Investigation of the ecological impact of Japanese knotweed (*Polygonum cuspidatum*) on the riparian plant community and its potential use as a food source for aquatic macroinvertebrates

Presented to the faculty of Lycoming College in Partial fulfillment of the requirements for Departmental Honors in Biology

by
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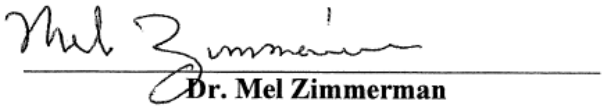
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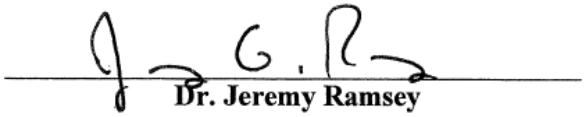
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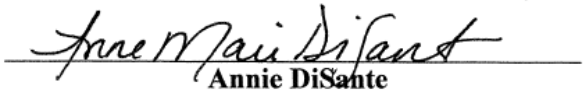
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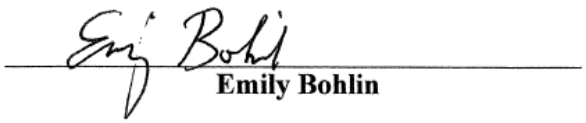

Emily Bohlin

Abstract

A major characteristic of invasive plants is their ability to grow quickly and aggressively, enabling them to spread and displace native species. Japanese knotweed (*Polygonum cuspidatum*) has become one of the most prolific invasive species in Pennsylvania riparian zones. This study focused on knotweed populations at two sites along the lower West Branch of the Susquehanna River in North Central PA. The ecological impact of Japanese knotweed on the riparian zone was assessed by collecting data on plant density and richness, light availability, tree populations, and soil characteristics. At each site, herbaceous plants were assessed using a transect and meter-squared plot method, and tree populations were assessed using the Point Quarter sampling method. A preliminary laboratory experiment testing kntoweed's allelopathic properties on Jewelweed (*Impatiens capensis*) seeds was completed; there was no germination in control or treatments, indicating the seeds were inviable, so no conclusions could be drawn. To test the potential use of knotweed as an aquatic macroinvertebrate food source, a comparative leaf decomposition study of knotweed versus Pin Oak (*Quercus palustris*) and Silver Maple (*Acer saccharinum*) was performed during the summer and fall. Three factors were assessed to determine the palatability of the leaves: leaf decomposition rate, fungal biomass colonization, and aquatic macroinvertebrate colonization. Results that showed knotweed, once established, is the dominant species at both sites, using its ability to grow quickly to outcompete other ground cover species by monopolizing the available light. It is a potential food source for aquatic macroinvertebrates, although it is a lower nutritional quality than other native leaf litter deposits.

Introduction

Japanese knotweed (*Polygonum cuspidatum*, Sieb. & Zucc. (syn: *Fallopia japonica*)) is an invasive species in the United States and Europe. Native to Asia, it was introduced to the United States in the Early 1800s as an ornamental plant (Stone 2010). It has become a serious problem and will continue to invade new areas due to its fast growth and lack of successful eradication techniques (Shaw et al. 2009).

Knotweed has a bamboo-like appearance, although it is in the Polygonaceae family and not the Poaceae family with bamboo. The stalk is erect and can grow up to and over 13 feet. It is smooth and hollow, and has an ochrea encircling the leaf node, an indicative trait of the Polygonaceae family. It has heart shaped leaves coming to a point at the tip. The stem can have a purple-ish hue to it, and the flowers are a creamy white. Flowers are found in a raceme. Knotweed has a rhizomatous root system that can spread laterally 23-65 feet. It reproduces both sexually, via seeds, but more commonly asexually, through vegetative regeneration from any part of the plant (Stone 2010).

It has been shown that knotweed produces secondary chemicals (Xiao et al. 2002) that could have an allelopathic effect on surrounding plants but more research needs to be done to determine the potential effects (Weston et al. 2005 and Fan et al. 2010). Knotweed is a strong competitor compared to other native species (Lecerf et al. 2007), using its numerous broad leaves to limit the amount of light reaching the soil surface and surrounding vegetation (Weston 2005). It is able to grow in a variety of conditions, so it is found in many areas like wetlands, disturbed areas, and riparian buffer zones.

Riparian Buffers, vegetative areas bordering surface water, are critical for keeping waterways clean. They are complex ecosystems that offer food and habitat for unique species while filtering out harmful materials before they enter the water. Areas where the riparian buffer has been ripped out for development or altered in some way often experience a decline in water quality, a direct effect of the buffer's presence and/or function (Hawes 2005). Riparian buffers decrease the amount of sedimentation that is deposited into the bordering water by stabilizing the stream bank. They also prevent nitrogen, phosphorous, and other nutrients and pollutants from entering the aquatic ecosystem by filtering the run-

off from surrounding areas. The plant cover also provides shade, cooling the water temperature and making a more ideal habitat for fish and aquatic macroinvertebrates.

The types of plants in riparian zones are closely tied to the adjacent aquatic ecosystem because aquatic macroinvertebrates depend solely on the litter deposited from the riparian plants (Cummins 1989). Shredders are the functional feeding group of macroinvertebrates that initially feed on this plant littler material by, as their name indicates, shredding it into small pieces for the other functional feeding groups: the collectors (gatherers, filters) and grazers.

In order for shredders to feed on plant material, it first needs to be conditioned, releasing the nutrients for easy availability. Aquatic fungi play an important role in the conditioning process, and therefore in the entire leaf litter food web (Gessner and Chauvet 1994). The fungi turn the plant litter material into a palatable food source for macroinvertebrates by releasing nutrients previously unavailable to them (Suberkropp 2001). Until fairly recently, quantifying fungal biomass was difficult and not standardized because of the fungi's ability to infiltrate the leaf substrate (Gessner and Chauvet 1993). A technique was developed in the early 1980's to quantify fungal biomass growth by quantifying the ergosterol content in a sample size. It has since been adjusted to yield best results (Newell et al. 1988) and appropriate conversion factors have been developed (Gessner and Chauvet 1993). Ergosterol, a sterol or steroid alcohol found in fungi, is an ideal indicator because it contains double bonds that are not common in sterols of vascular plants or microbes, and its concentration can be measured by ultra-violet spectroscopy (Newell et al. 1988).

Japanese knotweed is one of the most successful plant invaders but its impact on aquatic ecosystems, especially the plant litter food web is poorly understood (Lecerf et al. 2007). It is a concern that knotweed is invading critical riparian areas, hindering the native ecosystems ability to successfully buffer the adjacent waterways, and negatively impacting the aquatic ecosystem. Potential problems arising with a knotweed invasion include: shredders negatively impacted by the change in litter material, knotweed's secondary compounds inhibiting fungal decomposers, litter dynamics changing due to a high input of poor-quality food for leaf consumers. Finally, it is unknown whether native leaf consumers

could even adapt to non-native species because they lack the co-evolutionary history (Lecerf et al. 2007). Lecerf et al. (2007) found that invertebrates can utilize knotweed as a food source but also as the severity of a knotweed invasion increases, so does the possibility that the alteration it causes will influence the stream community and ecological function.

It is a concern that knotweed is invading these critical riparian areas and hindering the native ecosystems ability to successfully buffer the adjacent waterways by crowding out native species and thereby destabilizing the banks and taking up different nutrients. Furthermore, it is a concern that knotweed is negatively impacting the aquatic ecosystem. The objectives of this study were 1) to assess the ecological impact of Japanese knotweed on the riparian plant community at two sites along the West Branch of the Susquehanna River and 2) to assess whether or not Japanese knotweed is a potential food source for aquatic macroinvertebrates.

It is expected that in the two knotweed dominated riparian zones in question, knotweed will inhibit successful growth of the native plant community. It is expected that knotweed will be colonized by aquatic macroinvertebrates to some degree. Furthermore, it is expected that the aquatic macroinvertebrates will colonize knotweed leaves in similar distributions to native leaf species.

Methods

Study Sites – Population Monitoring

This study was conducted at two sites along the West Branch of the Susquehanna River. The first site is the Loyalsock Riverfront Park (LRP) located at [N 41.24425, W 76.95390]. The LRP site soil is comprised mostly of Linden loam (Lm), characterized by a dark brown loam surface layer, followed by a reddish brown loam and reddish brown fine sandy loam. Below those layers is a reddish brown and brown, friable and loose fine sandy loam and loamy fine sand. The permeability is moderately rapid to about 40 inches and rapid below that. This site has a channel dividing it into a mainland and island. The second site is the public park behind the Montoursville Airport (MA), located at [N 41.23341, W 76.92901]. The soil at this site is mostly made up of Udifluvents, loamy (Ud), which differ from area to

area but often consist of brownish silt loam, loam, or fine sandy loam deposits in the top 2-10 inches, followed by a reddish brownish loam, sandy loam, loamy sand, or sand. See Figure 1 showing both sites: the black star indicating the LRP site and the red star indicating the MA site.

Study Site – Leaf Decomposition Study

The leaf decomposition study leaf packs were incubated in Mill Creek, a third order stream, located at [N41.27677, W76.93232]. It is a tributary to the Loyalsock Creek, located one and a half miles above the confluence of the West Branch of the Susquehanna, near the MA site. The site was chosen because it is on a private property so non-natural disturbances would be minimized, it is relatively close to the two previously mentioned study sites, and it is representative of a stream in a knotweed invaded area.

Soil Sampling

Soil samples and leaf litter samples from a 12cm x 12cm x 3cm deep quadrat were gathered randomly at both sites and taken back to Lycoming College for processing following procedures by Cox (1976). At the LRP site, 12 spots were gathered from for a total of 36 samples (1 soil bag plus 2 leaf litter bags per spot), and at the MA site 6 spots were gathered from for a total of 12 sample bags (1 soil bag plus 2 leaf litter bags per spot). One leaf litter bag from each collected quadrat was used for Tullgren funnel extraction to collect arthropods; these arthropods were later identified and recorded. The second leaf litter bag was used for Baermann funnel extraction to collect nematodes; these were later counted and recorded.

The pH, Nitrogen, Potassium, and Phosphorous content of the soil were measured using a LaMotte Chemical Soil Test Kit. A hydrometer technique described by Cox (1976) and based on ideology by Bouyoucos (1927), was used to assess the soil texture or the particle size distribution. A small portion was taken from each soil sample, weighed, and rolled out to remove any large chunks. The coarse particles (> 2mm) were sieved out using a machine sieve, weighed and calculated as part of the total sample weight. The remaining sample was added to 1 L of deionized water and 'plunged' or mixed. Hydrometer readings were taken at 40 seconds and 2 hours to determine the percent sand, silt, and clay.

The percent organic content of the soil samples was quantified by drying out a portion, recording the weight, muffling it at 550°C for three hours in a muffle oven, and re-weighing it after.

Transect Sampling

At each site, various length transects were sampled to determine herbaceous species composition and density, tree importance values, and light availability above and below the knotweed canopy. Transects were established by running a meter tape from the river bank inland for 50 to 100 meters, depending on the extent of the knotweed invasion through the riparian buffer. At every 10 meters along the transect, a meter-squared plot was assessed for all present herbaceous plant species and their frequencies.

Depending on the length of the transect, one or two Point Quarter Sampling plots (Cottam et al. 1953) were completed to determine the relative importance of different tree species at the two sites. For the Point Quarter Sampling method, a second meter tape was run perpendicular to the existing transect, creating four quadrants. Within each quadrant the nearest tree to the origin was identified, the diameter at breast height was recorded, and the tree's distance from the origin was measured.

Finally, at every 10 meters on the transect, light meter readings, were taken using a handheld Extech Digital Light Meter (model 401025). Readings measured in lux were taken above the knotweed canopy and below it to determine the difference in available light for the knotweed and ground cover species below the knotweed.

Allelopathy Test

A preliminary test was completed in the greenhouse at Lycoming College to see if knotweed leaves generated any secondary compounds to prevent or suppress the growth of Jewelweed (*Impatiens capensis*). The procedure was based on Briggs (Unpublished Data). Knotweed leaves were collected from the LRP site in October 2015, soaked in water, and filtered through a Whatman #1 filter via water aspiration into an Erlenmeyer flask, creating an extract to pour over the jewelweed seeds. The extract was frozen until the experiment started, and in between treatments. Jewelweed seeds were collected from

the LRP site in October 2015 and refrigerated in a plastic bag to simulate a natural winter freezing period. One control group, and two experimental groups contained 10 pots each. Ten seeds were planted in each pot filled with peat-moss based grow mix (Farfard brand). The control pots were watered with only water and the experimental groups were watered with two varying concentrations of the knotweed extract every four days. In between watering, the soil was kept moist with water in all groups. The pots were kept in an environmental chamber, set to have 12 hours of light and 12 hours of darkness, at approximately 68°F. Unfortunately, there was no germination in any of the pots so this experiment was inconclusive and will not be discussed.

Leaf Decomposition Study

A study to assess the palatability of knotweed for aquatic macroinvertebrates was completed in the summer and fall of 2015. It included leaf pack incubation, macroinvertebrate identification, and ergosterol analysis to quantify the fungal biomass. There are various ways to carry out a leaf pack type of study, but we loosely followed the procedure of Gessner and Chauvet (1994). Leaf packs consisted of a coarse mesh bag (3 mm round holes) with a numbered brick and 10 leaves. In the summer, Silver Maple (*Acer saccharinum*), Pin Oak (*Quercus palustris*), and Japanese knotweed leaves were used, and in the fall study only Pin Oak and Japanese knotweed were used due to time restraints. For both the summer and fall assays, the leaves were taken directly off the plant and kept in a cold room until the packs were created; leaves were held for one day at the most. The surface area of each leaf was measured using a Li-Cor Surface Area Meter (model LI-3000A) and recorded. The leaves were secured together using a twist-tie but placed in the mesh bag freely with the numbered brick.

Each set of packs was incubated for a varying length of time in Mill Creek. In the summer, six packs individual packs for each of the three leaf species (18 packs), and a mixed pack with three leaves of each species (1 pack), were incubated for each period (5 periods), for a total of 95 packs. The packs were deployed on May 29, 2015. One set of packs was pulled after each of 5 incubation period: 7 days, 21 days, 41 days, 49 days, 82 days. The final set was collected August 19, 2015. In the fall, 3 packs of the

two species were incubated for each period, for a total of 30 packs. One set of packs was pulled weekly for 5 weeks, beginning on September 1, 2015, and ending on October 5, 2015. (see Tables 1 and 2 in Appendix III for reference water chemistry and macroinvertebrate data from this site)

After the incubation period was over, leaf packs were collected from the stream in buckets with stream water, and transported back to Lycoming College for processing. Macroinvertebrates were picked out of each pack that were then preserved in 70% Ethanol for later identification. All macroinvertebrates were identified down to family; most individuals were identified to genus. A population distribution of the macroinvertebrates, for each leaf species, was constructed based on their known functional feeding groups (Scrapers, Filtering Collectors, Gathering Collectors, Shredders, Predators).

The post-incubation surface area was recorded for each leaf. Then ten to twelve leaf disks were cut from random leaves in each pack using a 16-mm cork borer. The leaf disks were preserved in 25 mL of HPLC grade methanol to assess the fungal biomass on the leaves. The processing rates, or k-values, were calculated from the pre- and post-incubation surface area measurements and the rates were categorized as fast ($k > 0.010$), medium ($0.010 > k > 0.005$), or slow ($k < 0.005$) (Peterson and Cummings 1974). The water temperature of Mill Creek was collected daily using a Solinst Levelogger (model 3001).

The fungal biomass was determined through the use of High Pressure Liquid Chromatography (HPLC), following the procedure of Newell et al. (1988). The preserved leaf disks were transferred to a round bottom flask with the methanol and refluxed in an 80°C water bath for 30 minutes. Then, 5 mL of 10% potassium hydroxide (KOH) was added and refluxed for another 30 minutes. This process of saponification released ergosterol through the hydrolysis of sterol esters (Volker et al. 2000). The solution was cooled to room temperature and filtered via water aspiration into a filter flask through a glass frit (coarse, 40-60um) to remove any debris including the leaf disks. The filtrate was transferred to a 65-mL screw cap glass jar where ergosterol was extracted with pentane. First, 5 mL of 20% aqueous sodium chloride (NaCl) was added to induce separation. Then, three separate portions of pentane were added to the vial in the amounts 10 mL, 5 mL, and 5 mL. After each portion of pentane, the vial contents were

mixed vigorously, and once the solution settled, the top pentane layer containing ergosterol was extracted. The extracted portions were allowed to dry until all the pentane evaporated.

Following drying, the precipitate was redissolved in 1 mL of HPLC grade methanol, and sonicated for 30 minutes to dissolve any ergosterol residue. Not all of the precipitate crystals dissolved, so a standard sonication time was used to ensure continuity between samples. The point where there was no more change in the crystals was the sonication time chosen. Finally, the solution was filtered through a 13mm 0.45um nylon membrane Whatman filter directly into an HPLC vial.

The vials were labeled, set into the HPLC injecting plate, and analyzed on the HPLC instrument using the same methods used to create the standards. Ergosterol peaks were monitored at 282 nm, where there is maximum absorption. The Shimadzu LC-2010CHT instrument consisted of a Shimadzu pump, a Whatman Partisil 5 ODS3 5µ 85Å 25-cm x 4.6-mm column (catalog # 155271-PSP-ODS3; serial #201-15-84169) at room temperature, a 100 uL sample loop, and a Shimadzu SDD-10A photodiode array detector with Shimadzu LC Solution Software. Ergosterol eluted at a flow rate of 1.5 ml/min in methanol between 10 and 11 minutes.

Standard ergosterol concentrations were prepared from 0.1024 g of 98% ergosterol (ACROS ORGANICS catalog #AC117810250) diluted to 200 mL with HPLC-grade methanol for a stock concentration of 501.8 ug erg/mL MeOH. The stock standard was diluted with different volumes of methanol to generate the other standard concentrations which were then injected at the same volume. This method of injecting all at the same injection volume insures instrument measurement precision and repeatability. It also allowed the maximum injection volume of each sample so the peaks were within the sensitivity of the unit and were quantifiable. A standard curve was created by plotting the concentrations against their peak areas read from the HPLC chromatogram. A 182.6 g fungal biomass/g ergosterol conversion factor was used to find the amount of fungal biomass per leaf disc (Gessner and Chauvet 1993). Sample calculations, along with a sample chromatograph can be found in Appendix I.

Statistical analysis was run on the leaf decomposition rates using SPSS, a computer statistics program. A Two-Way Analysis of Variance (ANOVA) was completed with a significance level of $\alpha = 0.05$. Rank sum tests were completed by hand on select data using a significance level of $\alpha = 0.05$.

Results

The soil tests show both sites have similar soil characteristics. Using the USDA textural triangle to determine the soil textural class for both sites, Table 1 shows they are both a sandy loam based on the particle size distribution. The only parameters that are significantly different between the two sites are the percentage of silt (LRP= 6.69 ± 6.9; MA=18.45 ± 4.0) that makes up the soil and the phosphorous content, although phosphorous is very important to plants (LRP=14.8ppm; MA=5ppm). The biological characteristics (invertebrate and nematode density) were not significantly different between sites.

The change in herbaceous ground cover from when knotweed was young and less than two feet in height to when the knotweed was greater than two feet in height, is shown between Figure 2 and Figure 3. The dominant spring species starts as *Erythronium americanum* and changes to knotweed as the growing season progresses. The increase in species in Figure 4 is most likely due to more transects sampled at the peak growing season, rather than the knotweed having little effect on diversity. Most native species were still sparse once the knotweed is greater than two feet in height itself (Figure 3). Non-native species (*P. cuspidatum, Alliaria petiolate, and Microstegium vimenium*) comprised 50.96% of the relative density (25.14, 18.72, and 6.08 respectively), while all native species were under 4% relative density, except only two: *E. americanum* and *I. capensis* (8.27% and 6.76% respectively). All species common names can be found in Appendix II in tables 5 and 6.

Seen in Figure 4, the light availability after knotweed is greater than two feet in height decreases significantly underneath the knotweed canopy. All light readings were taken when the knotweed was greater than two feet tall, at the same time that the sampling for Figure 3 was done. On average, only 49.08% of the light penetrating the upper tree canopy is available to the ground cover plants through the knotweed.

Maple is the most important tree family at each site seen in Tables 3 and 4. At the LRP site, Silver Maple (*Acer saccharinum*) is the most important species with a value of 119.47, followed closely by Red Maple (*A. rubrum*) (98.69). At the MA site, Sugar Maple (*A. saccharum*) is the most important species (90.40).

The decomposition rate of all the leaf species were fast in the summer study (Table 6). The three species in the summer were significantly different from each other ($P = 0.000$) and the incubation time had no significant effect on decomposition rate ($P = 0.968$). Of the three summer species, knotweed is the fastest ($k = 0.0525 \pm 0.008$). Knotweed was also considered fast in the fall (0.01132 ± 0.0084), but the Two-Way ANOVA analysis showed its fall decomposition value is significantly different than the summer value ($P = 0.000$). The Pin Oak was categorized as slow in the fall (0.00198 ± 0.0021), and significantly differs from the fall knotweed value ($P = 0.017$) with no significant influence from incubation time ($P = 0.329$).

Fungal biomass was present in all the samples that were tested, including the knotweed, in both the summer and fall (Figures 5 and 6). There were no significant weekly trends of fungal biomass accumulation in the samples throughout the study duration. Table 7 shows the average fugal biomass for both seasonal incubation periods for Pin Oak and Japanese knotweed. Knotweed has higher fungal biomass in the summer (Summer=0.351 ± 0.215; Fall=0.216 ± 0.148) and Oak has more growth in the fall (Summer=0.438 ± 0.636; Fall=0.705 ± 0.995), but statistical analysis could not be completed due to the lack of data points so statistical significance is unknown.

The daily temperature collected by the data logger is given in Table 5. The May-August period corresponds with the time when the summer leaf packs were incubating, and the September-October period corresponds to the fall leaf pack incubation period. The May-August ($17.6°C \pm 3.3$) and September-October ($13.3°C \pm 2.1$) average temperatures differ significantly from each other.

The macroinvertebrate distribution in the leaf packs (Figures 7 and 8) is generally similar throughout the two incubation periods (summer and fall) and the species (summer=maple, oak, knotweed, and mixed; fall = oak and knotweed). The most abundant functional feeding group in all the populations

is the gathering collectors, followed by the scrapers. Figure 9 shows the distribution of macroinvertebrates in Mill Creek during September 2015 gathered from sampling completed separately from the leaf packs. The shredder abundance is similar to the leaf pack distributions but the gathering collector population was a lot smaller.

Discussion

Impact of Japanese knotweed on riparian buffer community

It was expected that the knotweed would be the dominant species throughout its growing season. As the herbaceous plant density results showed, knotweed was not dominant until it became tall enough to significantly outcompete other species for light. Knotweed is known to form a dense mat of dead stalks that are slow to decompose, preventing early spring growth of other species. Though there was a fairly dense mat of knotweed in the early stages of transect sampling, it did not prevent early spring ephemerals from successfully sprouting and having a healthy full life cycle, as seen by the density distribution in Figure 3, where *Erythronium americanum* is the dominant species. These results cannot be attributed to the presence or lack of secondary chemicals because our preliminary allelopathy test was inconclusive. It should be noted that knotweed is known to have secondary chemicals (Xiao et al. 2002; Weston et al. 2005; Fan et al. 2010), but the allelopathic properties of them are still in question, therefore all that can be concluded is at the two sites in this study early spring natives grew, no matter what the soil chemistry was.

Once growing over two feet tall, the knotweed is the dominant species as expected. The literature concerning knotweed invasions, and exotic invasions in general, has shown multiple negative effects that this could be having on the ecosystem. Knotweed slows down nitrogen cycling in the soil, and reduces the amount of inorganic nitrogen available in the spring affecting native species ability to germinate and grow but not its own because of its stored nutrients (Tharayil et al. 2013). Knotweed invasion is also known to cause loss of wild habitat and species diversity, as well as reduce the available water supply and carrying capacity of adjacent rivers and streams (Weston 2005).

One of knotweed's known mechanisms for outcompeting other ground cover plants is its ability to grow tall and monopolize the available light (Weston 2005), and the results (Figure 4) show how drastic this monopolization can be. Regardless of knotweed's other aggressive ecological factors, if a native individual is able to germinate and attempt to flourish during knotweed's peak growth, it will have an extremely hard time reaching maturity because of the lack of available sunlight underneath the knotweed canopy. This is detrimental to the ground cover plant community, and any organisms relying on it for food or habitat.

Japanese Knotweed as an aquatic macroinvertebrate food source

Most of the energy input into aquatic food webs is from allochthonous material, litter that originated from outside of the stream, (Cummins 1989) so riparian vegetation, the main source of that energy input, is closely tied to the success of aquatic macroinvertebrates. To see if Japanese knotweed, a common invasive plant in riparian areas, is palatable for macroinvertebrates, a leaf decomposition study was completed to analyze the leaf decomposition rate, aquatic fungi establishment, and macroinvertebrate colonization of knotweed compared to two native tree species.

The leaf decomposition rates were all significantly different between species in the summer, though they were all classified as fast (Peterson and Cummings 1974). This indicates that the knotweed is breaking down at a rate typical of native species and potentially supplying food to native aquatic macroinvertebrates. In the fall, knotweed and oak decomposed at significantly different rates from each other, and they were both significantly slower than summer decomposition rates. Decomposition rates are expected to be faster in the summer because of the warmer temperatures. The slower rates could also be due to the chemical components that develop in the leaves of knotweed and oak later in the summer, such as tannins and lignins (Gessner and Chauvet 1994). As demonstrated by these results, species and temperature cause decomposition rates to vary but so can factors such as water chemistry, leaf nutrients, habitat, and experimental protocol (mesh bags or no) (Ostrofsky 1997).

Aquatic fungi play a critical part in conditioning the allochthonous material for aquatic macroinvertebrates to consume (Gessner and Chauvet 1994). Studies have shown that leaf colonization by macroinvertebrates, specifically shredders, is strongly correlated with fungal biomass growth (Graca 2001), most likely because those are the most palatable and nutritious for the invertebrates (Cummins 1989). The study results showed no significant weekly trends of fungal biomass growth, in the summer or fall (Figure 5 and 6). A decreasing trend of fungal biomass growth is expected because the leaves start out large, with plenty of room for colonization but as time progresses, they break down due to fungal digestion and processing, leaving less area for fungal biomass to colonize. It has also been shown that as time and breakdown progresses, leaf colonization changes from fungi to bacteria (Suberkropp and Klug 1976).

When comparing the average fungal biomass over the entire study period for both incubation times, the knotweed values follow this trend, with higher fungal biomass in the warmer summer temperatures, and lower fungal biomass in the cooler fall temperatures. The Pin Oak fungal biomass values do not fit this trend, with a higher fungal biomass fall value. This could be due to an outlier in the data that when removed, decreased the standard deviation from 0.995 to 0.365, and resulted in a lower fall fungal biomass value (0.283± 0.365). It is important to note that in both seasons no matter the trend, there was fungal biomass present on knotweed leaves, so it is being colonized and therefore conditioned for the local macroinvertebrates.

The macroinvertebrates that feed on this conditioned allochthonous material are mainly shredders (Cummins 1989). When the leaf litter biomass is high, the shredder population is expected to be proportionally high as well, and once the biomass decreases, so too does the shredder count (Cummins 1989). As the results show, shredders did not constitute a large part of the population in any of the experimental conditions in this study. One explanation could be that the quality of Japanese knotweed is low for the macroinvertebrates, and studies have shown that shredders preferentially choose higher quality litter (Graca 2001), but that would also insinuate that the other two native species, Pin Oak and Silver Maple, are low quality litter, since they had similar distributions. Pin Oak does have a similar

tannin and lignin content to knotweed so it is also a low quality litter and would cause similar results. In contrast, Silver Maple does not have similar tannin and lignin levels so it should therefore not be the same quality, nor act in the same way. Another possibility is that the leaf pack method used limited the amount of oxygen circulating in the packs, creating an unsuitable environment for shredders (Cummins 1989) and deterring them from leaf colonization. It is potentially just a result of the normal stream distribution since the reference data (Figure 9) had a similar shredder population size.

Even though the proportion of shredders was low, it is clear that the leaves were decomposing from the decomposition rates and laboratory observations. This could be due to environmental factors such as temperature and flow rates, or there could be enough shredders in the system to process the material. Because the leaves are being broken down by one method or another, they are still a viable food option for gathering/filtering collectors, which would explain the high percentage seen in the results of this study (Figures 7 and 8). The high gathering collector and scraper proportions could also be due to the increased particles generated from the artificial substrate of the leaf packs (mesh bag and brick).

The fact that the fungal biomass is colonizing the knotweed leaves, paired with the presence of macroinvertebrates in knotweed packs similar to the native species' packs, and similar invasive and native decomposition rates indicates that knotweed is being incorporated into the aquatic food web in this ecosystem.

In conclusion, Japanese knotweed is invading two studied riparian areas along the West Branch of the Susquehanna River and establishing itself as the dominant plant species in the community by outcompeting native species for light, resulting in lower native species' densities. Further studies could investigate how the knotweed is changing the nutrient cycling, bank stability, and native organisms' habitat. In waterways with the adjacent riparian buffer areas being invaded, Japanese knotweed is a potential food source for aquatic macroinvertebrates, though the quality of the energy input is questionable. Overall, it is harmful that the knotweed is invading the terrestrial ecosystem, but the adjacent aquatic system seems to be able to incorporate the exotic food source, although it may be a low quality one.

Data Figures and Tables

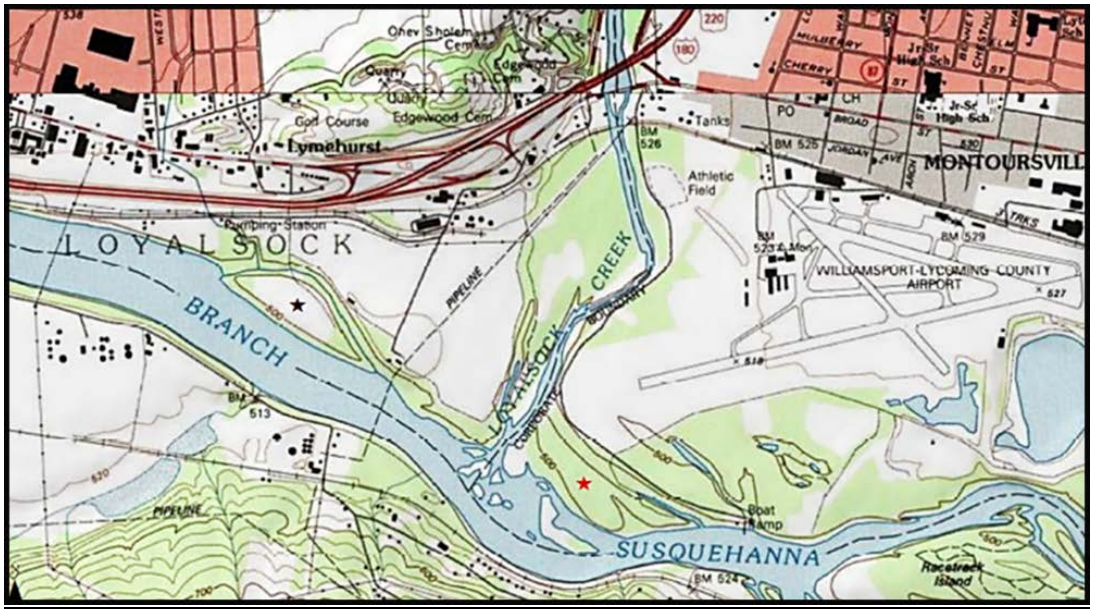


Figure 1. A Map including both study sites. The black star denotes the LRP site and the red star indicates the MA site.

Parameter	LRP	MA	Significant Difference
% Organic Content	12.26 ± 4.26	6.19 ± 2.65	Yes
% Sand	64.72 ± 7.7	73.00 ± 5.2	No
% Silt	26.69 ± 6.9	18.45 ± 4.0	yes
% Clay	8.59 ± 1.9	8.55 ± 1.8	No
% Coarse (> 2mm)	3.80 ± 3.0	5.20 ± 2.7	No
pH	4.99 ± 0.1	5 ± 0.0	No
Nitrate (ppm)	5 ± 0.0	5 ± 0.0	No
Phosphorous (ppm)	14.8 ± 18.9	5 ± 0.0	Yes
Potassium (ppm)	55.6 ± 5.3	78.3 ± 91.9	No

Table 1. Chemical/Physical data of the soil characteristics for both sites. Phosphorous and %silt are significantly different (α=0.05) according to a Rank Sum test.

Parameter	LRP	MA	Significant Difference
Nematode Density (org/m2)	2043 ± 2240	1319 ± 1048	No
Invertebrate Density (org/m2)	966 ± 1041	995 ± 611	No
Species Diversity - Shannon Weiner	2.40	2.25	

Table 2. Biological soil characteristics for both sites. A complete list of soil invertebrates can be found in Appendix I. A Rank Sum test (α=0.05), indicated no difference between sites.

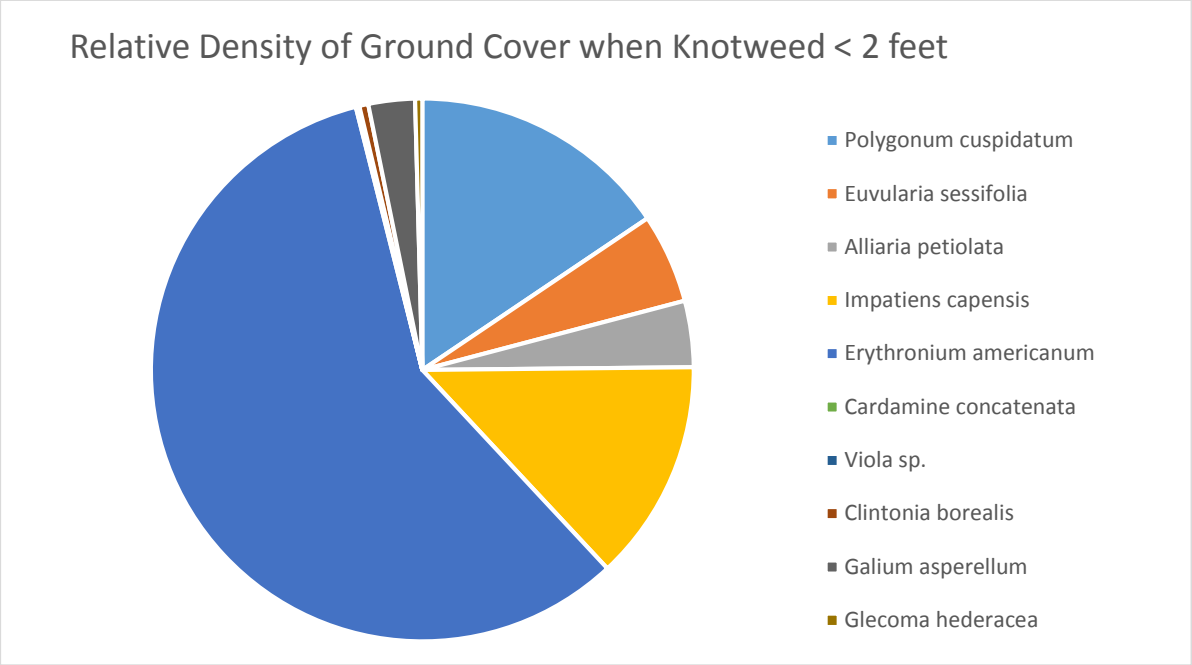


Figure 2. Relative density of herbaceous ground cover when the knotweed is below 2 feet. The dominant species is Erythronium americanum, known by the common name Yellow Trout Lily.

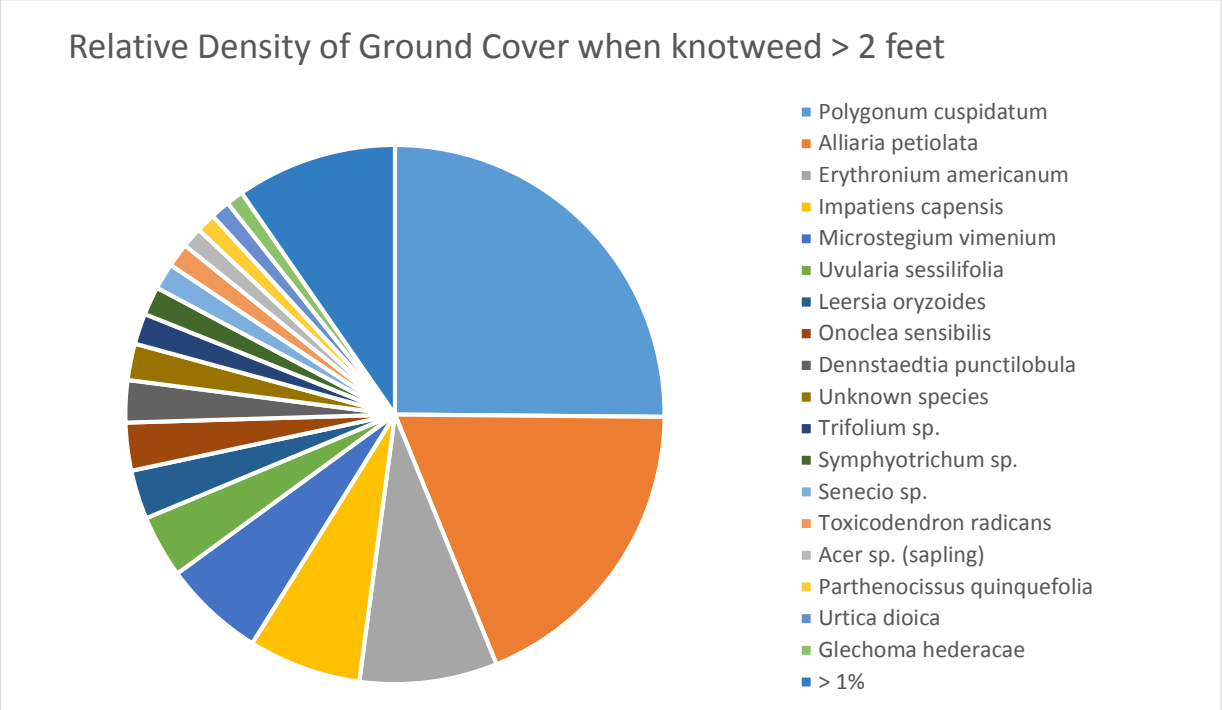


Figure 3. Relative density of herbaceous ground cover when the knotweed is 2 feet or above. Knotweed is the dominant species. The section labeled > 1% represents all the species that had a relative density less than 1 % (see Appendix II Table 6 for complete list).

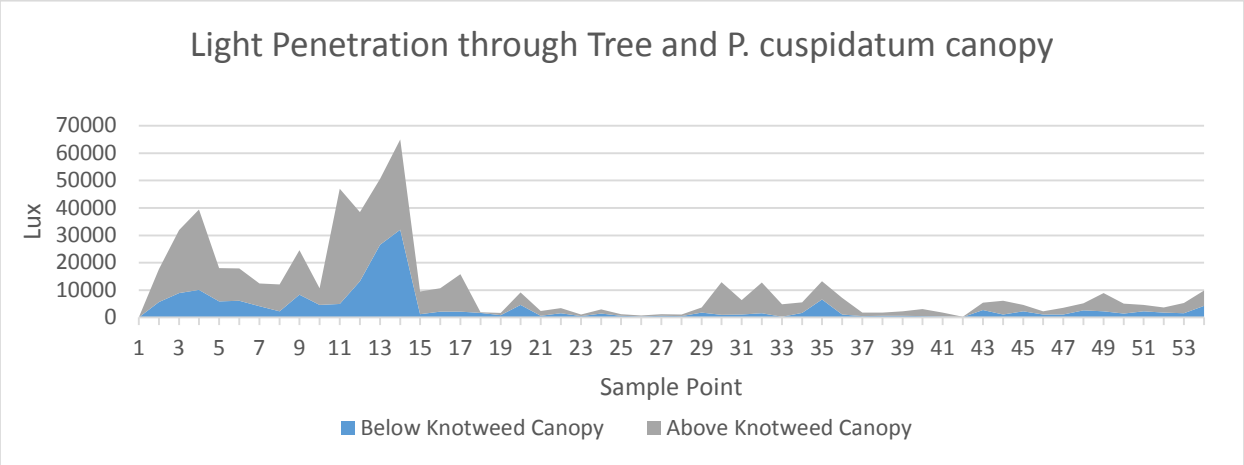


Figure 4. The light available above the knotweed is shown in grey and the light available below the knotweed is shown in blue. On average, only 49.08% of the light is available underneath the knotweed canopy.

LRP	
Species	**Importance Value**
Acer rubrum	98.69
Acer saccharinum	119.47
Acer saccharum	7.40
Acer sp.	12.50
Fraxinus pennsylvanica	9.73
Juglans nigra	7.35
Quercus prinus	9.85
Robinia psuedoacacia	10.17
Ulmus sp.	17.76
Vibernum plicatum	7.09
Total	300.00

Table 3. Includes all tree species found at the LRP site with their importance value.

MA	
Species	**Importance Value**
Acer rubrum	43.98
Acer saccharinum	38.21
Acer saccharum	90.40
Betula lenta	13.04
Celtis occidentalis	9.80
Fraxinus pennsylvanica	7.66
Liriodendron tulipifera	6.67
Pinus strobus	11.22
Platanus occidentalis	7.12
Robinia psuedoacacia	32.91
Tsuga canadensis	9.06
Ulmus fulva	9.92
Total	**280.00**

Table 4. Includes all tree species identified at the MA site with their importance value.

Average Daily Temperature (°C) of Mill Creek during the Leaf Pack Study		
Period	**Average °C ± SD**	**Significant Difference**
May 20 – August 15	17.6 ± 3.3	Yes
Sept 1 – October 31	13.3 ± 2.1	

Table 5. The average daily temperature (in °C) from the data logger during the leaf pack study.

	Summer			Fall	
Leaf species	**Average +/- SD**	**Speed**		**Average ± SD**	**Speed**
Silver Maple	0.0370 +/- 0.006	Fast		---	---
Japanese Knotweed	0.0525 +/- 0.008	Fast		0.01132 +/- 0.0084	Fast
Pin Oak	0.0167 +/- 0.001	Fast		0.00198 +/- 0.0021	Slow

Table 6. Leaf Decomposition rates (k-value) for the summer and fall leaf pack study. The species in the summer are significantly different from each other and the Knotweed and Oak are significantly different between seasons based on a Two-Way ANOVA (α=0.05).

Average ± SD Fungal Biomass (µg/leaf disc)		
	Pin Oak	**Japanese Knotweed**
Summer	0.438 ± 0.636	0.351 ± 0.215
Fall	0.705 ± 0.995	0.216 ± 0.148

Table 7. Average Fungal Biomass over the entire incubation period for Japanese knotweed and Pin Oak.

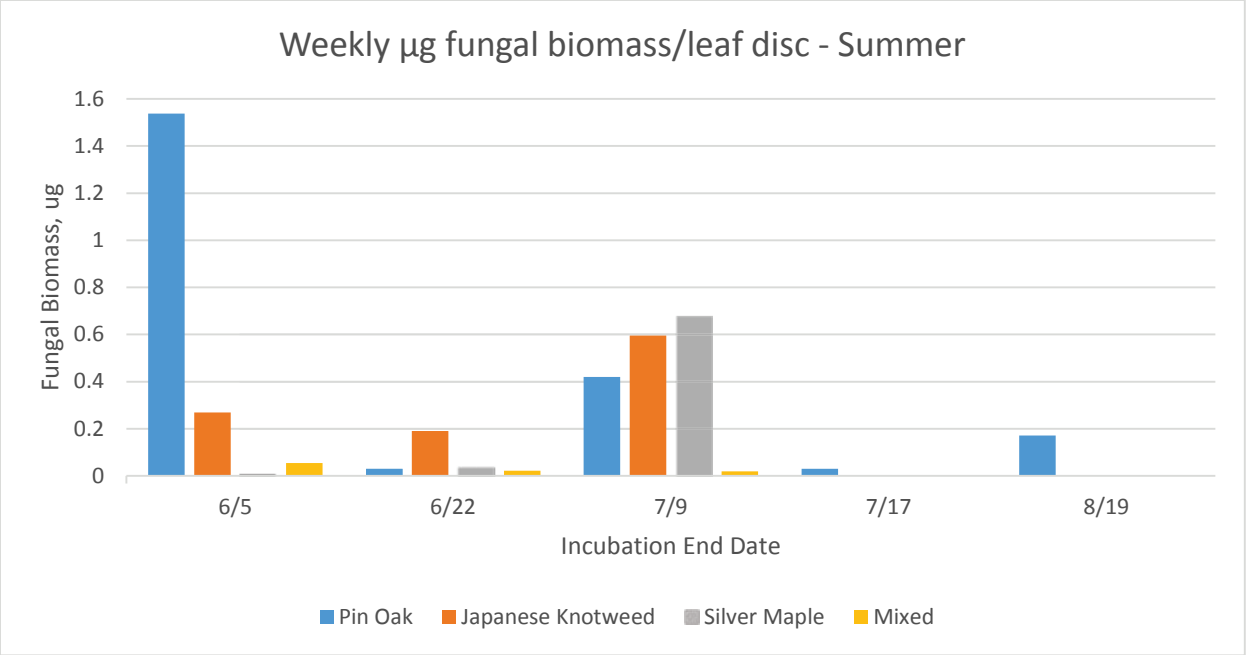


Figure 5. Fungal biomass after each incubation period for the summer leaf pack study. Leaf packs were deployed May 29. These values are converted from ergosterol concentrations (a full table of ergosterol values and a sample chromatograph can be found in Appendix II).

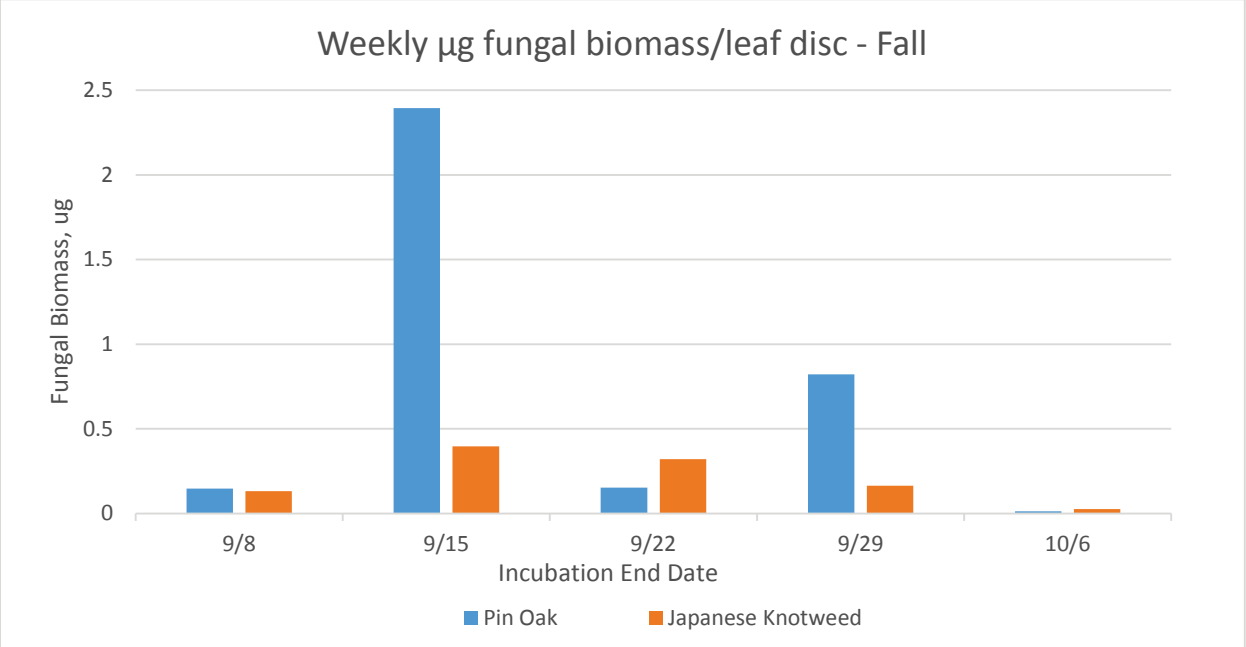


Figure 6. Fungal biomass after each incubation period for the fall leaf pack study. Leaf packs were deployed September 1.

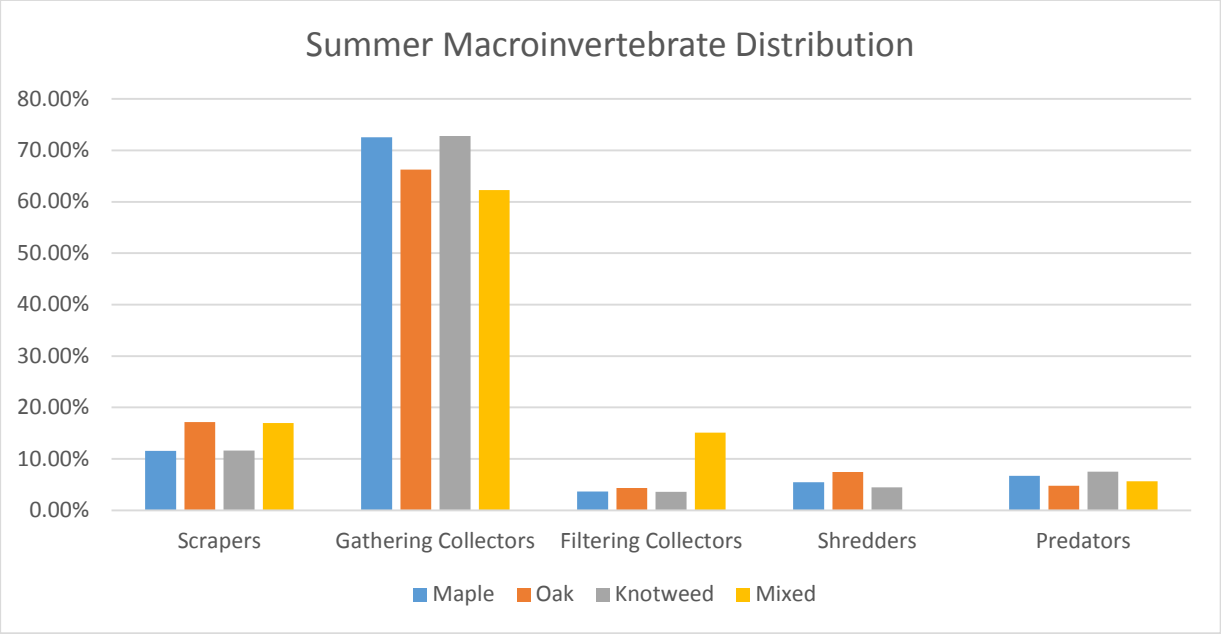


Figure 7. Macroinvertebrate distribution in the leaf packs during the summer incubation period. A full list of macroinvertebrates can be found in Appendix II)

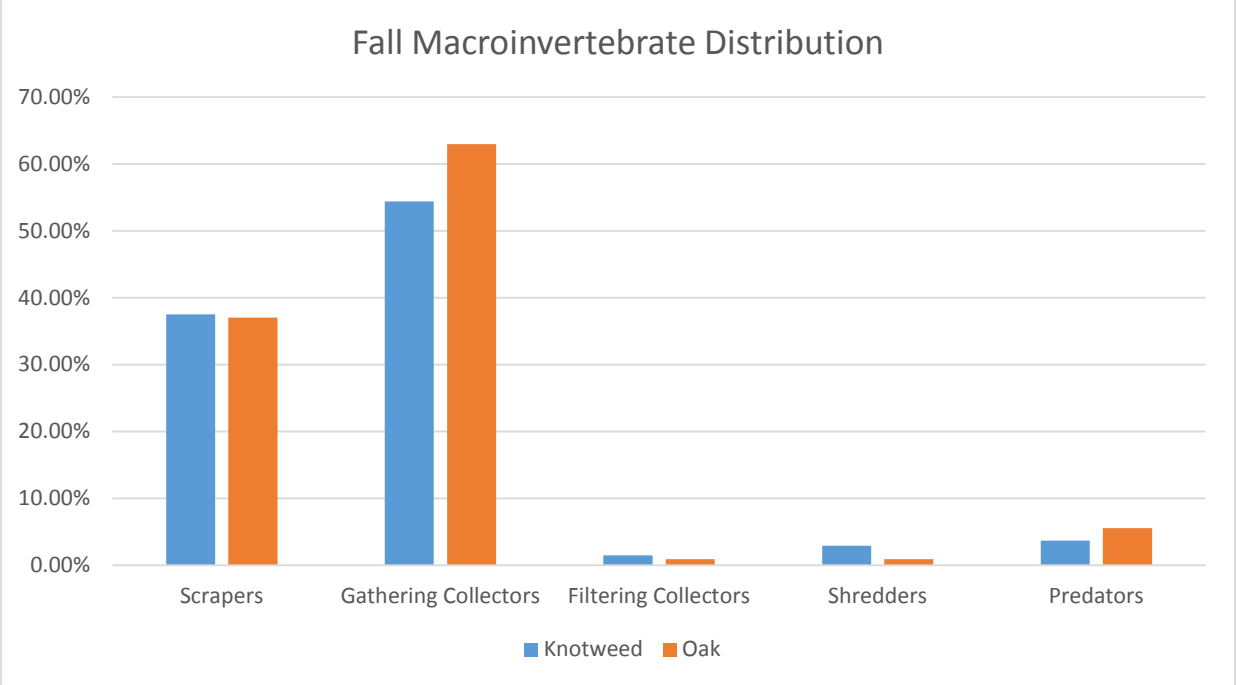


Figure 8. Macroinvertebrate distribution in the leaf packs during the fall incubation period.

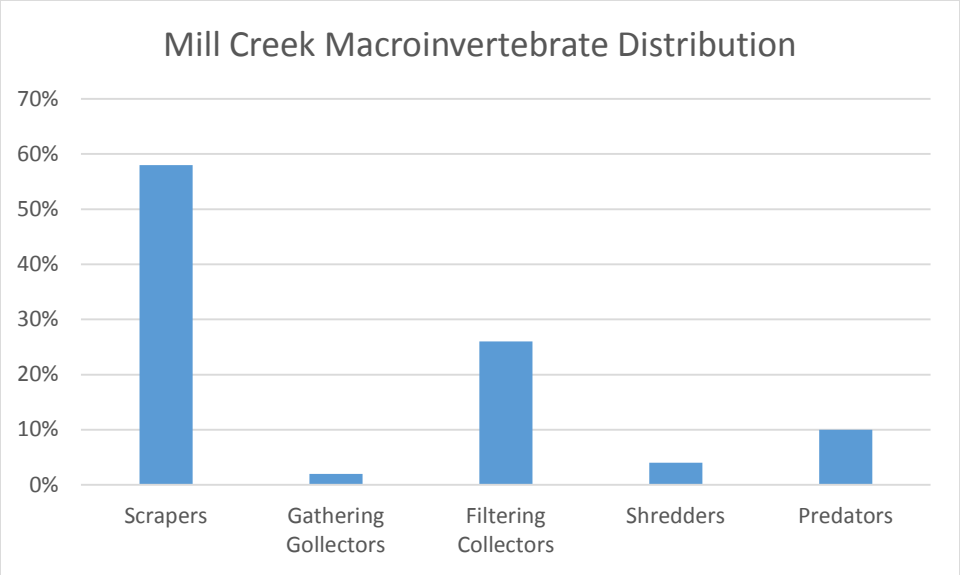


Figure 9. Reference macroinvertebrate distribution from in Mill Creek during September 2015.

References

Bouyoucos, BJ. 1927. The hydrometer as a method for the mechanical analysis of soils. Soil Science. 23: 343-353.

Cox, GW 1976. Laboratory manual of general ecology. Wm. C. Brown Co.: Dubuque, Iowa. 232p.

Cottam, G, Curtis JT, Hale BW. 1953. Some Sampling Characteristics of a population of randomly dispersed individuals. Ecology 34:741-757.

Cummins K, Wilzback MA, Gates DM, Perry JB, Taliaferro WB. 1989. Shredders and riparian vegetation: leaf litter that falls into streams influences communities of stream invertebrates. BioScience 39(1):24

Fan P, Hostettman K, Lou H. 2010. Allelochemicals of the invasive neophyte *Polygonum cuspidatum* Sieb. & Zucc. (polygonaceae). Chemoecology 20:223-227.

Gessner MO, Chauvet E. 1993. Ergosterol-to-Biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. 59(2): 502-507.

Gessner MO, Chauvet E. 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75(6): 1807-1817.

Graca M. 2001. The role of invertebrates on leaf litter decomposition in streams – a review. Internat. Rev. Hydrobiol. 86(4-5): 383-393.

Hawes E, Smith M. 2005. Riparian buffer zones: functions and recommended widths. Yale School of Forestry and Environmental Studies.

Lecerf A, Patfield D, Bioché A, Riipinen MP, Chauvet E, Dobson M. 2007. Stream ecosystems respond to riparian invasion by Japanese knotweed (*Fallopia japonica*). Can. J. Fish. Aquat. Sci. 64: 1273-1283.

Newell SY, Arsuffi TL, Fallon RD. 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. Appl. Environ. Microbiol. 54(7): 1876-1879.

Ofstrofsky ML. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J. N. Am. Benthol. Soc. 16(4): 750-759.

Peterson R, Cummins K. 1974. Leaf processing in a woodland stream. Freshwater Biol. 4:343-368.

Shaw RH, Bryner S, Tanner R. 2009. The life history and host range of the Japanese knotweed psyllid, Aphalara itadori Shinji: potentially the first classical biological weed control agent for the European Union. Biol. Control 49(2):105-113.

Stone, KR. 2010. Polygonum sachalinense, P. cuspidatum, P. x bohemicum. http://www.fs.fed.us/database/feis/plants/forb/polspp/all.html

Suberkropp K. 2001. Fungal growth, production, and sporulation during leaf decomposition in two streams. Appl. Environ. Microbiol. 67(11):5063-5068.

Suberkropp K, Klug MJ. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57(4): 707-719.

Tharayil N, Alpert P, Bhowmik P, Gerard P. 2013. Phenolic inputs by invasive species could impart seasonal variations in nitrogen pools in the introduced soils: a case study with *Polygonum cuspidatum*. Soil Biol. Biochem. 57:858-867.

Volker, EJ, DiLella K, Terneus K, Baldwin C, Volker I. 2000. The determination of ergosterol in environmental samples: An interdisciplinary project involving techniques of analytical and organic chemistry. J. Chem. Ed. 77(12): 1621-1623.

Weston LA, Barney JN, DiTommaso A. 2005. A review of the biology and ecology of three invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant Soil 277:53-69.

Xiao K, Xuan L, Xu Y, Bai D, Zhong D. 2002. Constituents from Polygonum cuspidatum. Chem. Pharm. Bull. 50(5): 605-608.

Appendix I

Equations and Ergosterol Standard Curve

Relative Density

$$Relative\ Density\ = \frac{\#\ of\ Individuals\ of\ a\ Species}{Total\ \#\ of\ Individuals\ of\ All\ Species} \times 100$$

Importance Value

$$Importance\ Value = Relative\ Density + Relative\ Dominance + Relative\ Frequency$$

Leaf Decomposition Rate (K-value)

$$\frac{-\{ln(post - incubation\ surface\ area/pre - incubation\ surface\ area)\}}{incubation\ time}$$

Ergosterol Stock Standard

0.1024g of 98% Ergosterol in 200mL of HPLC grade MeOH

$$\frac{0.104g\ erg\ \ x\ \ 0.98\ \ x\ \ (\mu g\ 10^6)}{200mL\ MeOH} = 501.8\mu g\ erg/\ mL\ MeOH$$

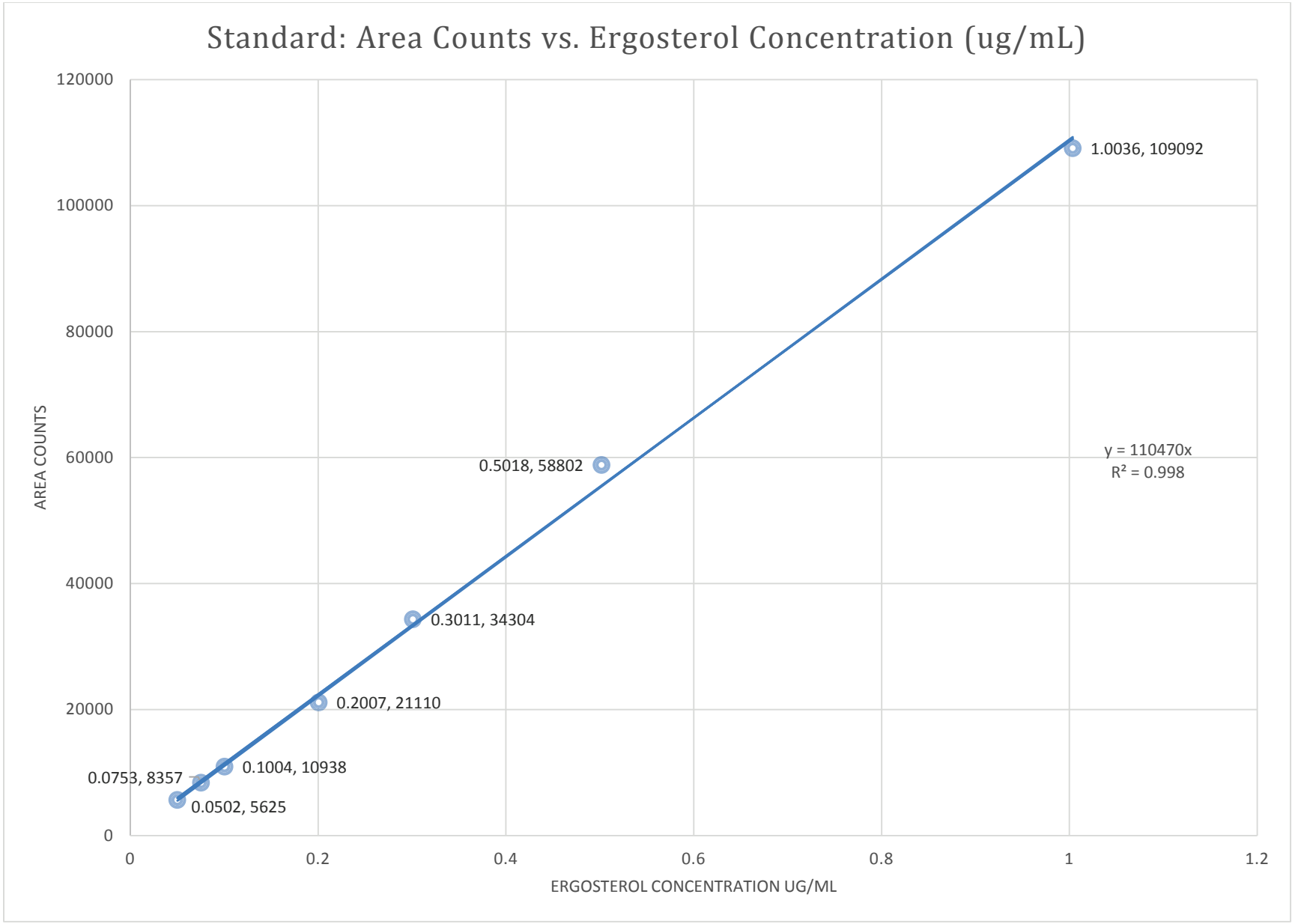
Ergosterol Standard Curve

Standards were made by diluting the stock standard (5.018µg erg/ml MeOH) to different concentrations in methanol. Each standard was injected at the same volume.

Intended Standard Concentration (µg erg/ mL MeOH)	**Volume of Stock Standard**	**Volume Diluted to with MeOH**	**Final Concentration (µg erg/ mL MeOH)**
1.0	10 mL	50mL	1.0036
0.5	10 mL	100mL	0.5018
0.3	6 mL	100mL	0.3011
0.2	4 mL	100mL	0.2007
0.1	2 mL	100mL	0.1004
0.075	1.5 mL	100mL	0.0753
0.05	1 mL	100mL	0.0502

Ergosterol to Fungal Biomass Conversion

$$Conversion\ Factor = \frac{182.6g\ fungal\ biomass}{g\ ergosterol}$$


Standard: Area Counts vs. Ergosterol Concentration (ug/mL)
120000
100000
80000
60000
40000
20000
0
AREA COUNTS
0
0.2
0.4
0.6
0.8
1
1.2
ERGOSTEROL CONCENTRATION UG/ML
1.0036, 109092
0.5018, 58802
0.3011, 34304
0.2007, 21110
0.1004, 10938
0.0753, 8357
0.0502, 5625
y = 110470x
R² = 0.998

Appendix II

Reference Data

Parameter	Average ± SD
pH	6.59 ± 0.20
Conductivity (uS)	100.17 ± 4.90
Alkalinity (ppm)	24.17 ± 2.14
Orthophosphate (ppm)	0.13 ± 0.04
Total Phosphorous (ppm)	0.21 ± 0.05
Nitrate (ppm)	1.03 ± 0.33
Nitrite (ppm)	0.003 ± 0.0008
Dissolved Oxygen (ppm)	8.81 ± 0.33
Temperature °C	16.50 ± 0.18
BOD (ppm)	2.27 ± 0.16
TDS (ppm)	50.40 ± 3.15
Turbidity (NTU)	2.08 ± 0.08

Table 1. Chemical/Physical data from Mill Creek. Data is from monthly grab samples during a 6 month period (May – September 2015).

Parameter	
Surber Density (org/m2)	693
Total Taxa	21
Species Diversity (Shannon-Weiner)	4.11

Table 2. Biological data for Mill Creek from July and September 2015.

Order/Class (common name)	LRP Site	MA Site
Order Aranea (spider)	3	8
Order Hymenoptera (ant)	3	2
Order Acari (mites and ticks)	42	28
Order Thysanura (bristletails)	4	2
Order Colomboloid (springtails)	73	37
Cranefly larvae	0	2
Black Fly Larvae	0	1
Moth Larvae	0	1
Order Coeleoptera (beetles)	3	1
Order Diptera (fly)	0	1
Order Annelida (earthworm)	7	1
Class Symphyla (garden centipede)	1	0
Order Protura	3	0
Order Isopoda (pill bugs)	6	2
Order Psuedoscorpiones	2	0
Class Chilopoda (centipede)	2	0
Class Gastropoda (snail)	1	0
Class Diplopoda (millipedes)	3	0
Order Lepidoptera (caterpillar)	1	0

Table 3. The number of each soil invertebrate at each site.

Trees	
Scientific Name	**Common Name**
Acer rubrum	Red Maple
Acer saccharinum	Silver Maple
Acer saccharum	Sugar Maple
Acer sp.	Maple
Betula lenta	Sweet Birch
Celtis occidentalis	Common Hackberry
Fraxinus pennsylvanica	Green Ash
Juglans nigra	Eastern Black Walnut
Liriodendron tulipifera	Tuliptree or Yellow Poplar
Pinus strobus	Eastern White Pine
Platanus occidentalis	American Sycamore
Quercus prinus	Chestnut Oak
Robinia psuedoacacia	Black Locust
Tsuga canadensis	Eastern Hemlock
Ulmus fulva	Slippery Elm
Ulmus sp.	Elm
Viburnum plicatum*	Japanese snowball

Table 4. The scientific and common name of all trees.

Herbaceous Plants	
Scientific Name	**Common Name**
Acer sp. (sapling)	Maple Tree Sapling
Alliaria petiolata	Garlic Mustard
Cardamine concatenata	Cutleaf toothwort
Clintonia borealis	Bluebead
Dennstaedtia punctilobula	Eastern Hayscented Fern
Erythronium americanum	Yellow Trout Lily
Uvularia sessilifolia	Sessileleaf Bellwort
Galium asprellum	Rough Bedstraw
Glechoma hederacae	Ground Ivy
Impatiens capensis	Jewelweed
Leersia oryzoides	Rice cutgrass
Microstegium vimenium	Japanese stiltgrass
Onoclea sensibilis	Sensitive Fern
Parthenocissus quinquefolia	Virginia Creeper
Polygonum cuspidatum	Japanese knotweed
Senecio sp.	Ragwort sp.
Symphyotrichum sp.	Aster
Toxicodendron radicans	Poison Ivy
Trifolium sp.	Clover
Urtica dioica	Stinging Nettle
Viola sp.	Violet

Table 5. The scientific and common name of all herbaceous plants.

Herbaceous Plants Less than 1%	
Scientific Name	**Common Name**
Ageratina altissima/aromatica	White Snakeroot
Ambrosia artemisiifolia	Common Ragweed
Arisaema triphylum	Jack-in-the-Pulpit
Aster sp.	Aster
Berberis thunbergii	Japanese Barberry
Boehmeria cylindrica	Smallspike False Nettle
Carex sp.	Sedge
Circaea lutetiana	Broadleaf Enchanter's-nightshade
Danthonia sp.	Oatgrass
Delphinium sp.	Larkspur
Dryopteris intermedia	Evergreen Woodfern
Dryopteris marginalis	Marginal Woodfern
Echinocystis lobata	Wild Cucmber
Eupatorium perfoliatum	Common Boneset
Galium sp.	Bedstraw
Gaultheria procumbens	Eastern Teaberry
Hosta sp.	Plantain Lily
Lindera benzoin	Northern Spicebush
Lysimachia nummularia	Creeping Jenny
Maianthemum racemosum	Feathery False Lily of the Valley
Oxalis stricta/europea	Common Yellow Woodsorrel
Poa sp.	Grass
Podophyllum peltatum	Mayapple
Polygonatum biflorum	Smooth Solomon's Seal
Polygonum sp.	Knotweed
Quercus sp. (sapling)	Oak Tree Sapling
Romulea rosea*	Rosy Sandcrocus
Rosa multiflora	Multiflora Rose
Rumex obtusifolius	Bitter Dock
Rumex sp.	Dock
Sanguinaria canadensis	Bloodroot
Staphylea trifolia	American Bladdernut
Symplocarpus foetidus	Skunk Cabbage
Thaspium trifoliatum	Purple Meadowparsnip
Viola cucullata	Marsh Blue Violet
Viola sp.	Violet

Table 6. List of species that are less than 1% relative density from Figure 4 in Appendix 1.

Sample	ug Ergosterol/Sample
Pin Oak 9/8/15	0.0072
Knotweed 9/8/15	0.0072
Pin Oak 9/15/15	0.1311
Knotweed 9/15/15	0.0217
Pin Oak 9/22/15	0.0004
Pin Oak Part 2 9/22/15	0.0079
Knotweed 9/22/15	0.0175
Pin Oak 9/29/15	0.0450
Knotweed 9/29/15	0.0090
Pin Oak 10/6/15	0.0007
Knotweed 10/6/15	0.0014
Pin Oak 6/5/15	0.1011
Knotweed 6/5/15	0.0177
Maple 6/5/15	0.0009
Mixed 6/5/15	0.0027
Pin Oak 6/22/15	0.0019
Knotweed 6/22/15	0.0125
Maple 6/22/15	0.0029
Mixed 6/22/15	0.0011
Pin Oak 7/9/15	0.0276
Knotweed 7/9/15	0.0033
Maple 7/9/15	0.0445
Pin Oak 7/17/15	0.0020
Mixed 7/17/15	0.0004
Pin Oak 8/19/15	0.0056

Table 7. Ergosterol concentrations.

Annelida	**CG**
Oligocheata	
Amphipoda	**SH**
Gammaridae	
Crangonyx	
Gammarus	
Isopoda	
Asellidae	
Caecidotea	SH
Lirceus	CG
Plecoptera	
Capniidae	SH
Leuctridae	SH
Leuctra	
Perlidae	P
Acroneuria	
Perlesta	
Perlodidae	P
Isogenoides	
Pteronarcidae	SH
Pteronarcys	
Coleoptera	
Elmidae	SC
Optioservus	
Promoresia	
Stenelmis	
Dysticidae	
Agabus	P
Psephenidae	SC
Ptilodactylidae	SH
Anchytarsus	

Ephemeroptera	
Baetidae	
Baetis	CG
Heterocleon	SC
Ephemerellidae	
Attenella	CG
Drunella	SC
Eurylophella	CG
Serratella	CG
Ephemeridae	CG
Ephemera	
Heptageniidae	
Heptagenia	SC
Leucrocuta	SC
Nixe	SC
Stenacron	CG
Stenonema	SC
Leptophlebiidae	CG
Leptophelbia	
Paraleptophlebia	
Polymitarcidae	CG
Ephoron	
Tricorythidae	CG
Tricorythodes	
Odonata	**P**
Coenagrionidae	
Argia	
Calopterygidae	
hetaerina	
Megaloptera	**P**
Corydalidae	
Sialidae	
Sialis	

Decapoda	
Cambaridae	CG
Orconectes	
Trichoptera	
Glossosomatidae	SC
Glossosoma	
Hydropsychidae	FC
Cheumatophsyche	
Hydropsyche	
Macrostemum	
Lepidostomatidae	SH
Limnephilidae	SH
Hydatophylax	
Polycentropidae	FC
Neureclipsis	
Polycentropus	
Rhyacophilidae	P
Diptera	
Athericidae	P
Chironomidae	CG
Culicidae	
Aedes	FC
Tabanidae	P
Tipulidae	
Antocha	CG
Dicranota	P
Hexatoma	P
Pedicia	P
Tipula	SH
Gastropoda	
Physidae	CG

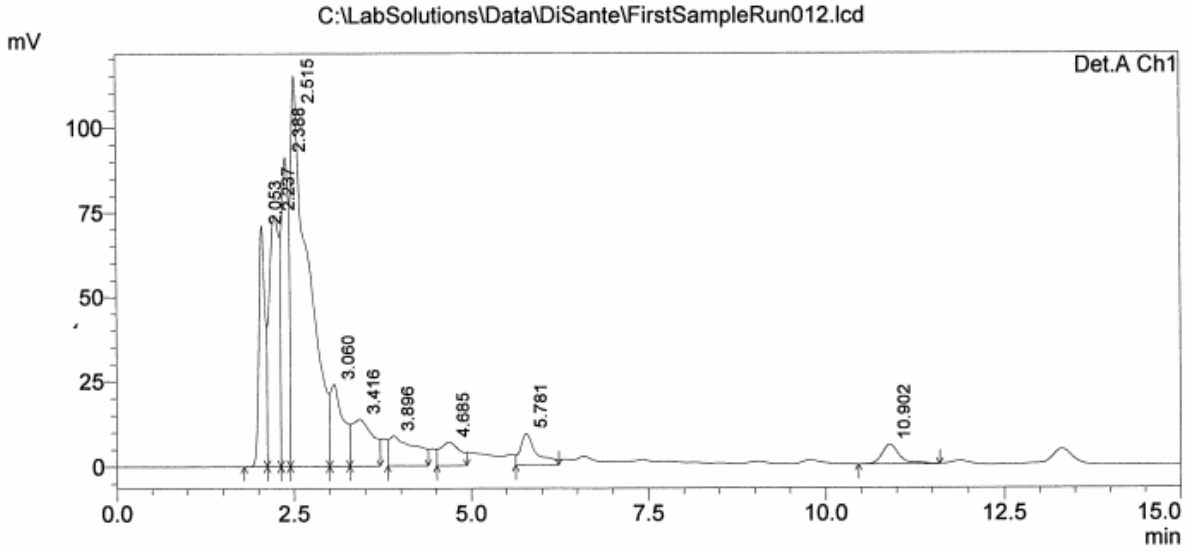
Table 8. Aquatic Macroinvertebrates found in the leaf packs and their corresponding feeding group. {SC=scrapers; SH=shredders; P=predators; CG=gathering collectors; FC=filtering collectors}

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\DiSante\FirstSampleRun012.lcd

Acquired by	: Admin
Sample Name	: Sample1
Sample ID	: Sample12
Tray#	: 1
Vail #	: 12
Injection Volume	: 100 uL
Data File Name	: FirstSampleRun012.lcd
Method File Name	: Standard ergosterol concentration.lcm
Batch File Name	: StandardCurve.lcb
Report File Name	: Default.lcr
Data Acquired	: 2/12/2016 12:10:17 PM
Data Processed	: 2/12/2016 1:20:26 PM

<Chromatogram>



1 Det.A Ch1/282nm

PeakTable

Detector A Ch1 282nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.053	432251	71175	8.736	16.930
2	2.237	737972	74384	14.916	17.694
3	2.388	654504	91364	13.229	21.733
4	2.515	1932595	115214	39.061	27.406
5	3.060	296844	23944	6.000	5.696
6	3.416	284332	13760	5.747	3.273
7	3.896	225976	8758	4.567	2.083
8	4.685	135507	6841	2.739	1.627
9	5.781	146561	9282	2.962	2.208
10	10.902	101106	5677	2.044	1.350
Total		4947648	420399	100.000	100.000