Urban Aquatic Ecology Research on Macroinvertebrates in Fort Collins, CO

2022-23 ESS SUPER Program
Skills for Undergraduate Participation in Ecological Research

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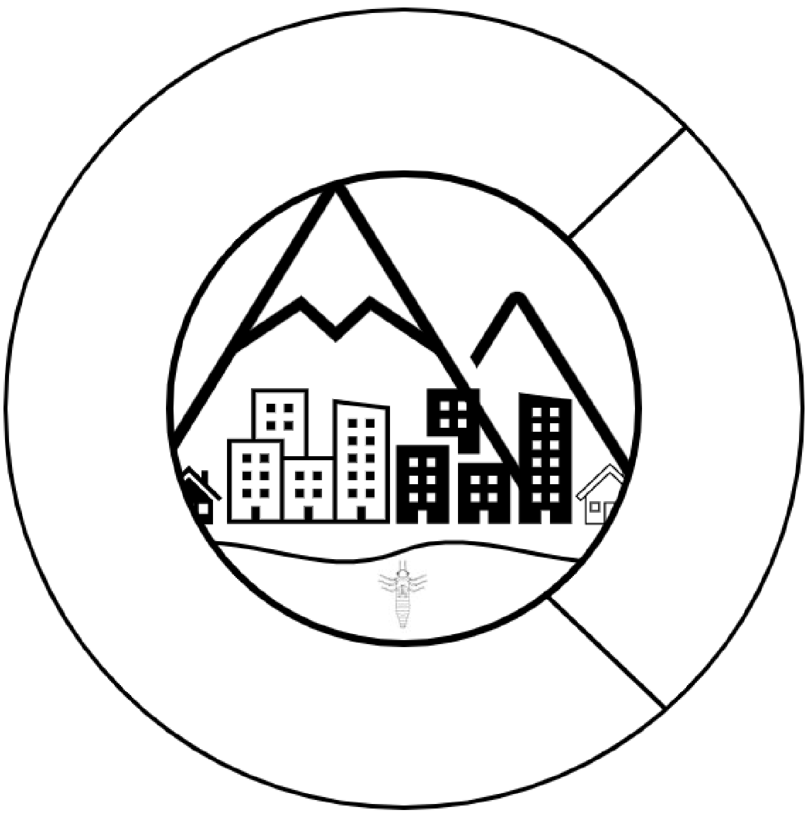

Abstract
As urbanized environments continue to grow, natural aquatic ecosystems become more impacted. To better understand consequences on aquatic environmental change, aquatic macroinvertebrate communities can be used as an indicator of ecosystem health. By sampling 18 different urban water bodies throughout Fort Collins, CO during the Fall of 2022, we were able to characterize macroinvertebrate community structures in ponds, streams, and canals/ditches. The samples were processed by IDing macroinvertebrates to the order level. Preliminary results indicate that species taxonomic richness and distribution are correlated to the different habitat types and the health of the habitat. Canals/ditches tend to have the highest density, while ponds and streams have lower density. Taxonomic richness appears to vary between the three site types. The differences in macroinvertebrate communities may stem from differences in local environmental factors and the amount of human disturbance. Further studies into community structure that include vertebrates and landscape variables are recommended to fully understand environmental change in urban areas.

Research Summary

Introduction
Urban Spaces
The 21st century has been marked by biodiversity loss caused by overconsumption, climate change, and urban development expansion. Enhancing the capacity for high biodiversity in anthropogenically impacted habitats is key to future sustainability, ecosystem services, and biodiversity conservation. A study by Chester and Robson (2013) defines this as 'reconciliation ecology'. This approach provides more resilient communities in the face of human and climatic changes. The human population is projected to increase, so it is imperative to gain knowledge about biodiverse habitats within urban blue space. Urban blue space refers to bodies of water, such as parks, lakes, rivers, canals, and coastal areas, that are found within urban environments. This type of blue space provides an important source of recreation and aesthetic enjoyment for urban residents. In addition, Oertli, B. and Parris, K. M. (2019) discovered that urban blue space can play a role in mitigating the negative impacts of habitat fragmentation and overall biodiversity enhancement.

Waterbody Types
Urban blue spaces can also support ecosystem health by providing insights into macroinvertebrate tendencies between ponds, streams, and canals/ ditches. Aquatic macroinvetebrate communities can track improved biodiversity and overall ecosystem health (Song, 2022). Streams, ponds, and canals/ditches are distinct aquatic ecosystems that differ in their physical and ecological characteristics. Ponds are still or slow-moving bodies of water with

low flow rates and lower oxygen content. They tend to accumulate nutrients and organic matter, leading to high levels of primary productivity and the growth of aquatic plants. These ecosystems rely on the various layers being shore, surface film, open water, and bottom water (DIEM Project, 2019), with different layers, as well as nutrient and oxygen availability, suiting different organisms. Streams are flowing bodies of water that have a higher oxygen content and a constant exchange of water that allows for the transport of nutrients and organisms. They serve as valuable connectivity between one habitat to another while sustaining their own communities of organisms. Streams are typically fed by either springs or runoff, with Fort Collins streams being primarily runoff fed from snow melt. Canals/ditches, which are human-made waterways, tend to be shallow with little or no vegetation, resulting in low primary productivity and low oxygen levels. They also tend to have high levels of nutrients and pollutants due to runoff from surrounding urban landscapes. Canals/ditches and streams experience higher flow rates, but typically include inorganic structures to guide direction. Canals/ditches are designed to assist in irrigation, drainage, or transportation within urbanized areas. While all three types of water bodies are important habitats for various aquatic organisms, they differ in terms of water depth, flow rate, and level of human intervention.

Figure 1: Collage of the three different water body types sampled. Each picture was taken on the day the site was sampled. **Figure 1A:** Picture of the pond at Homestead Natural Area (A. Hall). **Figure 1B:** Picture of the Fossil Creek Outlet stream (F. Carvallo). **Figure 1C:** Picture of the Dixon Canyon Lateral canal (A. Hall).

Aquatic Macroinvertebrates

Aquatic macroinvertebrates are an essential component of freshwater ecosystems, serving as primary or secondary consumers, decomposers, and nutrient cyclers. However, they are widely recognized as bioindicators that can be used to assess water quality and ecosystem health (Montilla et al., 2022). Their presence and abundance can provide valuable information about changes in environmental conditions, such as pollution or habitat degradation. Changes in their populations can signal changes in water quality and help identify potential environmental stressors or pollution sources. Aquatic macroinvertebrates are small organisms that lack a backbone and can be seen with the naked eye, such as insects, crustaceans, and mollusks. This study examines Ephemeroptera, Plecoptera, and Trichoptera because they are often used as indicator species due to their sensitivity to environmental stressors and importance as a fish food source. As such, monitoring changes in Ephemeroptera, Plecoptera, and Trichoptera populations can help identify and address potential threats to freshwater ecosystems. By utilizing macroinvertebrates as bioindicators, we can better understand the health of our freshwater systems and take necessary actions to protect them for future generations. Overall, aquatic macroinvertebrates are crucial contributors to the ecological health and biodiversity of freshwater ecosystems.

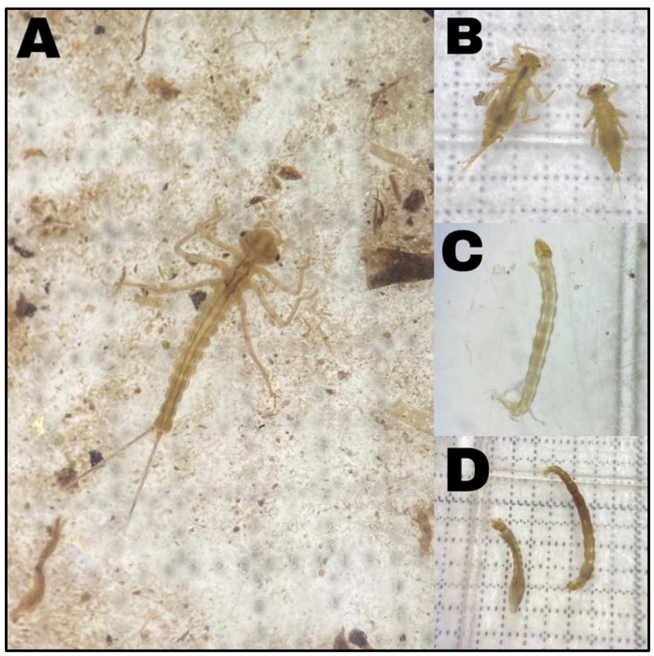

Figure 2: Collage of found macroinvertebrates sorted into individual order categories. **Figure 2A:** Individual Ephemorpotera (A. Hall). **Figure 2B:** Two Odanota (A. Hall). **Figure 2C:** One Diptera **Figure 1D:** Two Diptera of different families (A. Hall).

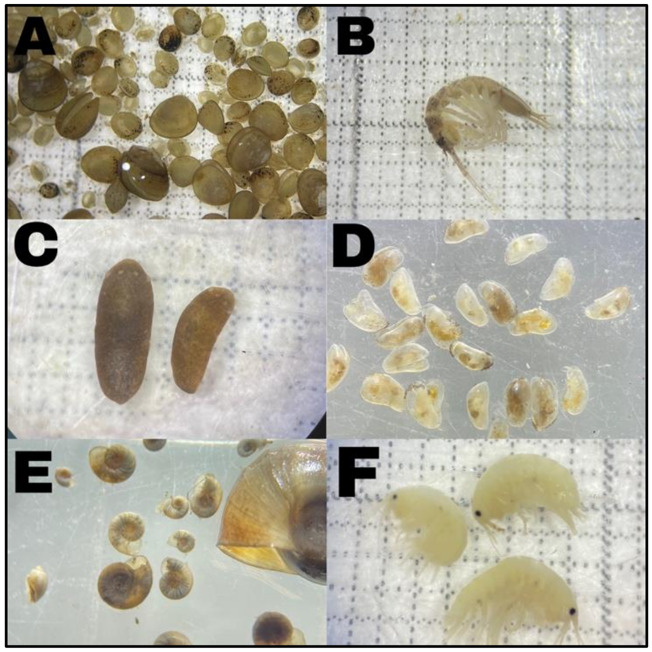

Figure 3: Collage of found macroinvertebrates sorted into the "other" category. **Figure 3A:** Sorted Bivalvia (A. Hall). **Figure 3B:** Single Isopoda (A. Hall). **Figure 3C:** Two Tricladida (A. Hall). **Figure 3D:** Sorted Ostrocoda (A. Hall). **Figure 3E:** Sorted Gastropoda (A. Hall). **Figure 3F:** Three Amphipoda (A. Hall).

Density, Taxonomic Richness, and Composition

Density, taxonomic richness, and composition are all metrics used in ecology to describe and understand the biodiversity of a given ecosystem. Density refers to the total number of individuals of study within the defined area. This paper defined density as total abundance of macroinvertebrates from one sample site. This metric can help identify patterns and trends of population dynamics within a defined habitat. Density and abundance can be found interchangeably, but for this study we will focus on density metrics. Taxonomic richness, on the other hand, is defined here as a measure of the number of different orders of macroinvertebrates present within one sample site. This metric is important because it provides insight into the diversity of an ecosystem and can be used to identify areas of high biodiversity that may require additional conservation efforts. Composition varies in that it takes the number of orders identified and their abundance and relates back to the overall role in the ecosystem. This metric allows insight into functional diversity of an ecosystem and helps explain the impacts of change to an order.

Research Questions & Hypotheses

Research question: How does macroinvertebrate (a) density, (b) taxonomic richness, and (c) composition within urban, freshwater bodies in Fort Collins, CO differ between ponds, streams, and canals/ditches?

Expected outcome, or research (alternative) hypothesis: We expect that there will be a significant difference in the (a) density, (b) taxonomic richness, and (c) composition of macroinvertebrates between the 3 habitat types (ponds, streams, canals/ditches). We expect species density to be highest in ponds and lentic ecosystems, while in lotic systems, such as streams and canals/ditches, it will be lower. We expect to see higher taxonomic richness in streams and canals/ditches compared to ponds. Lastly, we expect to observe similar composition across all three different habitat types.

Emergent null hypothesis: There will be no significant difference in the (a) density, (b) taxonomic richness, and (c) composition of macroinvertebrates between the 3 habitat types (ponds, streams, canals/ditcehs).

Explanation: We expect to see a significant difference in the density, taxonomic richness, and composition of macroinvertebrates between the 3 habitat types (ponds, streams, canals/ditches) because of the different environmental conditions of each habitat. We expect macroinvertebrate density to be highest in ponds due to the lentic nature of these habitats allowing for more stable environments that generates positive feedback loops and high abundance. We are anticipating this outcome due to the fact "ponds contribute a great deal to biodiversity at a regional level as networks of habitat patches that also act as 'stepping stones' to facilitate the movement of species through the landscape" (Hassall, 2014). Typically, the higher the taxonomic richness is in an ecosystem, the more likely it is to continue furthering the diversity of taxonomic richness in the said ecosystem. We expect to see higher taxonomic richness in streams and canals/ditches compared to ponds due to there being more change to the open-system layout of the habitat and different organisms may be passing through at different times. Lastly, we expect to observe similar composition across all three different habitat types because of the similar surrounding ecosystem each habitat resides in. Since all of the sites sampled are in urban settings, under similar climate and elevation, we expect to see a correlation between the three that could possibly be linked to the large-scale ecosystem that encompasses everything in Fort Collins, Colorado.

Methods

Study Sites

This report focuses on the urban city of Fort Collins, located in the front range of northern Colorado. This location has experienced rapid urbanization in the past 50 years, expanding infrastructure and housing across the plains. The map below (Figure 2) displays the specific sites we visited, color coded based on the type of water body. All of these locations were taken from a GPS unit and then the coordinates were used to create the map. Although there have been similar studies like this conducted throughout different urban areas all over the world, there has not been one in Fort Collins. This is why we are examining macroinvertebrate density, taxonomic richness, and composition within urban, freshwater bodies in Fort Collins, CO.

Figure 4: Map of study area with sites listed and coded. The purple represents canals/ditches, light green represents ponds, and blue represents streams (A. Hall).

Data Collection

We began our sample collection by first preparing our materials and equipment. We first gathered our needed supplies which included: waterproof datasheets to record our findings, small jars to hold the samples, and ethanol to preserve the samples. Equipment used included a D-net to collect the samples (net face area 8.25 m^2), and waders for protection. 18 sites were pre-selected by our mentor that consisted of 7 ponds, 6 streams, and 5 canals/ditches in urban areas of Fort Collins.

At each site we collected 3 replicate samples in best available habitat (BAH). BAH criteria included complex substrates such as vegetation, woody debris and gravel/cobble. Each of the 3 replicate samples were taken in different locations of the water body but within a relatively close distance, typically about three meters away from each other.

Depending on whether the water body was still or fast moving, we either chose to do a kick or sweep method of collection with the D-net. If the water was fast moving, the kick method of data collection was employed. This was done by placing the net onto the sediment surface of the sample area facing upstream and then disturbing the sediment within 1m upstream of the net by 'kicking' to release the macroinvertebrates that would then flow into the net. If the water was still, as in the case of ponds and some slow moving streams, the sweep collection method was used. The sample area was 'swept' by pulling the net towards yourself through a 1 meter long path making sure to disturb the sediment while remaining close to the sediment surface. There is potential for sampling bias between the two collection methods, however each method samples approximately the same area of habitat.

Once we completed our kick or sweep, we then transferred the contents from the D-net into our sample jars. The net was then rinsed and shaken out in the water where the collection took place to ensure a clean start for the next sample. We then repeated this process two more times per site to end with three samples from each location. We ended with 3 samples from 18 sites, accumulating 54 jars.

Data Entry and Processing

The first processing step was to separate the macroinvertebrates from the plant material. We then picked through the macroinvertebrate from the debris in each sample. An important note for our sampling is that hydrozoans were collected independently of all other macroinvertebrate species due to the high volume they occurred in. For this first sample, we would pick the Macroinvertebrates from the entire sample without subsampling.

Upon completion of the first sample, we transitioned to using a subsampling method to expedite sample processing. To subsample, we evenly spread the full sample's contents over a sieve tray that had 24 equal grid cells. We used a random number generator to select a grid cell, and then we scraped the contents off the selected cell into a tray to search for macroinvertebrates under a stereo microscope (Leica EZ4). We continued to randomly select new grid cells until we reached a minimum of 200 individuals. When picking, we separately counted Hydrozoans and did not include them in the count for 200 individuals. We chose to exclude Hydrozoans from the minimum count because they were so densely abundant in some samples that they would have drastically diminished the quality of those samples.

Once we reached 200 macroinvertebrates we completed picking the grid cell we were on. We kept note of how many cells we completed and what the exact count was in order to estimate the total abundance in the samples. Subsampling was used for dense samples, whereas individual scooping was preferred for samples with less material that would not cover the subsampling tray. Given the time frame of our research, we completed 1 replicate sample out of the 3 for the sample site.

The second phase of processing looked at the vials of total macroinvertebrates from each site for identification. We sorted the macroinvertebrates to order level while tracking abundance onto data sheets. The various orders and their abundance for each sample site were entered into excel for further analysis.

Data Analysis

In this study, data were entered into Excel, including the site name, site type (pond, stream, or canal/ditch), category of macroinvertebrate, taxonomic order, and total abundance. The abundance of macroinvertebrates subsampled during processing were extrapolated to represent the entire abundance had the sample been processed. This left us with representative data for all sites. To visualize the data, bar graphs were generated using Excel to show the average density and its standard error per site type, as well as the average taxonomic richness and standard error per site type. The community composition per site type was displayed as a proportionately stacked bar graph. A single-factor ANOVA test was run to determine statistical significance for density between site types and taxomonic richness between site types. Variance and P-values were generated and displayed below.

Results

Each habitat type exhibited different averages of density per site (Figure 5), with canals/ditches exhibiting the highest density per site (478.2), followed by ponds (155.7), and then streams (82.3). There is reasonable variability presented with the standard error (SE) of each site type as well, with canals/ditches experiencing 298.5, ponds 54.2, and streams 29.1 units of variability. A single-factor ANOVA test resulted in a P-value of 0.187, which shows statistical insignificance between the density of the 3 site types.

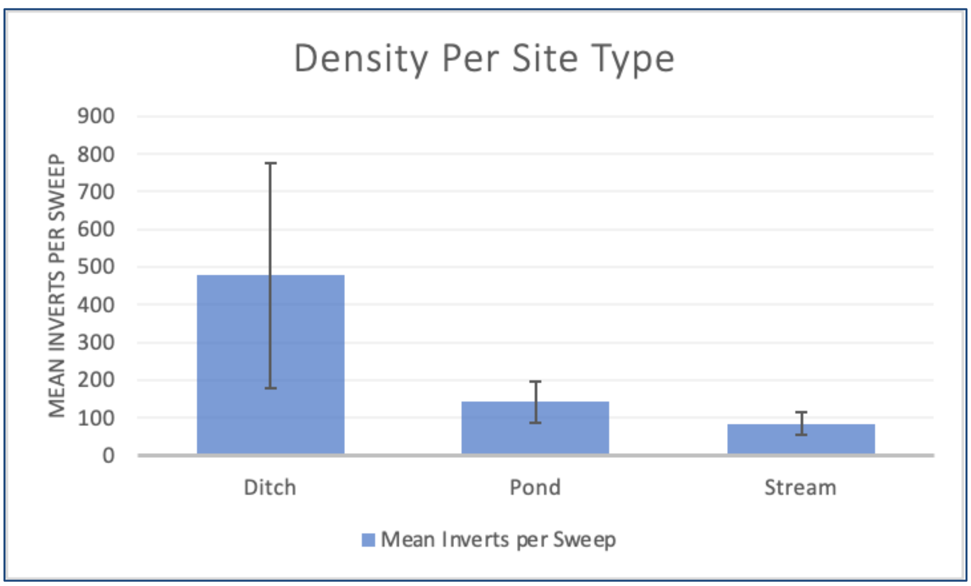

Figure 5: Mean density of macroinvertebrates collected per site type (A. Hall).

The taxonomic richness (Figure 6) shows an average of how many different orders of macroinvertebrates were present in each site type. We hypothesized that streams and canals/ditches would exhibit greater taxonomic richness than ponds, yet our data indicate otherwise. We found a relatively even taxonomic richness distributed amongst the three site types with canals/ditches and streams experiencing an average of 8 orders, while ponds averaged 7.85 orders. Standard error varied for each site type with canals/ditches at 2.25, ponds at 1.4, and streams at 0.6. Upon completion of a single-factor ANOVA test, we calculated a P-value of 0.99, which suggests confidence in the null hypothesis. This means that it is statistically unlikely that there is a difference in taxonomic richness between the 3 site types.

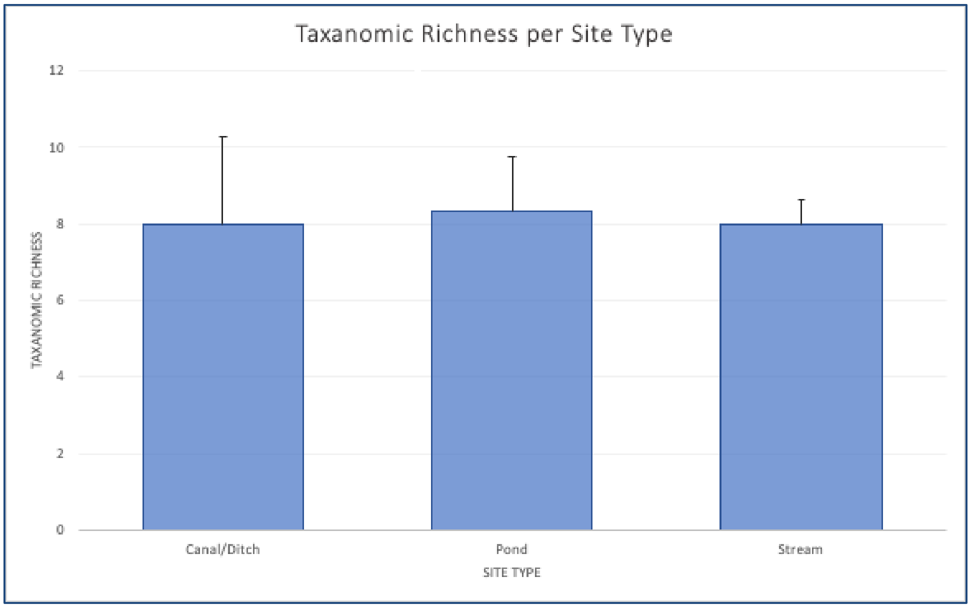

Figure 6: Average taxonomic richness of macroinvertebrate orders per site type (A. Hall).

In total, 22 orders of macroinvertebrates were collected from the 18 sample sites. Figure 7 below presents the community composition using either the order name or a category consisting of several orders with smaller abundance. The orders observed in samples were Coleoptera, Crustacea (Daphnia, Decapoda, Isopoda, Amphipoda, Ostracoda, Anomopoda, and Copepoda), Diptera, Ephemeroptera, Mollusca (Gastropoda and Bivalvia), Odonata, Oligochaeta, Other (Collembola, Tricladida, Hemiptera, Hirudinea, Nematoda, Anthoathecata, and Trombidiformes), and Trichoptera. Trichoptera were nearly exclusively found within canals/ ditches, whereas Odonata reside primarily in ponds. Other than Trichoptera and Odonata orders, the other orders were represented in all 3 water body types. There is overall reasonable proportion found between the composition of each site type.

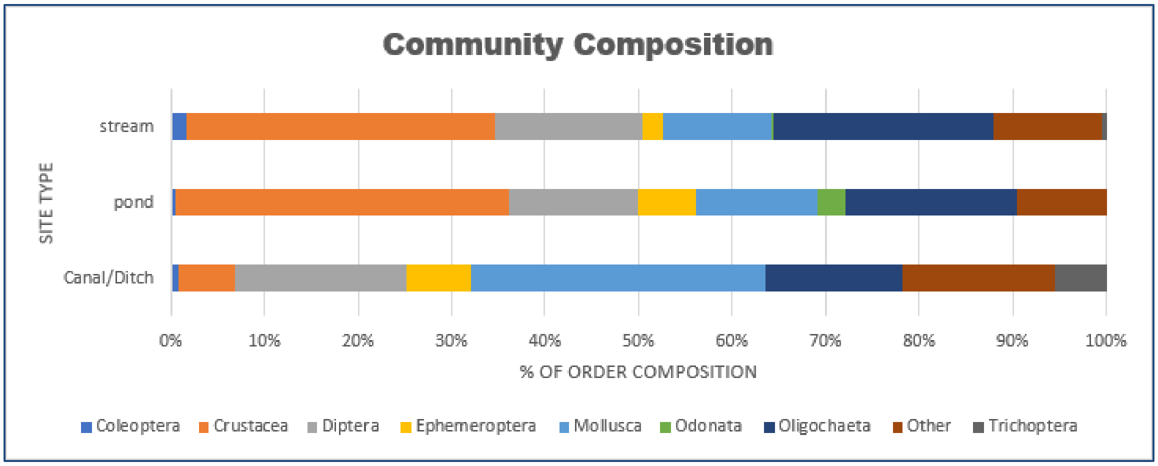

Figure 7: Community composition of macroinvertebrate orders per site type (C. Stevens).

Discussion

Density Per Site

Our findings indicate that the community composition of macroinvertebrates across the three habitat types demonstrated different patterns, with canals/ditches exhibiting the highest density per site, followed by ponds and then streams. This finding further emphasizes the complex nature of the relationship between habitat type and macroinvertebrate communities. The high density of macroinvertebrates observed in canals/ditches could be due to the high levels of nutrients and organic matter in these habitats, which can support a larger number of organisms with higher adaptability to anthropogenic change (Kim et al. 2016).

Canals/ditches often receive nutrient-rich runoff from agricultural or urban areas, which can lead to higher levels of primary production and subsequently support a greater number of macroinvertebrates (Yang Y. & Lusk M. 2018). In addition, the physical characteristics of canals/ditches, such as their relatively wide and deep channels, can create a habitat with a large amount of available space that can support high densities of organisms (Buss, 2014). This larger space may also allow for the coexistence of a greater number of species, each occupying a different niche within the canal/ditch ecosystem. This occurrance, remarked by Kim et al. (2016), is described as 'cluster preference', where certain freshwater macroinvertebrates' orders inhabit one section rather than occupying even distribution amongst their habitat. Cluster tendencies of macroinvertebrates helps explain the high variability of density per site within site type categories seen in this study.

The lower density of macroinvertebrates observed in streams may be due to a higher level of natural disturbances, such as floods or droughts, that can lead to a more dynamic and unpredictable environment for macroinvertebrates (O'Driscoll et al. 2010). Additionally, streams may be more susceptible to human disturbances, such as urbanization or agriculture, which can negatively impact macroinvertebrate communities by increasing habitat fragmentation.

Overall, the density of macroinvertebrates across the three habitat types are likely influenced by a combination of anthropogenic and environmental factors, such as nutrient availability, physical characteristics of the habitat, and the presence or absence of predators and competitors. Further research is needed to understand the mechanisms driving these patterns entirely and assess our findings' generalizability to other macroinvertebrate communities in different geographic regions and contexts.

Taxonomic Richness

Our results indicate that the taxonomic richness of macroinvertebrates was not significantly different across the three habitat types, with all three habitat types exhibiting similar taxonomic richness. This finding contradicts our initial hypothesis, which predicted that taxonomic richness would be highest in lentic systems like ponds and lowest in lotic systems like streams and canals/ditches. It is possible that the complexity of canals and ditches, with varying water flow rates and levels of human disturbance, contributed to the higher taxonomic richness observed in those habitats.

Canals and ditches often have complex hydrological characteristics, such as varying water flow rates, water depths, and sediment deposition patterns, which can create a range of microhabitats within the same system (Peacock, 2021). These microhabitats can provide unique environmental conditions that can support different species and contribute to the overall taxonomic richness observed in these habitats. For example, deeper sections of canals may provide habitat for species that require low flow rates, while shallower sections may support species that prefer faster currents (Meyer, 2004). Additionally, different types of substrate, such as sand, silt, or gravel, can support different species of macroinvertebrates (C. Chessman, & A. Williams, 1999).

Human disturbance can also play a role in shaping the taxonomic richness of canals and ditches. These habitats are often located in urban or agricultural landscapes, where they may be subject to pollution, nutrient enrichment, and physical alterations such as dredging or bank stabilization (Song, 2022). Song also finds that “urban blue-green space is the most severely disturbed ecosystem by human social and economic activities.” While some level of disturbance may negatively impact macroinvertebrate communities, it is also possible that certain species may be able to adapt to or benefit from human activity (Vermonden, 2009). For example, in Vermonden’s 2009 similar study, they found that “nutrient-poor urban water bodies harbored the highest numbers of macroinvertebrate taxa... as well as the highest number of red list species”. This could be due to some species of macroinvertebrates may be more tolerant to pollution or able to utilize human-made structures such as culverts or concrete-lined channels (Vermonden, 2009).

Overall, the higher taxonomic richness observed in canals and ditches in our study may be due to a combination of hydrological complexity and human disturbance, which can create a range of microhabitats and niche opportunities for different species. However, further research is needed to fully understand the mechanisms driving the observed patterns and to assess the generalizability of our findings to other canal and ditch systems in different geographic regions and contexts.

Community Composition

Our findings also indicate that the community composition of macroinvertebrates across the three habitat types demonstrate different patterns, with streams and ponds exhibiting similar community composition, while the composition canals/ditches differ slightly. This suggests that the ecological conditions of these habitats play a role in shaping macroinvertebrate communities.

Urbanization can have significant impacts on the ecological conditions of aquatic habitats. For example, increased impervious surfaces and stormwater runoff can result in altered water flow rates and sediment levels, which may affect the availability of food and shelter for macroinvertebrates (D. Booth & C. Jackson, 1997). Additionally, industrial and residential discharge can introduce pollutants and alter nutrient levels, further affecting the composition of macroinvertebrate communities ((D. Booth & C. Jackson, 1997). Booth and Jackson also found that "urbanization yields not only measure changes in specific elements of aquatic systems but also a decline in the overall function of those systems." These combining factors all contribute to community composition in urban blue spaces.

Our observations of slightly different macroinvertebrate communities in canals and ditches may be linked to the presence of urban influences. Canals and ditches are often found in urban areas and subjected to greater anthropogenic pressures, such as road runoff and impervious surfaces (Arjenaki et al. 2020). These pressures may result in a distinct community composition characterized by the presence of more tolerant species, which can thrive in conditions that are unfavorable to other macroinvertebrates. Clarence Goodnight found that "many workers have recognized the fact that some macroinvertebrates are more tolerant than others to pollution," which could correlate to our finsings.

Our findings highlight the importance of considering the influence of urbanization when studying macroinvertebrate communities in aquatic habitats. Conservation and management efforts should consider each habitat type's specific ecological conditions and anthropogenic pressures to ensure that macroinvertebrate communities are protected and sustained.

Conclusions

In conclusion, our study provides insight into macroinvertebrates' density, taxonomic richness, and composition in three habitat types (ponds, streams, and canals/ditches). Our findings suggest that each of these habitat types supports unique macroinvertebrate communities with distinct taxonomic composition and density. Specifically, canals/ditches exhibited the highest taxonomic diversity and density of macroinvertebrates, followed by ponds and streams

Our study also revealed unexpected findings, such as the insignificant difference in taxonomic richness per site type. While our findings failed to reject our null hypothesis, it continues to shed light on the complexity of environmental suitability and macroinvertebrate adaptability within anthropogenically impacted waterbodies. Urban runoff and habitat fragmentation ,an suit one sector of orders, while simultaneously relocating other orders, providing varied yet proportionate distibution of taxonomic richness between site type.

Overall, our study highlights the complex nature of the relationship between habitat type and macroinvertebrate communities. The factors that influence macroinvertebrates' taxonomic diversity and density are likely multifaceted and may include both anthropogenic and environmental facors. Our findings provide a foundation for further research into the mechanisms driving these patterns. There is potential for improved conservation efforts aimed at preserving macroinvertebrate diversity and functional ecosystems in different habitats.

References

Arjenaki, M. O., Sanayei, H. R., Heidarzadeh, H., & Mahabadi, N. A. (2020). Modeling and investigating the effect of the lid methods on collection network of urban runoff using the SWMM model (case study: Shahrekord City). *Modeling Earth Systems and Environment*, *7*(1), 1–16. https://doi.org/10.1007/s40808-020-00870-2

Booth, D. B., & Jackson, C. R. (1997). Urbanization of aquatic systems: Degradation Thresholds, stormwater detection, and the limits of mitigation. *Journal of the American Water Resources Association*, *33*(5), 1077–1090. https://doi.org/10.1111/j.1752-1688.1997.tb04126.x

Buss, D. F., Carlisle, D. M., Chon, T.-S., Culp, J., Harding, J. S., Keizer-Vlek, H. E., Robinson, W. A., Strachan, S., Thirion, C., & Hughes, R. M. (2014). Stream biomonitoring using macroinvertebrates around the globe: A comparison of large-scale programs. *Environmental Monitoring and Assessment*, *187*(1).

Goodnight, C. J. (1973). The use of aquatic macroinvertebrates as indicators of stream pollution. *Transactions of the American Microscopical Society*, *92*(1), 1. https://doi.org/10.2307/3225166

https://doi.org/10.1007/s10661-014-4132-8

C. Chessman, B., & A. Williams, S. (1999). Biodiversity and conservation of river macroinvertebrates on an expanding urban fringe: Western Sydney, New South Wales, Australia. *Pacific Conservation Biology*, *5*(1), 36. https://doi.org/10.1071/pc990036

Chester, E. T., & Robson, B. J. (2013). Anthropogenic refuges for freshwater biodiversity: Their ecological characteristics and management. *Biological Conservation*, *166*, 64–75. https://doi.org/10.1016/j.biocon.2013.06.016

Freshwater Pond and lake (FW) ecosystems. DIEM Project. (2019). Retrieved March 21, 2023, from https://diemproject.org/sensitive-ecosystems/freshwater-pond-and-lake-ecosystems/#:~:text=A%20pond%20or%20lake%20ecosystem,variability%20in%20the%20available%20habitats.

Hassall, C. (2014). The ecology and biodiversity of Urban Ponds. *WIREs Water*, *1*(2), 187–206. https://doi.org/10.1002/wat2.1014

Kim, D.-H., Chon, T.-S., Kwak, G.-S., Lee, S.-B., & Park, Y.-S. (2016). Effects of land use types on community structure patterns of benthic macroinvertebrates in streams of urban areas in the south of the Korea Peninsula. *Water*, *8*(5). https://doi.org/10.3390/w8050187

Johnson, P. T., Hoverman, J. T., McKenzie, V. J., Blaustein, A. R., & Richgels, K. L. (2012). Urbanization and wetland communities: Applying metacommunity theory to understand the local and landscape effects. *Journal of Applied Ecology*, *50*(1), 34–42. https://doi.org/10.1111/1365-2664.12022

Meyer, S. C. (2004). Analysis of base flow trends in urban streams, Northeastern Illinois, USA. *Hydrogeology Journal*, *13*(5-6), 871–885. https://doi.org/10.1007/s10040-004-0383-8

Montilla, V., Márquez, J. A., & Principe, R. E. (2022). Aquatic macroinvertebrates as bioindicators of the harvest effect on mountain streams afforested with Exotic Pines. *Limnologica*, *95*. https://doi.org/10.1016/j.limno.2022.125988

O’Driscoll, M., Clinton, S., Jefferson, A., Manda, A., & McMillan, S. (2010). Urbanization effects on watershed hydrology and in-stream processes in the Southern United States. *Water*, *2*(3), 605–648. https://doi.org/10.3390/w2030605

Oertli, B., & Parris, K. M. (2019). Review: Toward management of urban ponds for freshwater biodiversity. *Ecosphere*, *10* (7). https://doi.org/10.1002/ecs2.2810

Peacock, M., Audet, J., Bastviken, D., Futter, M. N., Gauci, V., Grinham, A., Harrison, J. A., Kent, M. S., Kosten, S., Lovelock, C. E., Veraart, A. J., & Evans, C. D. (2021). Global importance of methane emissions from drainage ditches and Canals. *Environmental Research Letters*, *16*(4), 044010. https://doi.org/10.1088/1748-9326/abeb36

Song, S., Wang, S., Shi, M., Hu, S., & Xu, D. (2022). Urban Blue–Green Space Landscape Ecological Health Assessment based on the integration of pattern, process, function and Sustainability. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-11960-9

Vermonden, K., Leuven, R. S. E. W., van der Velde, G., van Katwijk, M. M., Roelofs, J. G. M., & Jan Hendriks, A. (2009). Urban drainage systems: An undervalued habitat for aquatic macroinvertebrates. *Biological Conservation*, *142*(5), 1105–1115. https://doi.org/10.1016/j.biocon.2009.01.026

Yang, Y.-Y., & Lusk, M. G. (2018). Nutrients in urban stormwater runoff: Current state of the science and potential mitigation options. *Current Pollution Reports*, *4*(2), 112–127. https://doi.org/10.1007/s40726-018-0087-7

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Appendix 1: Methods Outline

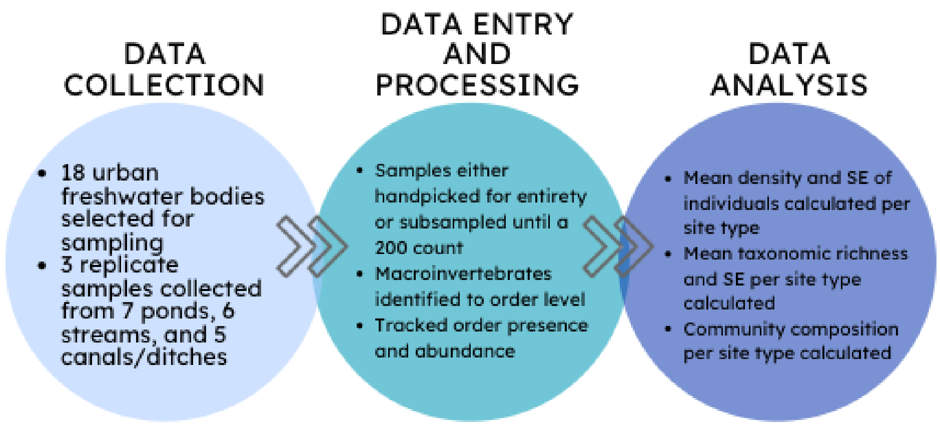

Figure 4. Project workflow and methodology: Extracting aquatic macroinvertebrates from different types of urban water bodies within Fort Collins, CO during the Fall of 2022.

Data Collection:

Data collection began in September 2022 and went until November 2022. Hall, A., Stevens, C., Carvallo, F., and Preston, D. went to 27 different freshwater sites to collect samples throughout

Fort Collins, CO. 7 being ponds, 6 being streams, and 5 being canals/ditches. This process used a D net that was either swept or kicked across the bottom surface. A sweep was implemented for standing water by lightly nudging the bottom surface while dragging the net for ~1 meter. A kick was used when water was flowing by placing the net in one position, and then nudging the bottom surface ~1 meter in front of the net. The nudges helped surface macroinvertebrates laying in the ground. Three samples were collected from each site, resulting in a total of 54 samples. Location within the water body was decided off Best Available Habitat (BAH). BAH consisted of sections of the water body with higher vegetation and/or movement of water. Material gathered inside the net was transferred to a labeled jar and filled with ethanol to preserve the samples. Notes regarding habitat vegetation, wildlife, nearby infrastructure, water quality, and other notable qualities were written on data sheets as well.

Data Entry & Processing:

The 54 jarred samples were brought back to the lab to begin processing. More dense samples were processed by subsampling, and lighter samples were picked by hand. Subsampling emptied the jar evenly onto a sieve that was divided and marked into 24 boxes. Using a random number generator, we would randomly select a cell to scoop and process under the microscope. Once 200 macroinvertebrates were identified, we would finish the entirety of the cell material, placing all macroinvertebrates into a small vial for storage. Picking samples by hand followed a similar pattern. We would scoop a spoonful of material to process under the microscope and place all identified macroinvertebrates into a storage vial. We scooped until the whole sample was processed, regardless of macroinvertebrate count. The next step involved categorizing the macroinvertebrates from each vial by order, tracking order presence and abundance for each site. These variables (site type, site name, order presence, and abundance) were entered into Excel to begin analysis.

Data Analysis:

The preliminary step of extrapolating subsamples to represent the entirety of the sample was completed before any statistics could be produced. The data analysis phase consisted of us generated averages, standard errors, and P-values for (a) density and (b) taxonomic richness between the three site types. The community composition of each site type was presented as a stacked bar graph with 100% ratio. These statistics were presented in the results section and further analyzed under the discussion.