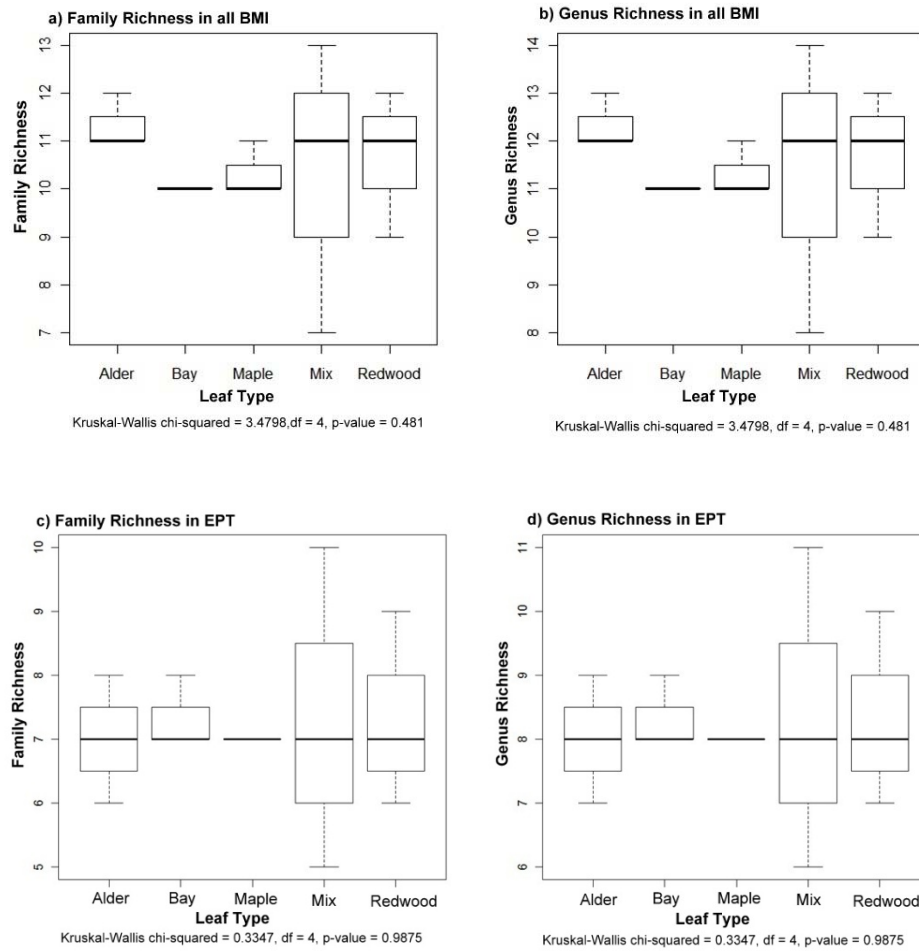
Figure 8.Family and Genus Richness across leaf types

Diversity

      I calculated the Shannon Diversity Index for each leaf type and studied the distribution of BMI diversity and EPT diversity across each leaf type .I found that BMI had highest diversity in Maple and lowest in Mix (Fig 9a). Similar results were found in EPT wherein Maple had highest diversity whilst Mix had the lowest (Fig 9.b). Both these distributions were tested for statistical significance using the Kruskal-Wallis test and it was found that BMI distribution had a p-value of 0.934 whilst EPT distribution had a p-value of 0.9702.
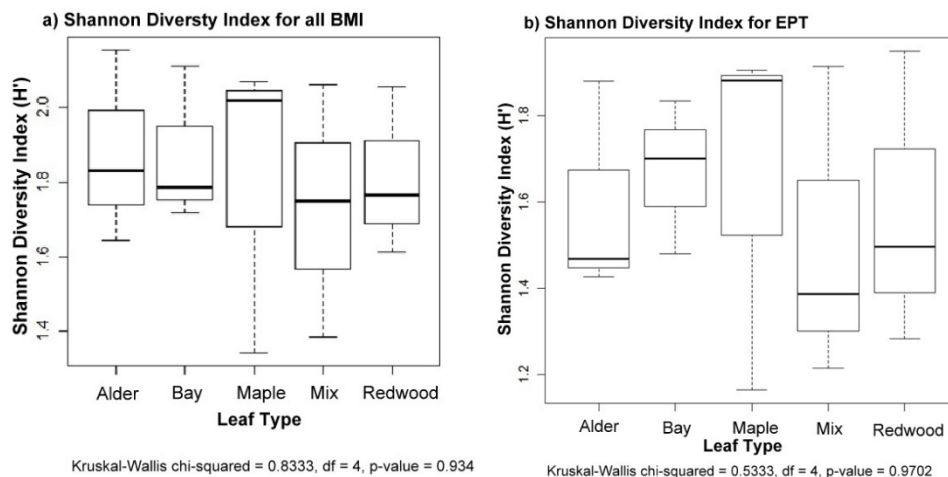
Figure 9.Shannon diversity index distribution across leaf types

Functional Feeding Groups Distribution and Site Differences

I examined the distribution of functional feeding groups solely through each leaf type. I found that gatherer/collectors, predators and scrapers were most abundant in Alder while shredders were most abundant in Maple. (Table.1) .I sorted the BMI based on functional feeding groups and then studied their distribution across each leaf type as well site. It was found that shredders were the most abundant functional feeders whilst predators were the least across all leaf types and sites. I also observed that site 1 had the largest number of individuals while site 3 had the least. (Table.2)

Table 1.Distribution of functional feeding groups based on leaf types

Leaf Type	Functional Feeding Group					Total
	Gatherer/Collector	Predator	Scraper	Shredder		Total
Alder	211 (45%)	34 (7%)	20 (4%)	208 (44%)		**473**
Bay	165 (43%)	16 (4%)	18 (5%)	184 (48%)		**383**
Maple	182 (40%)	12 (3%)	5 (1%)	258 (56%)		**457**
Mix	130 (34%)	13 (4%)	13 (4%)	215 (58%)		**371**
Redwood	146 (48%)	10 (3%)	7 (2%)	149 (48%)		**312**

Table 2: Distribution of functional feeding groups across each site

Site/Leaf Type	Functional Feeding Group Count				
	Gatherer/Collector	Predator	Scraper	Shredder	Total
SITE 1	**358**	**20**	**14**	**488**	**880**
Alder	109	3	2	100	214
Bay	64	7	0	59	130
Maple	54	2	1	167	224
Mix	52	6	8	88	154
Redwood	79	2	3	74	158
SITE 3	**156**	**17**	**9**	**287**	**469**
Alder	25	4	6	64	99
Bay	47	5	1	72	125
Maple	38	7	0	33	78
Mix	27	0	0	71	98
Redwood	19	1	2	47	69
SITE 5	**320**	**48**	**40**	**239**	**647**
Alder	77	27	12	44	160
Bay	54	4	17	53	128
Maple	90	3	4	58	155
Mix	51	7	5	56	119
Redwood	48	7	2	28	85
TOTAL	**834 (42%)**	**85 (4%)**	**63 (3%)**	**1014 (51%)**	**1996**

Leaf Litter Loss

  I measured the change in mass of leaf litter for all samples from sites 1 to 6. The differences in sites exposed for 4 weeks were compared with sites exposed for 2 weeks. I was observed that there was no pattern in differences between samples exposed for different time periods. I also studied the distribution of lost leaf litter mass across various leaf types and I found that Mix leaf bags lost slightly more mass than other bags but on an average all bags approximately lost the same amount of leaf litter mass.(Table.3)

Table 3: Change in leaf litter mass .Change in biomass was measured across each leaf type after being exposed for two different time scales, 4 weeks and 2 weeks.

Time Scale of Exposure	Site	Change in Mass per Leaf Litter Type (grams)						TOTAL
		Alder	Bay	Maple	Mix	Redwood		
4 weeks	1	3.777	3.212	2.188	2.395	1.998		**13.57**
4 weeks	3	2.815	3.169	2.422	3.031	1.458		**12.895**
4 weeks	5	3.168	3.799	3.514	2.267	2.315		**15.063**
2 weeks	2	1.234	2.451	2.889	3.139	3.901		**13.614**
2 weeks	4	3.233	2.675	2.984	3.097	3.945		**15.934**
2 weeks	6	1.982	1.234	2.304	3.096	3.028		**11.644**
	TOTAL	**16.209**	**16.54**	**16.301**	**17.025**	**16.645**		**82.72**

DISCUSSION

The objective of this study was to examine three general questions about colonization of benthic macroinvertebrates in riparian leaf litter. The first question examines BMI preference for leaf type and whether this preference is based on nutritional quality and or substrate quality of leaf species. I predicted that different leaf species would colonize different combinations and numbers of BMI based on differences in leaf litter quality as well as physical characteristics of each leaf species. The second question examines whether a mixture of leaf litter supports higher diversity in colonization of BMI than any single species of leaf litter. I examined diversity using the Shannon diversity index and by examining differences in functional feeding group for each leaf litter type. I also predicted that leaf bags with higher diversity would colonize BMI that would have a higher Shannon diversity index and higher functional feeding group richness. The third question examines the change in mass of leaf litter after two different time periods of colonization and whether there were variations in the rates of decomposition of different types of leaf litter. Because the leaf litter decomposition was a difficult variable to calculate, I predicted that different leaf species would have different amounts of biomass loss due to differences in their physical attributes.

Leaf bag treatments had graphical differences in abundance and richness; however these differences were not statistically significant suggesting that the sample size of this experiment might not have been large enough. Sites 1, 3 and 5 revealed that Maple had highest BMI abundance while Redwood had the lowest. I also found that Maple had highest EPT abundance and Redwood had lowest EPT abundance. However these distributions were not statistically significant ($p=0.615$ and $p=0.6667$ respectively) and all conclusions were made solely on

graphical observation of patterns in distribution. In my analysis of family richness and genus richness of BMI, I found that Alder, Mix, and Redwood had slightly higher family and genus richness than Maple and Bay by one family and two genuses. EPT family and genus richness did not vary between leaf type and no differences were statistically significant.

Leaf quality is a possible explanation for the variation in BMI abundance. Specifically leaf quality affects colonization in predictable ways based on leaf toughness, surface area and other generalizable patterns based on taxonomic families (Webster and Benfield 1986). Maple is likely able to support a larger and richer population of BMI due to its physical characteristics. Maple leaves were large in size, comparatively softer and had a wrinkled texture ideal for latching on to. Redwood possibly supported fewer individuals because of its tough, thin needles that broke down easily from stream turbidity and thus did not serve well as a source of nutrition or as a substrate. (Cummins and Klug 1979) The lack of variation in family and genus richness can be explained by the fact that all sites were in the same stream, geographically situated quite close to each other. Past research has shown that often the main source of variation in leaf litter assemblages of BMIs is due to differences between streams (Leroy and Marks 2006, Zilli et al 2007). These differences may be in physical characteristics of banks, canopy cover, pH and several other biotic as well as abiotic factors that affect streams (Leroy and Marks 2006).

Shannon diversity index was found to be highest in the Maple treatment and lowest in the Mix treatment for all BMI as well as EPT, however differences between treatments were not statistically significant. Thus conclusions can only be made based on graphical patterns of differences in treatment. The above results refute my initial hypothesis that higher diversity in leaf species (e.g., a mix of all four leaf species) would support a more diverse combination of BMI. Past research has shown that high diversity in leaf litter supports high diversity in BMI assemblage and thus diversity in riparian vegetation is essential to maintaining benthic invertebrate populations (Leroy and Mark 2006). I believe that these results can be attributed to the physical properties of Maple, because the replicates contained a higher count of BMI and a slightly higher diversity index. These results are not indicative of the overall in-stream potential diversity in colonization because my sample size was very small, the sites were geographically close to each other and the time scale for which the samples were exposed might not have been long enough to support colonization to its full extent.

The proportion of BMIs in their functional feeding groups had little or no variation across leaf types or sites. I found that four types of functional feeding groups, namely gatherers/collectors, shredders, scrapers and predators, were found in each leaf type and also at each site. Between leaf types there was variation in abundance of each functional feeding group. I found that Alder supported highest number of gatherer/collectors (45%), scrapers (20%) and predators (34%). Maple supported highest number of shredders (56%) and it was also found that shredders were the most abundant functional feeding group through all leaf types and sites. These variations can be attributed to two factors, the nutritional and substrate quality of Alder and the taxonomic characteristics of shredders. The feeding of shredders on riparian litter is source has been shown to be about 30% of total conversion of course organic particulate matter to fine organic particulate matter and thus they are often found in high numbers on leaf litter (Peterson and Cummins 1974, Short and Maslin 1977). My experimental findings contradict my initial hypothesis that higher diversity in leaf types would encourage higher functional feeding group richness.

Leaf litter loss of mass had little or no variation across leaf types suggesting that BMI colonization and time period of exposure may not be a source of variation in leaf litter decomposition. I observed that there was little or no difference in samples that had been exposed for 4 weeks as opposed to those exposed for only 2 weeks. Apart from this, I observed that sites 4 and 5 had a larger change in mass as opposed to other sites. Although invertebrate assemblages differ dramatically among streams, leaf decomposition is most affected by substrate quality and stream's water quality and not by stream-to-stream differences in shredder assemblage (Zilli et al.2007). Consequently, the variations observed at sites 4 and 5 compared to other sites were most likely a result of differences in the physical attributes of this site. The above findings contradict my initial hypothesis that different leaf types will have variations in leaf litter mass loss due to their physical attributes and there is reason to believe that the variations are caused by physical as well as biological attributes of sites.

The results obtained from abundance, richness, diversity, functional feeding groups and leaf mass loss showed differences but were found to be not statistically significant .The graphical differences observed support the hypothesis that different leaf types will colonize different numbers and combinations of BMI due to differences in leaf litter quality and physical attributes. The results contradict my prediction that more diversity in leaf types (e.g., the Mix leaf bags)

will colonize a more diverse assemblage of BMI with differences in functional feeding group proportions. It was observed that even though bags showed differences in proportions of functional feeding groups, diversity index was highest for Maple and not for Mix. I believe these results were directly related to some of the limitations of this experiment. I was only able to count and identify organisms from sites 1, 3, and 5 (4 week samples) because of time constraints and thus a lot of information about diversity may lie in the remaining samples (sites 2, 4 and 6 which were 2 weeks samples).Another important constraint of this experiment was that it was conducted in one stream and the study sites were geographically close to each other. My site selection was limited by time and budget constraints of the project and diversity in assemblage may have been better observed with a larger sample size to better capture the variability within the stream.(Needham and Usinger 1956) I also found that my hypothesis that different leaf types would have different amounts of mass loss due to differences in their physical attributes was contradicted. I found that the source of variations in leaf litter mass loss was more related to differences in sites and not leaf types. This result has been supported by past studies that show leaf decomposition is differs dramatically among streams as opposed to across leaf species (Sylvestre and Bailey 2005)

Future directions

I believe that in the future, with more time and equipment, this project can produce more conclusive data especially if the sample size was larger and more organisms were counted and analyzed. An important shortcoming of this experiment was the lack of BMI data from samples that had been exposed for 2 weeks .A good future project would be to sort, count and analyze BMI obtained from 2 weeks samples. I also believe that a wider range of BMI colonization diversity would be observed if a similar experiment was conducted in another stream and the data was compared with the above results. This would exemplify the site differences as opposed to just differences in colonization in different leaf species. The experimental setup of this research does not take into account the abiotic and microbial breakdown of leaf litter and assumes that the degree of non-BMI related breakdown of leaf litter is ubiquitous through the various leaf bags and thus safe to compare bags for just breakdown caused by BMI. This source of error could have been removed by having a sixth leaf bag as

control. It would be constructed from finer mesh that would prevent BMI related breakdown and thus account for other sources of leaf litter decomposition.

Conclusions

This project provides evidence that leaf litter plays essential roles in maintaining aquatic diversity and highlights the importance of conservation of riparian zones. It exemplifies the need for consideration of benthic macro invertebrates during riparian restoration projects and formulation of conservation strategies (Knopf et al.1988). This project also shows the need for native riparian plant species to support a healthy aquatic system as opposed to exotic species (Winfield and Hughes 2002).

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