AQUATIC INVERTEBRATE COLONIZATION AS A RIVER RESTORATION SUCCESS CRITERION: A CASE STUDY OF THE UPPER BLACKFOOT MINING COMPLEX SUPERFUND SITE

by

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DEDICATION

I dedicate my master's thesis to my beloved and remarkable family, Beryl, Rod, and Sandy Deyoe. Words can scarcely capture the profound impact you have had on my life, particularly throughout this arduous academic journey. Your unwavering love, boundless support, and unwavering belief in my abilities have been the bedrock upon which I have built my achievements. From the very beginning, you embraced my aspirations and dreams, offering unyielding encouragement and guidance. In moments of doubt, your reassuring presence reminded me of my inherent potential, instilling in me the confidence to persevere. Your unwavering faith in my abilities has been a constant source of inspiration, reminding me of the heights I can reach. I am forever grateful for the warmth and motivation you have provided me with, and it is with immense pride that I dedicate this thesis to you. Your support has been the catalyst for my success, and I am forever grateful for the immeasurable impact you have had on my life. May this dedication serve as a small token of my appreciation, a testament to the love, strength, and inspiration you have bestowed upon me.

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ABSTRACT

In 1975 the Mike Horse Dam partially collapsed, releasing 200,000 tons of cadmium, copper, iron, lead, manganese, and zinc into the streams and floodplains on the Upper Blackfoot Mining Complex (UBMC) in Montana, USA. The magnitude of the material that was toxic to humans from this event triggered the federal Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), which currently governs 1,329 sites across the USA. Portions of the $39 million lawsuit in 2008 with the American Smelting and Refining Company (ASARCO), funded the remediation and restoration of 37 hectares of floodplains, wetlands, and stream channels. Although CERCLA's success criteria focus on reducing risk to human health from hazardous substances, the Montana Natural Resource Damage Program was interested in aquatic invertebrate colonization of the restored river ecosystems, since they are monitoring progress of restoration. To answer this, I explored whether observations of invertebrate colonization could gauge restoration success and identify aquatic invertebrate-based tools for future restoration projects. Over three years, I compared invertebrate communities at five impacted "restored" sites on the UBMC with ten unimpacted "reference" sites. I then quantified colonization using seven indices: four statistical taxonomic diversity and similarity indices, the River Invertebrate Prediction and Classification System (RIVPACS), the Benthic Index of Biotic Integrity (B-IBI), and a new Stable Isotopic Colonization Index (SICI) which estimated isotopic complexity using metrics derived from $\delta^{15}N$ and $\delta^{13}C$ stable isotopes. Statistical diversity and similarity indices showed the restored sites were diversifying quickly. For example, from 2020 to 2023, the average (±1SD) Shannon Diversity of restored sites increased from 1.1 ±0.5 to 1.8 ±0.43 while reference was 2.1 ±0.3. The average B-IBI of restored sites increased from 11.1 ±4.8 in 2020 to 31.7 ±7.7 in 2023 while reference B-IBI was 65.7 ±4.5, indicating ongoing ecosystem recovery, but this index required taxonomic identification to the genus level. The average SICI for restored sites was 23.3 ±6.1 and reference was 54 ±9.2, and SICI required identification to the family level. Restoration efforts on the UBMC have resulted in a promising trajectory, but continuous monitoring is imperative to ascertain if restored streams have reached reference conditions.

CHAPTER ONE

INTRODUCTION

Mining is necessary for raw materials needed to fuel modern society (Coulson 2012). However, the long history of mining has left a trail of environmental impacts, including water pollution and habitat degradation (Wohl 2005). Mining waste can be particularly problematic as it often contains heavy metals and other toxic materials that can contaminate waterways, leading to the death of aquatic organisms, loss of ecosystem structure and function, and potential human health consequences (Vandeberg et al. 2011, Sun et al. 2018). To address these environmental concerns, the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund, was established in 1980 (USEPA 1980). CERCLA requires that the harm caused by mining activities be addressed to prevent the adverse effects of toxins from contaminated areas and affecting human health (Code of Federal Regulations 1985). This comprehensive approach under Superfund ensures that contaminated sites are thoroughly evaluated and remediated, safeguarding both the environment and the well-being of affected communities.

Superfund provides a framework to address contaminated sites that includes: 1) identifying hazardous substances, 2) assessing risks to human health and the environment, and 3) developing plans to eliminate the hazardous materials and restore the site to pre-contamination conditions (Switzer and Bulan 2002). Superfund also includes provisions for public involvement, community engagement, and liability and enforcement measures to hold responsible parties accountable for the costs associated with cleanup and restoration (USEPA 1980). Furthermore, Superfund encourages using innovative technologies and approaches for site characterization, remediation, and monitoring and promotes coordination among federal, state, tribal, and local agencies, stakeholders, and communities throughout the cleanup process (USEPA 1980).

CERCLA is complementary to the Clean Water Act (CWA), enacted in 1972 (USEPA 1972, Code of Federal Regulations 1978), which is focused on protecting the quality of the nation's water resources by regulating pollutant discharges into navigable waters, setting water quality standards, and regulating pollutant discharge permits (Sheridan 1986, Ofiara 2002). CWA’s mandate is to maintain our Nation's waters' chemical, physical, and biological integrity (USEPA 1972). Over the last several decades, that mandate has inspired approaches to assessment, permitting, and mitigation that maintains ecological processes (Karr and Dudley 1981, Fore et al. 1996, Karr and Chu 1997, Barbour et al. 1999, Barbour and Paul 2010). However, CERCLA traditionally only addresses hazardous materials, leaving many ecological functions unevaluated (Hird 1993). In the western United States, 40% of watersheds are impacted by abandoned mines (Olalde 2019) and potentially fall under the authority of CERCLA, not CWA. The success criteria that shape the outcome of the assessment, permitting, and mitigation will change the extent to which ecological functions are protected and restored in areas impacted by hazardous waste, abandoned mines, and other sources of contamination under the authority of CERCLA (Sheridan 1986, Ward 1998b, Ofiara 2002, Velleux and Lynch 2006). Assessing the biological response of river ecosystems following the remediation of a CERCLA site could provide crucial insights into the ecological recovery process.

Biological assessments (bioassessments) have been used for over a hundred years to evaluate aquatic ecosystems (Kolkwitz and Marsson 1908, Karr et al. 1986, Kleindl 1995). These are essential to monitoring the success of restoration strategies (Barbour and Paul 2010). Without bioassessments, there is a danger that practitioners may wrongly assume that restoration projects are successful and have returned to their original ecological state before disturbance (Bradshaw 1996, Lake et al. 2007). Aquatic invertebrates are often used for bioassessments because they are relatively easy to sample and can swiftly indicate biological changes in benthic stability, organic matter, and water quality (Reynoldson and Metcalfe-Smith 1992, Poff et al. 2006). Aquatic invertebrate-based bioassessment measures the divergence of attributes, such as diversity and composition (Fore et al. 1996, Barbour et al. 1999), at a site-of-interest from an ideal, unimpacted (reference) sites (Yount and Niemi 1990). Reference conditions are frequently defined as ecological characteristics and conditions of a minimally-impacted or unimpacted ecosystem that serves as a benchmark or reference point for comparison with an ecosystem that has been disturbed or impacted by human activities or other factors (Fore et al. 1996, Barbour et al. 1999).

The Upper Blackfoot Mining Complex (UBMC) near Lincoln, Montana, is an example of a Superfund site adversely affected by toxic waste (tailings) from an abandoned mine. In 1975, heavy rain caused the catastrophic collapse of the 21-meter-tall Mike Horse Dam, moving 200,000 tons of tailings, filled with toxic material, into the headwaters of the Blackfoot River (Spence 1997). The magnitude of this event triggered Superfund in 2008 where the American Smelting and Refining Company (ASARCO) funded $39 million (Montana Environmental Custodial Trust 2021) towards the removal (remediation) of tailings and restoration of thirty-seven hectares of floodplains, wetlands, and stream channels (River Design Group, Inc. et al. 2011). After remediation, streams were restored by utilizing channel reconstruction which lead to a novel ecosystem characterized by new physical, hydrological, and ecological interactions not present in the original ecosystem before the mining activities (River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). However, after the restoration was complete, it remained unclear whether the novel ecosystem resulted in an improvement of aquatic invertebrate communities as the newly constructed streams recover over time.

Following the completion of the restoration project at the UBMC in 2020, the Montana Natural Resource Damage Program (NRDP), who oversees the success of this effort, expressed interest in understanding the rate aquatic invertebrates were colonizing the novel ecosystem. Aquatic invertebrate colonization is the process by which invertebrates enter a new habitat, establish populations, and start reproducing (Mackay 1992). As colonization progresses, the trophic structure of the invertebrate community typically becomes more diverse, leading to the development of more feeding relationships over time (Merritt et al. 2017). invertebrates are selective and only colonize streams with acceptable biological conditions for them to thrive (Yount and Niemi 1990, Mackay 1992), thus indicating that colonization is an indicator of biological condition.

Most works (De Szalay and Resh 2000, Seger et al. 2012) have utilized diversity indices, such as the Shannon Diversity Index, to estimate colonization as invertebrate communities diversify. Although useful, diversity indices do not provide a comprehensive picture that more robust biological assessments can offer. These concepts of biological assessments and invertebrate colonization led me to explore how measurements of colonization using invertebrate communities could be used to quantify ecological success for Superfund stream restoration sites that create novel stream systems.

My thesis presents an investigation of the usability of biological assessments and invertebrate colonization rates to measure the ecological success of Superfund restoration projects, specifically the Upper Blackfoot Mining Complex. I aim to explore the effectiveness of various biological assessment tools that evaluate invertebrate diversity, quality, integrity, and isotopic complexity. My objective in applying bioassessments to the UBMC is to assess the viability of using invertebrate colonization to gauge restoration success and identify effective tools that can consistently produce robust results for future restoration projects.

I will address four research questions related to the utilization of invertebrate colonization to assess the restoration success of the UBMC Superfund project. I investigate (1) the applicability of the Shannon-Wiener, Gini-Simpson, and Inverse Simpson Diversity Indices in evaluating invertebrate community diversity and taxonomic similarity between restored and reference sites using the Bray-Curtis Similarity Index (2) the suitability of the River Invertebrate Prediction and Classification System (RIVPACS) for evaluating the overall biological quality between restored and reference streams (3) the effectiveness of a Benthic Index of Biotic Integrity (B-IBI) in assessing the biological condition of restoration and reference sites (4) the potential of a new Stable Isotope Colonization Index (SICI), which incorporates $\delta^{15}N$ and $\delta^{13}C$ stable isotopes, in measuring isotopic complexity.

CHAPTER TWO

LITERATURE REVIEW

Aquatic Invertebrate Ecology

Aquatic macroinvertebrates play a fundamental role in freshwater ecosystems, serving as indispensable components that help maintain ecological equilibrium and act as reliable indicators of water quality (Cummins 1973) and biological integrity, which is frequently defined as the “capacity of an ecosystem to sustain a well-integrated community of living organisms with a composition and functional structure comparable to that of the natural habitat in the area” (Karr and Dudley 1981). Aquatic macroinvertebrates are invertebrates larger than 500 microns that reside in lake (lentic) and river (lotic) systems. Aquatic invertebrate communities can be categorized into five types of functional feeding groups (FFGs) (Figure 2.1) in continuous lotic systems (Cummins 1973). The first are shredders that feed on coarse particulate organic matter (CPOM; >1mm), such as leaf litter, with a strong dependence on microbial biomass. The second are predators that capture and engulf their prey or tissues in their associated communities (>0.5mm). Gatherers collect fine (FPOM, 50µm-1mm) or ultra-fine particulate matter (UPOM 0.5-50µm) in depositional areas. Just like shredders, gatherers are also highly dependent on the microbial biomass associated with the particles for sustenance. Scraper’s shear attached periphyton (a complex assemblage of algae, bacteria, fungi, and mesofauna that are attached to submerged substrates) from rocks (0.01-1mm). Lastly, filterers feed on dissolved organic matter (DOM) in the water column (seston;0.5-0.1 µm) using labral fans. As an ecosystem develops, a wider variety of organic matter grants more opportunities to establish more FFGs, which leads to higher levels of taxa diversity and trophic complexity.

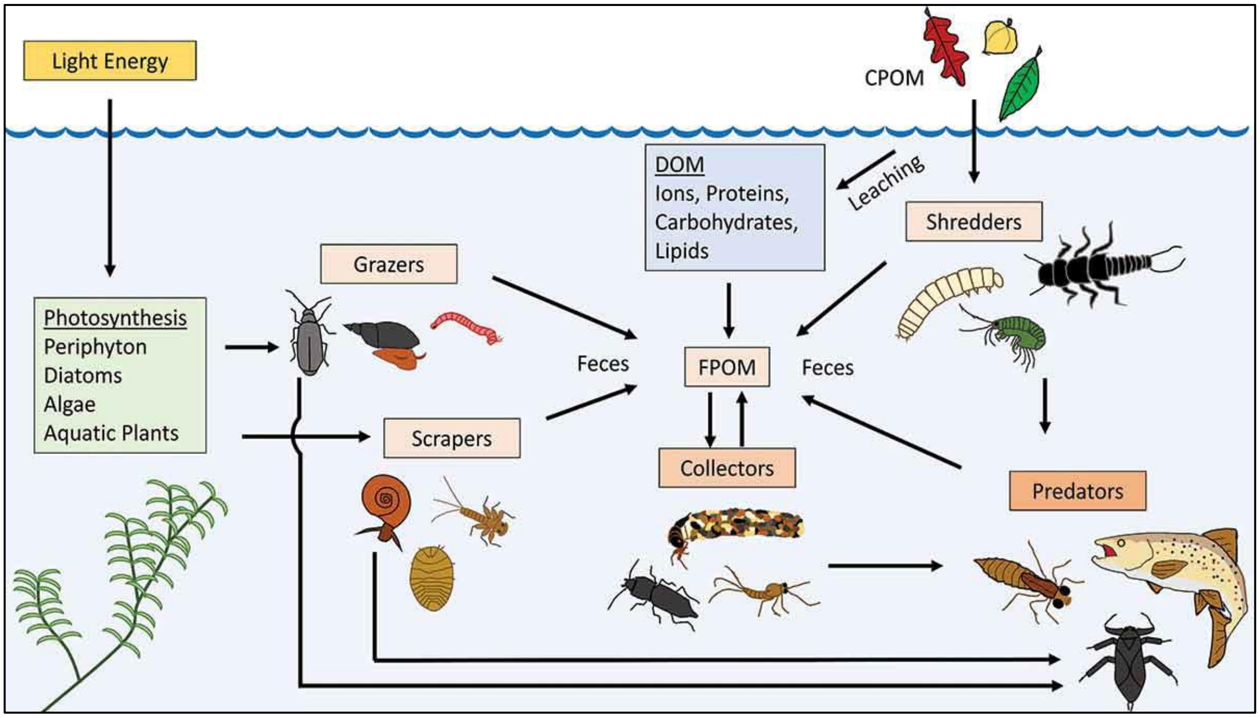

Figure 2.1. Aquatic invertebrate Food Web. Example of a simplified food web describing the roles of each FFG and the associated sustenance type (Hershey et al. 2010).

Like other organisms, aquatic invertebrates contain different taxonomic levels (Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species). The Trichoptera order in the Insecta class, commonly known as caddisflies, is a crucial component of freshwater ecosystems and plays a vital role in streams (Cardinale et al. 2004). These invertebrates are sensitive to changes in water quality and habitat conditions and are considered a good indicator of biological integrity (Hawkins et al. 2000). Caddisflies are also a keystone taxon in developing the river continuum concept (Vannote et al. 1980), as they are essential contributors to stream organic matter processing. Caddisfly larvae construct nets, retreats, and portable tubes or cases that serve as shelter and aid in obtaining food particles from the current (Cardinale et al. 2004). Due to their diverse case-making abilities and adaptations to various habitats, caddisflies are abundant and biologically diverse (Cummins 1973). Thus, they can serve as reliable water pollution indicators (Prommi 2018).

The Plecoptera order of insects, commonly known as stoneflies, are susceptible to changes in water quality and require clean, well-oxygenated water with a high level of dissolved oxygen for survival (Tierno de Figueroa and López-Rodríguez 2019). They are typically found in streams with lush riparian vegetation and diverse invertebrates. Stoneflies are important in stream ecosystems because they are major contributors to the nutrient cycle and provide food for other invertebrates, fish, and other aquatic organisms (Cummins and Klug 1979).

The family Heptageniidae, commonly known as the "flat-headed mayfly," is a family of invertebrates that play an essential role in freshwater ecosystems (Needham 1936). They are generally considered indicators of good water quality and are sensitive to changes in water temperature and flow (Vannote et al. 1980). While higher rates of upstream dispersal have been reported in Ephemeroptera, Heptageniidae contains the ability to disperse through the air and downstream drift as adults and colonize new habitats and is often one of the first families of invertebrates to recolonize a restored stream (Resh et al. 1988, Bilton et al. 2001). The presence of Heptageniidae also indicates the availability of food sources in the stream, as they feed on algal and detrital particles deposited on stones and vegetation (Needham 1936). Heptageniidae is highly sensitive to changes in water quality, and the presence of high pollution levels or other environmental stressors may prevent their survival and colonization (Mackay 1992). Therefore, the higher percent abundance of Heptageniidae may indicate better water quality and higher biological integrity.

Aquatic invertebrates also can be categorized based on their biological processes. For example, intolerant invertebrates are highly sensitive to environmental stressors such as pollution and habitat degradation and cannot survive in streams with high levels of these stressors (Reynoldson and Metcalfe-Smith 1992, Gresens et al. 2009). Clinger invertebrates live attached to rocks or other substrates in fast-moving water. They use their strong legs or other adaptations to cling onto the substrate and avoid being swept away (Needham 1936, Cummins and Klug 1979). Scrapers are a type of clinger that use specialized mouthparts to allow them to scrape algae off surfaces (Cummins and Klug 1979). The presence or absence of these invertebrates can help to understand stream ecosystem processes, nutrient cycling, and energy flow (Merritt et al. 2017).

Aquatic Invertebrate Colonization

The creation of new streams through channel reconstruction leads to the colonization of aquatic invertebrates from neighboring, unimpacted streams (Malmqvist et al. 1991). It is expected that the novel UBMC streams follow a colonization pathway described in the literature where the trophic ecology undergoes a shift over time as invertebrates gradually colonize and continue to develop (Merritt et al. 2017). In the early stages of colonization, invertebrate communities are limited in diversity, thus limiting trophic interactions (Mackay 1992). Initially, in-stream autochthonous species such as algae and aquatic plants dominate the ecosystem. As their biomass increases, these organisms begin to provide a food source for herbivorous invertebrates (Kelly and Harwell 1990). As the ecosystem develops, detritus from riparian vegetation becomes a more important food source for invertebrates, increasing the population of allochthonous detritivores and decomposers (Mninshall 1967, Anderson and Sedell 1979). This, in turn, can support higher trophic levels, such as predatory invertebrates (Tierno de Figueroa and López-Rodríguez 2019). In other words, during the ecological recovery of a river ecosystem after restoration, invertebrate communities become more diverse over time and increase in trophic complexity. Because invertebrate communities should follow this pathway, we can use these changes in composition to measure the ecological success of the restoration effort.

Invertebrate communities are often used to monitor stream response to restoration because they can sensitively indicate ecological changes in benthic stability, organic matter, and water quality (Reynoldson and Metcalfe-Smith 1992, Poff et al. 2006). Invertebrates conform to a state of energy equilibrium from the headwaters to the mouth of a stream (Vannote et al. 1980). They influence nitrogen and carbon cycling by being the intermediate step between primary producers and consumers (Vannote et al. 1980, Malmqvist 2002). They consume organic matter and end their life cycles in the stream or terrestrial landscapes of riverine zones, thus transferring carbon and nitrogen from the stream to the terrestrial habitat (Cummins 1973, Cummins and Klug 1979). They are also consumed by fish or birds, which further facilitates carbon and nitrogen from producers to larger consumers (Cummins 1973).

Stream Restoration

Stream restoration is a process aimed at improving the ecological functioning of streams, rivers, and their adjacent ecosystems which have been degraded (Wohl 2005, Sun et al. 2018). Restoration goes beyond mere hazard reduction; it aims to establish self-sustaining ecosystems that demand minimal attention once the process is finished (Wohl 2005, Sun et al. 2018), which the UBMC project aimed to do. The UBMC restoration project also aimed to establish self-sustaining ecological processes, including thriving aquatic invertebrate populations and riparian habitats for wildlife (River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). Common human activities, such as urbanization, agriculture, and mining, have led to land use changes, altered hydrology, increased sedimentation, and nutrient pollution (Henley et al. 2000, Bernhardt et al. 2005). Several approaches to stream restoration have been implored worldwide, including physical restoration, which involves reshaping the stream channel and its surrounding landscape to restore natural flow regimes and reduce erosion and sedimentation (Dyste and Valett 2019). Another approach is biological restoration, which involves reintroducing native plant species to a stream and surrounding habitat to improve ecosystem function (Jähnig et al. 2011). In the case of the UBMC project, a majority of hazardous material was transported offsite and new stream channels were physically reconstructed, thus creating a novel ecosystem (River Design Group, Inc. et al. 2011, River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). Specifically, 200,000 tons of contaminated sediments were transported to a lined repository seven miles away from the UBMC, and 37 hectares of floodplains and streams were constructed by 2020. Stream restoration involves the modification of a stream or its surrounding ecosystem to restore its natural functions and processes, while stream rehabilitation focuses on repairing or improving the physical structure and habitat of a degraded stream without necessarily restoring its natural state (Lake et al. 2007, Frainer et al. 2018).

Most CERCLA projects primarily prioritize the remediation of hazardous waste that poses a threat to human health. However, the UBMC project went above and beyond by not only addressing this concern but also taking significant measures to restore the river ecosystems

(River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). When work on a major stream restoration project is finished, a novel ecosystem (Figure 2.2) can have many trajectories of recovery (succession) back toward the original ecosystem condition (Bradshaw 1996). A novel ecosystem can either approach its original condition through restoration or rehabilitation, or it will remain degraded with diminished ecosystem structure and function if left neglected (Bradshaw 1996). When the original ecosystem no longer exists, the original condition can be assumed by surrounding reference sites that exhibit similar ecosystem conditions (River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). Using reference streams in assessing stream condition is a common practice when the original condition is unknown (Barbour et al. 1999, Wright et al. 2000, Dyste and Valett 2019).

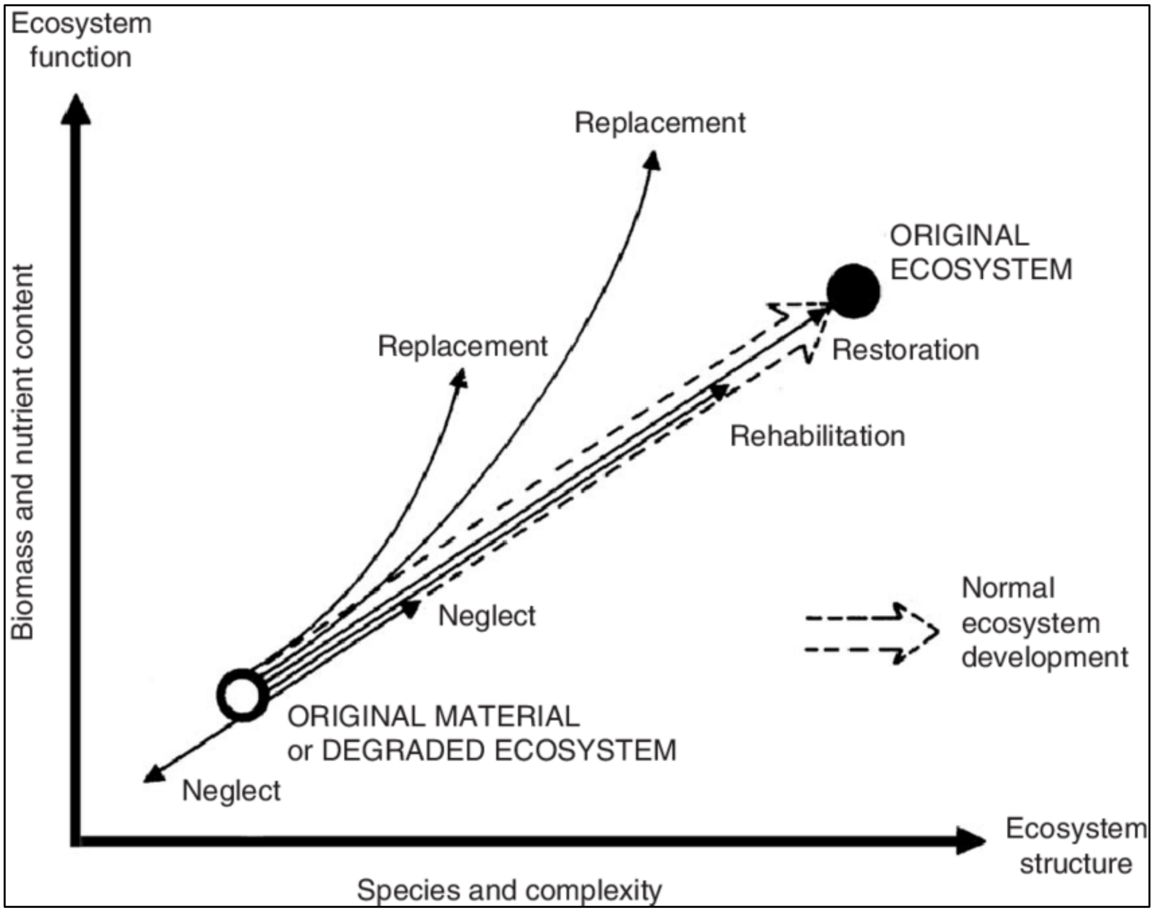

Figure 2.2. Ecological Succession. Example of a system undergoing ecological succession related to biological complexity and diversity (Bradshaw 1996).

Restoration success is often defined in terms of the mission, goals, and objectives of the restoration project. For restoration projects which result in novel ecosystems, success is usually considered to be achieved when the system has a similar ecosystem structure and function like reference conditions (Barbour and Paul 2010, Daniluk et al. 2013). One of the key challenges for a restoration project that resulting in a novel ecosystems is achieving long-term success, as stream restoration is a complex and dynamic process that depends on many factors, including the site-specific conditions of the stream, the effectiveness of restoration techniques, and the social and economic factors that influence the management and funding of stream restoration projects (Resh et al. 1988, Bradshaw 1996, Lake et al. 2007).

Diversity and Similarity Indices

Diversity indices are a commonly used tool for assessing the diversity of invertebrates in stream ecosystems (Mackay 1992, De Szalay and Resh 2000, Chapelsky et al. 2020). These indices provide a measure of the number and relative abundance of different taxa present in a sample and can be used to track changes in community structure over time. For example, the Shannon-Wiener index is a commonly used diversity index that considers both the number of taxa present and their relative abundance (Shannon 1948). Other diversity indices include Simpson's index, which is based on the dominance of taxa and considers the richness of a sample (Simpson 1949). Similarity indices have been used to compare the composition of invertebrate taxa between different sampling sites (Wilhm 1970, Wood et al. 2005, Çamur-Elipek1 et al. 2010). They provide a measure of the similarity or dissimilarity of the invertebrate communities at different sites. An example of a commonly used similarity index is the Bray-Curtis index, that measures the similarity of the abundance of different taxa between two sites (Bray and Curtis 1957).

There are also some limitations to the use of diversity and similarity indices. They do not provide information on the identity or function of individual taxa and are limited in their ability to detect changes in rare or uncommon taxa (Wilhm 1967). They may also be influenced by sampling efforts and site selection, which can affect the accuracy of the results (Wilhm 1970). Despite these limitations, diversity and similarity indices can provide important information on stream ecosystems' health and ecological condition (Hughes 1978). They can be used to identify changes in invertebrate communities in response to environmental stressors, evaluate the effectiveness of restoration efforts, and provide baseline data for long-term monitoring programs (Serrano Balderas et al. 2016).

Biological Assessments

Biological assessments (bioassessments) can provide valuable insights into the colonization patterns of invertebrate communities, shedding light on both the overall community colonization and its composition. First used in 1908, bioassessments are used to help characterize community structure (i.e., diversity, pollution tolerance) and evaluate the biological condition of an ecosystem by taking measurements of the resident biota (Kolkwitz and Marsson 1908). Bioassessments have been used after the completion of restoration projects to determine if restoration resulted in a successful outcome and is trending toward its original state (Bradshaw 1996, Lake et al. 2007). Although invertebrates have been long researched (e.g., Lindeman 1942), utilizing biological assessments to quantify colonization into novel ecosystems is very limited (Verdonschot et al. 2016). In the case of the UBMC, I am utilizing bioassessments to see if the recolonizing invertebrates on restored sites are trending toward the same biological condition as reference streams.

For my purpose here, reference streams are considered to be minimally disturbed systems that serve as a baseline for comparing the ecological condition of other streams (Harrelson 1994, Barbour et al. 1999). They provide a standard against, which the biological condition of restored streams can be assessed, helping to identify the extent of ecological degradation and the effectiveness of restoration efforts. When biologically assessing aquatic ecosystems, confirming which sites are reference and restored is required (Barbour et al. 1999). Although it might seem obvious, if a site is not correctly identified as reference or restored, that could affect which metrics are used as biological indicators when creating an MMI (Karr and Chu 1997, Barbour et al. 1999, Dahl and Johnson 2004). Reference sites are assumed to be in good ecological condition and serve as a benchmark for comparing with restored sites (Karr and Chu 1997) and provide a baseline for evaluating the biological condition of restoration sites. Restored sites, being novel artificial streams, will likely be stressed since they have not established the physical or hydrological conditions as reference sites (Wohl 2005).

Benthic Index of Biotic Integrity

Multimetric indices (MMIs) measure the biological condition of ecosystems by utilizing multiple metrics from various biological characteristics (Barbour et al. 1999). By incorporating multiple metrics, MMIs can comprehensively assess an ecosystem, representing various environmental types (Kleindl 1995). MMIs offer several advantages over single-metric approaches, such as a more holistic view of ecosystem conditions and identifying specific stressors that may impact biological communities (Karr and Dudley 1981). However, there are some concerns regarding the reproducibility of MMIs, as there may be differences in sampling methods, effort, number of metrics used, or the method of metric scoring (Ruaro et al. 2020). These issues can lead to variations in results, making it difficult to compare studies across different regions or periods, which is why each MMI must be calibrated for a specific region (Karr and Chu 1997, Wilhelm 2014).

A Benthic Index of Biotic Integrity (B-IBI) is a type of MMI commonly used to assess the biological integrity of streams (Karr and Dudley 1981). B-IBIs were developed in the 1980s to assess the biological integrity of aquatic ecosystems using the composition of benthic invertebrates as metrics (Karr and Dudley 1981). B-IBI has been used extensively in North America to evaluate the impacts of human activities such as mining, logging, and land development on stream health (Kerans and Karr 1994, Kleindl 1995, Fore et al. 1996). The index is based on a set of metrics that reflect the attributes of an invertebrate community and can be used to inform management and restoration efforts. However, because aquatic invertebrates are regionally diverse, B-IBI must be calibrated for specific regions (Dahl and Johnson 2004, Wilhelm 2014).

The River Invertebrate Prediction and Classification System

The River Invertebrate Prediction and Classification System (RIVPACS) uses the presence of different groups of invertebrates (taxa richness) to predict the expected biological quality for a given stream (Wright et al. 1993). RIVPACS is based on the premise that different types of invertebrates have different habitat requirements (Wright et al. 2000). The presence or absence of certain taxa can be used to predict the ecosystem's overall biological quality. RIVPACS has been widely used in Europe and North America and has been shown to be a reliable tool for predicting the expected biological community in streams (Wright et al. 1993, Clarke et al. 2003, Hargett et al. 2007a). Validation of RIVPACS has been an ongoing process, with studies comparing the predicted biological community from RIVPACS to the actual community observed in streams (Hargett et al. 2007b, Hawkins 2009). These studies have generally found that RIVPACS provides a reliable estimate of the expected biological community, with high levels of agreement between predicted and observed community structure. However, there are some limitations to the use of RIVPACS, including the need for a large dataset of stream samples to develop and validate the predictive model and the potential for the model to be less accurate in streams with very different environmental conditions than those in the original dataset (Clarke et al. 2003, Hargett et al. 2007a, Hawkins 2009).

δ^{15}N and δ^{13}C Stable Isotopes of Invertebrates

Stream biological assessments using stable isotopes of carbon and nitrogen in invertebrates have gained increasing attention in recent years (Vander Zanden et al. 2016, Hogsden and McHugh 2017, Smucker et al. 2018, Quinby et al. 2020, Kupilas et al. 2020, Motitsoe et al. 2022). The stable isotope ratio of carbon and nitrogen in invertebrates is influenced by their food source and their feeding habits. Delta-13-C, or δ^{13}C, of stream invertebrates reflect the sources of organic matter fueling the food web, which can be from autochthonous sources such as in-stream primary production, or allochthonous sources such as riparian vegetation (Choy et al. 2009). δ^{13}C refers to the measurement of the stable carbon isotope ratio, specifically the ratio of the stable carbon isotope ^{13}C to the more abundant isotope ^{12}C. The δ^{13}C value represents the difference in the ratio of ^{13}C to ^{12}C in a sample relative to a known standard. Delta-15-N, or δ^{15}N, is used to indicate the trophic structure of invertebrates in the food web (Anderson and Cabana 2007). δ^{15}N is a measurement used in stable isotope analysis to determine the relative abundance of the ^{15}N isotope compared to the more common ^{14}N isotope. The δ^{15}N values can be used to determine community food chain length, while δ^{13}C values can be used to determine the primary production source, although some macroinvertebrates may consume methanogenic organisms, making them very depleted in δ^{13}C (Sampson et al. 2019, DelVecchia et al. 2019). Both δ^{13}C and δ^{15}N are expressed as a deviation in parts per thousand (‰) from a standard reference material.

One study used δ^{15}N and δ^{13}C isotopes across eight restored and eight reference sites to assess the changes in vegetational resources and trophic ranges across ecosystems. (Kupilas et al. 2016). They used δ^{15}N and δ^{13}C isotopes to quantify isotopic complexity for each site. They found that stable isotope-based metrics are useful in identifying patterns in ecosystem processes related to river restoration management and that restoration influences the trophic structure of invertebrates depending on the size of the restoration effort (Kupilas et al. 2016). A more recent study used δ^{15}N and δ^{13}C isotopes from invertebrates across 22 sites to determine if restoration enhances aquatic-terrestrial linkages (Kupilas et al. 2020). Within the study, eleven restoration sites were compared to unrestored upstream references. At each site, the aquatic, riparian, and terrestrial areas were sampled. The results of this study indicated that using δ^{15}N isotope signatures was a satisfactory measurement to differentiate trophic lengths in the restored streams versus reference streams (Kupilas et al. 2020). These studies demonstrate the recent interest in using δ^{15}N and δ^{13}C isotopes of invertebrates to measure the isotopic complexity of restored streams (Kupilas et al. 2016, 2020).

δ^{13}C and δ^{15}N are commonly graphed in a bi-plot space (Abrantes et al. 2014, Kupilas et al. 2020) to display complex trophic and food web interactions of communities. Most studies collect a more comprehensive range of samples, such as fish, algae, invertebrates, and riparian vegetation, when using δ^{13}C and δ^{15}N to track the trophic organization of a community (Perkins et al. 2014a).

CHAPTER THREE

METHODS

Upper Blackfoot Mining Complex Description

The Upper Blackfoot Mining Complex (UBMC) is approximately 27 kilometers northeast of Lincoln, Montana, with a watershed area of 3,470 hectares. The UBMC resides in Lewis and Clark County and the Helena National Forest. In 1941, the Mike Horse Dam was built across Beartrap Creek (Figure 3.1) to withhold tailings containing significant amounts of cadmium, copper, iron, lead, manganese, and zinc, which were generated from a 150 tons-per-day flotation mill (Spence 1997). Heavy rains caused the partial failure of the dam in 1975, and 200,000+ tons of tailings were washed down the headwaters of the Blackfoot River, killing the aquatic organisms (Haaland 2010, Vandeberg et al. 2011).

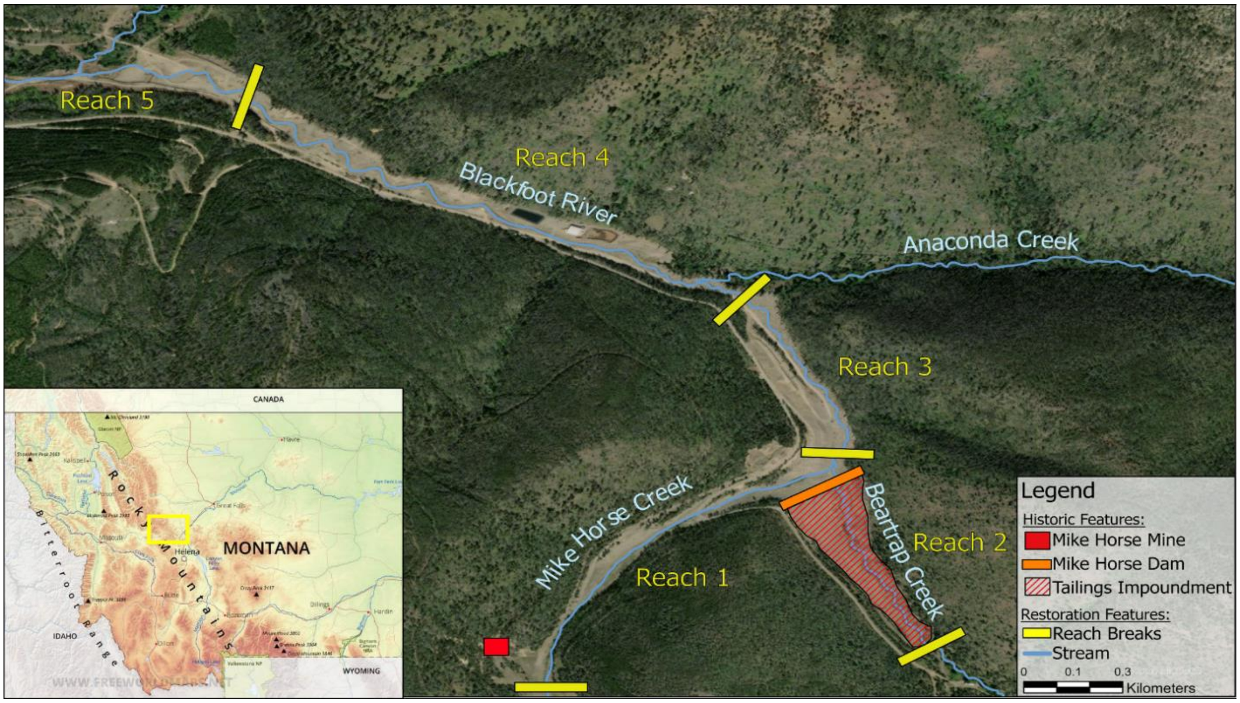

Figure 3.1. Upper Blackfoot Mining Complex. Historical and restoration features on the Upper Blackfoot Mining Complex. The Mike Horse Mine (red), Mike Horse Dam (orange), and tailings impoundment (red stripes) were removed before restoration. The UBMC was restored in sections (or reaches) beginning with Reach 1 and moving downstream to Reach 5.

The Montana Department of Environmental Quality (MT DEQ) was the lead agency for the effort to remediate the effects of this release and restore the stream and floodplains. This effort was conducted in collaboration with the U.S. Forest Service and the Montana Natural Resource Damage Program for the cleanup (Montana Environmental Custodial Trust 2021). The toxic sediment was transported to a lined repository approximately 13 kilometers from the restoration site (Haaland 2010). After 44,000 truckloads (~200,000 tons) of mine waste were removed (DEQ 2020), restoration occurred in five sections (or reaches) (see Figure 3.1) beginning at the Mike Horse Mine (Reach 1), to Beartrap Creek (Reaches two and three), and moving downstream into the Blackfoot River for Reaches four and five (see Figure 3.1). Instead of using substrate from natural streams, formed by rock and sediment deposited by running water (alluvium), the streams and floodplains on the UBMC were created using rock from an adjoining hillside (colluvium) (River Design Group, Inc. et al. 2011). This was necessary because the alluvium in the area had been contaminated with tailings and the only rock available was colluvium. Logs and willow cuttings from the hillsides were used to construct new stream channels and native vegetation was planted along the banks and in the floodplains (River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). After restoration was completed, the dry streams received water flow from the upstream sections that are out of the mining-impacted area.

Streams were reconstructed following a geomorphic stream classification system (Buffington and Montgomery 2013) to predict the most probable form of stream channels and floodplain ecosystems present prior to mining impacts. Each reach was constructed with physical characteristics consistent with cascading, step-pool, or pool-riffle geomorphic classes (River Design Group, Inc. et al. 2011, Buffington and Montgomery 2013). Geomorphic classes describe the physical characteristics of streams and rivers. A series of small waterfalls characterize a cascading stream, whereas a step-pool stream has a series of steps or ledges followed by deeper pools (Montgomery and Buffington 1997). In contrast, a pool-riffle stream has a series of alternating shallow riffles and deeper pools (Montgomery and Buffington 1997). The differences between these classes are important because they affect the stream's hydrology, habitat, and ecological processes involving the organisms that live in and depend on these aquatic environments (Cummins and Klug 1979). Consequently, we expected these classes to impact our invertebrate sampling results.

Invertebrate Sampling Sites

I sampled invertebrates across five restoration and 10 reference sites to quantify invertebrate colonization in the years following restoration. Invertebrates were sampled at five sites on three different streams on the UBMC (hereafter designated as restored sites) collected on the five obvious anthropogenic impacted restoration reaches (Figure 3.2). These are named by the type of site (Rest for restored), an acronym of the stream name, and the reach number at the end of the site name. For example, Mike Horse Creek is a restored site in Reach 1 and has the name Rest.MH1.

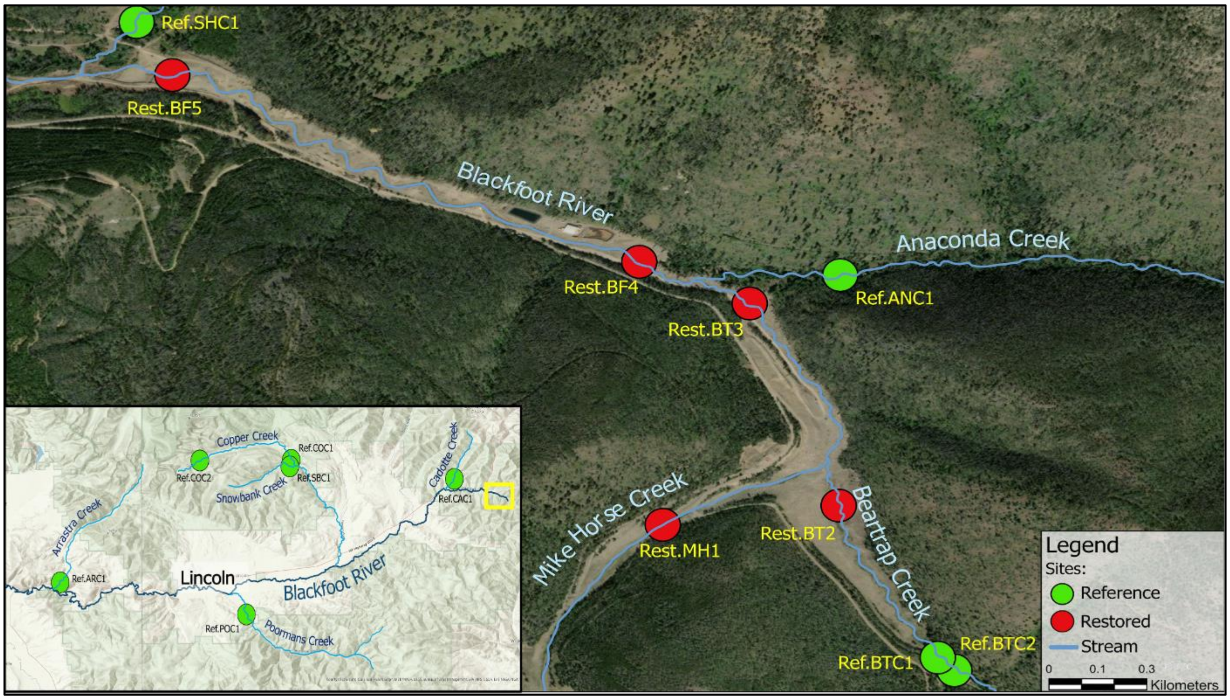

Figure 3.2. Invertebrate Sampling Locations. Sampling locations of five restored sites on the UBMC (Red) and ten reference sites in the greater Blackfoot River watershed (Green).

I selected reference sites in the upper Blackfoot River watershed that have similar geomorphic characteristics and whose impacts were limited to ambient regional perturbations such as logging, exurban development, roads, and natural lands. However, I avoided sites that were immediately adjacent to anthropogenic impacts. Most reference sites were selected by restoration design contractors and were in their initial design report (River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). The report provided four streams as reference sites, but more sites were needed to represent every geomorphic class on the UBMC. To find additional sites, I used stream lines, property ownership, and ensured the inclusion of sites with public access. I selected an array of reference streams across different watersheds to represent a range of disturbances typical to the region (e.g., some degree of logging, exurban development, roads, and natural lands) (Harrelson 1994, Daniluk et al. 2013). I sampled ten reference sites on eight streams (hereafter designated as reference sites) (see Figure 3.2) to compare newly restored streams to reference conditions. Reference sites are assumed to be in good ecological condition and serve as a benchmark for comparing with restored sites (Karr and Chu 1997) and provide a baseline for evaluating the biological condition of restoration sites. Reference sites also have a similar naming convention, but the number at the end corresponds to the stream locations if multiple samples were collected on the same stream. For example, Copper Creek collected two samples, and the upstream sample is named Ref.COC2, while the downstream sample is Ref.COC1.

Physical Site Description

I collected physical data on all streams to describe site characteristics and determine geomorphic classes of each reach (Montgomery and Buffington 1997). I established 200-meter reaches approximately centered on invertebrate sample locations. Walking with a hip chain, I kept track of the linear length of pools, riffles, runs, vegetation type, large wood jams, and bank hydromodifications while progressing up the stream channel. A biodegradable string replaced the string inside the hip chain, and most had been collected post-surveying (Loegering 1997). I classified streams as cascade, step-pool, or pool-riffle based on the stream ratio of pools, riffles, and runs (Leopold and Wolman 1957, Buffington and Montgomery 2013). For example, Beartrap Creek at Rest.BT3 was classified as a pool-riffle because, in the 200-meters sampled, there were 70% pools, 30% riffles, and no runs (or a ratio of 7: 3: 0).

Additionally, I took cross-sections, pebble counts, and stream slopes at exact sampling locations (Harrelson 1994) to characterize the streams (Table 3.1). Cross-sectional data provides information on the shape, depth, and width of the stream channel in meters, while pebble counts indicate the size and composition of the streambed (Wolman 1954). Pebble counts were taken by randomly measuring 100 samples of benthic grain: sand (<2mm), gravel (3-45mm), cobble (64-181mm), or boulders (>256mm) using a ruler across the intermediate axis (Wolman 1954) and calculating abundance of each in percentage. Slopes were calculated using a clinometer and expressed in percentage. I used the watershed tool in ArcGIS with a Digital Elevation Model from the USGS (USGS 2018) to calculate the contributable watershed area in hectares for sample locations. I also used the measuring tool in ArcGIS to calculate the sinuosity of streams (Khan et al. 2018).

Table 3.1. Physical Descriptions of Streams. Distances are reported in meters and slopes in percentage. Using physical site data, sampling sites were described as cascade, step-pool, or pool riffle geomorphology.

Site	Stream Name	Class	Pool: Riffle: Run Ratio	Mean Pool Depth	Mean Riffle Width	Stream	Slope	Sinuosity
Rest.MH1	Mike Horse Creek	Cascade	1: 0: 9	0.2	0.8	1	7	1.04
Ref.COC1	Copper Creek	Cascade	1: 2: 7	0.5	4	5.5	6	1.13
Ref.COC2	Copper Creek	Cascade	1: 1: 8	0.3	4.5	6	7	1.07
Ref.SHC1	Shaue Creek	Cascade	1: 3: 6	0.3	1.3	3	6	1.18
Rest.BF4	Blackfoot River	Pool-riffle	5: 4: 1	0.6	3.4	4.4	2	1.37
Rest.BF5	Blackfoot River	Pool-riffle	4: 5: 1	0.9	3.6	4.2	1	1.34
Ref.ANC1	Anaconda Creek	Pool-riffle	3: 5: 2	0.3	1.8	2.2	2	1.34
Ref.ARC1	Arrastra Creek	Pool-riffle	4: 5: 1	0.8	4.1	5	2	1.81
Ref.POC1	Poorman's Creek	Pool-riffle	4: 5: 1	0.6	4.2	4	1	1.23
Ref.CAC1	Cadotte Creek	Pool-riffle	5: 4: 1	0.6	2.3	4	1	1.8
Rest.BT2	Beartrap Creek	Step-pool	7: 2: 1	0.4	1.4	2.3	5	1.15
Rest.BT3	Beartrap Creek	Step-pool	7: 3: 0	0.6	1.5	2	5	1.15
Ref.BTC1	Beartrap Creek	Step-pool	9: 1: 0	0.2	1	1	5	1.12
Ref.BTC2	Beartrap Creek	Step-pool	9: 1: 0	0.2	1	1	5	1.12
Ref.SBC1	SnowBank Creek	Step-pool	8: 2: 1	0.8	2.2	2.7	2.3	1.12

Site Land Use/Land Cover

To help characterize the reference and restored sites, I calculated land use/land cover (LULC) surrounding each sampling location. Using 2018 areal imagery (USGS 2018), I applied a 500-meter buffer in ArcGIS around each site (Johnson and Zelt 2005). The buffer area was delineated into land cover categories and classified as coniferous/herbaceous vegetation, developed land/bare ground, and river/side channels (Figure 3.3). The total and percent land area of each category was calculated by summing the area of the polygons representing that LULC type.

Figure 3.3. ArcGIS Buffers to Quantify LULC. 500-meter radial circles surrounding reference site Ref.ARC1 (left) and restored site Rest.BT2 (right). The area inside of the circle was characterized as coniferous/herbaceous vegetation (dark/light green), developed land/bare ground (red/tan), and river/side channels (dark/light blue).

Descriptions of Restored Sites

Located within the UBMC, Mike Horse Creek, Beartrap Creek, and the Blackfoot River form a dynamic sequence, flowing in succession from upstream to downstream. The project area and encompassing watershed exhibit rugged terrain and are predominantly covered by dense forests of *Pinus contorta* (lodgepole pine) and *Pseudotsuga menziesii* (Douglas fir). The elevations vary within the range of 2,286 meters at the headwaters on the continental divide, gradually descending to 1,584 meters downstream of the UBMC project area.

Table 3.2. Restored Sites Characteristics. Characteristics present on restored sites. Substrata is reported in percent for sand (<2mm), gravel (2-45mm), cobble (64-181mm), or boulders (>256mm). The watershed area is in hectares.

Site	**Stream Name**	**Class**	**Restored/ Reference**	**Watershed Area**	**Sand**	**Gravel**	**Cobble**	**Boulders**	**Aspect**
Rest.MH1	Mike Horse Creek	Cascade	Restored	93.9	2	88	10	0	NE
Rest.BT2	Beartrap Creek	Step-pool	Restored	356.6	3	90	7	0	N
Rest.BT3	Beartrap Creek	Step-pool	Restored	769.2	10	79	11	0	NW
Rest.BF4	Blackfoot River	Pool-riffle	Restored	1535.4	9	85	6	0	NW
Rest.BF5	Blackfoot River	Pool-riffle	Restored	1815.1	2	84	14	0	W

Mike Horse Creek (Rest.MH1)

Figure 3.4. Photo of Mike Horse Creek. Photo looking upstream of Mike Horse Creek and sampling site Rest.MH1.

The highest stream on the UBMC project is Mike Horse Creek (see Figure 3.4), which flows immediately beneath the Mike Horse Mine. Mike Horse Creek is in Reach 1 and was completed in 2018. A narrow riparian zone (~2 meters) of vegetation borders both sides of the stream. Vegetation includes shrubs in the riparian zone consisting of *Salix planifolia* (diamond-leaf) and *S. sitchensis* (Sitka) willow. Rest.MH1 is a first order stream (Strahler 1957) and has the geomorphological classification of cascade (Montgomery and Buffington 1997) and the channel substrate is angular and primarily gravel (see Table 3.2).

Beartrap Creek (Rest.BT2 and Rest.BT3)

Figure 3.5. Photos of Beartrap Creek. Photos of sampling sites Rest.BT2 (left) and Rest.BT3 (right) on Beartrap Creek.

Beartrap Creek flows where the tailings impoundment resided above Mike Horse Dam. Two sampling locations are along Bear Trap Creek (see Figure 3.5), both being second order streams (Strahler 1957). Both have a geomorphic class of a step-pool. The vegetation growing along the banks is primarily alder and willow in the terrestrial zone. Rest.BT2 is 50 meters upstream of the confluence with Mike Horse Creek, restored in 2017, and is in Reach 2. Rest.BT3 is 100 meters downstream of the confluence with Mike Horse Creek, restored in 2018, and is in Reach 3. The channel substrate at Rest.BT2 and Rest.BT3 is angular, consisting mainly of gravel (see Table 3.2).

Blackfoot River (Rest.BF4 and Rest.BF5)

Figure 3.6. Photos of Blackfoot River. Photos of sampling sites Rest.BF5 (left) and Rest.BF4 (right) on the Blackfoot River.

There are two sampling locations along the Blackfoot River (see Figure 3.6), formed by the confluence of Anaconda and Beartrap Creeks making it a third order river (Strahler 1957). Completed in 2019, Rest.BF4 lies 30 meters below the confluence and is in Reach 4. Completed in 2020, Rest.BF5 is 200 meters downstream of Rest.BF4 and is in Reach 5. Rest.BF4 and Rest.BF5 are classified as pool-riffle streams, and the channel substrate is angular, consisting mainly of gravel (see Table 3.4). Vegetation on both streams has alder and willow in riparian areas.

Descriptions of Reference Sites

Table 3.3. Reference Sites Characteristics. Characteristics of reference sites. Substrata is reported in percentage for sand, gravel, cobble, or boulders. The watershed area is in hectares.

Site	Stream Name	Class	Restored/ Reference	Watershed Area	Sand	Gravel	Cobble	Boulders	Aspect
Ref.SHC1	Shaue Gulch Creek	Cascade	Reference	842.7	10	78	12	0	SW
Ref.COC1	Copper Creek	Cascade	Reference	7786.7	9	40	46	6	NW
Ref.COC2	Copper Creek	Cascade	Reference	880.4	6	63	30	1	NW
Ref.BTC1	Beartrap Creek	Step-pool	Reference	248.9	18	70	12	0	NW
Ref.BTC2	Beartrap Creek	Step-pool	Reference	249.1	18	70	12	0	NW
Ref.SBC1	SnowBank Creek	Step-pool	Reference	1918.5	7	57	36	0	SE
Ref.ARC1	Arrastra Creek	Pool-riffle	Reference	6173	1	68	31	0	SW
Ref.CAC1	Cadotte Creek	Pool-riffle	Reference	1526.3	7	83	10	0	S
Ref.ANC1	Anaconda Creek	Pool-riffle	Reference	739.2	3	51	45	1	SE
Ref.POC1	Poorman’s Creek	Pool-riffle	Reference	10328.7	5	93	2	0	N

Shaue Gulch Creek (Ref.SHC1)

Figure 3.7. Photo of Shaue Gulch Creek. Photo looking downstream of Shaue Gulch Creek and sampling site Ref.SHC1.

Shaue Gulch Creek is also located on the UBMC but outside the mining-impacted area (see Figure 3.7). Shaue Gulch Creek flows into the Blackfoot River 120 meters below the sampling site Rest.BF5. Shaue Gulch Creek is a second order stream (Strahler 1957) and is classified as cascade. Vegetation along the creek consists of alder willow in the terrestrial zone and alpine fir and spruce trees in the upland. The channel substrate at Ref.SHC1 is sub-angular and consists mainly of gravel (see Table 3.3).

Copper Creek (Ref.COC1 and Ref.COC2)

Figure 3.8. Photos of Copper Creek. Photos of sampling sites Ref.COC2 (left) and Ref.COC1 (right) on Beartrap Creek.

Copper Creek is located 27.6 kilometers northwest of the UBMC and is a primary tributary to the Blackfoot River. Both sites have alder and spruce trees in the riparian zones, with grass and lodgepole pine in the uplands (see Figure 3.8). Ref.COC2 is upstream of Ref.COC1 by 6.4 kilometers and is much steeper, being classified as a cascading and a first order stream (Strahler 1957). Ref.COC2 channel substrate is sub-angular and consists mainly of gravel and cobble (see Table 3.3). Ref.COC1 is classified as a cascade and a second order stream (Strahler 1957). Ref.COC1 channel substrate is rounded and consists of mainly gravel and cobble with a few boulders.

Upper Beartrap Creek (Ref.BTC1 and Ref.BTC2)

Figure 3.9. Photos of Beartrap Creek Reference. Photos of sampling sites Ref.BTC1 (left) and Ref.BTC2 (right) on Beartrap Creek.

Two reference sampling locations on Beartrap Creek are upstream of the restoration and away from any potential Mike Horse Mine-associated ecological legacies (see Figure 3.9). Ref.BTC1 and Ref.BTC2 are both classified as step-pool and a first order stream (Strahler 1957). The channel substrate is rounded and consists of gravel, sand, and cobble (see Table 3.3). Ref.BTC1 is downstream of Ref.BTC2 by 90 meters and the vegetation is thick and dominated by alder, with willows in the terrestrial zone and cottonwoods in the upland.

SnowBank Creek (Ref.SBC1)

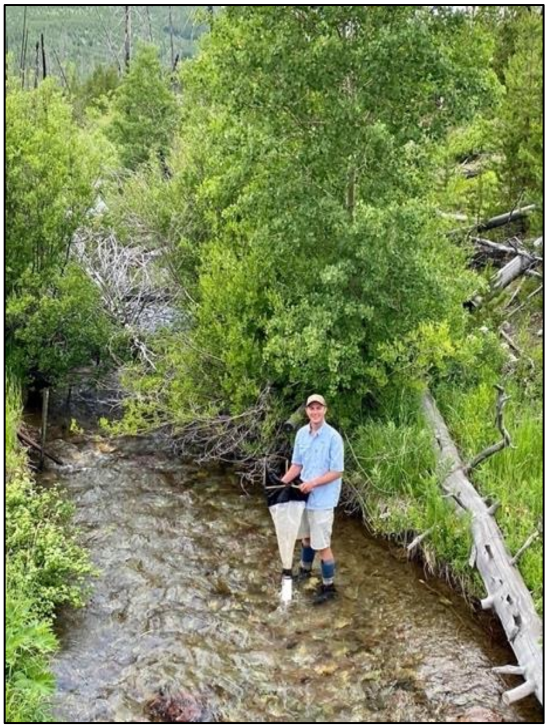

Figure 3.10. Photo of Snowbank Creek. Photo of me standing in Snowbank Creek sampling site Ref.SBC1.

Snowbank Creek is located 26.2 kilometers northwest of the UBMC and flows into Copper Creek. Ref. SBC1 is a first order stream (Strahler 1957) and is classified as a step-pool. The channel substrate is sub-rounded and consists mainly of gravel and cobble (see Table 3.3). There are shrubs, willow, and alder in the riparian zone, with lodgepole pines in the upland (see Figure 3.10).

Arrastra Creek (Ref.ARC1)

Figure 3.11. Photo of Arrastra Creek. Photo looking downstream of Arrastra Creek and sampling site Ref.ARC1.

Arrastra Creek is in the greater Blackfoot River watershed but 41 kilometers west of the UBMC. It contains thick alder and willows in the terrestrial zone and pine trees in the upland (see Figure 3.11). The channel has multiple secondary side channels developed on active floodplain surfaces and wetlands before entering the Blackfoot River. The creek is a first order stream (Strahler 1957) and is classified as a pool-riffle. The channel substrate consists primarily of gravel and cobble (see Table 3.3).

Cadotte Creek (Ref.CAC1)

Figure 3.12. Photo of Cadotte Creek. Photo looking downstream of Cadotte Creek and sampling site Ref.CAC1.

Cadotte Creek is located 5.6 kilometers northwest of the UBMC and is dominated by alder, willow grasses in the terrestrial zone, alpine fir, and spruce trees in the upland (see Figure 3.12). There are meadows interspersed around the stream. The channel form is a primarily single thread with secondary side channels developed on active floodplain surfaces. The channel substrate is sub-rounded and consists mainly of gravel (see Table 3.3). The creek is a first order stream (Strahler 1957) and is classified as a pool-riffle stream.

Anaconda Creek (Ref.ANC1)

Figure 3.13. Photo of Anaconda Creek. Photo looking downstream of Anaconda Creek and sampling site Ref.ANC1.

Anaconda Creek flows into Bear Trap Creek to create the headwaters of the Blackfoot River. Ref.ANC1 is located 150 meters upstream of the confluence with Bear Trap Creek and away from the mining-impacted area (see Figure 3.13). Ref.ANC1 is classified as a pool-riffle and a second order stream (Strahler 1957). Although approximately 20 meters of Anaconda Creek appears to have been impacted by the mine, most of the stream appears to be unimpacted. The vegetation along Anaconda Creek is heavily dominated by alder, grass, and cottonwoods in the terrestrial zone, along with alpine fir and spruce trees in the upland. The channel substrate is sub-angular and consists mainly of gravel and cobble (see Table 3.3).

Poorman's Creek (Ref.POC1)

Figure 3.14. Photo of Poorman's Creek. Photo looking upstream of Poorman's Creek and sampling site Ref.POC1.

Poorman's Creek is located 27 kilometers southwest of the UBMC and is a second order stream (Strahler 1957). The vegetation consists of mature cottonwood, alder, and willows in the riparian zones, along with alpine fir and spruce trees in the upland (see Figure 3.14). Ref.POC1 is classified as pool-riffle; the channel substrate is rounded and mainly consists of gravel (see Table 3.3).

Invertebrate Sampling Procedure

Invertebrates were collected in 2020 by Adrian Massey, and I collected invertebrate samples in 2021 and 2022 (Table 3.4) to document the colonization of invertebrates on the UBMC over multiple seasons. High-frequency sampling was conducted on the restored sites due to rapid colonization that can occur within the first few years of a novel ecosystem's establishment (Gore 1982, Malmqvist et al. 1991, Chapelsky et al. 2020). Most aquatic invertebrates are univoltine (one generation per year), therefore early autumn is usually the best time to collect aquatic invertebrates because, many species have nearly completed the juvenile phase of their life cycle and are in their largest body form, making them easier to identify (Çamur-Elipek1 et al. 2010, DEQ 2012, Hogsden and McHugh 2017). While this is true, most streams on the UBMC are first order streams (Strahler 1957), and are dry by autumn, making the ideal sampling period to be late summer. After learning this in 2020, I sampled a month earlier for the spring and summer seasons in 2021.

I established more reference than restored sites to develop an array of reference conditions across a range of different disturbance regimes (Ward 1998a, Mack and D'Antonio 1998, Peckarsky et al. 2014). Most reference samples were collected during the autumn of 2020, as I initially believed it to be the optimal period for long-term sampling on the UBMC, but later realized that most UBMC stream channels became dry by September.

Table 3.4. Sampling Timing and Location. The sequence of when invertebrate samples were collected. Sites were sampled for invertebrate composition (X) or δ^{13}C and δ^{15}N isotopes (I). Some planned samples at the UBMC could not be taken (NS) due to drought in the late fall seasons.

Location	Site	2020			2021			2022
		June	August	September/October	May	July	September	July
Restored	Rest.MH1	X	X	NS	X	X	NS	I
	Rest.BT2	X	X	X	X	X	NS	I
	Rest.BT3	X	X	NS	X	X	NS	I
	Rest.BF4	X	X	X, I	X	X	I	I
	Rest.BF5	X	X	NS	X	X	NS	X, I
Reference	Ref.ARC1		X	X				I
	Ref.ANC1			X, I			X, I	I
	Ref.BTC1			X				I
	Ref.BTC2			X				I
	Ref.CAC1			X				I
	Ref.COC1			X				I
	Ref.COC2			X				I
	Ref.POC1						X	I
	Ref.SBC1			X				I
	Ref.SHC1					X		I

I used a Surber sampler (Surber 1937) to collect invertebrates because it has guide dimensions of 0.09 square meters on the bottom, which helps provide a consistent measurement across sites. It is used by placing the sampler in the streambed (preferably a riffle) while having the net facing upstream to catch the invertebrates (Needham and Usinger 1956). I sampled at mid-riffle locations at water depths of 10-40cm with consistent flow rates. Stirring up the substrata with a metal spike inside the sampler, at a depth of 10 cm, allowed invertebrates to be carried by water into the collection receptacle at the tail of the conical collection net. I cleaned larger rocks within the frame with both hands to detach any clinging organisms and debris. The sampler was lifted out of the water and "rinsed" three times, with the opening still facing upstream, to wash the organisms into the receptacle at the tail of the net. I then detached the receptacle from the net and poured them into a 25x35 cm sorting tray. I separated the invertebrates from the substrate by stirring the tray's contents and ran the floating organic matter into a NO.40 425 µm U.S.A. Standard Testing soil sieve. Invertebrates were poured into small polyvinyl chloride containers containing 70% ethanol for preservation (Sarakinos et al. 2002, Hogsden and McHugh 2017). I continued rinsing and pouring them into the sieve until all apparent invertebrates were removed. Finally, the tray and the Surber net were closely inspected for remaining insects, which were carefully removed with tweezers and placed in the collection jar.

Invertebrate Attributes and Taxonomic Effort

Aquatic invertebrates contain a suite of physical, behavioral, and ecological attributes, such as size, shape, mobility, feeding habits, and tolerance to pollution or environmental stressors (Cummins 1973, Gresens et al. 2009). Some of these respond to perturbations to the natural disturbances regime and can act as indicators of those perturbations. For example, the presence of invertebrate taxa known to be sensitive to changes in suspended sediments, eutrophication, or temperature can be used as an indicator of a stream with high water quality (Surdick and Gaufin 1978). Conversely, the presence of more pollution-tolerant species can indicate poor water quality (Prather et al. 2013).

Invertebrates collected to calculate taxonomic diversity and similarity indices, RIVPACS, and B-IBI were sent to EcoAnalysts, Inc. (Moscow, Idaho) for professional taxonomic identification to the lowest possible taxonomic level possible, predominantly to the genus level. invertebrate attribute data (community compositions, biological processes, functional feeding groups) was provided by EcoAnalysts, Inc. and found in other previous works (Cummins 1973, Cummins and Klug 1979, Fore and Wisseman 2012). Since most were identified to the genus level, invertebrates from genus were matched to attribute data using R (R Core Team 2023). I identified invertebrates for the SICI to the family level using a taxonomic key (Merritt and Cummins 2008). FFGs were matched to families using the attribute data from EcoAnalysts, Inc.

Taxonomic Diversity and Similarity Indices

I utilized the Shannon-Wiener (or Shannon), Simpson, and Inverse Simpson Diversity indices to assess if restored stream's invertebrates are diversifying to the same extent as reference sites (Shannon 1948, Simpson 1949). I chose these three to measure taxonomic diversity because I found that they were the most used (De Szalay and Resh 2000, Seger et al. 2012) and easy to calculate using R. The Shannon index considers the number of taxa and their relative abundance in each community or ecosystem. The Gini-Simpson Diversity Index, on the other hand, only considers taxa richness (the number of taxa present in a sample). A higher Gini-Simpson and Shannon Diversity index value closer to 1 indicates a more diverse community. The Inverse Simpson Diversity Index (or Simpson Reciprocal Index) measures diversity by considering the number of taxa present in a community and their relative abundances. I used the Vegan package in R to calculate Shannon, Inverse Simpson, and Simpson Diversity indices (R Core Team 2023). Using these three diversity indices measures the biological diversity of invertebrates in restored and reference sites.

Lastly, I utilized the Bray-Curtis similarity Index to assess how similar the taxa on restored sites are compared to reference sites (Bray and Curtis 1957). This index measures the similarity between sites based on the taxa present and their abundance, making it a valuable tool for comparing the taxonomic composition of different sites. I utilized this index to determine how similar restored streams are to reference sites regarding the invertebrate communities present. I used R to calculate Bray-Curtis Similarity (R Core Team 2023).

Shannon-Wiener Diversity Index

The Shannon-Wiener Diversity index (Shannon 1948) (H': Equation 3.1) takes the negative sum of the proportion of individuals in each taxon multiplied by the natural logarithm (2.72) of that proportion. This value was then multiplied by the total number of species:

$$\mathrm{H}' = -\sum_{i=1}^{S} p_i * logn_b p_i, \qquad \text{(Equation 3.1)}$$

where p_i is the proportion of taxa i, S is the number of taxa, and b is the natural log.

Gini-Simpson Diversity Index

The Gini-Simpson Diversity index was calculated (D: Equation 3.2) by summing the proportion of individuals in each taxon, squaring the values, and then subtracting by 1:

$$D = 1 - \sum_{i=1}^{S} p_i^2, \qquad \text{(Equation 3.2)}$$

where p_i is the proportion of taxa i, and S is the number of taxa.

Inverse Simpson Diversity Index

The Inverse Simpson Diversity Index ($\frac{1}{D}$) (Equation 3.3) is simply the reciprocal of the Simpson Diversity Index:

$$\frac{1}{D} = \frac{1}{1-\sum_{i=1}^{S} p_i^2}, \qquad \text{(Equation 3.3)}$$

where p_i is the proportion of taxa i, and S is the number of taxa. This index ranges from 1 to the number of taxa in the community, with higher values indicating greater diversity.

Bray-Curtis Similarity Index

The Bray-Curtis similarity index (Bray and Curtis 1957) (BC: Equation 3.4) calculates the minimum abundance of each taxon shared between the two communities, sums these minimum abundances for all shared taxa, divided by the total abundance in both communities, and multiplied by 2. Finally, this value is then subtracted from 1:

$$BC = 1 - \frac{2C_{ij}}{S_i + S_j}, \qquad \text{(Equation 3.4)}$$

For the application here, C_{ij} is the sum of the minimum abundance of each taxon shared between a restored and reference site of similar geomorphic type, *Si* is the total number of taxa of the restored site of interest, and *Sj* is the total number of reference site taxa. The Bray-Curtis Similarity index values range from 0 to 1, with a higher value indicating greater similarity to reference sites.

Biological Assessments

I utilized three biological assessments to quantify colonization and find a long-term monitoring tool for the UBMC. Each index captures different aspects of biological condition and can comprehensively assess the biological state of the streams. The first index I used was the River Invertebrate Prediction and Classification System (RIVPACS), which was developed by a team of scientists at the Institute of Freshwater Ecology (now the Centre for Ecology and Hydrology) in the United Kingdom (Wright et al. 1993). RIVPACS utilizes invertebrate data and GIS-derived environmental variables to assess the biological condition of rivers based on taxonomic richness. The second index I used was the Benthic Index of Biotic Integrity (B-IBI), which incorporates various invertebrate metrics to measure departure from a reference condition, providing a scoring system for comparing biological conditions across streams. Finally, I introduced an index called the Stable Isotopic Colonization Index (SICI) that utilized carbon and nitrogen stable isotope compositions to estimate invertebrate colonization. SICI aimed to simplify the assessment of colonization by providing a straightforward scoring approach like an IBI, thus facilitating ecosystem management decisions.

River Invertebrate Prediction and Classification System

RIVPACS, since its inception in 1993, has gained significant recognition as a powerful tool for assessing the biological condition of rivers worldwide, primarily by evaluating the richness of invertebrate populations (Clarke et al., 2003). While its use has been more widespread in the United Kingdom and Europe, it has also found application in the western USA, albeit to a lesser extent (Hawkins et al., 2000; Hargett et al., 2007b; Hawkins, 2009; DEQ, 2012). The method employed in RIVPACS relies on gathering invertebrate assemblage data and incorporating habitat characteristics from a diverse range of sites to describe the biological condition of rivers (Wright et al., 1993; Wright et al., 2000).

RIVPACS uses invertebrate assemblage data and habitat characteristics at a wide range of sites to describe the biological condition of rivers (Wright et al. 1993, 2000). The observed (O) condition is the invertebrate taxa richness of a site of interest. The expected (E) condition is the taxa richness computed using invertebrate data from hundreds of streams and environmental predictor variables (DEQ 2012) describing the region. A RIVPACS index score of O/E can then be calculated by dividing the Observed over the Expected condition. An O/E ratio that approaches 0 indicates a disturbed aquatic system that does not display the taxonomic richness of a similar region.

A study in Wyoming used aquatic invertebrate and GIS-derived predictor variables at 925 stream monitoring sites to successfully validate predictor variables to use for RIVPACS (Hargett et al. 2007a). To find predictor variables (Appendix A), I followed protocols from Hargett et al. (2007) to calculate each site's latitude, longitude, watershed area, and terrain roughness. The air temperature was noted during sample collection. I worked with Rithron Associates, Inc. in Missoula, Montana, to compute Expected values from the predictor variables I found using GIS and field observations.

Benthic Index of Biotic Integrity

I developed a Benthic Index of Biotic Integrity (B-IBI) specific to the Upper Blackfoot watershed to estimate the biotic integrity of restored and reference sites (Kerans and Karr 1994, Kleindl 1995). I identified 60 invertebrate attributes, which could measure departure from a reference condition (Barbour et al. 1999). I evaluated if those attributes were ecologically meaningful and then statistically tested those attributes to discover if they were appropriate indicators between restored and reference sites (Gosset 1908, Levene 1960, Dunn 1961, Krishnamoorthi et al. 2023). I then used a scoring system that assigns each stream a score ranging from 0 to 100, which can be used to compare the biological condition of different streams (Wilhelm 2014).

B-IBI Metric Selection.

Since the B-IBI is an MMI, selecting correct invertebrate attributes as metrics is essential for ensuring that is an indicator of the biological condition of the aquatic invertebrate community. It is important to consider the assessment goals when selecting metrics for a multi-metric index (Kerans and Karr 1994). For the UBMC, I aimed to choose metrics for the B-IBI, which represent a restored stream recovery to an ambient disturbance regime of the Upper Blackfoot watershed.

To find metrics that represent ambient disturbance conditions for the UBMC, I compared 60 invertebrate attributes between restored and reference sites (Table 3.5). Attributes included different types of invertebrate taxa richness, community compositions, biological processes, and functional feeding groups. When testing for statistical significance, site attributes were first tested for equal variance using Levene’s Test (Levene 1960). If indicators displayed unequal variance, Welch’s two-sample T-test was used (Welch 1947), otherwise, a two-sample T-test assuming equal variance was used. I found in the literature whether I should expect each to increase or decrease to an increase in disturbance (Table 3.5) (Needham 1936, Anderson and Sedell 1979, Cummins and Klug 1979, Cardinale et al. 2004, Gresens et al. 2009, Merritt et al. 2017, Prommi 2018).

Table 3.5. Invertebrate Attributes Tested for B-IBI. 60 invertebrate attributes were tested for statistical significance between reference and restored sites.

B-IBI Metrics Tested	**Expected Response to Increased Disturbance**
Invertebrate Taxa Richness and Community Composition	
Taxa Richness	Decrease
% Richness EPT Taxa	Decrease
EPT Richness	Decrease
% Richness Ephemeroptera	Decrease
Ephemeroptera Richness	Decrease
% Ephemeroptera Abundance	Decrease
% Richness Plecoptera	Increase
Plecoptera Richness	Decrease
% Plecoptera Abundance	Decrease
% Richness Trichoptera	Decrease
Trichoptera Richness	Decrease
% Trichoptera Abundance	Decrease
% Richness Diptera	Increase
Diptera Richness	Increase
% Abundance Diptera	Increase
% Richness Coleoptera	Decrease
Coleoptera Richness	Decrease
% Abundance Coleoptera	Decrease
% Richness Heptageniidae	Decrease
Heptageniidae Richness	Decrease
% Abundance Heptageniidae	Decrease
% Richness Baetidae	Increase
Baetidae Richness	Increase
% Abundance Baetidae	Increase
Invertebrate Biological Processes	
% Richness Clinger	Decrease
Clinger Richness	Decrease
% Abundance Clinger	Decrease
% Richness Semivoltine	Decrease
Semivoltine Richness	Decrease
% Abundance Semivoltine	Decrease
% Richness Intolerant	Decrease

Intolerant Richness	Decrease
% Abundance Intolerant	Decrease
% Richness Sprawler	Increase
Sprawler Richness	Increase
% Abundance Sprawler	Increase
% Richness Univoltine	Increase
Univoltine Richness	Decrease
% Abundance Univoltine	Decrease
% Richness Multivoltine	Increased
Multivoltine Richness	Decrease
% Abundance Multivoltine	Increase
Invertebrate Functional-Feeding Group	
% Richness Predator	Increase
Predator Richness	Decrease
% Abundance Predator	Decrease
% Richness Gatherers	Increase
Gatherers Richness	Decrease
% Abundance Gatherers	Increase
% Richness Shredders	Decrease
Shredders Richness	Decrease
% Abundance Shredders	Decrease
% Richness Scrapers	Decrease
Scrapers Richness	Decrease
% Abundance Scrapers	Decrease
% Richness Filterers	Increase
Filterers Richness	Increase
% Abundance Filterers	Increase
Invertebrate Abundance	
Total Abundance	Decrease
% EPT Abundance	Decrease
EPT Abundance	Decrease

Redundant indicators were also excluded from the final B-IBI. For instance, while Trichoptera richness and EPT richness (Ephemeroptera, Plecoptera, and Trichoptera) displayed strong significance (Appendix B), Trichoptera richness was the primary driver for EPT richness to be highly significant. Therefore, EPT richness was excluded, and Trichoptera richness was used. To reduce variability, four metrics are expressed as percentages relative to taxa abundance or richness. For example, the percentage of abundance represents the taxa in a particular group divided by the total abundance. In contrast, the percentage of richness represents the number of taxa in a specific group divided by the full richness.

<u>B-IBI Scoring.</u> The B-IBI for the UBMC should represent a collective biological response and a culmination of indicators selected (Barbour and Paul 2010). To portray this numerically, the ten indicators chosen for the B-IBI were scored, thereby becoming metrics, from 0 to 10 using the 10^{th} and 90^{th} percentiles from each indicator (Appendix C) (Wilhelm 2014). Indicators were scored from 0 to 10 because 10 metrics were used for the B-IBI. If a site's B-IBI indicator value was above the 90th percentile, it received a score of 10. If it was below the 10th percentile, it received a score of zero. When an indicator value was between the 10th and 90th percentiles, it was applied to the respective scoring formula displayed in Table 3.6.

The metrics are simply added, without weights, for the total B-IBI score that ranges from 0 to 100. A score that is closer to 100 represents a stream with high biological integrity and pristine conditions for the Blackfoot River watershed. A score that is closer to 0 represents a stream that exhibits lower biological integrity than the other streams in the Blackfoot River watershed. This scoring method also allows streams that exhibit invertebrate attributes that become more present with less disturbance to trend toward 100. All B-IBI scores can be found in Appendix D.

Table 3.6. B-IBI Scoring. Scoring method when scoring metrics. A metric above the 90th percentile receives a score of 10, while a metric below the 10th percentile receives a score of 0. If a metric falls between the 10th and 90th percentiles, it is applied to the scoring formula.

Metric Response with Disturbance	Score for Values <10th Percentile	Score for Values >90th Percentile	Scoring Formula for Values >10th and <90th Percentile
Decrease with Human Disturbance	**0**	**10**	$= \frac{10\ X\ (Observed\ Value\ -\ 10th\ Percentile)}{90th\ Percentile -\ 10th\ Percentile}$

Stable Isotopic Colonization Index

Carbon and nitrogen stable isotope compositions (δ^{13}C and δ^{15}N) are commonly used in trophic studies (Abrantes et al. 2014). Stable carbon isotopes change very little from producers to consumers (DeNiro and Epstein 1978), therefore δ^{13}C performs well as an indicator of carbon sources across trophic levels. δ^{15}N is generally used to track relative trophic positions for the trophic fractionation changes +1.9-3‰ per trophic link (McCutchan et al. 2003, Kupilas et al. 2016). The δ^{13}C and δ^{15}N values are expressed in ‰ (per mil or parts per thousand) relative to reference material and are used to track the flow of energy and nutrients through food webs (Layman et al. 2007b).

After invertebrate taxonomic identification to family and assigning FFGs, I calculated the δ^{13}C and δ^{15}N compositions of invertebrates in the community (Kupilas et al. 2020) to quantify isotopic complexity (Kupilas et al. 2016). To do this, I used invertebrates from every FFG in the sample. When I selected one representation of each FFG, I chose the invertebrates that had the highest abundance when samples contained multiple families for one FFG. For example (Figure 3.15), a stream sample was sorted into seven families, but six represent three FFG. In this instance, I choose the families with the most abundant invertebrates and assumed they represented more of the collected sample (Kupilas et al. 2020).

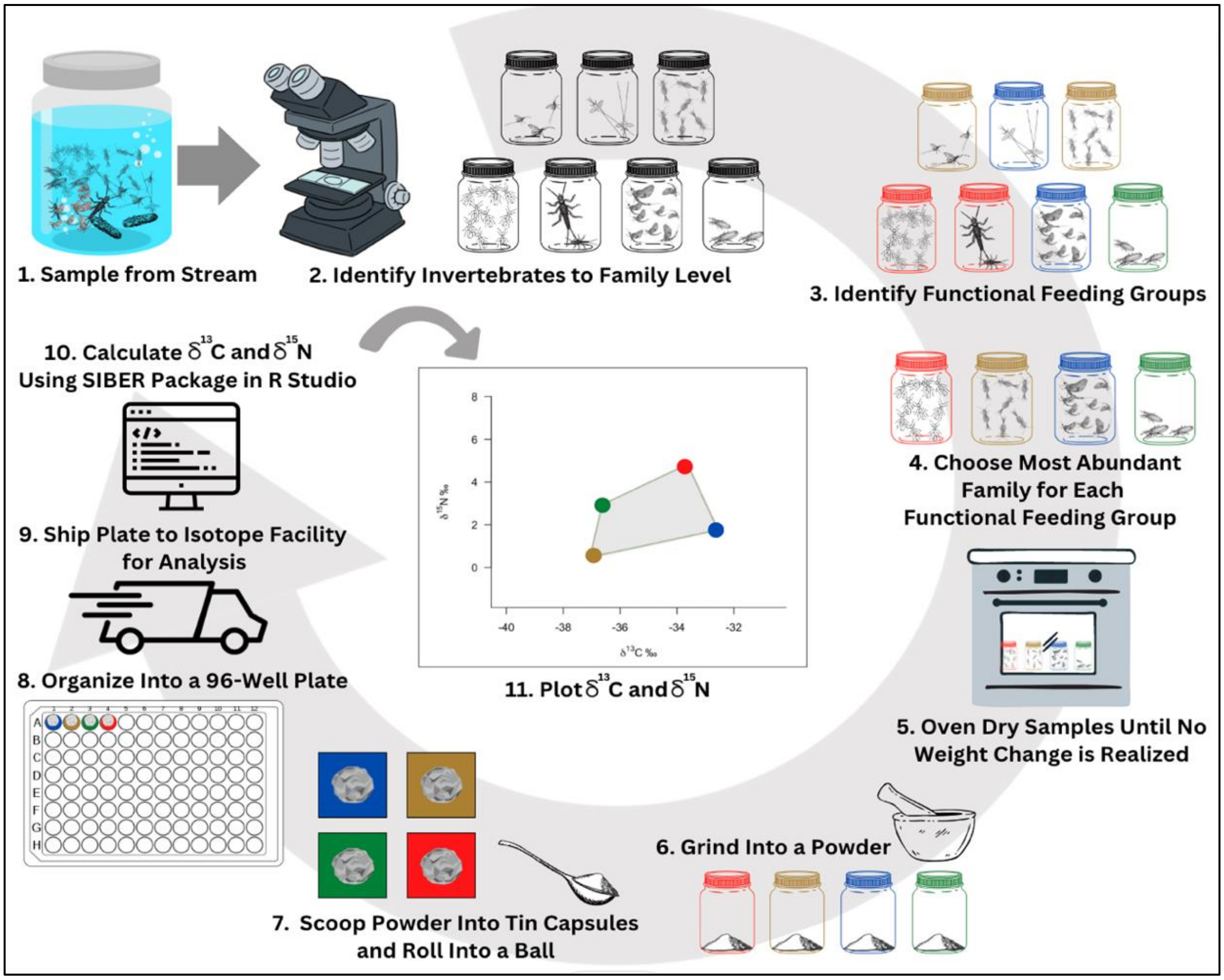

Figure 3.15. Sample Processing for Stable Isotopes. Diagram of methods to calculate δ^{13}C and δ^{15}N starting from a stream sample.

The selected families were then oven-dried until the sample weight did not change to ensure no ethanol or water remained in the samples (Kupilas et al. 2016). I then ground the samples to a powder using a mortar and pestle and enclosed them in tin (Sn) capsules in preparation for analysis by the UC Davis Stable Isotope Facility (Brandeberry 2020a). For proper analysis, 0.5-1.25mg of each sample was submitted (Brandeberry 2020b), which requires approximately five invertebrates to be ground into a powder. The homogenized samples were analyzed for δ^{15}N and δ^{13}C using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer. Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier flowed through a water trap (magnesium perchlorate and phosphorous pentoxide). N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS (Brandeberry 2020a). The equation:

$$\delta^{13}\mathrm{C},\delta^{15}\mathrm{N} = [(\frac{\mathrm{Rsample}}{\mathrm{Rstandard}})\text{-}1] \times 1000, \qquad \text{(Equation 3.5)}$$

where $R_{\mathrm{sample}} = \frac{13_C}{12_C}$ or $\frac{15_N}{14_N}$ and $R_{\mathrm{standard}} = \frac{13_C}{12_C}$ or $\frac{15_N}{14_N}$ was used to calculate ‰ (parts per thousand) of δ^{13}C and δ^{15}N. The national R_{standard} values used were $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{Air}$ for carbon and nitrogen respectively (Brand et al. 2014). Measures of standards placed throughout samples exhibited acceptable instrument reproducibility of ±0.13‰ for δ^{15}N and ±0.11‰ for δ^{13}C.

After receiving δ^{13}C and δ^{15}N values from UC Davis, eight community-wide attributes that estimate invertebrate trophic levels (NR), assimilated carbon sources (CR), niche spaces

(TA), trophic diversity (CD), FFG density (MNND), and evenness (SDNND) (Layman et al. 2007a) were computed. Nitrogen Ranges (NR) were calculated as maximum δ^{15}N minus minimum δ^{15}N and represented the trophic length. Carbon Ranges (NR) were calculated by maximum δ^{13}C minus minimum δ^{13}C and represented assimilated carbon sources (Layman et al. 2007b). Total Areas (TA) were computed by a polygon (convex hull) drawn around all FFGs in the δ^{15}N, and δ^{13}C biplot, representing the isotopic niche space of the invertebrate community (Layman et al. 2007a). Mean Distance to Centroid (CD) is the mean distance of points to the centroid of the biplot space and represents the average degree of trophic diversity (Layman et al. 2007a). The CD is one of the best assessments of trophic diversity, as FFG outliers influence it less than other metrics (Layman et al. 2007a). By relying on the mean values of all FFGs, CD provides a more comprehensive and accurate picture of the trophic structure of the invertebrate community. Mean Nearest Neighbor Distances (MNND) and Standard Deviation of Nearest Neighbor Distances (SDNND) were calculated by computing the mean Euclidean distance to each FFG and the standard deviation of the nearest FFG distance (Layman et al. 2007a). These two represent the overall density and evenness of FFG packing, respectively. I will describe each metric used for SICI in the results. Several R packages (SIBER, tRophicPosition, ggplot) were used to calculate and graph FFG community metrics in bi-plot space (Jackson et al. 2011, Wickham 2016, Quezada-Romegialli et al. 2018).

<u>SICI Metric Selection.</u> The eight community-wide attributes (Layman et al. 2007a) were tested between reference and restored sites (Table 3.7), and after reading the literature, I expected all to decrease with the addition of a disturbance, except SDNND (Needham 1936, Anderson and Sedell 1979, Cummins and Klug 1979, Cardinale et al. 2004, Gresens et al. 2009,

Merritt et al. 2017, Prommi 2018). Like the B-IBI, each indicator is essential for ensuring that the attribute accurately indicates the biological condition of the ecosystem and should show statistical significance between reference and restored sites. When I tested for statistical significance, metrics were first tested for equal variance using Levene's Test (Levene 1960). If indicators displayed unequal variance, the Welch's two-sample T-test was used (Welch 1947), otherwise, a two-sample T-test assuming equal variance was used. By sampling all FFGs in the stream, I assumed that the invertebrate community is captured when graphing δ^{13}C and δ^{15}N in bi-plot space.

Table 3.7. SICI Metrics Tested. Eight metrics were tested for the SICI to estimate the biological condition of reference and restored sites.

SICI Metrics Tested	**Expected Response to Increased Disturbance**
Total Area	Decrease
Standard Ellipse Area	Decrease
Standard Ellipse Area corrected for small sample size	Decrease
Nitrogen Range	Decrease
Carbon Range	Decrease
Mean Distance to Centroid	Decrease
Mean Nearest Neighbor Distance	Decrease
Standard Deviation Nearest Neighbor Distance	Increase

SICI Scoring. The SICI for the UBMC should represent a collective biological response and a culmination of indicators selected (Barbour and Paul 2010). To portray this numerically, the three indicators chosen for SICI were scored, thereby becoming metrics, from 0-33.3 using the 10th and 90th percentiles from each indicator (Appendix E) (Wilhelm 2014). Indicators were scored from 0-33.3 because three metrics were used for the SICI. If a site's SICI indicator value was above the 90th percentile, it received a score of 33.3 (Table 3.8). If it was below the 10th percentile, it received a score of zero. When an indicator value was between the 10th and 90th percentiles, it was applied to the respective scoring formula displayed in Table 3.10.

The metrics are simply added, without weights, for the total SICI score that ranges from 0-99.9. A score that is closer to 100 represents a stream with high isotopic complexity and pristine conditions for the Blackfoot River watershed. A score that is closer to 0 represents a stream that exhibits lower isotopic complexity than the other streams in the Blackfoot River watershed. This scoring method also allows streams that exhibit invertebrate attributes that enhance with less disturbance to trend toward 100. All SICI scores can be found in Appendix F.

Table 3.8. SICI Scoring. SICI scoring method when scoring metrics. A metric above the 90th percentile receives a score of 33.3, and a metric below the 10th percentile gets a score of 0. It is applied to the scoring formula if it falls between the 10th – 90th percentiles.

Metric Response with Disturbance	**Score for Values <10th Percentile**	**Score for Values >90th Percentile**	**Scoring Formula for Values >10th and <90th Percentile**
Decrease with Human Disturbance	**0**	**33.3**	$= \frac{33.3\ X\ (Observed\ Value\ -\ 10th\ Percentile)}{90th\ Percentile -\ 10th\ Percentile}$

CHAPTER FOUR

RESULTS

Confirming Reference and Restored Sites

LULC data (Figure 4.1) from ArcGIS was used (USGS 2018) to find the common environmental indicator between reference and stressed sites. Combining bare ground and developed land was the best distinguishing disturbance factor between reference and restored sites (Welch two-sample t-test p-value < 0.01). Bare ground/developed land averaged (±1SD) 5.4% ±0.7 and 21.3% ±2.9 for reference and restored sites, respectively. As the restored sites develop over time, I expect the bare ground will trend toward the reference conditions as vegetation recolonizes the floodplains.

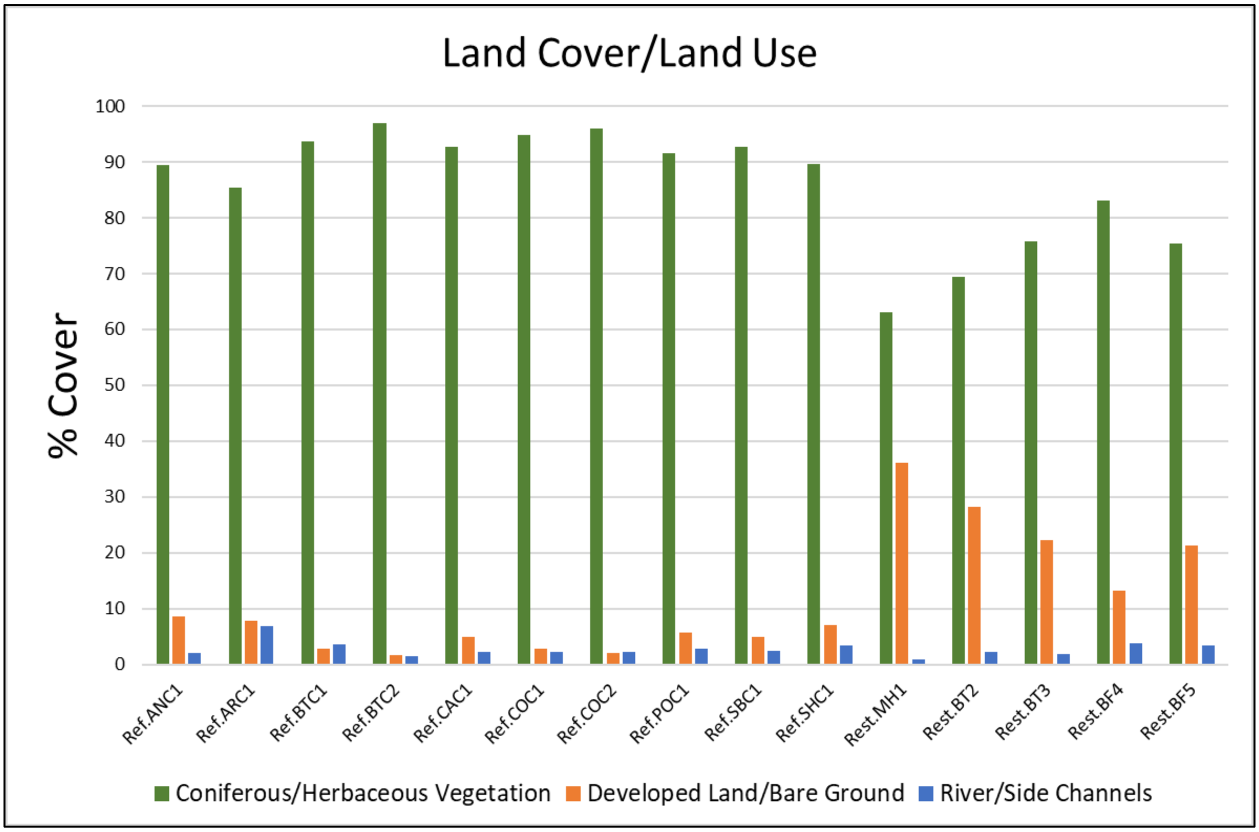

Figure 4.1. Land Cover Distribution. Land cover/land use categories of sites. Each category is reflected as the area of the categorized land divided by the total area (%).

Taxonomic Diversity and Similarity Indices

Gini-Simpson Diversity Index

Reference sites (0.82 ±0.05) had significantly higher (±1SD) Simpson diversity scores than restored sites (0.63 ±0.19; $p < 0.01$), indicating that reference sites consistently exhibited higher levels of diversity, both across different years and locations.

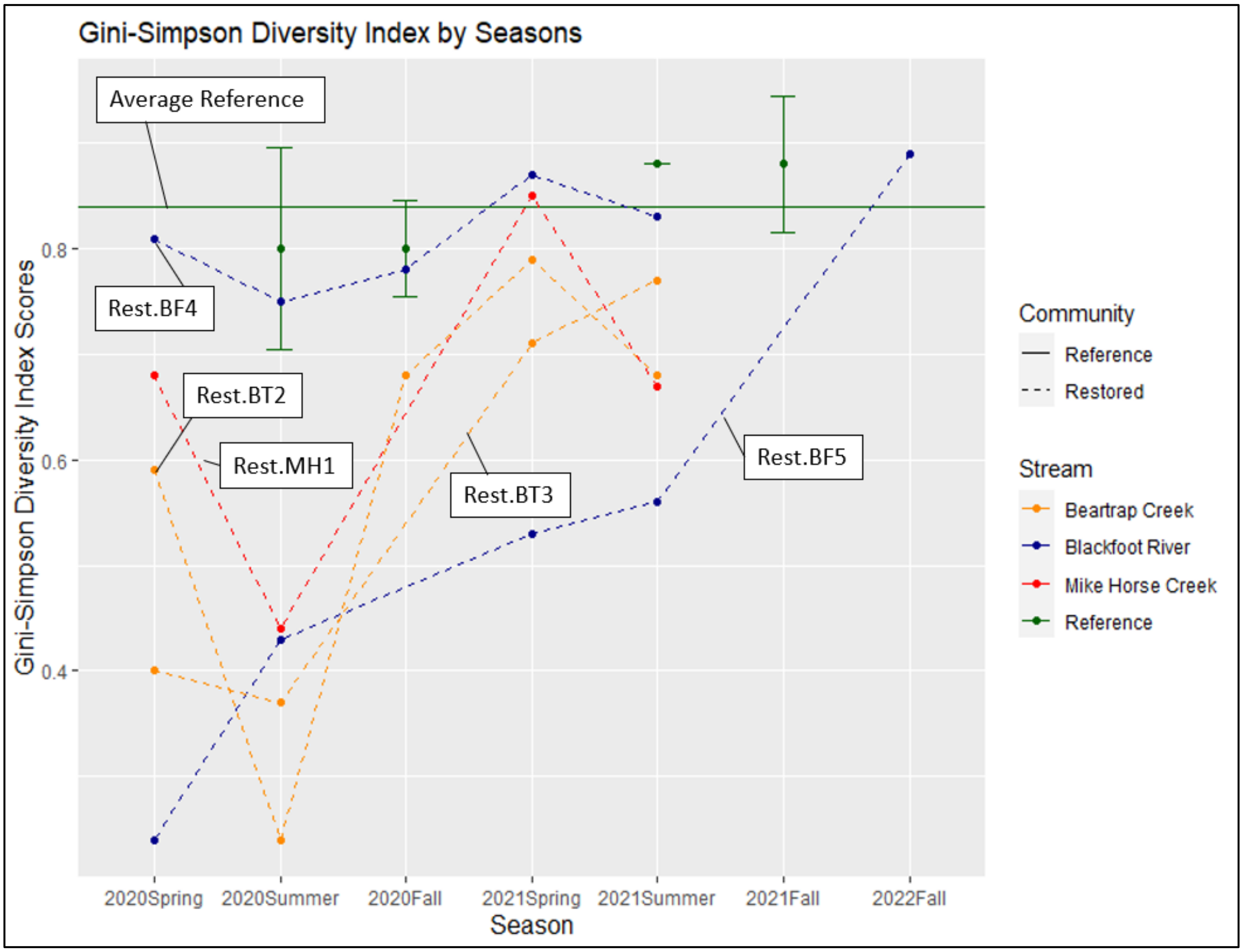

Figure 4.2. Gini-Simpson Diversity Index. Simpson diversity index scores across all seasons. Reference sites had significantly higher scores (two-sample t-test p-value < 0.01), with an average (±1SD) of 0.82 ±0.05 compared to only 0.63 ±0.2 for restored sites. The average score of restored sites was 0.53 ±0.2 in 2020, which increased to 0.74 ±0.1 in 2021 and 2022.

Despite this, there is evidence of progress in the restoration process. I found a significant increase in restored sites' Simpson diversity index scores from 2020 to 2021 and 2022 (two-sample t-test p-value < 0.01). In 2020, the average (±1SD) score of restored sites was 0.53 ±0.2, which increased to 0.74 ±0.1 in 2021 and 2022. Reference site diversity did not significantly increase (p-value = 0.07) (±1SD) from 0.8 ±0.05 in 2020 to 0.88 ±0.05 in 2021.This indicates that the restoration efforts are showing promising results, and the diversity of the restored sites is gradually increasing over time.

Inverse Simpson Diversity Index

Inverse Simpson diversity index scores of reference sites were significantly higher than that of restored sites, with reference sites averaging 6.3 ±2.5 and restored sites being 3.7 ±2.1 (two-sample t-test p-value < 0.01). This disparity in diversity levels implies that the restoration efforts still need to achieve the full diversity of the reference sites. However, restored sites Inverse Simpson diversity index scores increased significantly from 2020 to 2021 and 2022 (two-sample t-test p-value < 0.01). Specifically, the average (±1SD) score increased from 2.6 ±1.3 in 2020 to 4.8 ±2.3 in 2021 and 2022, indicating that the restoration efforts are starting to bear fruit. Reference site diversity did not significantly increase (p-value = 0.22) (±1SD) from 5.4 ±1.5 in 2020 to 8.9 ±3.6 in 2021.

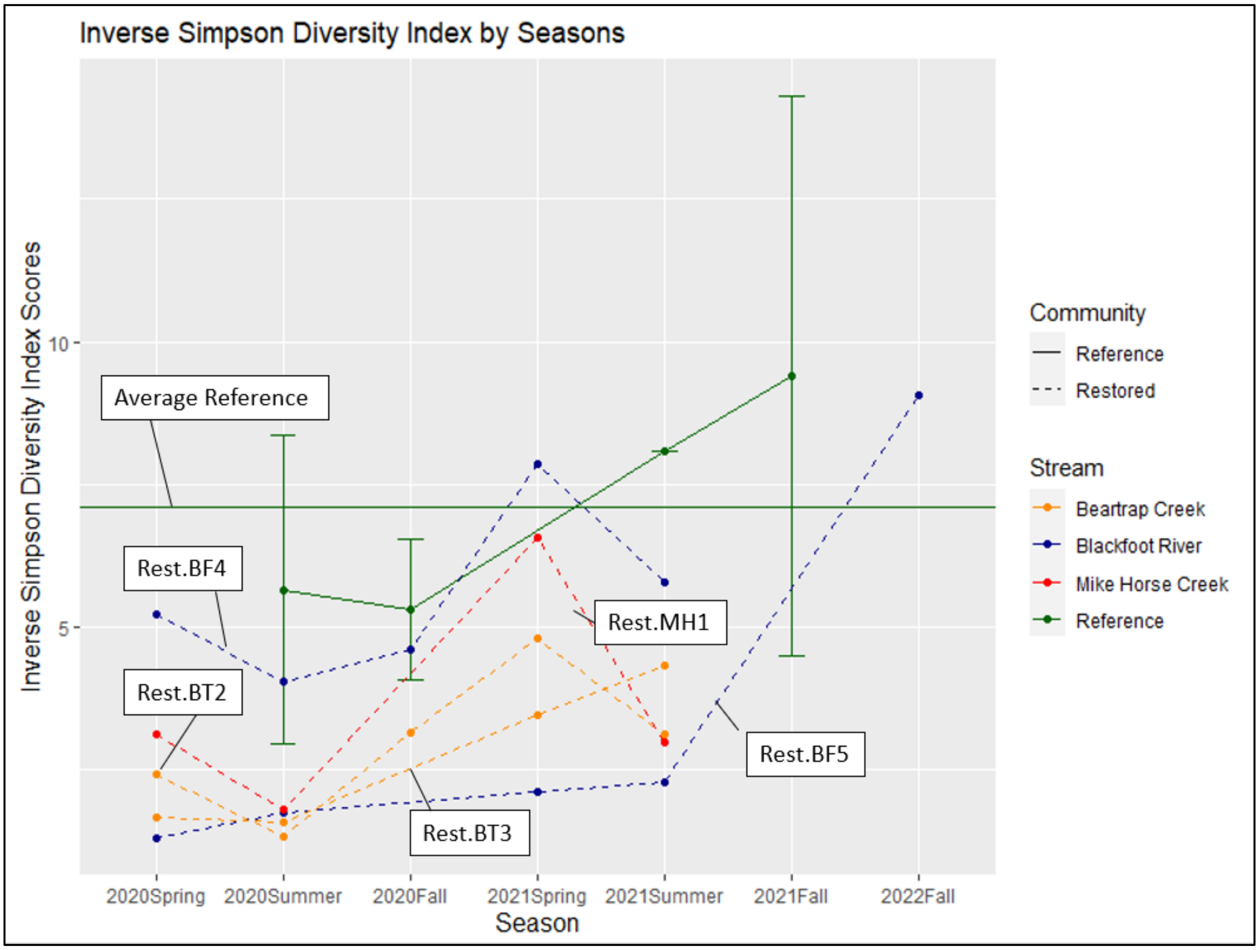

Figure 4.3. Inverse Simpson Diversity Index. Inverse Simpson diversity index scores across all seasons. The average score of reference sites was significantly higher than that of restored sites, with reference sites (±1SD) scoring 6.3 ±2.5 and restored sites scoring 3.7 ±2.1 (two-sample t-test p-value < 0.01). The average score of restored sites increased from 2.6 ±1.3 in 2020 to 4.8 ±2.3 in 2021 and 2022. The high standard error bars for the 2020 summer and 2021 fall reference are from only collecting one and two samples in those seasons, respectively.

Shannon-Wiener Diversity Index

Reference sites (±1SD) showed significantly higher Shannon diversity than restored sites, (2.1 ±0.3 vs 1.5 ±0.6, respectively; two-sample t-test p-value < 0.01), but only when diversity was averaged across restored sites and years (Figure 4.2).

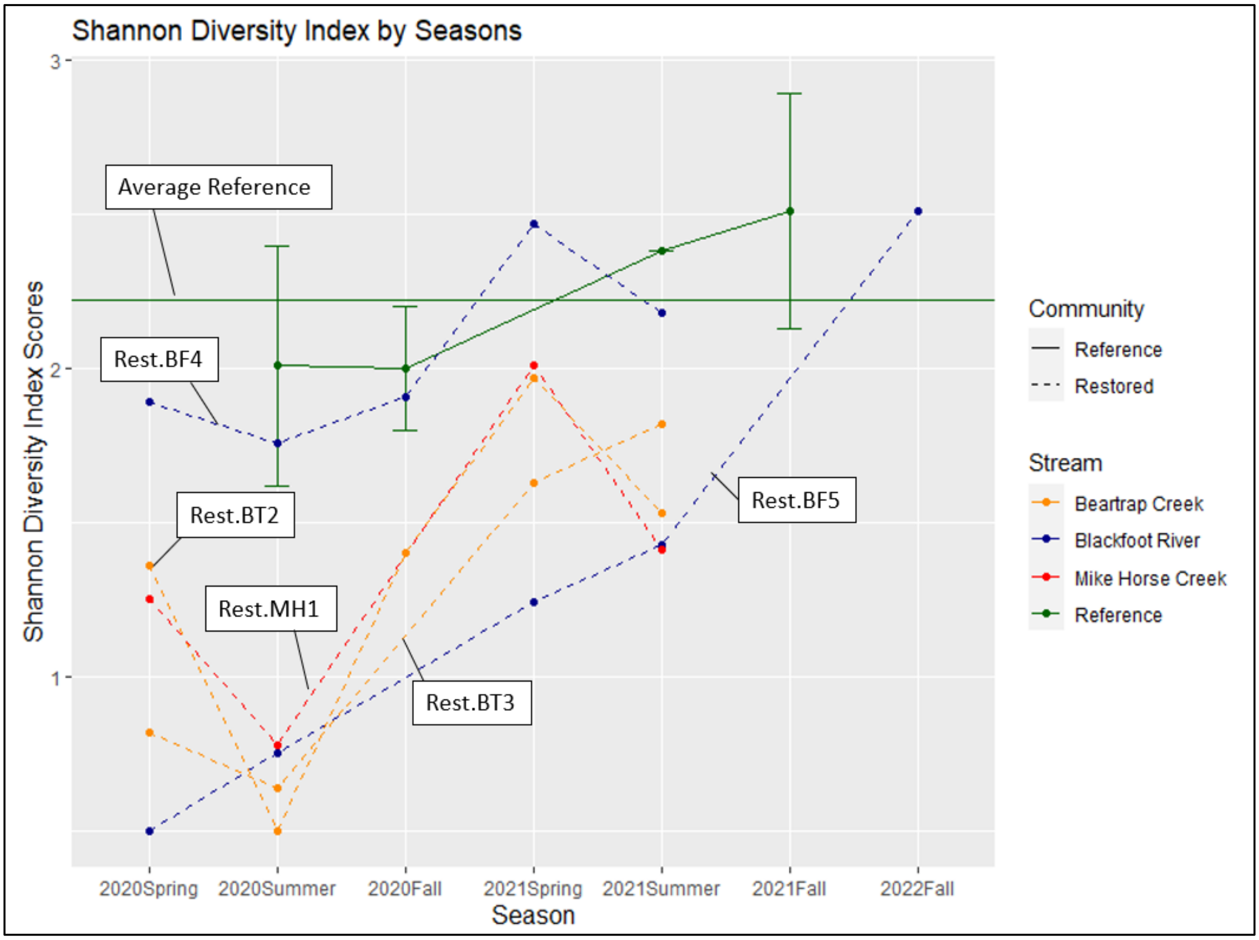

Figure 4.4. Shannon-Wiener Diversity Index. Shannon diversity index scores across seasons. Reference sites were significantly higher than restored sites, with reference sites scoring 2.1 ±0.3 and restored sites scoring 1.5 ±0.6 (two-sample t-test p-value < 0.01). The average (±1SD) score of restored sites increased from 1.1 ±0.5 in 2020 to 1.8 ±0.4 in 2021 and 2022.

Diversity also increased at restored sites from 2020 to 2021 and 2022 ($p < 0.01$). Specifically, the average score of restored sites increased from 1.1 ±0.5 in 2020 to 1.8 ±0.43 in 2021 and 2022. This indicates that while restoration efforts may still be underway, a positive trend in Shannon's diversity of the restored sites is apparent. Keeping in mind that seven of the ten reference sites were collected in 2020, reference site diversity did not significantly increase (p-value = 0.08) (±1SD) from 2 ±0.2 in 2020 to 2.4 ±0.3 in 2021.

Bray-Curtis Similarity Index

I employed the Bray-Curtis similarity index (Bray and Curtis 1957) to assess the similarity between the invertebrate taxa of restored sites and reference sites (Figure 4.5). Only 17.7% (index score of 0.17) ±0.1 of invertebrate taxa from restored sites (±1SD) were like those found in reference sites. Furthermore, the similarity between the taxa of restored and reference sites declined between 2020 and 2023. Specifically, the average index score decreased from 0.25 ±0.2 in 2020 to 0.09 ±0.1 in 2022 and 2023 (two-sample t-test p-value < 0.01). While restored sites seem to be diversifying, they still have a different level of taxonomic similarity than reference sites. Although this represents a significant decrease, it is crucial to note that a high degree of seasonal variation was observed.

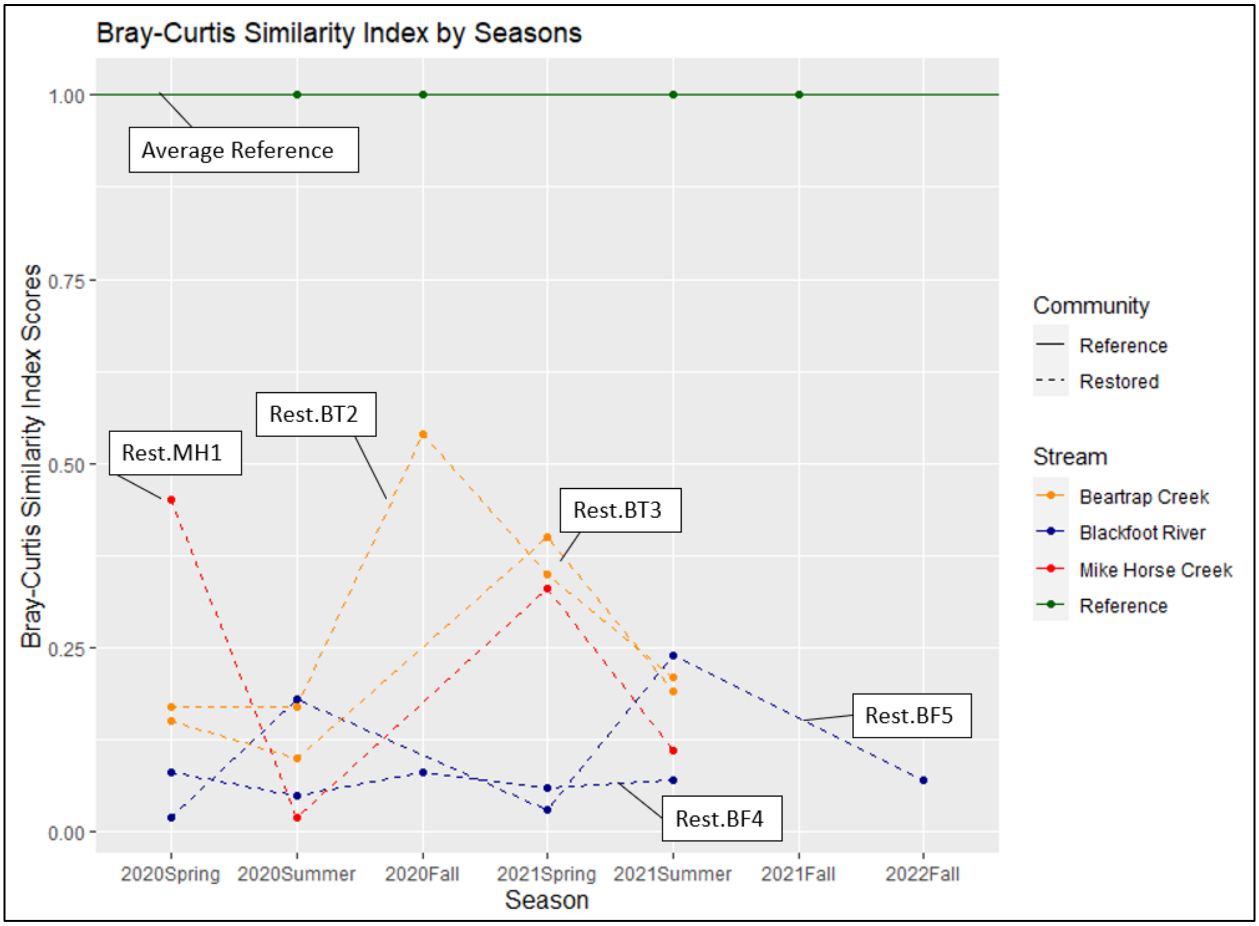

Figure 4.5. Bray-Curtis Similarity Index. Bray-Curtis similarity index scores across all seasons. An average of 17.7% (index score of 0.17) ±0.1 of invertebrate taxa from restored sites were like those found in reference sites. The average (±1SD) index score decreased from 0.25 ± 0.1 in 2020 to 0.09 ±0.1 in 2022 and 2023 (two-sample t-test p-value < 0.01).

River Invertebrate Prediction and Classification System

I collaborated with Rithron Associates, Inc. in Missoula, Montana, to develop RIVPACS (Wright et al. 1993) that was calibrated to the Upper Blackfoot watershed to calculate observed-to-expected (O/E) scores based on taxa richness and predictor variables (Figure 4.6). The results show that the average O/E score for reference sites (±1SD) was 0.57 ±0.2, while restored sites scored an average of 0.39 ±0.2. Unfortunately, the expected scores did not meet the Montana

Department of Environmental Quality reference standard, which requires reference streams in the western Rocky Mountain region to have a score higher than 0.9 (DEQ 2012). One possible reason for this RIVPACS analysis failure is that the Expected condition calculation typically requires a much larger reference condition dataset than what was available for Montana (Hawkins et al. 2000, Hargett et al. 2007, Hawkins 2009). While there is no existing data set for similar predictor variables as the Upper Blackfoot watershed, sampling hundreds of reference sites across Montana with similar predictor variables was not feasible for this project.

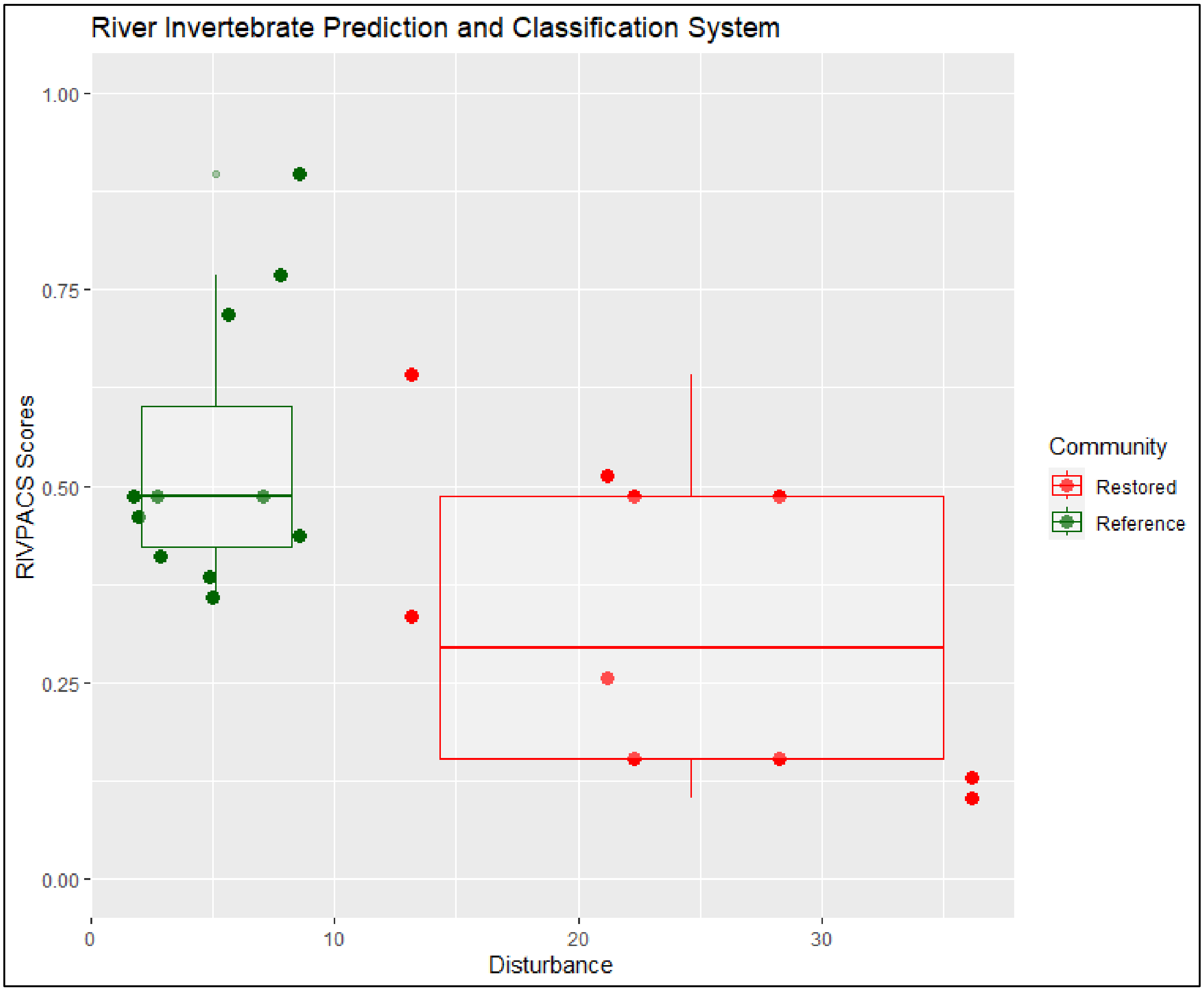

Figure 4.6. RIVPACS scores of restored and reference sites. The figure is not a robust estimate of the biological quality of reference or restored sites due to not meeting the Montana Department of Environmental Quality standard, which requires reference streams in western Montana to receive a score higher than 0.9. This is most likely due to a lack of sufficient data on reference sites.

Benthic Index of Biotic Integrity

Out of the 60 invertebrate attributes tested, ten were selected for the B-IBI as suitable indicators of change in stream condition, by exhibiting differences between reference and restored sites. Other attributes displayed high inconsistency and/or low significance and were therefore deemed unreliable indicators of disturbance.

Table 4.1. B-IBI Metrics. Taxa richness and community composition attributes displayed statistical differences between restored and reference sites.

B-IBI Metric	Response to Increased Disturbance	P-Value
Invertebrate Taxa Richness and Community Composition		
Taxa Richness	Decrease	0.04
Trichoptera Richness	Decrease	<0.01
% Abundance Plecoptera	Decrease	<0.01
Heptageniidae Richness	Decrease	0.02
% Abundance Heptageniidae	Decrease	<0.01
Intolerant Richness	Decrease	<0.01
% Abundance Intolerant	Decrease	<0.01
Invertebrate Biological Processes and Functional-Feeding Group		
Clinger Richness	Decrease	0.01
Scrapers Richness	Decrease	<0.01
% Abundance Scrapers	Decrease	<0.01

Taxa Richness and Community Composition Metrics

Taxa richness and community composition of invertebrate metrics (see Table 4.1) can provide valuable information about an ecosystem's biological integrity and stability (Karr and Chu 1997). Taxa richness refers to the number of different species within a stream. A higher level of taxa richness indicates a diverse ecosystem, while a lower level may suggest a more degraded or disturbed ecosystem (Cummins and Klug 1979). Community composition refers to the relative abundance and diversity of different taxa within a community. Taxa richness and community composition metrics have been proven to work in many multi-metrics indexes (Fore et al. 1996, Karr and Chu 1997, Barbour et al. 1999).

Taxa Richness. Taxa richness is a widely used metric to evaluate the diversity of invertebrate communities in freshwater ecosystems (Fore et al. 1996, Barbour et al. 1999). I used the taxa richness metric to compare the richness of invertebrate communities at reference and restored sites. The results show that the reference sites (±1SD) had a significantly (Welch two-sample t-test p-value = 0.04) higher taxa richness (22.5 ±1.2) compared to the restored sites (15.5 ±2.6) (Figure 4.7).

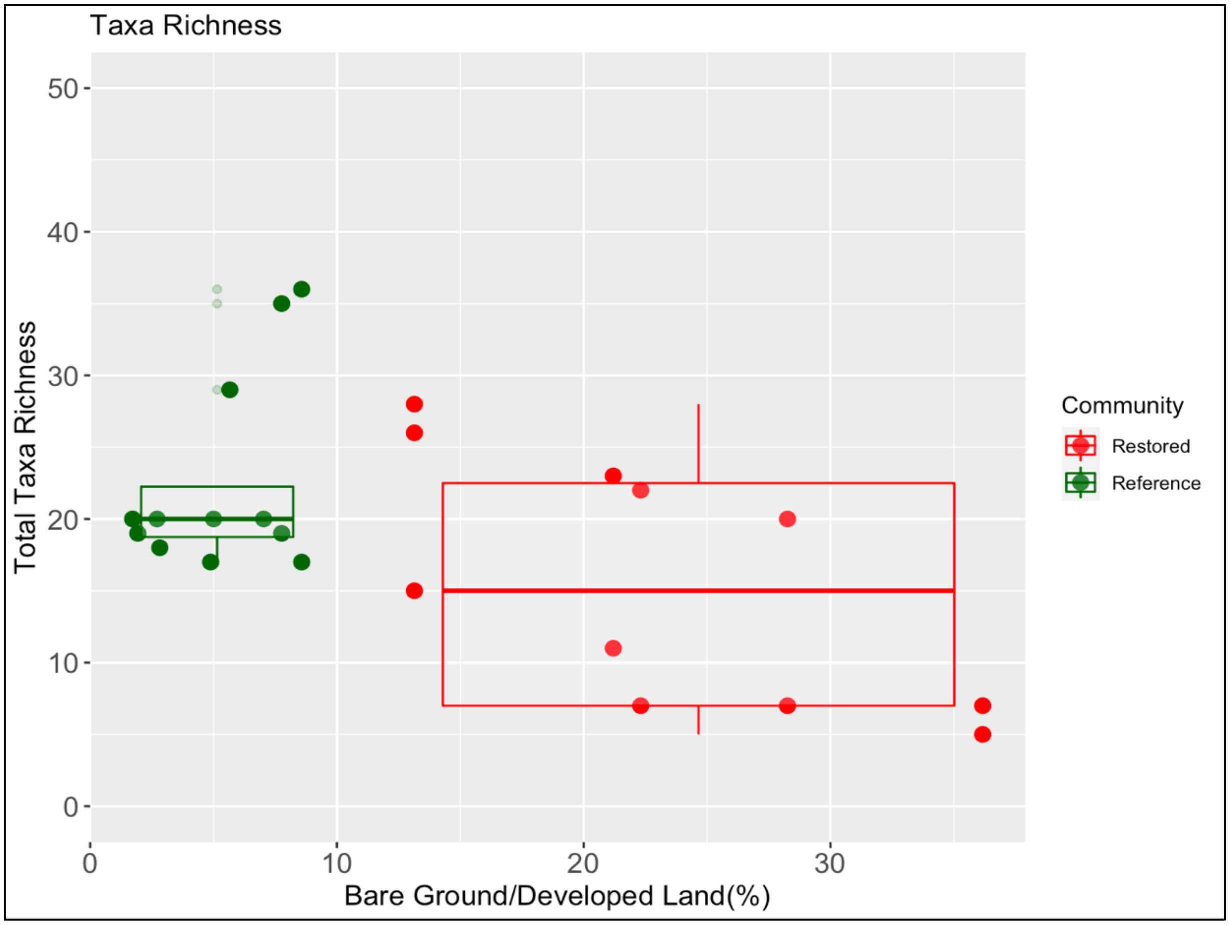

Figure 4.7. Taxa Richness Metric. Taxa richness displayed statistical differences (Welch two sample t-test p-value = 0.04) between restored and reference sites. Taxa richness also decreased with the addition of bare ground/developed land cover (disturbance).

The higher level of taxa richness observed in the reference sites could be attributed to various factors. For instance, reference sites most likely have more diverse habitat types, which can support a greater variety of invertebrate taxa. Additionally, reference sites may have better water quality or more excellent connectivity to other freshwater ecosystems, contributing to a higher diversity level.

Trichoptera Richness. Using the number of Trichoptera taxa present as a measure of Trichoptera richness, I found that Trichoptera richness (±1SD) across reference sites (4 ±0.4) was significantly richer (two-sample t-test p-value < 0.01) than restored sites (1 ±0.4) (Figure 4.8). The lower Trichoptera richness in the restored sites may indicate that the habitat conditions are less favorable for these sensitive invertebrates or that restoration efforts have yet to lead to the colonization of Trichoptera from neighboring streams or ponds.

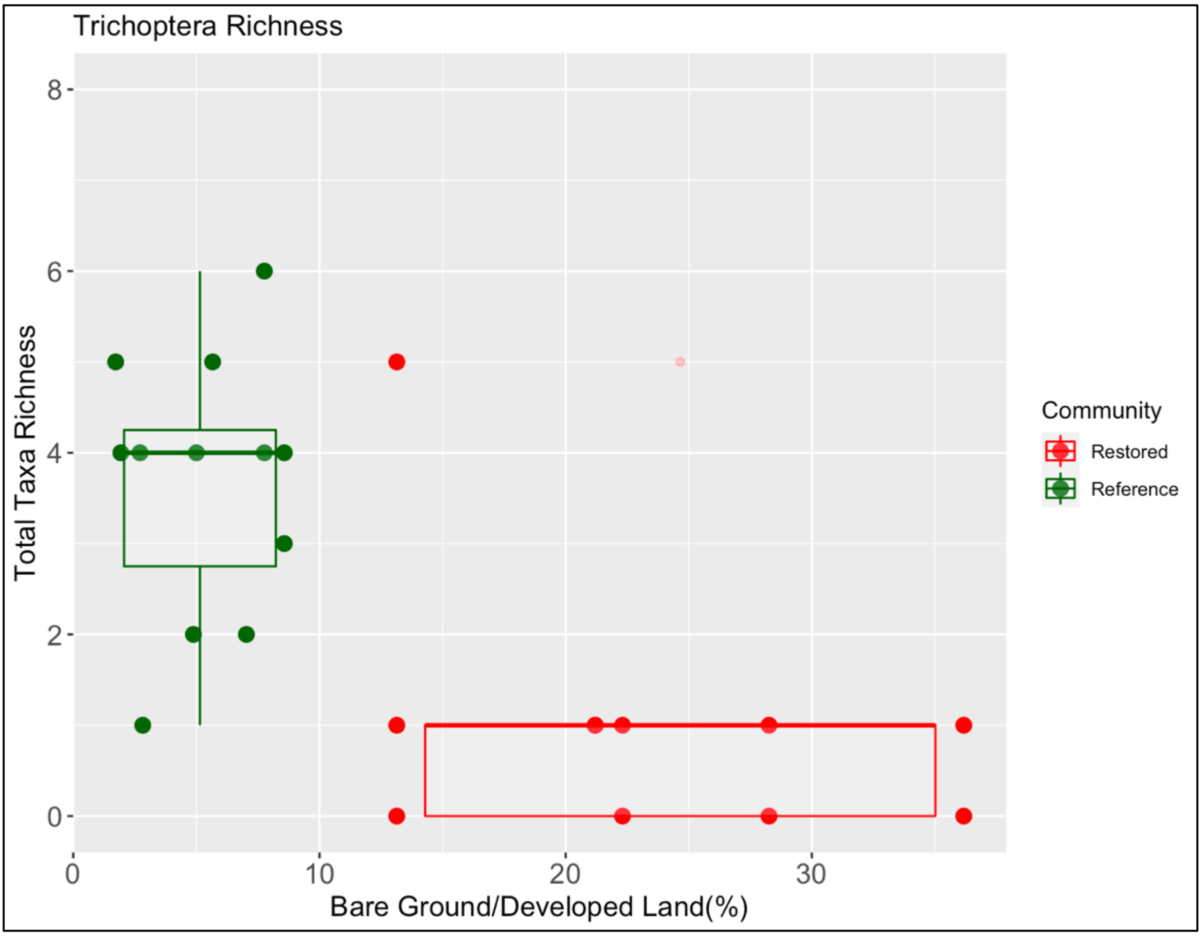

Figure 4.8. Trichoptera Richness Metric. Trichoptera richness displayed statistical differences (two sample t-test p-value < 0.01) between restored and reference sites. Trichoptera richness also decreased with the addition of disturbance.

Percent Abundance Plecoptera. I defined the percent taxa abundance Plecoptera metric as Plecoptera taxa abundance divided by total abundance. The results show that the percent abundance of Plecoptera in reference sites (26.3% ±3.9) was significantly higher (two sample t-test p-value < 0.01) than in the restored sites (±1SD) (10.7% ±3.3) (Figure 4.9). The higher abundance of stoneflies observed in reference sites may indicate that reference sites have better-dissolved oxygen levels, lower levels of sedimentation, and more appropriate water temperatures, all of which are required for the survival and reproduction of stoneflies (Surdick and Gaufin 1978).

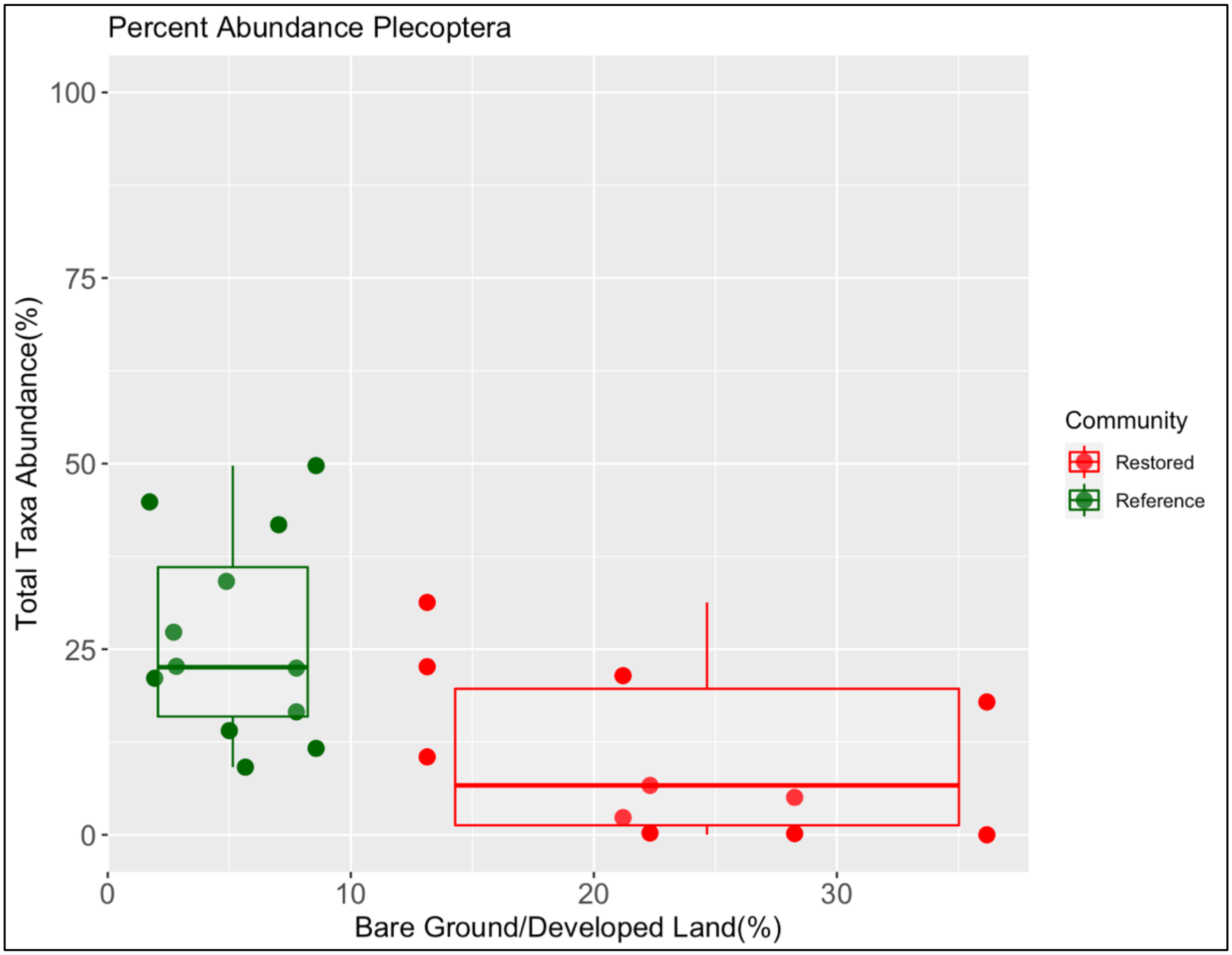

Figure 4.9. Percent Abundance Plecoptera Metric. Percent abundance of Plecoptera displayed statistical differences (two sample t-test p-value < 0.01) between restored and reference sites. The percentage abundance of Plecoptera also decreased with the addition of disturbance.

Heptageniidae Richness. I define the Heptageniidae richness metric as the number of Heptageniidae taxa present, which provides insight into the diversity of this group of invertebrates. I found that there was a higher richness (two sample t-test p-value = 0.02) of Heptageniidae in reference sites (±1SD) (2.3 ±0.4) compared to the restored sites (1.4 ±0.4)

(Figure 4.10). This could suggest that the restored sites may still need to fully recover their invertebrate communities due to slower colonization rates, limited dispersal of specific taxa, or the presence of environmental stressors that continue to affect invertebrate communities in restored sites. The higher richness of Heptageniidae may also suggest a greater availability of food resources in reference sites than in restored sites. The lower richness of Heptageniidae in restored sites highlights the importance of continued monitoring and management efforts to support the recovery of invertebrate communities in restored streams.

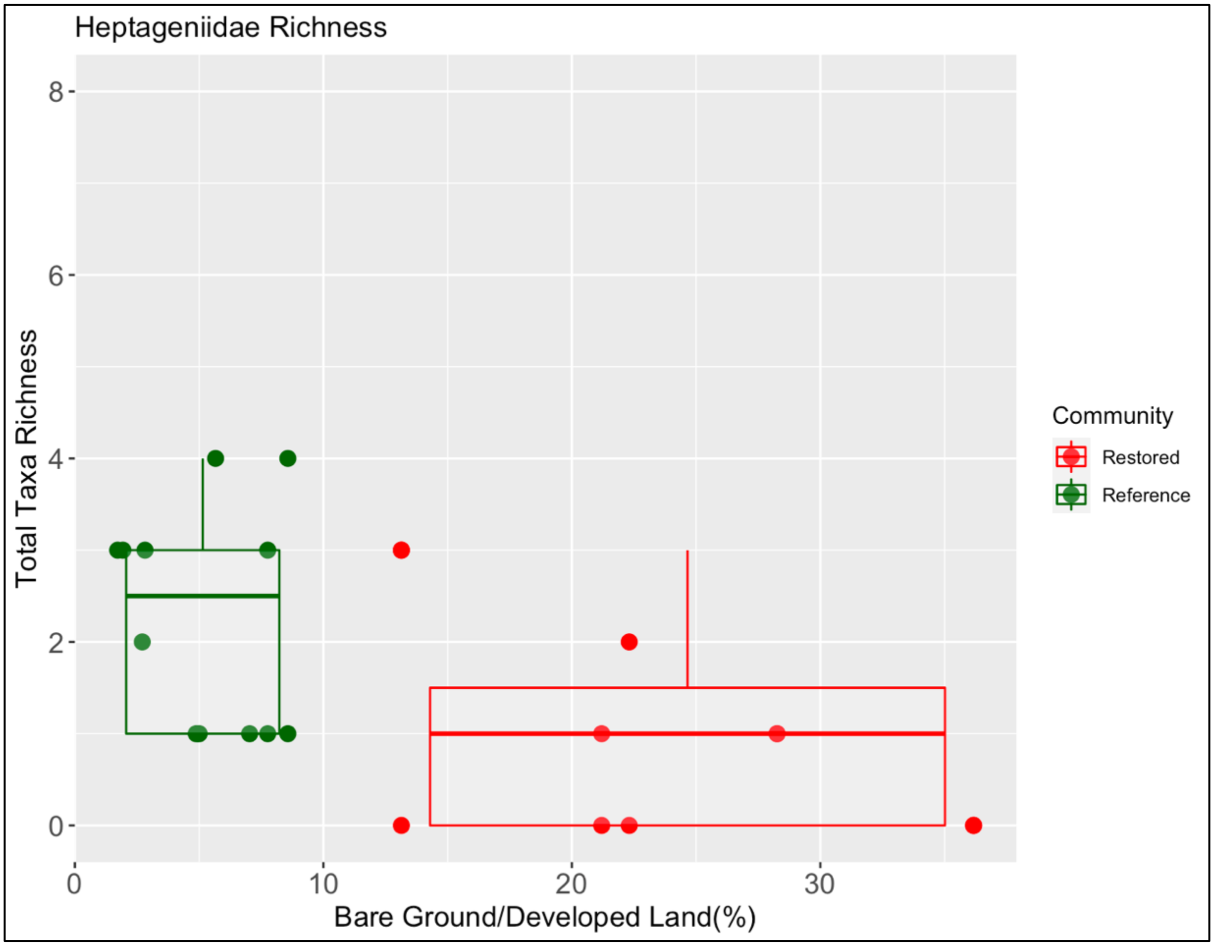

Figure 4.10. Heptageniidae Richness Metric. Heptageniidae richness displayed statistical differences (two sample t-test p-value = 0.02) between restored and reference sites. Heptageniidae richness also decreased with the addition of disturbance.

Percent Abundance Heptageniidae. The percentage abundance of Heptageniidae is an important metric as it reflects the relative contribution of this family to the total invertebrate community. I define percent abundance Heptageniidae as Heptageniidae taxa abundance divided by total abundance. I found that percent abundance (±1SD) was significantly (Welch two sample t-test p-value < 0.01) higher in reference sites (21.9% ±4.4) compared to restored sites (0.6% ±0.2) (Figure 4.11).

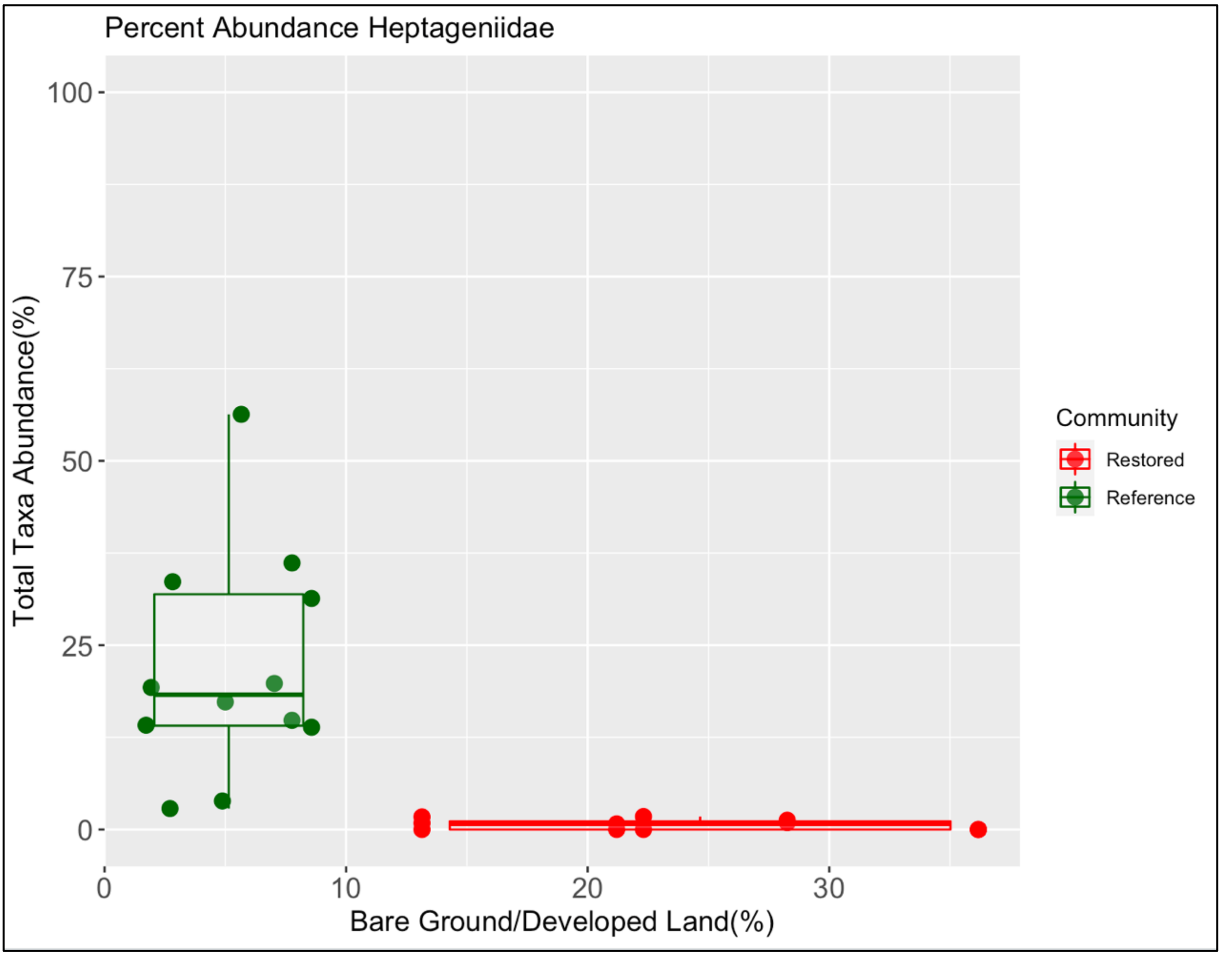

Figure 4.11. Percent Abundance Heptageniidae Metric. Percent abundance of Heptageniidae displayed statistical differences (Welch two sample t-test p-value < 0.01) between restored and reference sites. The percentage abundance of Heptageniidae also decreased with the addition of disturbance.

Intolerant Richness. To quantify the impact of environmental stressors on invertebrate communities, the intolerant richness can be used as a metric. Intolerant richness refers to the number of taxa that are intolerant to pollution or other stressors in a stream. The results show that the intolerant richness (±1SD) of reference sites (4.8 ±0.4) was significantly higher (two-sample t-test p-value < 0.01) than that of the restored sites (2.7 ±0.7) (Figure 4.12). This finding suggests that the restored streams may still be experiencing environmental stressors limiting the survival of intolerant invertebrate taxa.

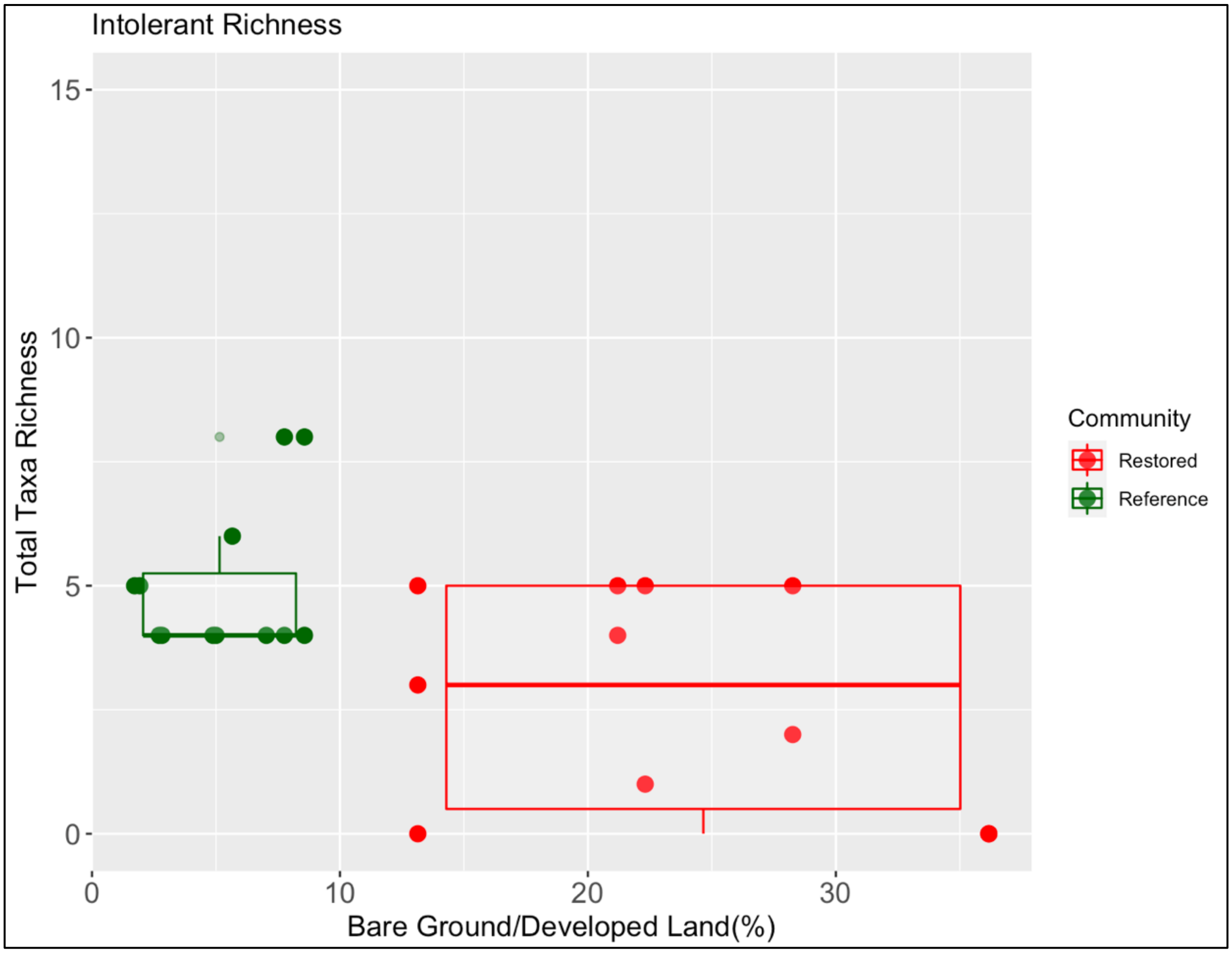

Figure 4.12. Intolerant Richness Metric. Intolerant richness displayed statistical differences (two sample t-test p-value < 0.01) between restored and reference sites. Intolerant richness also decreased with the addition of disturbance.

Percent Abundance Intolerant. The percent taxa abundance intolerant is a metric that provides insight into the relative abundance of these organisms compared to the total invertebrate population (Reynoldson and Metcalfe-Smith 1992). This metric is calculated as the intolerant taxa abundance divided by the total abundance. I found that the reference sites (±1SD) had a significantly higher percent abundance intolerant (35% ±4.4) compared to the restored sites (5.4% ±1.9), as determined by a Welch two-sample t-test with a p-value of <0.01 (Figure 4.13). This indicates that reference sites have a greater abundance of intolerant invertebrates and are, therefore, likely to have better water quality and biological integrity.

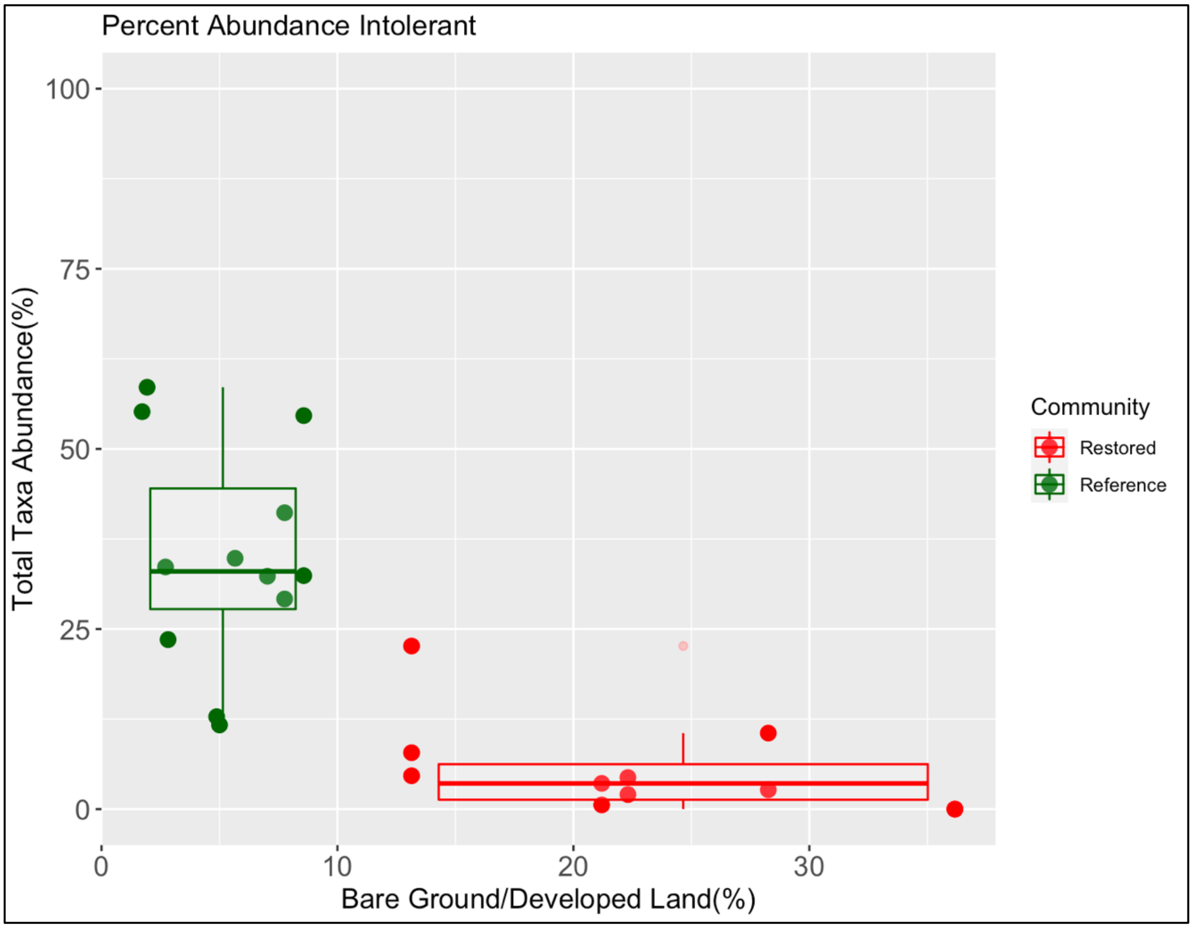

Figure 4.13. Percent Abundance Intolerant Metric. Percent abundance intolerant displayed statistical differences (Welch two sample t-test p-value < 0.01) between restored and reference sites. The percentage abundance intolerance also decreased with the addition of disturbance.

Biological Processes and Functional Feeding Group Metrics

Thirty-six invertebrate biological processes and FFG attributes were tested between reference and restored sites. Three (Table 4.1) were statistically significant and ecologically meaningful for the B-IBI. Clingers and scrapers were the biological processes and FFGs that differed most between restored and reference sites.

Clinger Richness. I define clinger richness as the taxa richness that was clingers. Clinger richness (±1SD) across reference sites (13.5 ±1.4) was significantly richer (two sample t-test p-value = 0.01) than the restored sites (8 ±1.7) (Figure 4.14). The diversity of clingers found in reference sites could suggest they contain cleaner water and more stable substrate than restored sites since clingers require a stable streambed, a continuous supply of oxygenated water, and the right conditions for feeding and reproduction (Cummins and Klug 1979). Most clingers of the restored sites were found on the Blackfoot River, indicating a better streambed for clinger colonization.

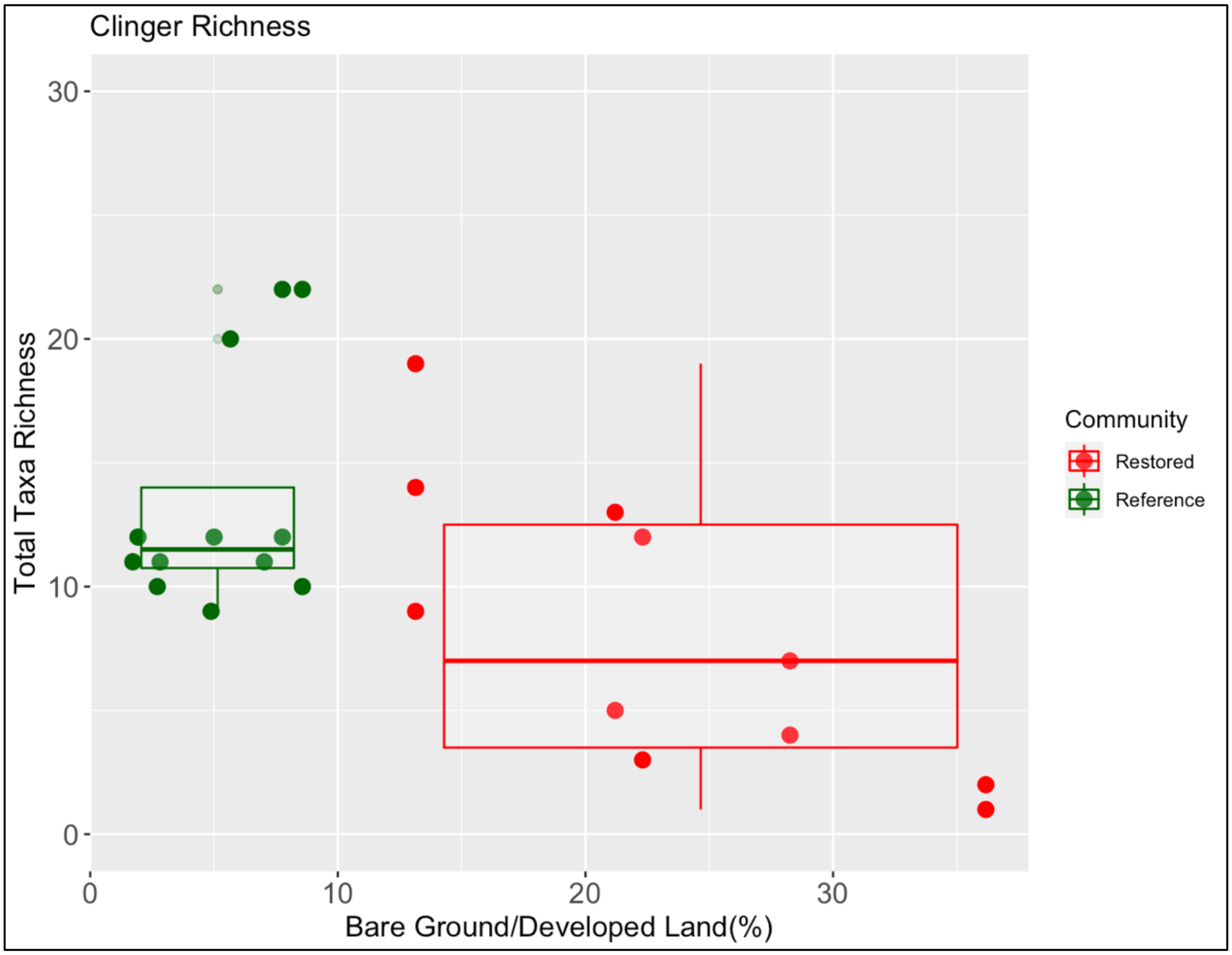

Figure 4.14. Clinger Richness Metric. Clinger richness displayed statistical differences (two sample t-test p-value = 0.01) between restored and reference sites. Clinger richness also decreased with the addition of disturbance.

Scraper Richness. Results indicate that the scraper richness (±1SD) of reference sites (4.7 ±0.5) was significantly richer than that of the restored sites (2.18 ±0.6) (Figure 4.15), as determined by a two-sample t-test with a p-value of <0.01. This suggests that the reference sites have a higher abundance of invertebrates adapted to scraping and are better equipped to utilize the available resources in the stream ecosystem.

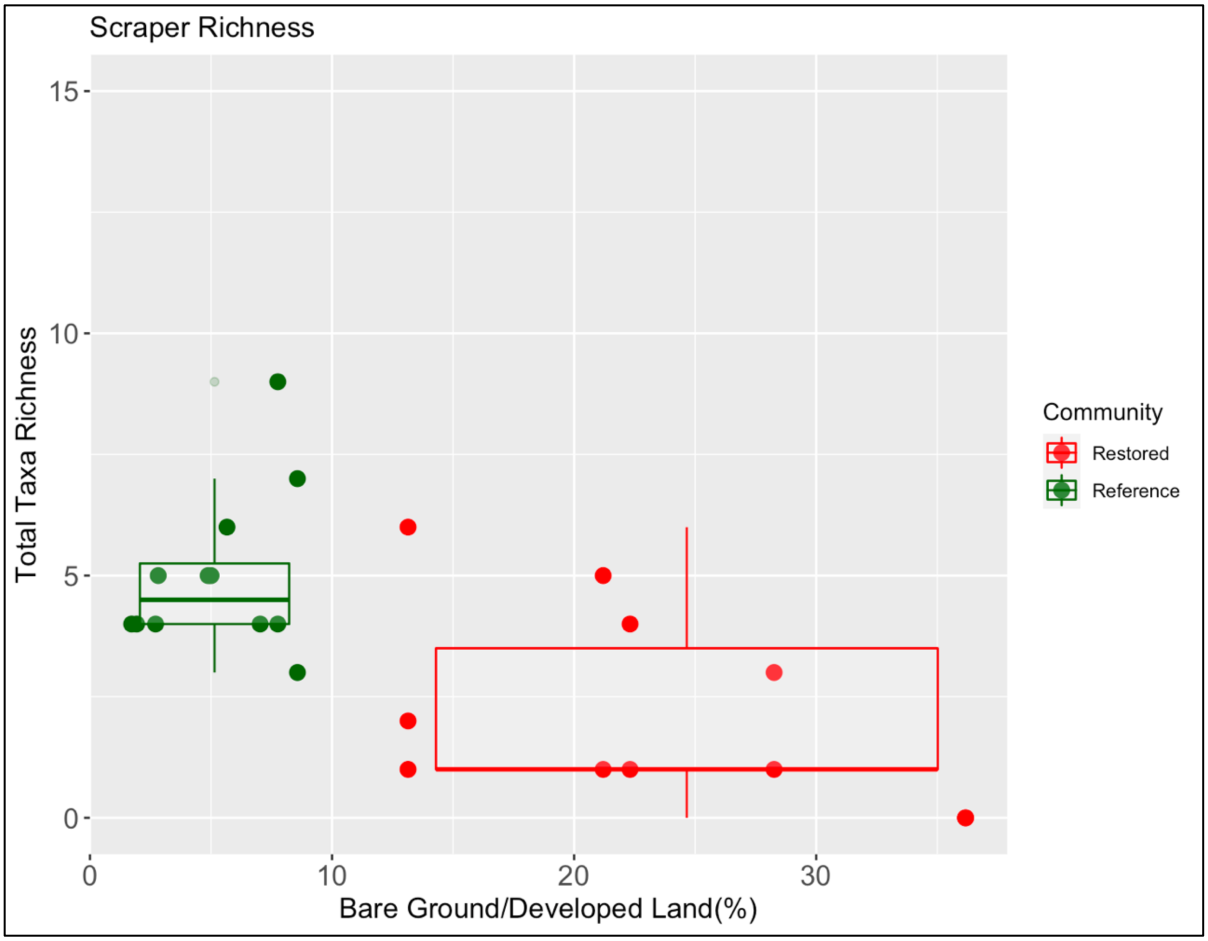

Figure 4.15. Scraper Richness Metric. Scraper richness displayed statistical differences (two sample t-test p-value < 0.01) between restored and reference sites. Scraper richness also decreased with the addition of disturbance.

Percent Abundance Scrapers. The abundance of scraper invertebrates is a common indicator of stressed streams (Kondratieff et al. 1984). The abundance of scraper invertebrates in streams is a good indicator of biological integrity (Anderson and Sedell 1979, Prommi 2018), and the percentage of taxa abundance composed of scrapers can provide valuable insights into stream ecosystems. I define the percent taxa abundance scraper as the abundance of invertebrates adapted to scraping divided by the total abundance of invertebrates in each stream.

The results show that the percent abundance of scrapers (±1SD) in reference sites (27.8% ±4.7) was significantly higher than in restored sites (4.3% ±1.5) (Figure 4.16), as determined by a Welch two-sample t-test with a p-value of <0.01. The higher abundance of scrapers in reference sites suggests that these streams have higher biological integrity, with a greater abundance of invertebrates that play an essential role in nutrient cycling and energy flow.

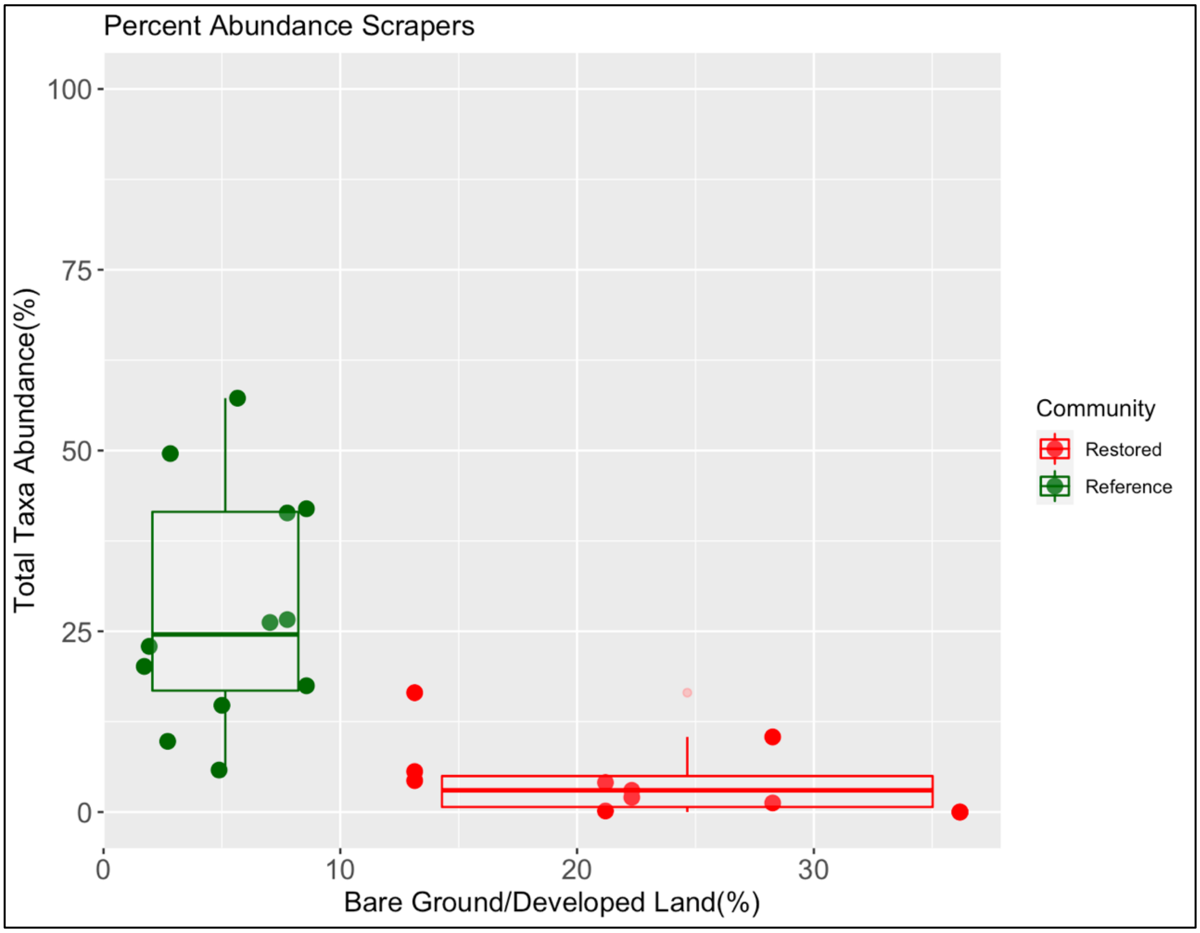

Figure 4.16. Percent Abundance Scraper Metric. Percent abundance scraper displayed statistical differences (Welch two sample t-test p-value < 0.01) between restored and reference sites. The percent abundance scraper also decreased with the addition of disturbance.

<u>B-IBI Scores</u>

This index provided a comprehensive evaluation of the biological integrity of stream ecosystems by assessing the composition and abundance of invertebrates. The results show that the total B-IBI scores (±1SD) of reference sites (65.7 ±4.5) were significantly higher than those of restored sites (23.8 ±5.5) by a magnitude of 2.7 (Figure 4.17), as determined by a two-sample t-test with a p-value of <0.01. The lower B-IBI scores of restored sites suggest that these streams still contain low biological integrity, which is expected since restoration was just completed. The

B-IBI scores also highly correlated to the LULC indicator of bare ground/developed land, having an R-squared value of 0.67.

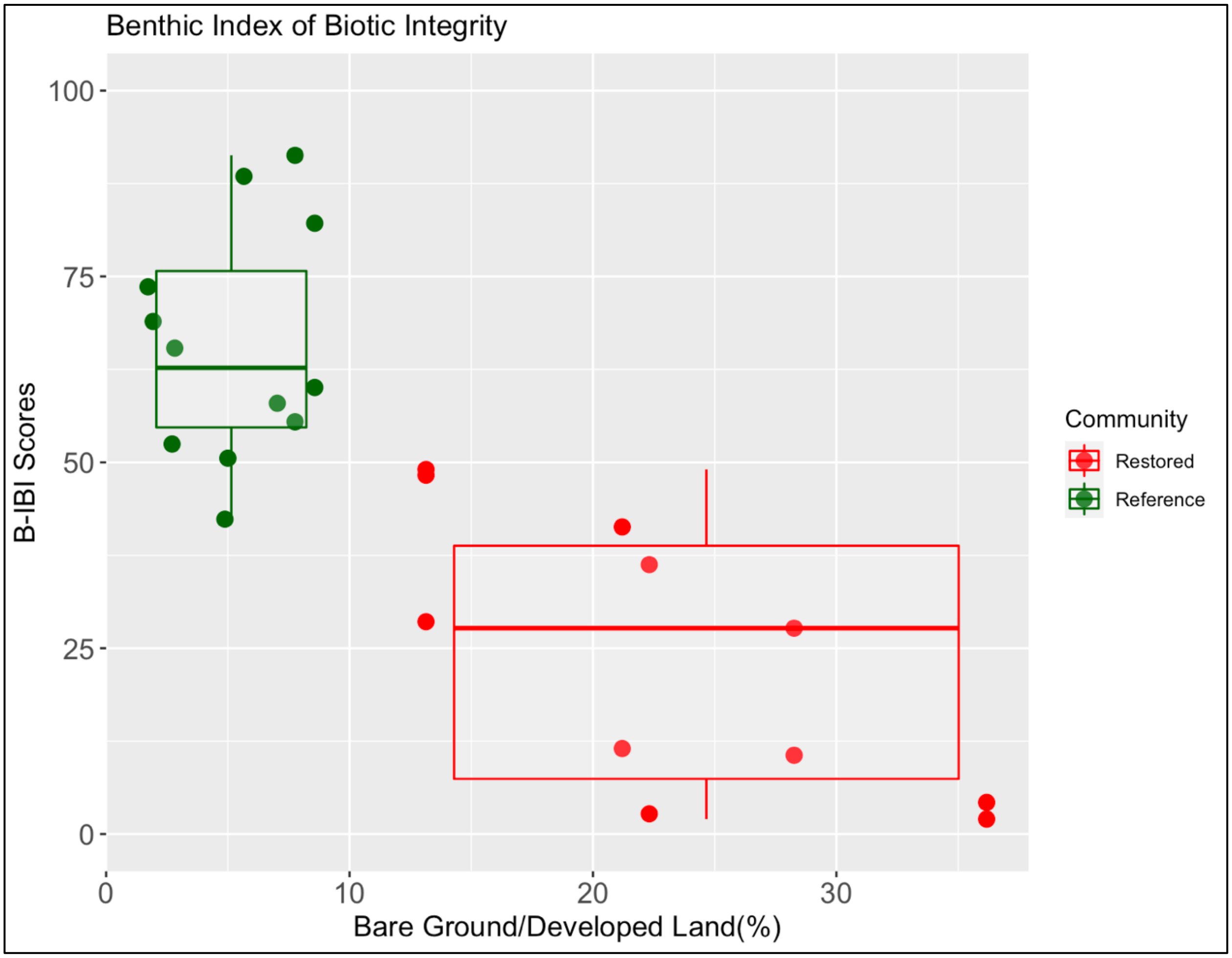

Figure 4.17. B-IBI Scores. B-IBI scores displayed statistical differences (Welch two sample t-test p-value < 0.01) between restored and reference sites. B-IBI also decreased with the addition of disturbance (Adjusted $R^2 = 0.67$).

I utilized the Analysis of Variance (ANOVA) test to see the B-IBI variability between reference and restored streams and each individually (Fisher 1935). The "Between Groups" row (Table 4.3) shows the sum of squares, degrees of freedom, mean square, F-value, and p-value for the variability between reference and restored sites. The "Within Groups" row shows the same values for the B-IBI variability within each restored and reference group.

The sum of squares (SS) represents the total amount of deviation in the data (Fisher 1935). Precisely, it measures the difference between each data point and the average of all data points, squared and then summed up. Degrees of Freedom (Df) is the number of observations in a sample that can vary after the sample statistics (e.g., mean, variance) have been computed (Fisher 1935). The Mean Square (MS) is obtained by dividing the SS by the Df (Fisher 1935). The F-value is the ratio of the MS between groups to the MS within groups (Fisher 1935). The Sum of Squared Errors (SSE) is calculated as the sum of the squared differences between each observed value and its corresponding predicted value (Fisher 1935). Mean Square Error (MSE) is calculated by dividing SSE by the Df of the residuals (Fisher 1935).

For B-IBI scores, the MSE value of 301.4 suggests relatively low within-group variability in the data. This means that the observations within each group are not far spread out around the group mean. The ANOVA results (Table 4.2) show that there is a significant difference between groups in terms of SICI (F = 35.09, p < 0.01). This indicates that the mean values of SICI across the different groups are different and that the difference is unlikely to be due to chance alone.

Table 4.2. B-IBI Scores ANOVA test. ANOVA test between reference and restored sites (Between Groups) and each individually (Within Groups).

Source	SS	Df	MS	F-value	p-value
Between Groups	10,071	1	10071	35.09	<0.001
Within Groups	6,028	20	287		
Total	16,099	21			

The results also show that all restored streams improved from 2020 to 2021, as the average (±1SD) B-IBI score increased from 11.1 ±4.8 to 31.7 ±7.7 (Figure 4.14). This improvement suggests that restoration efforts are having a positive impact on biological integrity over time. However, the biological integrity of these streams is still much lower than that of reference sites (Figure 4.18).

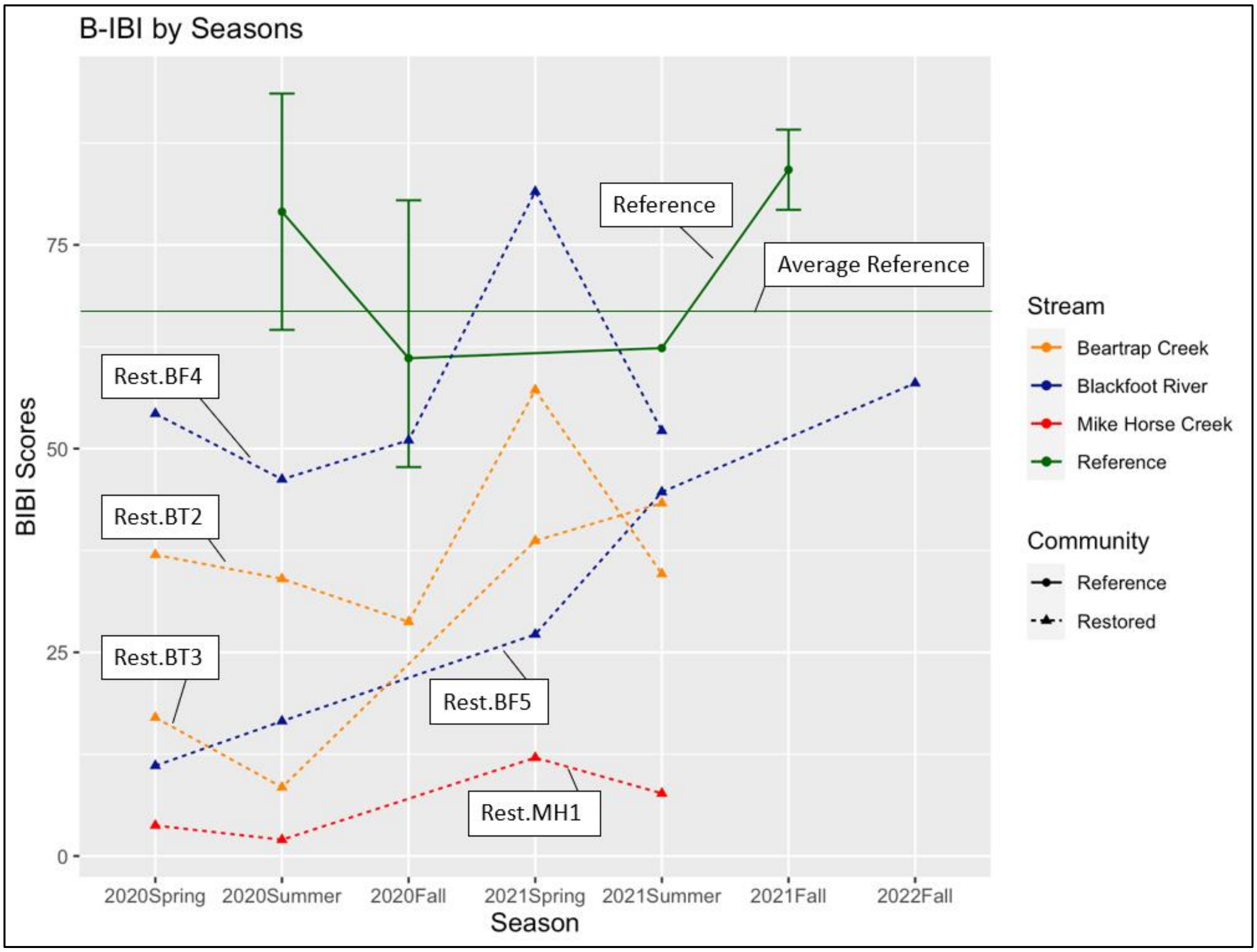

Figure 4.18. B-IBI Scores. B-IBI scores of all samples collected across a temporal gradient. Colors represent the three restored streams (orange, blue, and red) and reference streams (green). Dashed lines are restored streams, and the solid line is the reference.

δ^{13}C and δ^{15}N of Restored and Reference Sites

I examined δ^{13}C and δ^{15}N of invertebrates to understand the trophic relationships of reference sites and restored sites (Figure 4.19). A lower δ^{13}C value indicates that the organism is incorporating carbon from primary producers, while a higher δ^{13}C value suggests that the organism is containing carbon from higher-trophic level organisms. A higher δ^{15}N value indicates that the organism is further up the food chain and has incorporated nitrogen from higher-trophic level organisms.

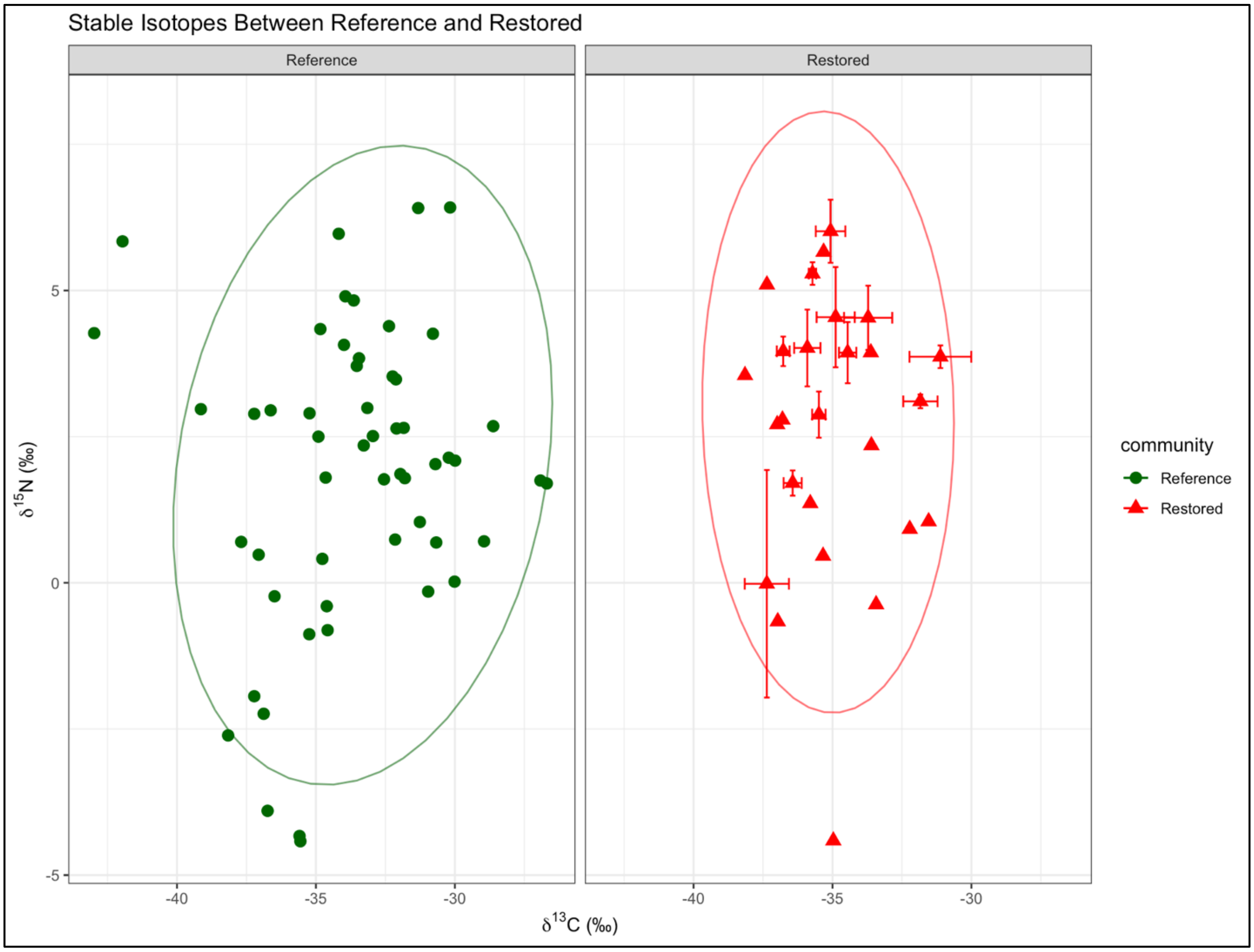

Figure 4.19. Stable Isotopes of Invertebrates Between Reference and Restored Sites. δ^{13}C and δ^{15}N compositions of all reference and restored sites. 95% prediction intervals (ellipses) were added to calculate Standard Ellipse Area (SEA) and for visual aid.

Standard error bars displayed in Figure 4.15 are from multiple samples of the same site of each FFG. For example, site Rest.BF4 contained four FFGs, and three of each functional feeding group were sent for δ^{13}C and δ^{15}N analysis for twelve total. This was done for three sites to estimate the standard error when calculating δ^{13}C and δ^{15}N. It is important to note; Figure 4.15 can be used to see the general δ^{13}C and δ^{15}N compositions found on reference and restored sites. While good as a visual aid, community-wide metrics cannot be calculated from these bi-plots alone because (Kupilas et al. 2016) grouping different stream δ^{13}C and δ^{15}N compositions and calculating metrics from those would be ecologically incorrect when assuming each stream is its community (Pauly 1998, Jennings et al. 2002, Anderson and Cabana 2007). I compared communities of similar geomorphic classes (Figure 4.20) to understand the trophic distributions across a geomorphic class gradient. It appeared that δ^{13}C and δ^{15}N values were similar between reference and restored sites, but the δ^{13}C and δ^{15}N ranges were more extensive for reference sites. Pool-riffle streams appeared to be more enriched in δ^{15}N indicating that invertebrates in these streams consumed matter that was higher on the food chain

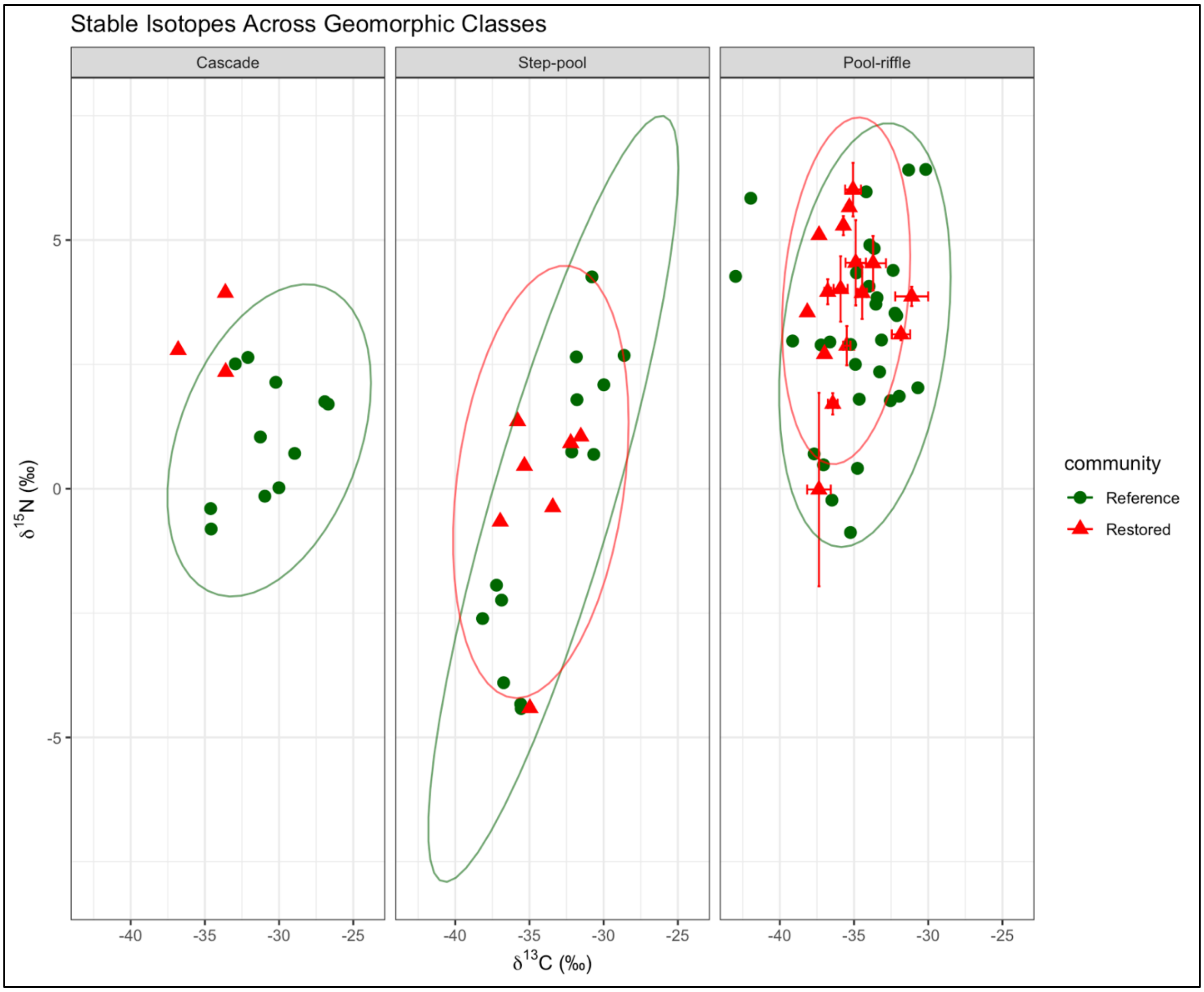

Figure 4.20. Stable Isotopes Across Geomorphic Classes. δ^{13}C and δ^{15}N FFG compositions of different geomorphic classes. 95% prediction intervals (ellipses) were added to calculate SEA and for visual aid. Mike Horse Creek had three FFGs and ellipses cannot be generated from only three data points for the restored cascade sample.

It is generally understood that trophic diversity exists across FFGs of river communities (Cummins 1973). To see this at reference and restored sites, I looked at the δ^{13}C and δ^{15}N of FFG communities at different geomorphic classes to see if there were similarities (Figure 4.21). The FFGs between reference and restored sites contained similar structure overall and it appears that predators were at the top of the food chain (Lindeman 1942, Cummins 1973), and scrapers were consuming more δ^{13}C- depleted food such as algae, which is consistent with the literature

(Perkins et al. 2014b). Other than one sample, restored streams did not contain filter feeders, which could indicate there is a lack of dissolved organic matter contributing to the new streams (Cummins and Klug 1979). δ^{13}C and δ^{15}N isotopic signatures did not differ between restored and reference sites because the invertebrates consume similar carbon sources.

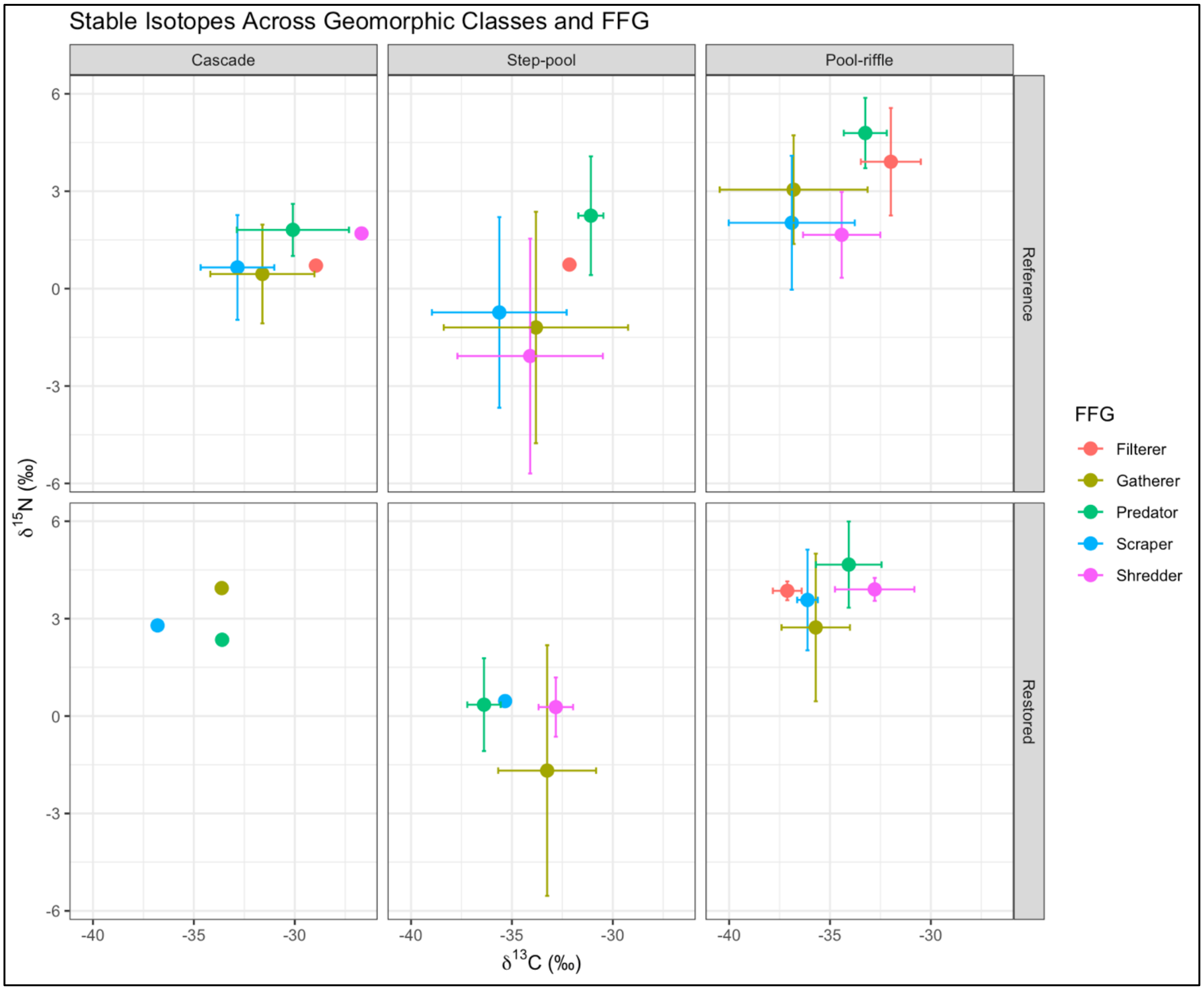

Figure 4.21. Stable Isotopes of FFGs. δ^{13}C and δ^{15}N compositions of FFGs in different geomorphic classes. Error bars represent a range of δ^{13}C and δ^{15}N invertebrate compositions for a sample.

Stable Isotopic Colonization Index

I created the stable isotope colonization index (SICI) to index the biological response of invertebrate communities to restoration efforts. By using stable isotope ratios of δ^{13}C and δ^{15}N, SICI was able to provide information on the isotopic complexity of the invertebrate community, which is particularly important for understanding the role of these organisms in ecosystem functioning. Three of the eight stable isotope indicators displayed pronounced statistical differences between reference and restored sites (Table 4.3). Other indicators exhibited low statistical significance as indicators between restored and reference sites. This highlights the importance of selecting appropriate indicators when using stable isotopes to assess ecosystems.

Table 4.3. SICI Indicators Tested. Indicators for the SICI displayed differences between restored and reference sites.

SICI Indicators	**Response to Increased Disturbance**	**P-Value**
Carbon Range (CR)	Decrease	0.03
Mean Distance to Centroid (CD)	Decrease	0.04
Nitrogen Range (NR)	Decrease	0.09
Total Area (TA)	Decrease	0.20
Standard Deviation Nearest Neighbor Distance (SDNND)	Increase	0.20
Standard Ellipse Area (SEA)	Decrease	0.44
Standard Ellipse Area Corrected (SEAc)	Decrease	0.66
Mean Nearest Neighbor Distance (MNND)	Decrease	0.97

SICI Metrics

I computed metrics for each stream using δ^{13}C and δ^{15}N isotopes and compared restored and reference sites (see Table 4.3). As I was developing this index, I wanted to ensure that the statistical significance of each metric was considered. While a p-value of 0.05 or less is often used as a threshold for statistical significance, I believed using slightly higher p-values for these metrics still showed statistical evidence between restored and reference sites. I carefully considered the ecological meaningfulness of each metric to ensure that the resulting index would be a reliable indicator of isotopic complexity. I found that carbon range (CR), nitrogen range (NR), and mean distance to the centroid (CD) were the best stable isotope metrics that displayed relative differences between reference and restored sites (Figure 4.22).

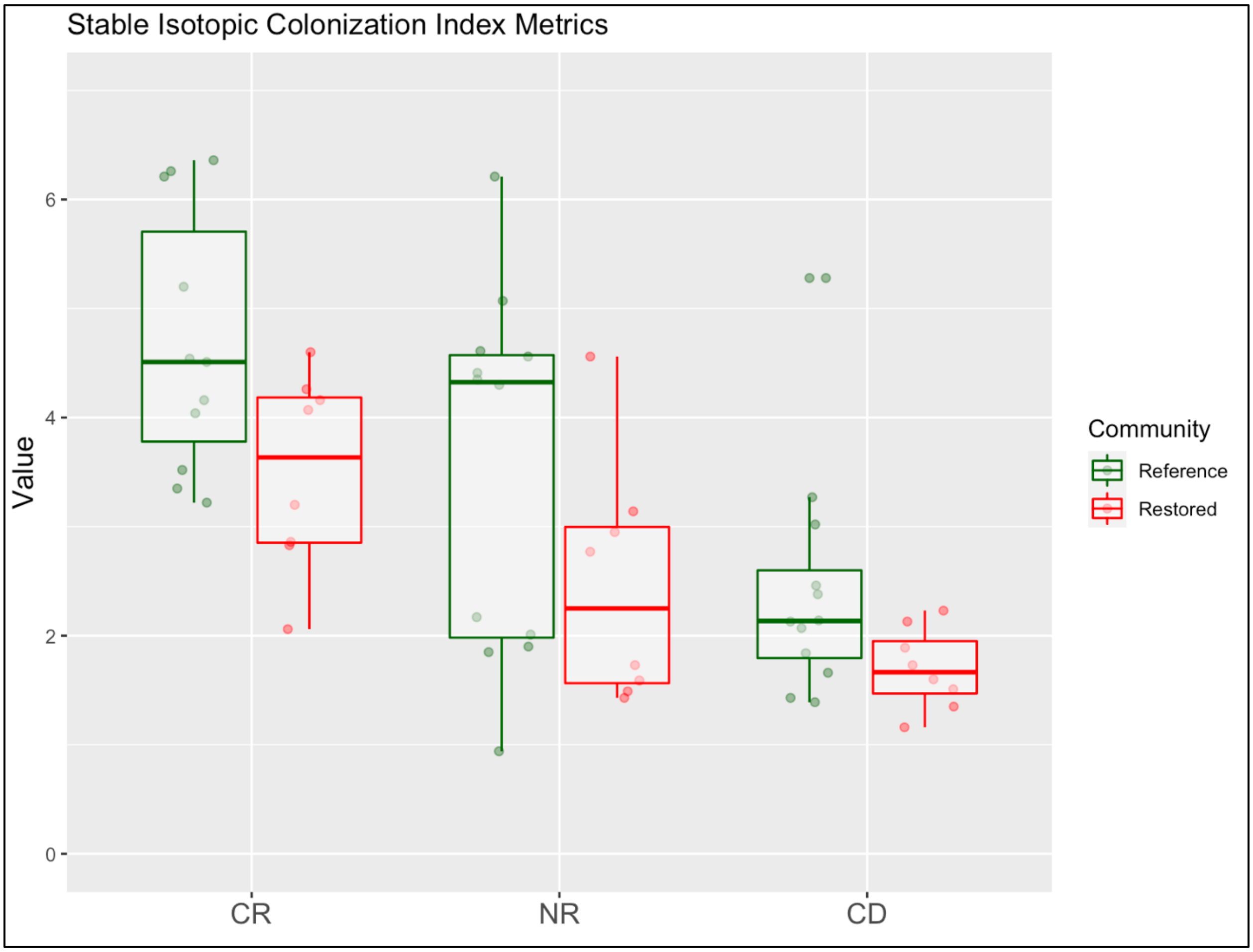

Figure 4.22. SICI Metrics. Selected metrics for the SICI that displayed differences between restored and reference sites.

I aimed to create an index that would provide a comprehensive biological assessment from 0 to 100 of the ecosystems. While two of the three SICI metrics contained a p-value less than the traditional value of 0.05, the carbon range, nitrogen range, and mean distance to the centroid contained much lower p-values than other metrics tested.

δ^{13}C Range. The Carbon Range (CR) is an essential ecological metric providing insight into the assimilated carbon sources consumed by a community (Layman et al. 2007a). The δ^{13}C values can vary depending on the carbon source, with different vegetation sources having different δ^{13}C values. For example, δ^{13}C of Doritos is -18‰ whereas oatmeal is -27‰. Thus, an increase in CR would indicate a higher diversity of carbon sources with varying δ^{13}C values, which can provide important information about what vegetation is entering the stream of a particular community (Layman et al. 2007a). In this study, CR was measured at both restoration and reference sites, and the results show that the restoration sites had an average (±1SD) CR of 3.5‰ ±0.8, which was lower than the CR at the reference sites, which was 5.4‰ ±0.3 (see Figure 4.22).

δ^{15}N Range. Nitrogen Range (NR) is an ecological metric that provides insight into the trophic length of the invertebrate community associated with a particular ecosystem. NR was measured at both restoration and reference sites, restoration sites had an average (±1SD) NR of 2.4‰ ±0.4, and reference sites had a NR of 3.5‰ ±0.5 (see Figure 4.22). This indicates that reference sites have one additional trophic level in the invertebrate community than restored sites (McCutchan et al. 2003). More trophic levels allow for more nutrient cycling and greater energy flow through the food web (Dolédec and Statzner 2010).

Mean Centroid Distance. The Mean Distance to Centroid (CD) is a key metric used to assess the trophic diversity of food webs (Layman et al. 2007a). An increase in CD suggests an increase in the average distance between FFGs, which implies greater trophic diversity (Layman et al. 2007b). The CD was measured at both restoration and reference sites, and restoration sites had an average CD (1±SD) of 1.7‰ ±0.1, which was lower than the CD at the reference sites, being 2.4‰ ±0.3 (see Figure 4.22). This small difference may have implications for ecosystem functioning, as greater trophic diversity can enhance nutrient cycling and energy flow within the food web (Cummins 1973, Vannote et al. 1980).

SICI Scores

The SICI measures invertebrates' overall degree of isotopic complexity in each ecosystem. This index is calculated based on the $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios of the FFGs present in the system. It reflects the extent that these taxa have colonized the available habitat. SICI scores were calculated for both reference and restored sites, and reference sites had significantly higher SICI scores than the restored sites (Welch two sample t-test p-value = 0.02). Specifically, the mean (±1SD) SICI score for reference sites was 51 ±9.2, significantly higher than the mean SICI score for restored sites, which was 23.3 ±6.1. This difference in SICI scores represents a magnitude of 2.3 (Figure 4.23), indicating a substantial difference in isotopic complexity between reference and restored sites.

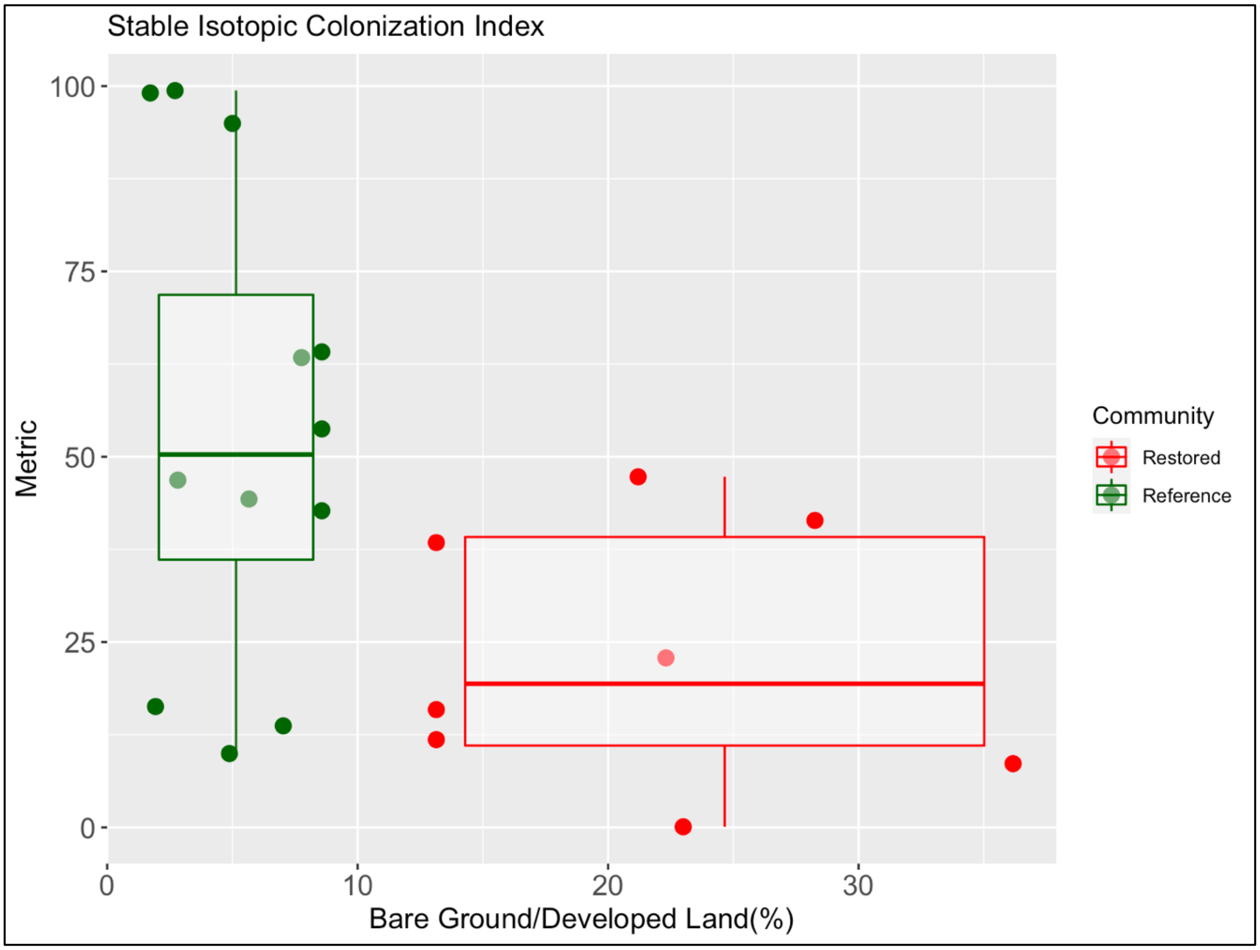

Figure 4.23. SICI Scores of Reference and Restored Sites. SICI scores of restored and reference sites across a disturbance gradient. The mean (±1SD) SICI score for reference sites was 54 ±9.2, while the restored score was 23.3 ±6.1.

For SICI, the MSE value of 738.4 suggests relatively high within-group variability in the data. This means that the observations within each group are somewhat spread out around the group mean, indicating that other factors may contribute to the variability in the data beyond the effect of the independent variable(s) or element (s) included in the ANOVA test. Despite this within-group variability, the ANOVA results (Table 4.4) show a clear significant difference between groups regarding SICI (F = 6.14, p = 0.023). This indicates that the mean values of SICI across the different groups are different and that the difference is unlikely to be due to chance alone.

Table 4.4 SICI Scores ANOVA test. ANOVA test between reference and restored sites (Between Groups) and each individually (Within Groups).

Source	SS	Df	MS	F-value	p-value
Between Groups	4,536	1	4,536	6.14	0.023
Within Groups	13,291	18	738		
Total	17,827	19			

These SICI findings suggest that efforts to restore ecosystems may take years to fully establish an invertebrate isotopic complexity comparable to that of reference sites. Understanding the factors that contribute to invertebrate colonization in restored ecosystems is essential for guiding restoration efforts and ensuring the long-term success of these efforts. Additionally, the SICI provides a helpful tool for biologically assessing streams and can serve as a valuable indicator of ecosystems.

Cost-Effectiveness

I compared the cost-effectiveness of using the four diversity and similarity indices, SICI, RIVPACS, and B-IBI (Table 4.5). Evaluating the cost-effectiveness of different approaches informs assessment decisions. Cost-effectiveness can help me determine the efficient use of limited time and budget resources. I evaluated the costs and time for collecting invertebrates, sample processing, taxonomic identification (including time to receive results), laboratory analysis (including time to receive results), and data analysis for the assessments. I used an estimated pay rate of $320/day ($40/hour for 8 hours including per diem) for invertebrate collection, and $200/day ($25/hour for 8 hours) for sample processing and analysis of data. I also present actual laboratory analysis costs from receipts from UC Davis Stable Isotope Facility and EcoAnalysts, Inc. The time to perform each step was based on an environmental science graduate student background or equivalent experience working for a full 8-hour day.

Table 4.5. Cost-effectiveness to Develop Assessment Approaches. Estimated time and costs to perform the diversity/similarity indices and bioassessments on fifteen streams. Hourly pay rates of $320/day (*) and $200/day (**) are noted.

	SICI	**Diversity & Similarity Indices**	**RIVPACS**	**B-IBI**
Streams Assessed	15	15	15	15
Time (Days)				
Invertebrate Collection	3	3	3	3
Sample Processing	8	6	6	6
Taxonomic Identification	2	105	105	105
Laboratory Analysis	100	0	0	0
Analysis of Data	4	2	8	12
Total Time (Days)	**117**	**116**	**124**	**128**
Costs ($)				
Invertebrate Collection*	960	960	960	960
Sample Processing**	1,600	1,200	1,200	1,200
Taxonomic Identification	400	2,460	2,460	2,460
Laboratory Analysis	938	0	0	0
Analysis of Data**	800	400	1600	2400
Total Cost for 15 Streams:	4,698	5,420	6,220	7,020
Cost per Stream:	**$313.20**	**$361.33**	**$414.66**	**$468. 00**

CHAPTER FIVE

DISCUSSION

Diversity and Biological Assessments

My thesis investigated the use of aquatic invertebrate colonization to assess restoration success and to identify assessment tools to quantify this. To do this, I utilized seven indices (four taxonomic diversity and similarity indices, RIVPACS, B-IBI, and SICI) to assess the success of the $39 million restoration of UBMC streams. Using taxonomic diversity indices showed invertebrate communities at restored sites diversifying quickly as the streams are being colonized, but still did not have similar taxa as reference sites. B-IBI showed that the biological integrity of restored sites was lower, but trending towards reference streams. SICI showed that isotopic complexity was lower on restored sites than reference, only required identification to family, and was the most cost-effective method.

Diversity and Similarity Indices

The invertebrate communities in the restored streams are becoming more diverse over time, as suggested by the Shannon, Gini-Simpson, and Inverse Simpson Diversity indices. This indicates that invertebrates are colonizing the restored streams over time and finding new niches. While diversity is increasing, the low taxonomic similarity between the UBMC and reference sites could be due to the lack of environmental conditions (substrate, vegetation, habitat) not present on the UBMC yet. This is a promising finding, but it is important to note that there is still a difference in the invertebrate taxa found in the restored streams compared to the reference sites. While they are diversifying, invertebrate taxa still do not resemble those of reference, nor are they trending toward reference conditions. Rest.BT2 did have the highest similarity, possibly due to being immediately downstream of Ref.BT1 and Ref.BT2. Previous research indicates that establishing the same taxonomic similarity can take months to multiple years, depending on the restored stream surroundings (Gore 1982, Malmqvist 2002, Chapelsky et al. 2020). Gore 1982 emphasizes the influence of the distance between the colonizing stream and other waterways on the rate that invertebrates colonize, most beginning rapidly first and then leveling off.

While all three indices had similar results, I believe that the Gini-Simpson diversity index was the best of the three, as reference streams tended to have lower standard deviations, and provided diversity scores from zero to one, making it easy to categorize (Table 5.1). Based on the Gini-Simpson scores, the diversity of restored sites was higher in the spring than summer for both 2020 and 2021. I believe this is due to the water flows on the UBMC peaking in the spring, and the rapid reduction of flows after snowmelt. It appears that there was no pattern for diversity of when restoration was completed, or which reach the sample was collected. Site Rest.BF4 did have the lowest seasonal variability, and averaged the highest diversity, potentially from being located below the confluence of Anaconda and Beartrap Creeks. Reference streams averaged excellent biological diversity, while restored increased to good diversity by 2022. If invertebrates continue this trend, they will likely attain similar biological diversity as reference sites by 2023.

Diversity indices used in the analysis effectively estimated invertebrate diversification. I found that they were not the most cost-effective method among the four evaluated due to the cost of taxonomic identification required. Nonetheless, the results still provide valuable insights into the progress of colonization and the potential for continued diversification over time. Furthermore, these indices can be compared with values from historical Mike Horse monitoring efforts from the late 1960s (Spence 1975) and early 1970s (Spence 1997) before the tailings dam collapse. Assuming no major disturbances, my initial findings of invertebrate diversity suggest that restored sites will reach excellent biological diversity (Table 5.1) by 2024.

Table 5.1. Categories of Biological Diversity. Qualitative categories of biological diversity. Modified (Karr and Dudley 1981) and updated with 0-1 scoring.

Biological Diversity	Description	Score Range
Excellent	Like reference condition with minimal disturbance; ecosystem displays a significant presence of various taxa and overall taxonomic diversity is high.	0.80-1
Good	Slight departure from reference condition with minimal disturbance; ecosystem displays absence of some taxa. Slight decline of taxa diversity.	0.60-0.79
Fair	Moderate departure from reference condition with minimal disturbance; taxa diversity is reduced.	0.40-0.59
Poor	Considerable departure from reference condition with minimal disturbance; taxa diversity is depressed.	0.20-0.39
Very Poor	Far departure from reference condition with minimal disturbance; overall taxa diversity very low.	0-0.19

River Invertebrate Prediction and Classification System

I measured an expected RIVPACS average score of for the 10 reference sites, which fell short of DEQ's reference standard minimum of 0.9. Nonetheless, valuable insights can still be gleaned from employing this method. Among the 10 reference sites I selected, reference sites averaged as fair (Table 5.2), which did not align with the outcomes of the other three methods, which were good to excellent. The average UBMC site was also rated as fair but exhibited very high standard deviations. The alternative approaches highlighted a disparity between reference and restored sites, whereas my RIVPACS assessment suggested that the restored sites possessed the same biological quality as the reference sites. This discrepancy may be attributed to RIVPACS considering taxonomic richness as the primary variable, while other methods that use MMI take multiple attributes into account. This study underscores the importance of incorporating a greater number of reference sites to establish meaningful reference conditions that encompass similar environmental predictor variables. This approach enables the inclusion of a more diverse range of environmental predictor variables, which in turn provides a more accurate assessment of the ecological status. By expanding the reference site pool, the study outcomes can offer more robust and reliable conclusions for effective ecosystem management and restoration efforts.

Table 5.2. RIVPACS Categories of Biological Quality. Qualitative categories of biological quality. Modified (Karr and Dudley 1981) and updated with RIVPACS 0-1 scoring.

Biological Quality	Description	Score Range
Excellent	Like reference condition with minimal disturbance; ecosystem displays a significant presence of various taxa and overall taxa richness is high.	0.80-1
Good	Slight departure from reference condition with minimal disturbance; ecosystem displays absence of some taxa. Slight decline of taxa richness.	0.60-0.79
Fair	Moderate departure from reference condition with minimal disturbance; taxa richness is reduced.	0.40-0.59
Poor	Considerable departure from reference condition with minimal disturbance; taxa richness is depressed.	0.20-0.39
Very Poor	Far departure from reference condition with minimal disturbance; overall taxa richness very low.	0-0.19

Benthic Index of Biotic Integrity

The B-IBI method emerged as a valuable tool for assessing the biological integrity of both reference and restored streams by utilizing various invertebrate attributes as metrics. The B-IBI method demonstrated notable advantages, including the utilization of a comprehensive set of ten invertebrate attribute-derived metrics. These metrics produced results that contained lower coefficient of variations compared to other the other assessments. Very similar to the diversity indices, B-IBI increased over years, indicating that invertebrates are colonizing the restored streams. This is interesting, as this indicates that as biological integrity of the invertebrate assemblage changes, this also could reflect how the invertebrates colonize the streams. As stream conditions improve, it is reasonable to expect that the restored streams become more hospitable environments for invertebrates to colonize. As restoration sites recover, the B-IBI scores may indicate whether the conditions have improved and whether the restored streams are becoming more suitable for invertebrate colonization.

Like diversity indices, the B-IBI scores of the restored sites exhibited an upward trend over time, indicating an improvement in the biological integrity of the streams. Notably, during the spring of 2021, there was a jump in B-IBI scores. This surge could potentially be attributed to favorable water flows experienced that year, which likely contributed to enhanced ecological conditions. However, the subsequent seasons of summer and fall in 2021 deviated from the norm, characterized by abnormal dryness and heat. Consequently, mid-summer in 2021 witnessed a lack of water flow, potentially impacting B-IBI scores.

Reference sites within the region generally exhibited good or excellent biological integrity, as indicated in Table 5.3. In contrast, the restored sites demonstrated poor to very poor biological integrity. When considering the overall UBMC, it is noteworthy that the biological integrity of invertebrate communities within the restored streams displayed an improvement, transitioning from a very poor rating in 2020 to a poor rating in 2021. These findings suggest that the restoration efforts have had a positive impact on the recovery of the novel ecosystem, resulting in the gradual enhancement of the biological integrity of the streams. Assuming no major disturbances on the UBMC occur, I would expect that the UBMC would begin to see good biological integrity by 2024.

The insights gained from the B-IBI method underscore its efficacy in capturing and monitoring changes in the biological conditions of the streams. For example, Mike Horse Creek consistently contained the lowest biological integrity than any other site, being poor. By identifying the streams that require further attention and observing the positive outcomes of restoration actions, this method facilitates informed decision-making and effective management strategies for the continued improvement and preservation of stream ecosystems.

Table 5.3. B-IBI Categories of Biological Integrity. Qualitative categories of biological condition. Modified (Karr and Dudley 1981) and updated with B-IBI 0-100 scoring.

Biological Integrity	**Description**	**Score Range**
Excellent	Like reference condition with minimal disturbance; ecosystem displays a significant presence of various taxa, which are scraper, clinger, and intolerant. Overall taxa richness is high. Furthermore, the population of Plecoptera and Heptageniidae is relatively abundant.	80-100
Good	Slight departure from reference condition with minimal disturbance; ecosystem displays absence of some taxa, which are scraper, clinger, and intolerant. Slight decline in taxa richness, Plecoptera, and Heptageniidae abundance.	60-79
Fair	Moderate departure from reference condition with minimal disturbance; taxa richness is reduced particularly intolerant, Plecoptera, and clinger taxa, relative abundance of scraper declines.	40-59
Poor	Considerable departure from reference condition with minimal disturbance; taxa richness is depressed, proportion of scrapers greatly reduced as is Trichoptera and Heptageniidae taxa richness.	20-39
Very Poor	Far departure from reference condition with minimal disturbance; overall taxa richness very low. Trichoptera, Heptageniidae, clinger, scraper, and intolerant taxa largely absent; relative abundance of Plecoptera is very low.	0-19

Stable Isotopic Colonization Index

The ability to develop a straightforward score to assess a river that can be easily translatable for the management application (Barbour et al. 1999) is highly sought after when ecological data can be too complex (Underwood 1995). While $\delta^{13}C$ and $\delta^{15}N$ stable isotopes of aquatic invertebrates have been used to quantify trophic structure (Layman et al. 2007a, Motitsoe et al. 2022), and measure aquatic-terrestrial linkages (Kupilas et al. 2020), I know of no scholarly work that uses these metrics as a scored colonization index of stream restoration success.

To address this gap, I combined the community-wide attributes from $\delta^{13}C$ and $\delta^{15}N$ isotopic bi-plots (Layman et al. 2007a) and the same scoring technique used for the B-IBI (Wilhelm 2014). SICI was developed based on the premise that as invertebrates colonize, more trophic interactions develop, which has been established in the literature (Mackay 1992, Cummins and Merritt 1996, Merritt et al. 2017). By providing a simplified colonization score for rivers like a B-IBI, SICI could offer a more accessible and straightforward approach to assessing invertebrate colonization, which has the potential to improve ecosystem management decisions. Since I combined individual SICI metrics into an index, the significance between the reference and restored conditions became even more pronounced.

Based on the SICI scores, restored sites seemed to have poor or very poor (Table 5.4) isotopic complexity, and reference sites contained fair to excellent complexity. Although isotopic complexity was statistically lower in restored sites, this finding is consistent with the existing literature, which suggests that establishing an ecosystem with complex trophic interactions can take several years (Gore 1982, Kondratieff et al. 1984, Milner 1987, Yount and Niemi 1990, Malmqvist et al. 1991).

Table 5.4. SICI Categories of Isotopic Complexity. Qualitative categories of isotopic (δ^{13}C and δ^{15}N) complexity. Modified (Karr and Dudley 1981) and updated with SICI 1-100 scoring.

Isotopic Complexity	Description	Score Range
Excellent	Like reference conditions with minimal disturbance; ecosystem displays a significant presence of various taxa, which are tropically diverse, consuming a wide range of carbon source, and contains excellent trophic structure.	80-100
Good	Slight departure from reference condition with minimal disturbance; ecosystem displays slightly reduced isotopic complexity, consuming fewer resources, and relatively good trophic structure.	60-79
Fair	Moderate departure from reference condition with minimal disturbance; invertebrate isotopic complexity is reduced, few carbon sources are being consumed, and some trophic structure.	40-59
Poor	Considerable departure from reference condition with minimal disturbance; isotopic complexity is low, minimal invertebrates consuming carbon, and trophic structure not as developed.	20-39
Very Poor	Far departure from reference condition with minimal disturbance; isotopic complexity is very low, minimal invertebrates consuming carbon, and trophic structure is faint.	0-19

Community attributes from stable isotopes remain the most widely used methods for evaluating trophic interactions and are anticipated to remain vital tools for assessing the impact of restoration efforts on ecosystems (Lock et al. 2020). SICI also had the extra benefit of measuring invertebrate trophic length, assimilated carbon sources, niche spaces, degree of trophic diversity, and evenness of FFG packing (Layman et al. 2007a). In sampling all FFGs in the stream, I assumed that most of the invertebrate community is captured when graphing δ^{13}C and δ^{15}N in bi-plot space. While SICI was used to assess the isotopic complexity of this novel ecosystem, it could be applied to any wadable stream in theory.

Indices Comparisons

Applying taxonomic diversity methods allowed for a more comprehensive understanding of how the invertebrate communities colonized the streams over the three-year sampling period. At the same time, B-IBI and SICI allowed for a bioassessment of biological integrity and isotopic complexity. The standard deviation in SICI was 48% higher than the B-IBI reference scores, most likely due to using only three broader community-based metrics instead of using ten invertebrate attribute-based metrics. I also found that there was greater variation in SICI (coefficient of variation of reference scores [CV]: 0.59) than in B-IBI (CV of reference scores 0.22): If I were to choose an assessment method that has been widely used and contains less variability, it would be the B-IBI. While SICI worked well to assess the invertebrate community at a lower price than other methods, the B-IBI provided an assessment with less variability. Although the RIVPACS method did not meet DEQ’s standards, the other methods provided valuable insight into the success of the restoration efforts.

RIVPACS, B-IBI, and diversity indices required taxonomic identification to the genus level, which required a taxonomic specialist. For SICI, fewer invertebrates needed to be taxonomically identified and only to the family level. This saved time and cost, being the cheapest assessment method of the four. While the costs reflected in the results reflected a “starting from scratch” estimate, monitoring costs for the UBMC would only need to include sampling the restored sites and exclude any assessment development costs. More research must be done to see if SICI can provide the same level of accuracy and consistency that a B-IBI can provide. Which method to utilize for a restoration project heavily depends on the restoration goals and outcomes desired.

Implications for the Upper Blackfoot Mining Complex

The biological assessments performed at the UBMC revealed a promising trend in the colonization of restored reaches by the invertebrate communities. Taxonomic diversity and biological integrity are approaching those of reference sites, indicating that the extensive and expensive stream restoration efforts have successfully fostered positive recovery towards reference conditions. This achievement is particularly meaningful given that restoring aquatic ecosystems can be challenging due to heavy anthropogenic impacts and environmental stressors (Resh et al. 1988, Bradshaw 1996). The restoration efforts at UBMC highlight the importance of adopting a comprehensive and integrated approach that considers the entire ecosystem and surrounding landscape in stream restoration projects.

The lower carbon range (CR) of restoration sites (~9‰) suggests that there is lower diversity of vegetation sources being consumed by the invertebrate communities than for reference sites (~16‰), which could have negative implications. For instance, when there is a reduction of assimilated carbon sources, the invertebrate communities residing in restored sites face heightened susceptibility to environmental disturbances (Mackay 1992). Moreover, this limited availability of carbon sources may discourage the colonization of the stream by a diverse range of invertebrate species (Lake et al. 2007). Rest. BF5 did have the largest CR (4.6‰) of restored sites, likely due to being the furthest site downstream potentially capturing more carbon sources. The lower nitrogen range (NR) at restoration sites may suggest that the trophic structure of the invertebrate community is less developed than at reference sites (Layman et al. 2007a). Rest. BF4 contained the highest NR (4.6‰) of other restored sites, potentially from to being below the confluence of Anaconda and Beartrap Creeks, leading to more trophic interactions.

Furthermore, lower centroid distance (CD) at the restoration sites may indicate that these sites still need to restore the same level of trophic relationships as reference sites. This could be due to the limited availability of suitable habitats or the slow colonization of invertebrate communities following stream restoration.

The taxonomic diversity, biological integrity, and isotopic complexity among the three streams at UBMC exhibited a few notable differences. Mike Horse Creek demonstrated the lowest taxonomic diversity, biological integrity, and isotopic complexity relative to reference reaches, while Beartrap Creek and the Blackfoot River had comparable diversity and biological integrity relative to reference reaches. This could be due to Mike Horse Creek being the smallest stream and being a first-order stream, thus having the smallest and shortest flow season. However, Rest.BT3 had more similar taxa to reference sites than the Blackfoot River, potentially due to being beneath the confluence of Beartrap Creek and Mike Horse Creek. Restored streams also had almost no (0.6%) abundance of Heptageniidae, indicating that there is poorer water quality and have not colonized the restored sites. This finding is consistent with previous research that suggests it takes many years for Heptageniidae to recolonize an artificial stream (Malmqvist et al. 1991).

It is worth noting that the biological diversity, integrity, and isotopic complexity, were not dependent on the restoration reach that the site was located on (see Figures 4.3 and 4.18). This also indicates that colonization is not dependent on when the streams were restored as well. This finding suggests that the restoration efforts were reflected across the entire site, and the restoration strategies successfully achieved the desired outcomes.

The primary objective of the UBMC restoration project was to establish self-sustaining ecological processes, including thriving aquatic invertebrate populations and riparian habitats for wildlife (River Design Group, Inc. and Geum Environmental Consulting, Inc. 2014). If I had to give a letter grade for the UBMC restoration project, I would give it a B+. The fact that the restored ecosystem's invertebrate populations are diversifying to those of reference streams is a positive indication that the project is on track to achieving these objectives set before restoration began. While they are diversifying, invertebrate taxa still do not resemble those of reference, nor are they trending toward reference conditions. They are meeting CERCLA criteria by reducing hazardous materials to humans and the environment and have restored the streams to where they are self-sustaining. While my study did not focus on wildlife, in my experiences sampling at the UBMC, there have been noticeable increases in vegetation along the floodplain, which should positively impact habitat for wildlife recruitment. However, it is essential to note that attaining self-sustaining ecological processes can be complex and ongoing, requiring continuous monitoring and management.

I recommend continuous biological monitoring to determine whether invertebrate assemblages fully achieve reference conditions to ensure the long-term success and sustainability of the restoration efforts. If wanting to monitor every stream on the UBMC, I would suggest sampling yearly, in early July, at restored sites Rest.MH1, Rest.BT3, and Rest.BF5. By implementing this approach, comprehensive monitoring of all geomorphic types, the majority of reaches, and spanning various periods of restoration on the UBMC can be ensured. If the most accurate monitoring method is desired, I recommend using the B-IBI. If wanting a comprehensive evaluation of the invertebrate communities, I would use SICI or the Gini-Simpson indices, since they were the most cost-effective approaches.

CHAPTER SIX

CONCLUSIONS

The mining industry is critical in modern society, providing essential materials for various applications. However, it is no secret that the industry has also had significant environmental impacts, including habitat degradation and water pollution. Superfund was enacted to regulate the mining industry's activities and mandate restoration efforts to mitigate these adverse effects. These projects aim to restore the impacted ecosystems to their pre-mining conditions or as close to them as possible. This thesis aimed to investigate whether invertebrate colonization could serve as a criterion for evaluating the success of Superfund restoration projects. I utilized various diversity and similarity indices and bioassessments to assess invertebrate diversity, quality, integrity, and isotopic complexity to achieve this goal. These included the Shannon Diversity Index, Gini-Simpson Diversity Index, Inverse Simpson Diversity Index, Bray-Curtis Similarity Index, River Invertebrate Prediction and Classification System (RIVPACS), Benthic Index of Biotic Integrity (B-IBI), and a new Stable Isotope Colonization Index (SICI).

Using diversity and similarity indices and bioassessments were able to quantify invertebrate colonization in the restored streams at the UBMC. Using four statistical taxonomic diversity and similarity indices showed invertebrate communities identified at restored sites were diversifying quickly but still did not have similar taxa as reference sites. The RIVPACS approach required sampling hundreds of streams to establish expected conditions acceptable to the Department of Environmental Quality standard. B-IBI was able to estimate the biological integrity of restoration and reference sites with the least variability but required the taxonomic identification to genus. SICI only required identification to family, was the most cost effective method, and estimated isotopic complexity using metrics derived from $\delta^{15}N$ and $\delta^{13}C$ biplots. Applying these methods allowed for a comprehensive understanding of how the invertebrate communities recolonized the streams over the three-year sampling period. Findings showed that the restored streams were approaching comparable biological diversity and integrity but still differed in isotopic complexity compared to reference streams. This indicates that the extensive stream restoration efforts fostered positive recovery towards reference conditions.

The restoration efforts at UBMC highlight the importance of adopting an integrated approach to stream restoration that considers the entire ecosystem and the surrounding landscape. However, achieving self-sustaining ecological processes can be complex and ongoing, requiring continuous monitoring and management. Therefore, I recommend continuous biological management to determine if populations have achieved reference conditions, which is critical to the long-term success and sustainability of the restoration efforts at UBMC. I would suggest sampling yearly, in early July, at restored sites Rest.MH1, Rest.BT3, and Rest.BF5. By implementing this approach, comprehensive monitoring of all geomorphic types, the majority of reaches, and spanning various periods of restoration on the UBMC can be ensured. If the most accurate monitoring method is desired, I recommend using the B-IBI. If wanting a comprehensive evaluation of the invertebrate communities, I would use SICI or the Gini-Simpson index, since they were the most cost-effective. Overall, using invertebrate colonization as a restoration success criterion for Superfund projects is a promising approach that can help ensure the effective restoration of a novel ecosystem.

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APPENDICES

APPENDIX A

RIVPACS PREDICTOR VARIABLES

Appendix A. RIVPACS Predictor Variables. ArcGIS was used to calculate latitude, longitude, watershed area, SD of elevation. Average air temperatures were found by a USGS weather station. Temperature is in Celsius, and area is in hectares.

Sample	Latitude	Longitude	Area	Air Temperature(1971-2000)	SD of Elevation	Air Temperature(1961-1990)	Julian Day
ANCR01.Fall.2020	47.03	-112.36	7.39	127.8	132.87	126.7	275
ANCR01.Fall.2021	47.03	-112.36	7.39	127.8	132.87	126.7	261
ARCR01.Fall.2020	46.95	-112.90	61.73	127.8	279.07	126.7	274
ARCR01.Summer.2020	46.95	-112.90	61.73	127.8	279.07	126.7	228
BFS4.Fall.2020	47.04	-112.36	15.35	127.8	78.63	126.7	274
BFS4.Spring.2020	47.04	-112.36	15.35	127.8	78.63	126.7	164
BFS4.Spring.2021	47.04	-112.36	15.35	127.8	78.63	126.7	147
BFS4.Summer.2020	47.04	-112.36	15.35	127.8	78.63	126.7	221
BFS4.Summer.2021	47.04	-112.36	15.35	127.8	78.63	126.7	206
BFS5.Spring.2020	47.04	-112.37	18.15	127.8	112.04	126.7	164
BFS5.Spring.2021	47.04	-112.37	18.15	127.8	112.04	126.7	147
BFS5.Summer.2020	47.04	-112.37	18.15	127.8	112.04	126.7	221
BFS5.Summer.2021	47.04	-112.37	18.15	127.8	112.04	126.7	206
BTCR01.Fall.2020	47.02	-112.35	2.49	127.8	134.86	126.7	275
BTCR02.Fall.2020	47.02	-112.35	2.49	127.8	130.66	126.7	281
BTS2.Fall.2020	47.03	-112.35	3.57	127.8	131.36	126.7	275
BTS2.Spring.2020	47.03	-112.35	3.57	127.8	131.36	126.7	162
BTS2.Spring.2021	47.03	-112.35	3.57	127.8	131.36	126.7	147
BTS2.Summer.2020	47.03	-112.35	3.57	127.8	131.36	126.7	221
BTS2.Summer.2021	47.03	-112.35	3.57	127.8	131.36	126.7	208
BTS3.Spring.2020	47.03	-112.36	7.69	127.8	66.28	126.7	164
BTS3.Spring.2021	47.03	-112.36	7.69	127.8	66.28	126.7	147
BTS3.Summer.2020	47.03	-112.36	7.69	127.8	66.28	126.7	221
BTS3.Summer.2021	47.03	-112.36	7.69	127.8	66.28	126.7	207
CACR01.Fall.2020	47.05	-112.42	15.26	127.8	101.09	126.7	274
COCR01.Fall.2020	47.07	-112.61	77.87	127.8	254.56	126.7	274
COCR02.Fall.2020	47.08	-112.72	8.80	127.8	151.33	126.7	281
MHS1.Spring.2020	47.03	-112.36	0.94	127.8	110.84	126.7	162
MHS1.Spring.2021	47.03	-112.36	0.94	127.8	110.84	126.7	147
MHS1.Summer.2020	47.03	-112.36	0.94	127.8	110.84	126.7	221
MHS1.Summer.2021	47.03	-112.36	0.94	127.8	110.84	126.7	206
POCR01.Fall.2021	46.92	-112.66	103.29	127.8	179.97	126.7	244
SBCR01.Fall.2020	47.07	-112.61	19.19	127.8	192.54	126.7	274
SHCR01.Summer.2021	47.04	-112.37	8.43	127.8	136.93	126.7	210

APPENDIX B

B-IBI ATTRIBUTES TESTED

Appendix B. B-IBI Attributes Tested. Invertebrate attributes tested between reference and restored sites. My expected response to disturbance based on what is common in the literature and the p-value showing significance is shown.

B-IBI Attributes Tested	Expected Response to Increased Disturbance	P-value
Invertebrate Taxa Richness and Community Composition		
Taxa Richness	Decrease	0.033
% Richness EPT Taxa	Decrease	0.046
EPT Richness	Decrease	0.007
% Richness Ephemeroptera	Decrease	0.75
Ephemeroptera Richness	Decrease	0.051
% Ephemeroptera Abundance	Decrease	0.299
% Richness Plecoptera	Increase	0.636
Plecoptera Richness	Decrease	0.136
% Plecoptera Abundance	Decrease	0.004
% Richness Trichoptera	Decrease	0.006
Trichoptera Richness	Decrease	<.001
% Trichoptera Abundance	Decrease	0.233
% Richness Diptera	Increase	0.02
Diptera Richness	Increase	0.377
% Abundance Diptera	Increase	0.002
% Richness Coleoptera	Decrease	0.015
Coleoptera Richness	Decrease	0.032
% Abundance Coleoptera	Decrease	0.052
% Richness Heptageniidae	Decrease	0.01
Heptageniidae Richness	Decrease	0.004
% Abundance Heptageniidae	Decrease	0.002
% Richness Baetidae	Increase	0.143
Baetidae Richness	Increase	0.751
% Abundance Baetidae	Increase	0.144
Invertebrate Biological Processes		
% Richness Clinger	Decrease	0.024
Clinger Richness	Decrease	0.012
% Abundance Clinger	Decrease	0.158
% Richness Semivoltine	Decrease	0.008
Semivoltine Richness	Decrease	0.007
% Abundance Semivoltine	Decrease	<0.001

% Richness Intolerant	Decrease	0.269
Intolerant Richness	Decrease	0.021
% Abundance Intolerant	Decrease	<0.001
% Richness Sprawler	Increase	0.004
Sprawler Richness	Increase	0.137
% Abundance Sprawler	Increase	<0.001
% Richness Univoltine	Increase	0.459
Univoltine Richness	Decrease	0.173
% Abundance Univoltine	Decrease	0.863
% Richness Multivoltine	Increased	0.335
Multivoltine Richness	Decrease	0.1
% Abundance Multivoltine	Increase	0.666
Invertebrate Functional-Feeding Group		
% Richness Predator	Increase	0.441
Predator Richness	Decrease	0.1
% Abundance Predator	Decrease	0.618
% Richness Gatherers	Increase	0.408
Gatherers Richness	Decrease	0.261
% Abundance Gatherers	Increase	0.111
% Richness Shredders	Decrease	0.751
Shredders Richness	Decrease	0.11
% Abundance Shredders	Decrease	0.587
% Richness Scrapers	Decrease	0.005
Scrapers Richness	Decrease	0.007
% Abundance Scrapers	Decrease	0.001
% Richness Filterers	Increase	0.229
Filterers Richness	Increase	0.708
% Abundance Filterers	Increase	0.284
Invertebrate Abundance		
Total Abundance	Decrease	0.999
% EPT Abundance	Decrease	0.012
EPT Abundance	Decrease	0.2

APPENDIX C

B-IBI SUMMARY STATISTICS

Appendix C. B-IBI Summary Statistics. Summary statistics calculated using Excel for each invertebrate metric selected for the B-IBI.

Metric	Mean	Minimum	Maximum	10th Percentile	90th Percentile
% Abundance Plecoptera	17.8	0.0	49.7	1.2	56.3
% Abundance Intolerant	21.0	0.0	58.5	0.0	56.0
% Abundance Scrapers	16.4	0.0	57.2	0.0	46.5
% Abundance Heptageniidae	11.7	0.0	56.3	0.0	32.3
Taxa Richness	18.6	5.0	36.0	5.1	25.9
Trichoptera Richness	2.0	0.0	6.0	0.0	5.0
Clinger Richness	10.2	1.0	22.0	3.0	15.0
Intolerant Richness	4.1	0.0	8.0	0.0	6.0
Scrapers Richness	3.7	0.0	9.0	0.0	3.0
Heptageniidae Richness	1.6	0.0	4.0	0.0	3.0

APPENDIX D

B-IBI SCORES

Appendix D. B-IBI Scores. Computed scores for every sample collected, and the final B-IBI score out of a hundred.

Site/Metric	% Plecoptera Abundance	% Abundance Intolerant	% Abundance Scrapers	% Abundance Heptageniidae	Taxa Richness	Trichoptera Richness	Clinger Richness	Intolerant Richness	Scrapers Richness	Heptageniidae Richness	**B-IBI**
ANCR01.Fall.2020	1.90	9.76	9.02	9.72	5.72	6.00	5.83	6.67	10.00	3.33	67.95
ANCR01.Fall.2021	8.84	5.81	3.77	4.32	10.00	8.00	10.00	10.00	10.00	10.00	80.74
ARCR01.Fall.2020	3.89	5.25	5.76	4.63	6.20	8.00	7.50	6.67	10.00	3.33	61.23
ARCR01.Summer.2020	2.84	7.46	9.03	10.00	10.00	10.00	10.00	10.00	10.00	10.00	89.33
BFS4.Fall.2020	9.31	2.44	0.75	1.16	6.68	4.00	5.00	8.33	10.00	3.33	51.02
BFS4.Spring.2020	6.97	9.06	10.00	5.43	3.32	2.00	2.50	5.00	6.67	3.33	54.28
BFS4.Spring.2021	3.16	7.83	7.87	10.00	10.0	6.00	10.00	10.00	10.00	6.67	81.53
BFS4.Summer.2020	8.49	8.58	7.53	0.00	3.80	2.00	4.17	5.00	6.67	0.00	46.23
BFS4.Summer.2021	2.05	0.99	1.44	0.63	9.57	0.00	9.17	8.33	10.00	10.00	52.18
BFS5.Fall.2022	6.06	1.55	1.03	0.30	9.09	10.00	10.00	6.67	10.00	3.33	58.03
BFS5.Spring.2020	0.36	0.28	0.34	0.49	0.43	0.00	0.83	1.67	3.33	3.33	11.08
BFS5.Spring.2021	10.00	0.18	0.22	0.32	3.32	4.00	0.83	1.67	3.33	3.33	27.20
BFS5.Summer.2020	0.35	0.14	0.04	0.00	2.36	2.00	1.67	6.67	3.33	0.00	16.55
BFS5.Summer.2021	3.72	0.65	0.89	0.24	7.16	2.00	8.33	8.33	10.00	3.33	44.66
BTCR01.Fall.2020	4.84	6.13	2.15	0.90	6.68	8.00	5.83	6.67	10.00	6.67	57.86
BTCR02.Fall.2020	8.06	10.00	4.41	4.46	6.68	10.00	6.67	8.33	10.00	10.00	78.61
BTS2.Fall.2020	3.31	1.74	1.87	4.94	1.88	0.00	1.67	3.33	6.67	3.33	28.74
BTS2.Spring.2020	0.12	10.00	0.83	1.20	3.32	4.00	2.50	8.33	3.33	3.33	36.97
BTS2.Spring.2021	1.41	5.39	5.89	0.61	9.57	6.00	5.00	10.00	10.00	3.33	57.19
BTS2.Summer.2020	0.00	10.00	10.00	2.76	0.43	0.00	0.83	3.33	3.33	3.33	34.02
BTS2.Summer.2021	0.33	0.28	0.16	0.17	6.68	2.00	3.33	8.33	10.00	3.33	34.62
BTS3.Spring.2020	5.76	2.03	0.00	0.00	1.88	4.00	1.67	1.67	0.00	0.00	17.00
BTS3.Spring.2021	10.00	0.89	0.49	0.56	5.24	4.00	2.50	5.00	6.67	3.33	38.69
BTS3.Summer.2020	0.00	0.45	0.55	0.00	0.43	2.00	0.00	1.67	3.33	0.00	8.43

BTS3.Summer.2021	1.43	1.07	0.88	0.74	6.68	0.00	7.50	8.33	10.00	6.67	43.30
CACR01.Fall.2020	2.22	3.49	3.15	3.78	4.28	2.00	3.33	6.67	10.00	3.33	42.25
COCR01.Summer.2020	3.97	4.28	10.00	10.00	5.24	2.00	6.67	6.67	10.00	10.00	68.82
COCR02.Fall.2020	3.71	10.00	5.06	6.13	6.20	8.00	7.50	8.33	10.00	10.00	74.93
MHS1.Spring.2020	0.00	0.00	0.00	0.00	0.91	2.00	0.83	0.00	0.00	0.00	3.75
MHS1.Spring.2021	5.12	1.05	0.00	0.00	1.39	2.00	0.83	1.67	0.00	0.00	12.07
MHS1.Summer.2020	0.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	2.00
MHS1.Summer.2021	7.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.69
POCR01.Fall.2021	1.45	6.25	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	87.70
SBCR01.Fall.2020	6.16	2.36	1.29	1.24	4.76	4.00	5.00	6.67	10.00	3.33	44.81
SHCR01.Summer.2021	7.38	5.79	5.66	6.16	6.68	4.00	6.67	6.67	10.00	3.33	62.34

APPENDIX E

SICI SUMMARY STATISTICS

Appendix E. SICI Summary Statistics. Summary statistics calculated using Excel for each invertebrate metric selected for the SICI.

Metric	Mean	Minimum	Maximum	10th Percentile	90th Percentile
Nitrogen Range	3.42	0.94	7.00	1.48	5.10
Carbon Range	4.85	2.83	12.81	2.86	6.27
Mean Distance to Centroid	2.18	1.16	5.28	1.39	3.04

APPENDIX F

SICI SCORES

Appendix F. SICI Scores. Computed scores for every sample collected, and the final SICI score out of 99.9.

Site	NR	CR	CD	**SICI Score**
Ref.COC1	0.00	33.20	13.64	46.85
Ref.COC2	3.84	11.54	0.92	16.30
Ref.SHC1	3.37	4.81	5.52	13.70
Ref.ANC1 (2020)	4.85	22.86	15.01	42.72
Ref.ANC1 (2021)	26.42	16.13	21.59	64.14
Ref.ANC1 (2022)	25.96	12.71	15.07	53.75
Ref.ARC1	26.98	16.42	19.97	63.37
Ref.CAC1	28.36	33.30	33.30	94.96
Ref.POC1	28.82	6.47	9.01	44.29
Ref.BTC1	33.30	33.30	32.79	99.39
Ref.BTC2	33.06	32.71	33.30	99.07
Ref.SBC1	6.32	3.54	0.10	9.96
Rest.MH1	0.98	3.35	4.29	8.61
Rest.BF4 (2020)	0.00	11.84	0.00	11.84
Rest.BF4 (2021)	13.52	0.00	2.37	15.89
Rest.BF4 (2022)	28.36	0.00	10.06	38.42
Rest.BF5	15.24	17.04	15.01	47.29
Rest.BT2	11.81	12.66	16.94	41.41
Rest.BT3	2.27	13.69	6.91	22.87