

**The Relationship Between Aquatic Macroinvertebrates, Riparian Vegetation,
and Sediment Nitrogen and Carbon levels in Streams and Irrigation Ditches**

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Abstract

The purpose of our research was to determine how submerged and emergent vegetation in water bodies impact the density and diversity of aquatic macroinvertebrates in the streams and ditches of the Great Plains area. We ran multiple linear regressions between macroinvertebrate population data and vegetation data (width of emergent vegetation, width of submerged vegetation, and biofilm presence), as well as between populations and sediment nutrient loads and water quality parameters. We found that the EPT- Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)- measurements were significantly impacted by the width of the water body and if biofilm was found, but not by how much submerged or emergent vegetation was at the site. However, the number of scuds counted in a sample was significantly affected by the width of the submerged vegetation. Due to this significance, we can see that riparian vegetation is one major parameter that can be used as a tool for improving aquatic health and macroinvertebrate richness, though the parameters other than vegetation were also seen to significantly influence the organisms found in water bodies, Further studies on riparian vegetation and aquatic macroinvertebrates could develop a broader understanding of their relationship, especially by identifying the types of vegetation found, classifying only macroinvertebrates found hiding in the vegetation and ignoring those in the substrate, and developing a more exact method of measuring how much vegetation is present in the whole sample site.

Introduction:

Benthic macroinvertebrates have been increasingly used as an indicator of water health due to their interaction with both water bodies and bottom sediments during their lifecycle, their relative

immobility compared to other organisms like fish, and their relative ease of identification (Hannaford and Resh, 1995). They are also ubiquitous, abundant even in small streams and irrigation ditches, and consist of thousands of species that require different water quality conditions to survive—thus, they are differentially sensitive to changes in water quality. For example, certain macroinvertebrates require high levels of dissolved oxygen (generally indicative of clean, healthy streams) to survive, and others thrive in lower levels of dissolved oxygen (found in more polluted streams) (Bonada et al. 2006). So, by looking at which macroinvertebrates can survive in the ecosystem, we can gain an understanding of the health of the system (Berger et al. 2017).

Macroinvertebrates can also indicate the biological makeup of the ecosystem outside of water quality, as different species have different conditions that they require in order to survive, and knowing these conditions would lend insight into the conditions of the ecosystem. Shredders (Plecoptera and Trichoptera) shred and chew leaves and bark for food; collectors (Ephemeroptera, Plecoptera, Trichoptera, midges, and blackflies) gather or filter primarily detritus; scrapers (Ephemeroptera and Trichoptera) graze biofilm and algae off of exposed rocks and riparian vegetation; predators (Plecoptera, flat bodied Ephemeroptera, and free living Trichoptera) attack and engulf other insects and macroinvertebrates.

Swimmers are characterized by their tendency to cling to the surfaces of rocks in between bouts of swimming from one rock to another. Families in this category include Small Minnow Mayflies (family *Baetidae*), Prong-gilled Mayflies (family *Leptophlebiidae*), and Combmouthed Minnow Mayflies (family *Ameletidae*). Clingers will attach themselves to surfaces with suckers,

claws, or silk. One type of clinger family is the Flatheaded Mayflies (family *Heptageniidae*), which are good indicators of water quality, as the larvae are intolerant of nutrient pollution in the water. The richer in nutrients the habitat is, the greater the diversity of macroinvertebrates that live there. In northwestern Montana, many lotic ecosystems are impacted by various land uses such as agriculture that can structure aquatic macroinvertebrate communities. Streams and modified irrigation ditches occur throughout this arid landscape, which beckons several ecological questions related to stream health.

Since the different macroinvertebrate families have different survival needs, any variations in water conditions found between streams and ditches would likely have an effect on the macroinvertebrate populations found. Astudillo et al. (2016) studied cloud forests in Mexico and found patterns in the abundance of the various genera of aquatic macroinvertebrates depending on the land cover in the catchment area and the riparian vegetation. Streams with similar forest coverage showed slight differences in their abundance, suggesting that greater forest coverage is associated with slight seasonal changes in the structure and composition of insect types because they provide more shelter and breeding grounds for adults of most aquatic macroinvertebrates and buffer the variations in some water chemical variables. Their findings suggest that insect diversity is closely related to land cover in the catchment area and the characteristics of riparian vegetation, though different insect types are more directly affected by water temperature, chemical composition, and hardness.

Other research done by Masaru et al. in 2012 found that in areas with deer browsing of understory vegetation, aquatic insect assemblages and stream environments were altered due to

increased overland flow and sandy sedimentation of the streambed from the erosion and runoff usually buffered by the vegetation. This increased sediment loading can decrease the surface area of exposed rocks, which decreases the amount of biofilm that grow and can be used by scrapers for food. Carlson et al. found that distance of vegetation to the stream bed was significant for Dipteran family richness and abundance, which both declined with increasing distance, and more so at the agricultural sites than at the forested sites.

Due to previous correlations found between the presence of riparian vegetation and benthic macroinvertebrates, our research was aimed at determining if decreases in vegetation caused by anthropogenic influences and by agriculture could impact the organisms found in water bodies on the Flathead Indian Reservation. We also aimed to look at how vegetation was influenced by the water quality and sediment nutrients, and also how macroinvertebrates were influenced by sediment nutrients and water quality. By understanding these interactions, we can also better understand how changing one parameter of the water body can change those connected to it.

Methods:

Our study took place on the National Bison Range and the Flathead Indian Reservation in Northern Montana. Study sites were chosen based on a visual assessment of the area, where water that flowed alongside agricultural fields and/or were fed by irrigation runoff from fields were considered to be irrigation ditches and highly impacted by anthropomorphic influences, while water bodies that were fed from natural sources (e.g. snowmelt), were not adjacent to farmlands, and/or were filtered by a wetland were considered to be the least disturbed condition (LDC) (Stoddard, 2006). Based on this classification scheme, we chose 12 agricultural ditches

and 2 LDC streams in Moiese Valley. One stream site was fed partly by irrigation ditches, but that water was filtered through a wetland area and therefore the stream was still considered to be pristine. 7 LDC streams were chosen on the National Bison Range, as well as 1 LDC site on the Upper Jocko River, 1 LDC site on the Lower Jocko River, 1 LDC site near Schwartz Lake, 1 LDC site on Magpie Creek, 1 LDC site at the Ninepipes Wildlife Refuge, and 1 irrigation ditch that runs through a ranching property in Dixon County. Figure 1 shows a map of sample sites.

Macroinvertebrate Sampling

Macroinvertebrates were collected manually using a Dip Net at all sites during June and July. Depending on the conditions of the site, a length between 1-3m was chosen at the site, and we agitated the sediment and rocks with our feet for 5 minutes in several spots along this length in order to kick up the macroinvertebrates. We then caught the macroinvertebrates in the Dip Net that had its net submerged slightly downstream. If present, we also chopped at and agitated submerged or emergent vegetation found in the water or near the bank in order to also capture macroinvertebrates that could be found there. In areas with rockier substrate, we also picked up larger rocks and manually picked off macroinvertebrates that adhered to them as these were difficult to dislodge with our feet. Any macroinvertebrates caught were transferred into plastic containers, to which a 70% ethanol solution was added to kill them so that they could be stored and later identified to the taxonomic level of family under a microscope according to Dr. J. Reese Voshell, Jr.'s 'A Guide To Common Freshwater Invertebrates of North America.' Any macroinvertebrates that were too badly damaged to be keyed out or were terrestrial in origin were removed from the sample and not considered in final counts. **Figure 3.a-aa** shows a breakdown of the macroinvertebrate populations at the sampling sites.

Water Quality Measurements and sediment sampling

Using a probe, we also measured dissolved oxygen (DO) levels, total dissolved solids (TDS in ppm), water temperature (*C), pH, and conductivity. Due to time constraints, DO, TDS, temperature, and conductivity was not measured at Schwartz or Magpie, and pH was not measured at Upper Jocko, Jerry's Ditch, Moiese Wetland, and Pauline Creek. Sediment samples were collected at the Moiese Irrigation Ditch, Stipe Ditch, Diagonal Ditch, Cow Ditch, Coyote Trail Ditch, Farmer Dan's Ditch, the NBR Visitor Ditch, Triangle Upper, as these sites had substrate that was mainly composed of sand and silt while other sites were mainly pebbles or larger rocks. The sediment samples were stored in a vacuum sealed bags, stored taken to the University of Montana Department of Environmental Biogeochemistry lab and tested for total carbon and total nitrogen levels. We only tested one sediment sample at each site as we assumed that soil conditions would not vary significantly along the length of our transect since the habitat and substrate type did not change.

Vegetation Assessment

Vegetation was measured by taking the width of the water body, the width of the emergent vegetation, and the width of the submerged vegetation at one place. Emergent vegetation was classified as vegetation that grew from the water but still had a part of the plant out of the water, while submerged vegetation was fully covered by the water. Biofilm was classified in a binary manner, where bodies of water that have a majority of biofilm on the rocks were giving a "1" and those that had little to no biofilm (mainly ditches that had a silt/clay substrate) were given a "0."

Statistics

Before statistics were run on our data, we calculated the Hilsenhoff Biotic Index (HBI) for all sites. The HBI is a common metric used to assess stream health, where a tolerance rating is given to aquatic macroinvertebrate species to indicate their tolerance to polluted water conditions (1 is very sensitive, 10 is very resilient), and so averaging the scores for all organisms caught at a site gave us a pollution level for the site based on the composition of the resident organisms. We also calculated the EPT, as all the species of EPT are indicators of the high quality of stream water, and species richness (the number of unique taxa found) for the sites (Liu et al. 2017). **Figure 1** shows the EPT counts for all sites, and **Figure 2** shows the taxa richness values for all sites. Using the statistical program RStudio Version 0.99.902, we then ran multiple linear regressions insect populations and stream quality, sediment nutrients, and stream vegetation and width, as well as between vegetation and stream health and sediment nutrients.

Results

Overall, we collected macroinvertebrates at 27 sites, tested sediment nutrient loads at 11 sites, measured water quality parameters at 25 sites, and measured pH at 23 sites. The distribution of macroinvertebrate species, the EPT, species richness, and the HBI index was determined for all sites where macroinvertebrates were collected. In total, 7,165 aquatic macroinvertebrates were identified under a microscope, with counts per site ranging from 6 (Parallel Ditch) to 785 (Thistle Stop).

Multiple linear regressions ran between EPT and aquatic vegetation

We ran a multiple linear regression using EPT data as the response variable and site width, width of the submerged vegetation, width of the emergent vegetation, and if biofilm was present as predictor variables. We found no significance between EPT and the width of the submerged vegetation ($p=0.191$) or the width of emergent vegetation ($p=0.11$). However, we did find a significant relationship between EPT and the width of the water body ($p=0.00545$) and the presence of biofilm ($p=0.02359$).

Multiple linear regressions ran between EPT and water quality

We ran a multiple linear regression between EPT and the water quality variables that we measured, and found that TDS was not significant ($p=0.384$), conductivity was not significant ($p=0.951$), temperature was not significant ($p=0.123$), DO was not significant ($p=0.426$), and pH was not significant ($p=0.488$).

Multiple linear regressions ran between macroinvertebrates and sediment nutrients

We ran multiple linear regressions between EPT and sediment nutrient loads and found that EPT values were not significantly affected by total N ($p=0.4852$) or by total C ($p=0.6337$) measured in the sediment sample. We also found no significance between soil nutrients and Coleoptera, Odonata, Diptera, snail, and scud populations. However, Chironomids were significantly related to sediment nitrogen ($p=0.0169$) and carbon ($p=0.0215$).

Multiple linear regressions ran between vegetation, and sediment nutrients and water quality

We ran multiple linear regressions between vegetation data and total N and C levels in the sediment, and found significance between the width of submerged vegetation in the water and

nitrogen ($p=0.00406$) and carbon ($p=0.00064$). Our regressions between the width of emergent vegetation and carbon and nitrogen did not show significance ($p=0.699$, $p=0.573$), nor did our regression between whether biofilm was present and the carbon and nitrogen ($p=0.575$, $p=0.504$). No significance was when we ran regressions between any of the submerged vegetation, emergent vegetation, and biofilm and any of the water quality measurements. We did find that whether or not biofilm was present was impacted by how much submerged vegetation was present ($p=0.0173$)

Multiple linear regressions ran between HBI and taxa richness, and vegetation

We ran a multiple linear regression between HBI and riparian vegetation and found significance for stream width ($p=0.0013$) and the width of the emergent vegetation ($p=0.171$), but not the width of submerged vegetation ($p=0.9509$) or the presence of biofilm ($p=0.7543$). There was no significance between the taxa richness and any of the vegetation parameters.

Multiple linear regressions ran between HBI and taxa richness, and sediment nutrients

We found no significance when running a multiple linear regression between HBI and sediment nutrients or between taxa richness and sediment nutrients.

Discussion

Multiple linear regressions ran between EPT and aquatic vegetation

We found a significant relationship between EPT levels and the width of the water body and the presence of biofilm, but not between EPT and the width of emergent vegetation and the width of submerged vegetation. This could be because Ephemeroptera and Tricoptera are scrapers that eat

biofilm, so they would be present in greater abundance at the sites where biofilm is present, which was found to be influenced by the amount of submerged vegetation. The lack of influence of the submerged vegetation despite the fact that it provides protection from predators, a food source, stability in fast moving water, and are known to filter polluted water, could be because it was harder to fully aggravate the vegetation and collect from anywhere but the fringes, so our sample most likely did not fully encompass the true population found in the vegetation.

Multiple linear regressions ran between EPT and water quality

We did not find that the population of EPT found in our sample was significantly influenced by water quality. This could be because we took these measurements after we had collected and identified our insect samples, and so natural climate variations could have caused changes in these parameters between when macroinvertebrates were collected and when measurements were taken. Also, the probe needed to be professionally calibrated, so the accuracy of these measurements is questionable.

Multiple linear regressions ran between macroinvertebrates and sediment nutrients

Regressions ran between macroinvertebrate populations and the total nitrogen and total carbon levels in the sediment found that the nutrient levels affected only chironomids. Since chironomids are generally found in more anoxic and polluted waters, it would make sense that they can be found in high nutrient sediments that receive runoff from fertilized fields, which more sensitive macroinvertebrate types could not tolerate. Also, sediment from only 11 sample locations were tested, so the results may not fully represent the response of macroinvertebrates to

nutrients, especially since only the sites with mainly a sediment substrate were chosen and so would not reflect the macroinvertebrates that live on rocks and biofilm.

Multiple linear regressions ran between vegetation, and sediment nutrients and water quality

Since we found significance between some aspects of vegetation and the macroinvertebrate community, we looked at which aspects would affect the vegetation. The width of the submerged vegetation was found to be influenced by both the nitrogen and carbon levels, which could be because nitrogen is a fertilizer nutrient that encourages growth in water bodies and carbon is naturally added to water bodies by detritus such as submerged vegetation (Meyer and O'Hop, 1983). The lack of significance between riparian vegetation and biofilm presence could be because the riparian vegetation and biofilm may be more greatly influenced by other parameters such as nutrients in the soil on the bank of the water body or in the water. The insignificance of the water quality measurements could once again be because the readings from the probe were not fully reliable, or were not important for the species of vegetation or biofilm that was growing. Future studies that classified the vegetation and biofilm may find significance.

Multiple linear regressions ran between HBI and taxa richness, and vegetation

When we ran a multiple linear regression between HBI and riparian vegetation, we found significance for stream width and the width of the emergent vegetation. This could be because wider streams generally were LCD streams like the Upper Jocko, which we assumed to be healthier than the narrower irrigation ditches. Also, wider streams were rougher and harder to cross, so macroinvertebrates would rely more on emergent vegetation for stability and food. However, if wider streams were more turbulent, then it is less likely that sediment would settle

and allow submerged vegetation or biofilm to grow and macroinvertebrates that need these conditions to live, so it is unlikely that significance could be found with these variables and the width and emergent vegetation parameters for HBI.

Multiple linear regressions ran between HBI and taxa richness, and sediment nutrients

We found no significance when running a multiple linear regression between HBI and sediment nutrients or between taxa richness and sediment nutrients, possibly because we did not have enough sediment samples to run this analysis. Our samples excluded rockier sites and the macroinvertebrates that may be found only there.

The results of this research could aid in conservation efforts because it will lead to a better understanding of the overall effects that stream changes- such as changes in vegetation due to climate changes and human activities, water temperature, nutrient loadings, DO and organic matter from decomposing and respiring plants, pH levels, and sediment nitrogen and carbon levels- have on the life in the stream. So, if stream conservation measures involve replenishing vegetation and improving stream conditions, and aquatic insect populations are correlated with these factors, then conservationists can use aquatic insect density as a template for measuring the success of their efforts.

Considerations:

Certain methods needed to be modified for some sites where fences crossed over the irrigation ditches, as we had to limit the length of the transect that we made in order to stay outside the gate. We also could not sample across the entire width of certain sites due to current and sheer

size, such as with the Upper Jocko, which could have influenced which macroinvertebrates we found and the sample counts by limiting them to those found only in calmer, shallower waters. Improvements on this project would include focusing more exclusively on classifying vegetation and collecting macroinvertebrates only from the riparian vegetation, while trying to minimize variations in the other variables. However, our study did show that riparian vegetation has some influence on the microorganisms in the stream, and that it can be a parameter of interest in classifying the effectiveness of water rehabilitation efforts.

Acknowledgements

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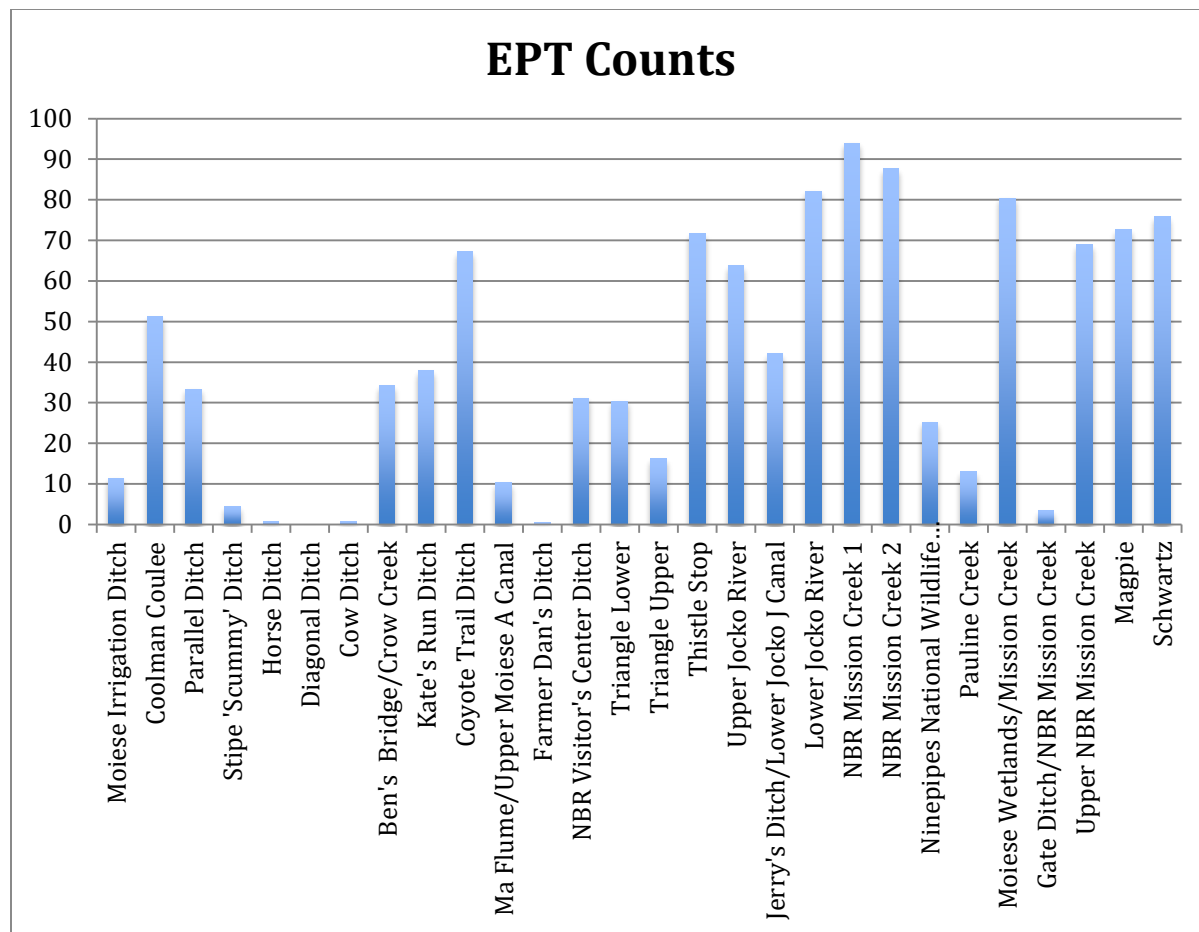


Figure 1: EPT (Ephemeroptera, Plecoptera, Tricoptera) counts for all sampling sites

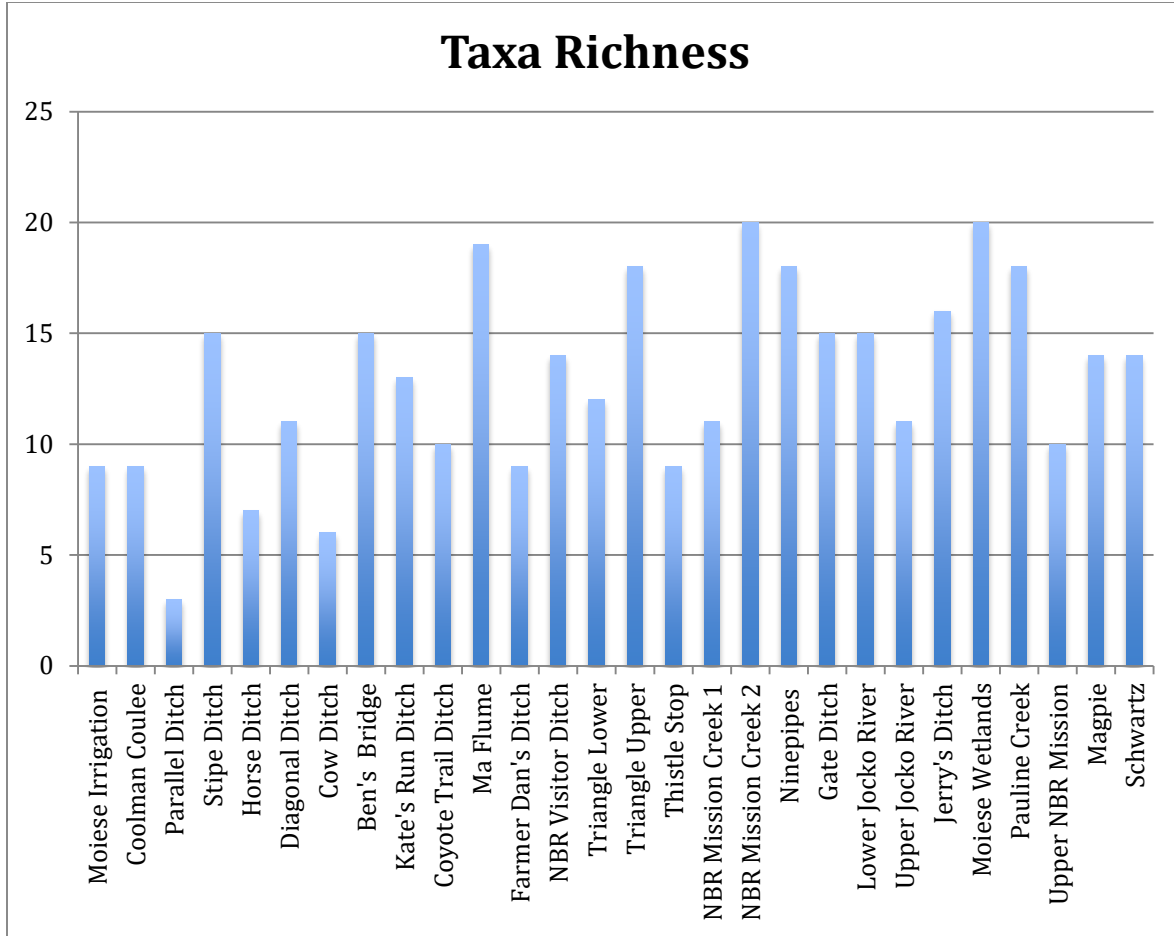
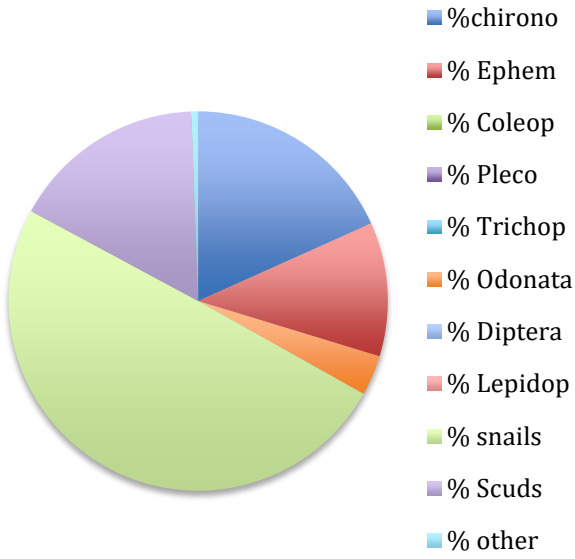
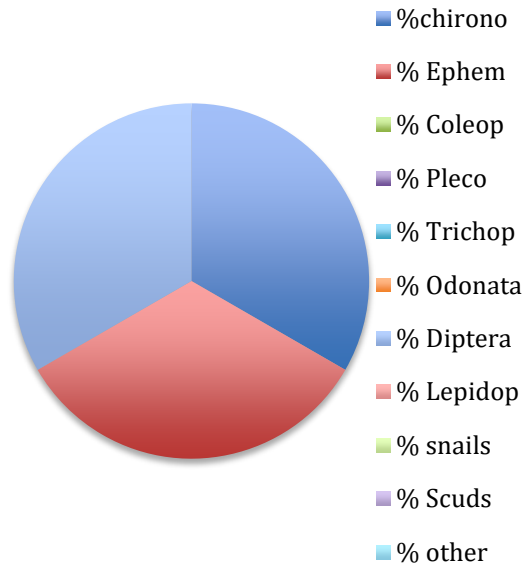


Figure 2: Taxa Richness of macroinvertebrates collected at all sites

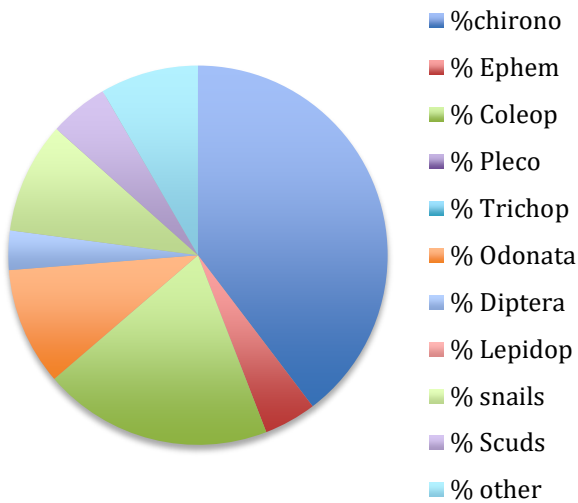
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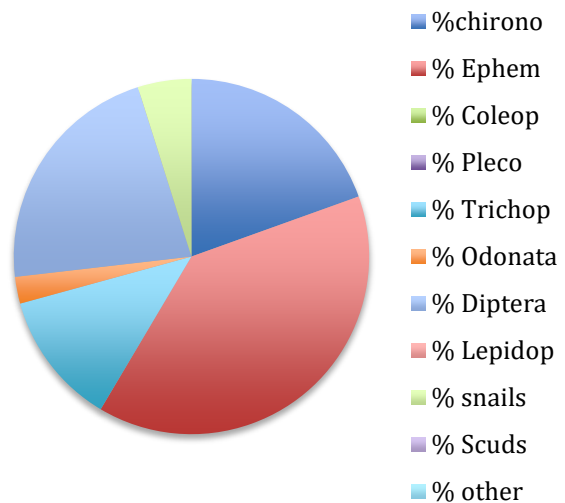
b. Parallel Ditch



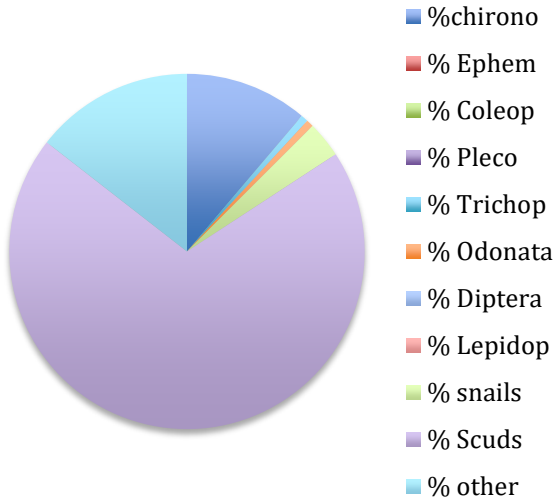
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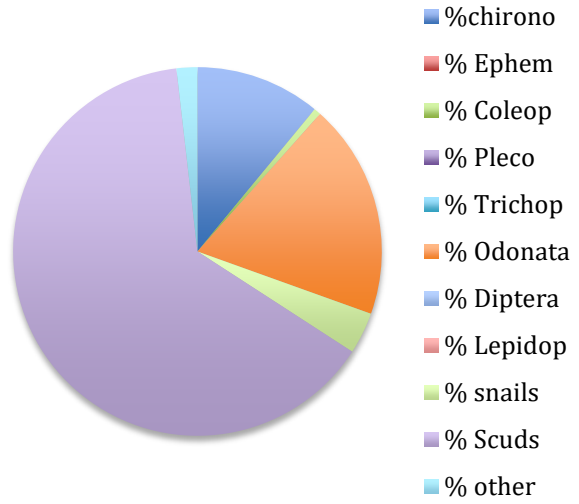
d. Coleman Coulee



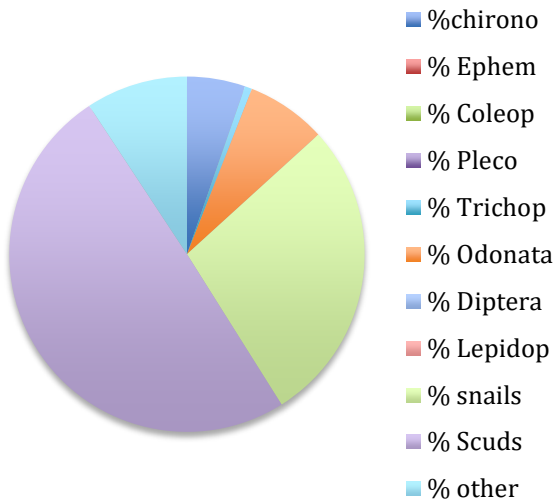
e. Horse Ditch



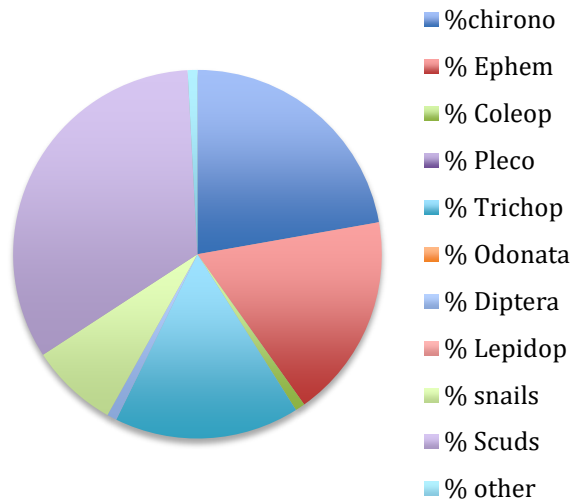
f. Diagonal Ditch



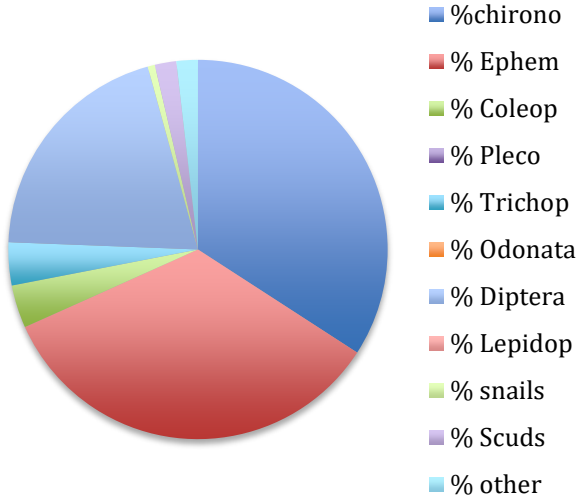
g. Cow Ditch



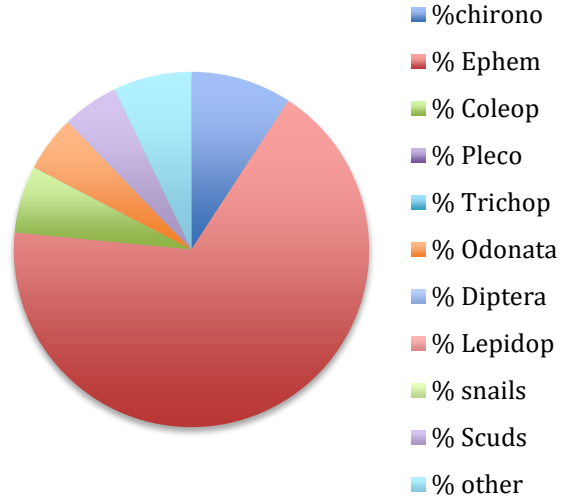
h. Ben's Bridge



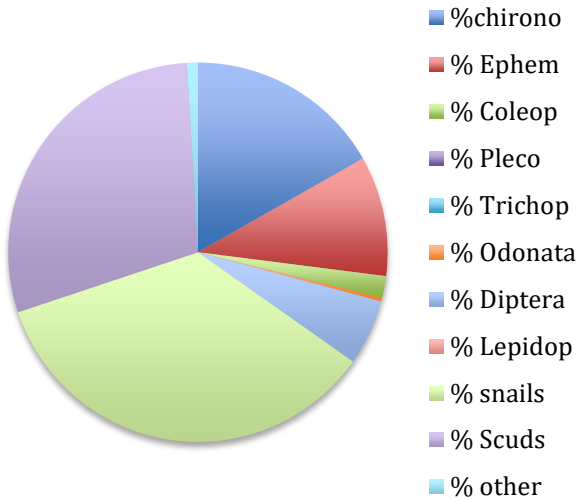
i. Kate's Run Ditch



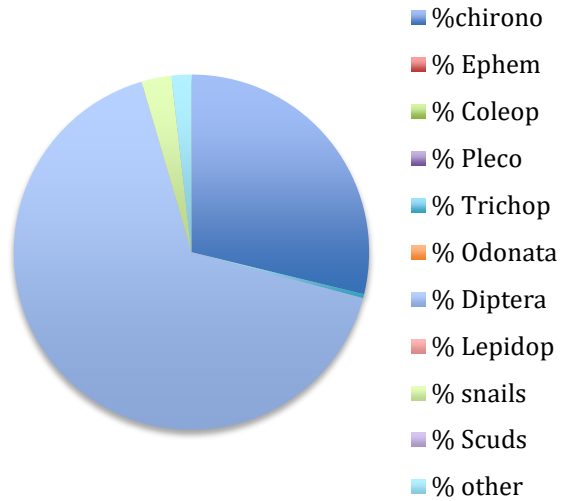
j. Coyote Trail Ditch



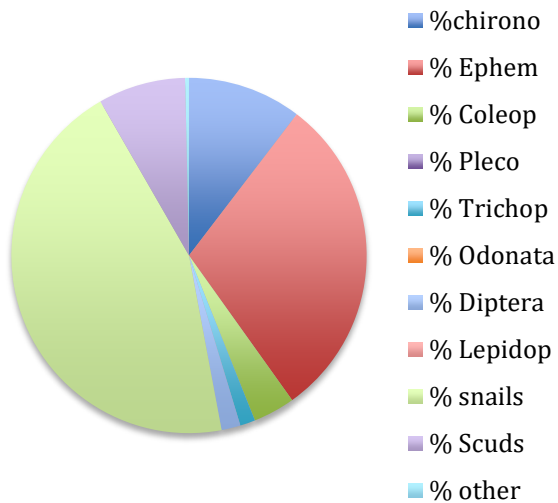
k. Ma Flume



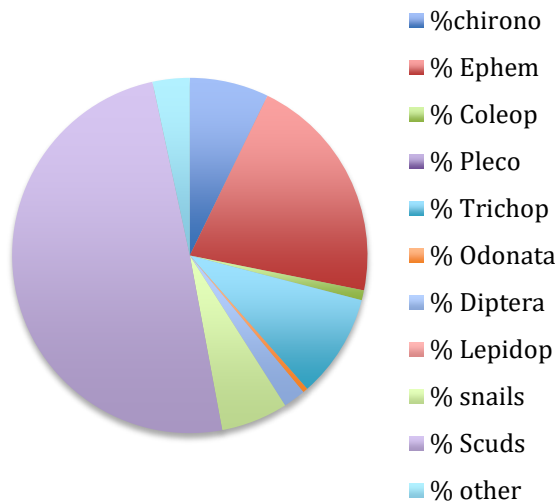
l. Farmer Dan's Ditch



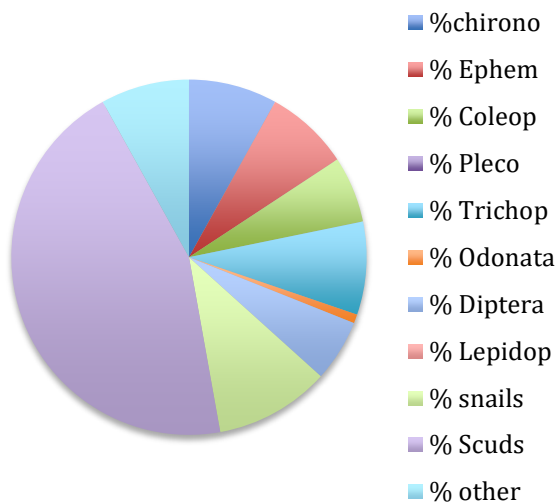
m. NBR Visitor Ditch



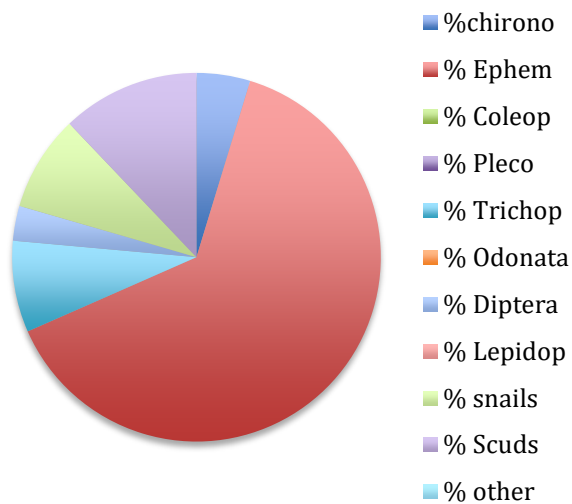
n. Triangle Lower



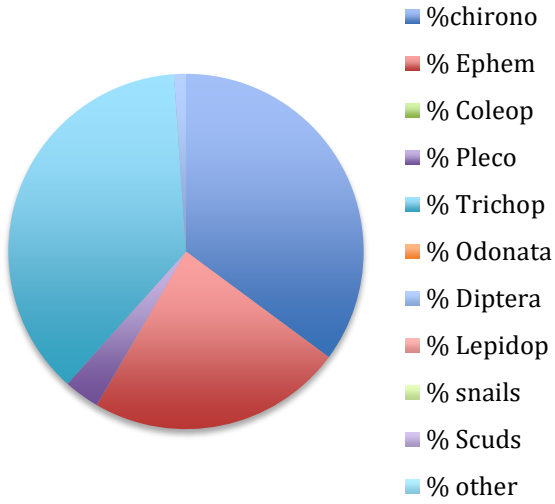
o. Triangle Upper



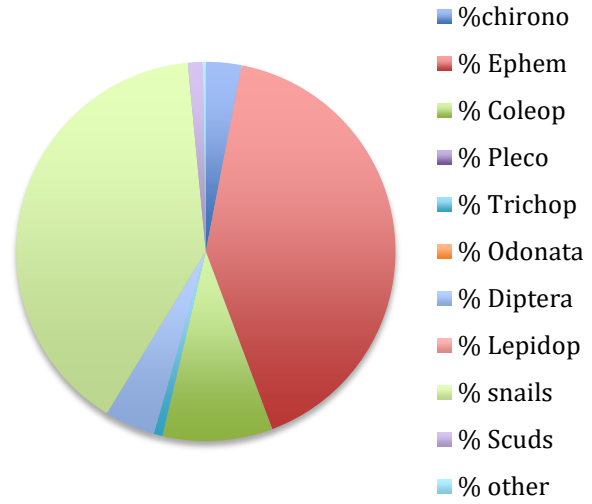
p. Thistle Stop



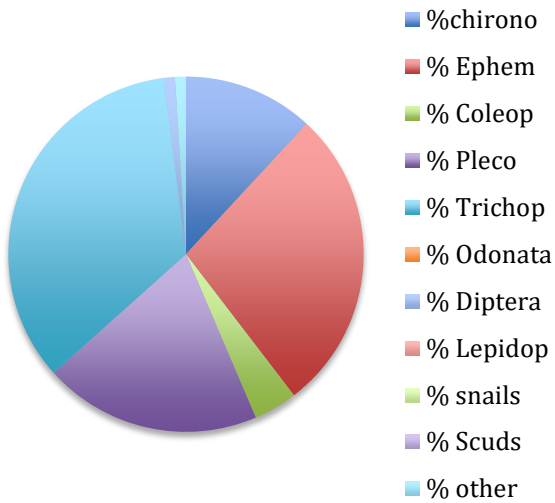
q. Upper Jocko



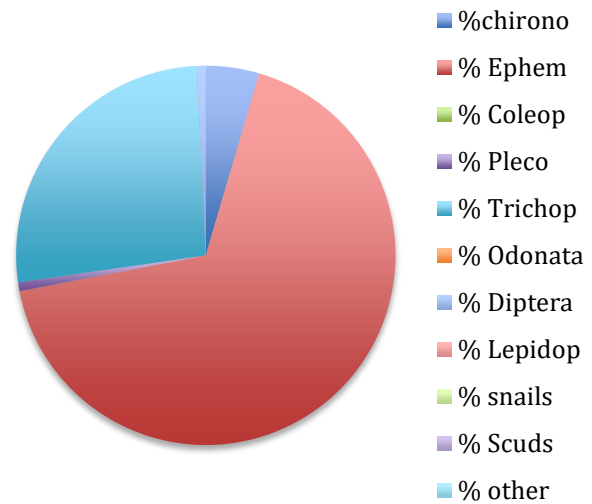
r. Jerry's Ditch



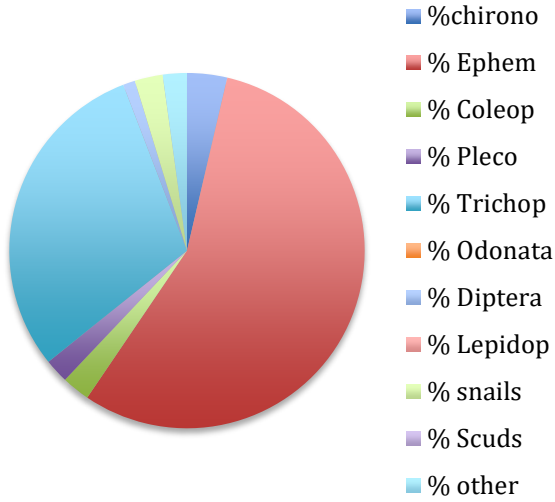
s. Lower Jocko



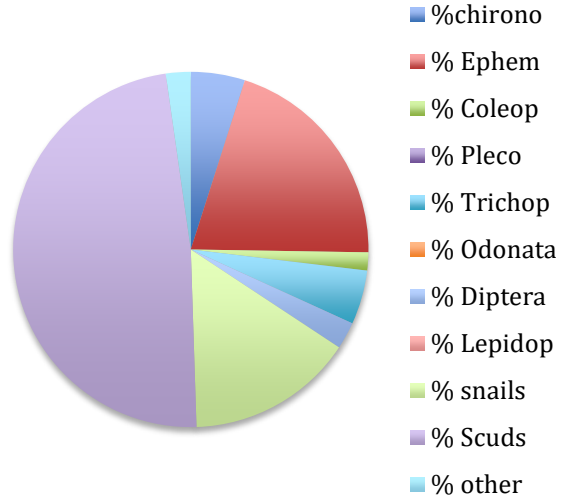
t. NBR Mission Creek 1



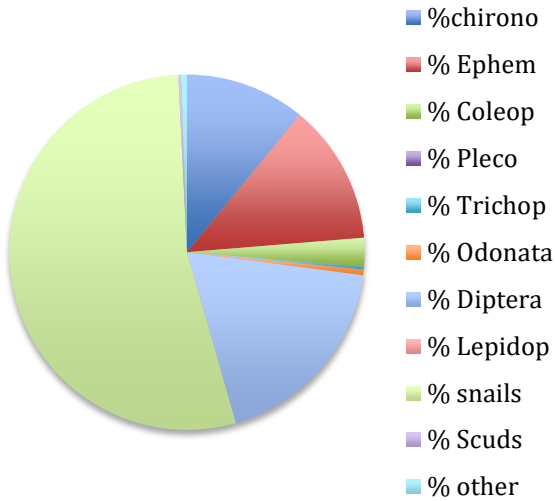
u. NBR Mission Creek 2



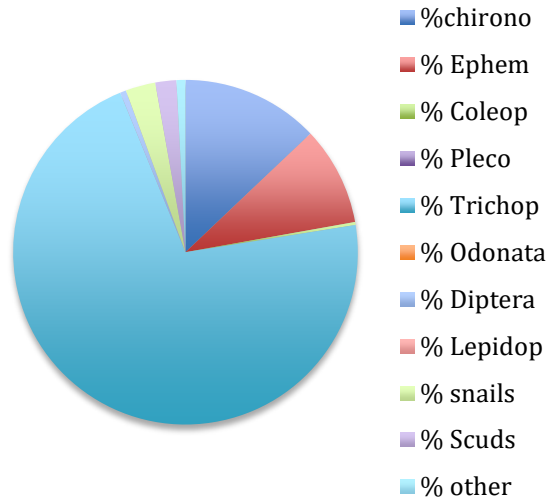
v. Ninepipes Refuge



w. Pauline Creek



x. Moiese Wetland



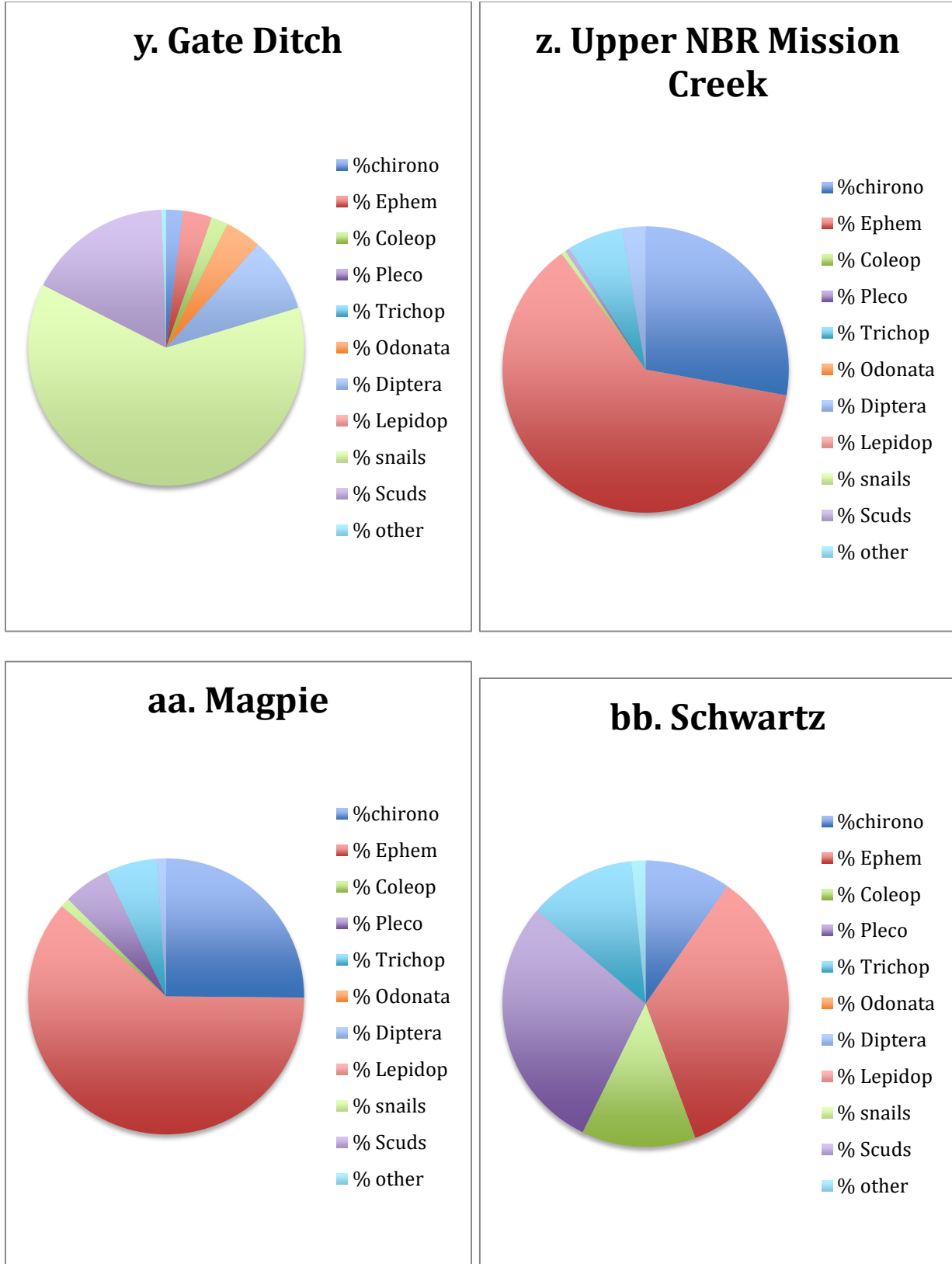


Figure 3.a-aa: Breakdown of macroinvertebrates found at each site

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