MACROINVERTEBRATE DIVERSITY, COMMUNITY STRUCTURE, AND DISPERSAL ARE AFFECTED BY TRIBUTARY IDENTITY AND CONFLUENCE CONDITIONS IN A REGULATED RIVER

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2023

DEDICATION

For Amanda.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Lindsey Albertson, for the support, guidance, and expertise at every step of the research process. She is the reason I know what an ecologist truly is. I’d also like to thank my committee members Dr. Wyatt Cross, and Dr. Christopher Guy for their time, direction, and thoughtful edits. All of my lab-mates spent countless hours selflessly providing feedback and assisting with fieldwork while creating a great environment to learn. I would also like to thank Matt Jaeger and Jon Hanson for their help in the initial stages of the project. I would like to thank Montana State University, NorthWestern Energy, the Madison River Foundation, Sitka©, and the Montana Water Center for their funding that made this research possible.

Lastly and most importantly, thanks to my mother, Nurse Kate, who read to me every night from birth until high school and instilled in me a love and appreciation for the written word as well as a strong imagination. Gratitude, admiration, and respect must be given to my father, John, who flew out to Montana three separate times after he retired to be my lead field technician. He has been the best role model a young man could ever ask for and continues to be the benchmark against which I measure my actions. He saved my life once again.

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CHAPTER ONE

INTRODUCTION

Please refer to chapter two for the introduction.

CHAPTER TWO

MACROINVERTEBRATE DIVERSITY, COMMUNITY STRUCTURE, AND DISPERSAL ARE AFFECTED BY TRIBUTARY IDENTITY AND CONFLUENCE CONDITIONS IN A REGULATED RIVER

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Contributions: LKA helped conceptualize the research, helped design the study and write the manuscript.

Manuscript Information

Zachary J. Maguire, Lindsey K. Albertson

River Research and Applications

Status of Manuscript:
__X__ Prepared for submission to a peer-reviewed journal
______ Officially submitted to a peer-reviewed journal
______ Accepted by a peer-reviewed journal
______ Published in a peer-reviewed journal

John Wiley & Sons Ltd.

ABSTRACT

Tributaries are essential components of freshwater ecosystems, playing a crucial role in maintaining connectivity and providing habitat for a diverse array of aquatic organisms. The role of tributaries in creating heterogeneity in physical conditions and food resources for fishes could be critical, yet little is known about how variable conditions in different tributaries in regulated river systems influence the mainstem. Using field observations in five tributaries on the Madison River, Montana, we found that tributaries in the same network and within relatively short distances of 60km varied greatly in their environmental conditions, macroinvertebrate densities, and macroinvertebrate community structure. Downstream of confluences macroinvertebrate richness increased overall, and per capita weight of drifting macroinvertebrates decreased overall. These findings suggest that confluences may act as hotspots for biodiversity in regulated rivers and introduce smaller bodied macroinvertebrates to the drift. The amount that a tributary influenced benthic richness and mean per capita weight in the drift downstream of its confluence was related to land use and abiotic factors within that tributary; both macroinvertebrate metrics significantly increased in magnitude downstream of confluences with higher percentage of US Forest Service land, cooler temperatures, decreased discharge, and increased elevation loss (i.e. steeper watershed slope). In contrast, tributaries that had a larger proportion of agricultural land, warmer temperatures, and higher discharge more strongly influenced benthic macroinvertebrate metrics. These tributaries supported higher benthic density and biomass downstream of confluences. Our results offer insight into the ways that tributaries can create heterogeneous habitats that in turn structure macroinvertebrate communities in mainstem rivers and suggest that conservation and restoration of these essential components of freshwater ecosystems is a well-spent endeavor in rivers with regulated mainstems. Future research will need to test the ubiquity of the patterns we observed in other river networks and under other global changes such as pollution, invasive species, and drought. Continued understanding of the importance of heterogeneity imparted by tributaries and their confluences on diversity, availability, and quality of food for threaten fishes is needed to guide restoration efforts aimed at improving river condition and resilience.

Introduction

Changes to climate, land use, and flow regimes are altering macroinvertebrate and fish populations and their interactions in freshwater ecosystems worldwide (Wallace and Webster 1996, Strayer and Dudgeon 2010, Reid et al. 2019, Skoglund et al. 2023). Dams in particular homogenize the flow regime and reduce flooding, which impedes the redistribution of benthic substrate and floodplain connection (Poff et al. 2007). Effects of dams have been extensively documented and linked to altered sediment transport regimes, changes in temperature and flow, and even the prevention of upriver dispersal of plant and animal species (Poff and Hart, 2002). In contrast, heterogeneous flow regimes conserve species diversity, endangered fish, and riparian vegetation by increasing habitat diversity and inundating floodplains (Pickett et al. 1992, Poff 1997, Tonkin et al. 2018, Stanford 1994, Toth 1995). As a result, management actions that mitigate potential negative effects by increasing habitat heterogeneity are becoming increasingly important to understand (Céreghino and Lavandier, 1998, Patterson & Smokorowski, 2011, De Jalon et al. 1994).

Tributaries within a river network often express unique physical conditions compared to each other and to the mainstem due to changes in geology, geomorphology, and climate across the network. Dams, and the resulting reduction in transport of organic and inorganic material downstream, create discontinuities that unregulated tributaries could aid in restoring (Ward and Stanford 1983). Tributaries and their confluences thus act as biologically important areas where heterogeneity is introduced to the system. For example, tributary streams have been found to increase coarse benthic organic matter, large woody debris, and substrate diversity below confluences (Kiffney et al. 2006, Roy and Bergeron 1990). Inputs from tributaries have also been found to increase thermal heterogeneity in river systems (Milner and Petts, 1994). Although these findings show a possible benefit to habitat heterogeneity in regulated rivers and therefore a direction for restoration efforts, additional co-occurring constraints such as land use and riparian disturbance within tributary basins might temper the magnitude and direction of the tributary’s influence (Gőethe et al. 2019). While tributaries may be distinguishable in a network based upon underlying abiotic conditions, these human influences will likely also be having a stronger effect on some tributaries compared to others (Jackson et al. 2022). Additionally, network position likely influences how degraded a particular tributary is and thus what the conditions will be like and how they will influence the mainstem (Slawski et al. 2008).

Heterogeneity in physical conditions can drive differences in macroinvertebrate density, biomass, and richness, which all have consequences for consumer abundance, growth, and behavior. In free-flowing rivers, macroinvertebrate richness has been found to increase downstream of tributary confluences, and community structure has been found to shift towards a higher proportion of large bodied, filter-feeding caddisflies where substrate size and bed stability increased downstream of tributaries (Knipsel and Castella 2003, Rice et al. 2001). Coarse benthic organic matter and macroinvertebrates flowing out of tributaries could be providing subsidies for drift feeding fish and other primary consumers in the mainstem (Wipfli and Gregovich 2002, Kiffney et al. 2006). Within-tributary habitat could also be acting as a refuge for fish during high flows and other disturbance events when the tributaries themselves are being used (Tsuboi et al. 2022). For example, tributary use and patterns in diets between mainstem and tributary locations during a high flow event recently provided improved understanding of coexistence between native and invasive fish species (Cox et al. 2023). Together, these findings clearly suggest that heterogeneity introduced from tributary confluences is critical to structuring biological communities and ecosystem processes in unregulated rivers.

In a regulated river, however, little is known about how tributaries influence macroinvertebrates in the benthos or drifting in the water column and subsequently their availability as food for fishes (Kiffney et al. 2006, Rice et al. 2001). A reduction in density or shift in community structure of a major food source such as macroinvertebrates for stream fishes are understudied but potentially critical limitations that could affect fish population size, body condition, or habitat use (Wipfli 1997, Wipfli and Baxter 2010). In the Rocky Mountains, rising water temperatures and increased fine sediment inputs are negatively correlated with macroinvertebrate abundance and body size (Anderson 2019a, Anderson 2019b, Kowalski & Richer 2020). Because drifting and benthic macroinvertebrates are essential prey items for many fish species, understanding how they respond to tributary inputs that exist on a spectrum of degradation will provide insight into how tributary condition influences ecosystem function in the mainstem (Naiman et al. 2012, Wallace et al. 1996).

Ecosystem-based management that maintains or aims to restore heterogeneity has developed exciting ways to support macroinvertebrate and fish populations and for restoring freshwater ecosystems experiencing anthropogenetic influences, including damming (Radinger et al. 2023, Poff & Zimmerman 2010, Tickner et al. 2020). For example, connection to unregulated tributaries and implementing flushing flows have reversed reductions in habitat size and homogeneity in habitat conditions caused by dams (Katano et al. 2009, Robinson et al. 2003). In the Missouri River, an unregulated tributary confluence downstream of an impoundment positively impacted young of the year paddlefish CPUE (Pracheil et al. 2009);

another study showed tributaries that flowed through US Forest Service property and conservation areas increased the abundance of sculpin, large caddisflies, and juvenile salmonids at or slightly downstream of confluences with the mainstem (Kiffney et al. 2006). Enacting flushing flows that artificially replicate the natural flow regime has also resulted in positive effects on biota by increasing densities of both macroinvertebrates and fish (Pander et al. 2019). However, in rivers where flows cannot be altered because of energy production and agricultural demands, tributaries that experience a more natural flow regime by existing outside of the control of mainstem dams could be contributing important abiotic conditions and increasing habitat heterogeneity. A recent study contends that investing in tributaries with diverse ecological conditions within a watershed can provide numerous benefits to the survival and restoration of large river fish populations in the face of anthropogenic change (Bouska et al. 2023). As such, information about the condition and variability of tributaries in regulated rivers to inform and supplement restoration goals around environmental flows will likely be an important addition to management strategies (Ciotti et al. 2021).

To investigate how tributaries affect macroinvertebrates, which are major food resources for fish, we paired physical habitat characteristics with benthic and dispersing (drifting) macroinvertebrate density, biomass, and diversity surrounding five tributary confluences on the regulated Madison River, Montana, USA. Our objectives were to determine how habitat and macroinvertebrates differ (1) among neighboring tributaries and (2) among confluence sites (upstream, downstream, and within) at any single tributary. We hypothesized that tributary identity would influence physical habitat characteristics and macroinvertebrates because of underlying variation in conditions such as position within the watershed and land use. We further hypothesized that tributary confluences would have downstream effects on macroinvertebrates, resulting in differing macroinvertebrate community structure upstream of a confluence compared to downstream. And finally, we hypothesized that physical characteristics of tributaries and their confluences would create heterogeneity in habitat, resulting in increased beta diversity in the watershed and variable magnitudes of influence on mainstem macroinvertebrates depending on tributary identity. Our findings highlight the importance of maintaining connection to tributaries for macroinvertebrates and their predators, as well as why funding habitat restoration projects in tributaries that produce food resources for fish could continue to be viable management options in regulated systems when a mainstem natural flow regime cannot be accomplished.

Methods

Study Site

The Madison River begins at an elevation of 2,072 m in the northwestern portion of Yellowstone Nation Park, Wyoming at the confluence of the Firehole and Gibbon rivers and flows 212 km northward to Three Forks, Montana where it joins the Jefferson and Gallatin rivers to form the Missouri River (Figure 1). The Madison River watershed is located east of the Gravelly Mountain Range and west of the Madison Mountain Range and drains approximately 6690 km^2. Vegetation throughout the valley ranges from mostly coniferous Lodgepole pine (*Pinus contorta*), Douglas fir (*Pseudotsuga menziesii)*, Engelmann spruce (*Picea engelmannii*), Rocky Mountain juniper (*Juniperus scopulorum*), and Aspen (*Populus tremuloides)* at higher elevations, to mixed prairie grasses and sagebrush steppe at lower elevation montane foothills, to cottonwood (*Populus spp.*), willow (*Salix spp.*), water birch (*Betula nigra)*, grass (*Poaceae*), and sedge (*Cyperaceae*) in the riparian corridor. Dominant land use in the Madison River valley is agricultural/rangeland for livestock grazing. Sprinkler and flood irrigation are the main methods of agricultural water use. The river is renown for its game fish (Brown (*Salmo trutta*), Rainbow (*Oncorhynchus mykiss*), and Westslope cutthroat trout (*Oncorhynchus clarkii*)), and also supports other species such as Longnose dace (*Rhinichthys cataractae*), Longnose sucker (*Catostomus catostomus*), White sucker (*Catostomus commersonii*), Mountain sucker (*Catostomus platyrhynchus*), Stonecat (*Noturus flavus*), and Rocky Mountain sculpin (*Cottus bondi*). The Madison River flows through two dams, Hebgen dam and Madison dam at 177 and 59 km, respectively. Hebgen is an embankment dam that creates Hebgen Reservoir, and Madison is a run-of-the-river hydroelectric dam that creates Ennis Reservoir.

Sampling Locations

The sampling locations in this study (Figure 2, Table 1) surrounded the confluences of each of five tributaries that merge with the Madison River between Hebgen and Ennis reservoirs: Jack Creek, Odell Creek, Indian Creek, Ruby Creek, and Standard Creek. Jack Creek originates in the northern Madison Range at an elevation of 2,047 m, flowing 21.24 km westward through 23% USFS, 60% agriculture, and 17% residential land before joining the Madison River near Ennis, Montana at an elevation of 1,482 m. Two sections of streambank encompassing a 1,000m reach were restored between 2018 and 2020 to reestablish a connection to the floodplain and produce a vegetated riparian buffer near the confluence. Odell Creek is spring-fed and originates in the Madison Valley at an elevation of 1,561 m, flowing 14.49 km northeastward through 79% agriculture and 21% residential land before joining the Madison River south of Ennis, Montana at an elevation of 1,491 m. Historically, the headwaters of Odell encompassed large wetland and spring creek habitats that were ditched and partially drained for agricultural/ranchland at the turn of the century. A project supported by multiple stakeholders starting in 2005 and continuing into the present has reconstructed 20km of new stream channel in the headwaters and enhanced 780 acres of wetland habitat. Indian Creek originates in the Madison Range at an elevation of 2,471 m, flowing 20.95 km westward through 82% USFS, 15% agriculture, and 3% residential before joining the Madison River near Ennis, Montana at an elevation of 1,653 m. Ruby Creek originates in the Gravelly Range at an elevation of 2,689 m, flowing 21.48 km eastward through 79% USFS, 11% agriculture, and 10% residential before joining the Madison River south of Ennis, Montana at an elevation of 1,682 m. Standard Creek originates in the Gravelly Range at an elevation of 2,714 m, flowing 20.28 km eastward through 92% USFS, 5% agriculture, and 3% residential before joining the Madison River South of Ennis, Montana at an elevation of 1,773 m.

We established three sampling locations at each tributary confluence for a total of 15. The three locations were (i) 20 m upstream from the mainstem confluence, (ii) 20 m downstream from the mainstem confluence, and (iii) 20 m within the tributary. Sampling events occurred four times: in July 2020, November 2020, April 2021, and July 2021, and no sampling locations were skipped.

Environmental Characteristics Sampling

Water temperature (ºC) was recorded at 30-minute intervals from July 15th, 2020 – July 28th, 2021 at each sampling location using a HOBO Pro v2 temperature logger (Onset Computer, Bourne, Massachusetts) secured to a 15 kg concrete block placed near the thalweg and protected with a UV-safe PVC tube. Water temperature is reported as mean weekly maximum temperature (MWMT). Pebble counts were conducted at each location during the first week of sampling (July 20th – July 26th, 2020) by walking perpendicular to flow across random transects of riffles and picking up the particle making contact with the big toe at each step. Each particle was measured on the b-axis using a gravelometer and D_{50} was calculated using Wolman's method, resulting in n = 4 (Wolman 1954). The riparian corridor (n = 1) was assessed using the NRCS (Natural Resources Conservation Service) riparian assessment method at each location over a distance of 1 km starting from the confluence and upstream, downstream, or into the tributary in early July 2020 (Environment Technical Note No. MT-2, 2012). NRCS scores range from 0 – 100; a value of 80 and above is considered sustainable and in good ecological condition, a value between 50 – 80 at risk, and below 50 as not sustainable.

Discharge was measured within in each tributary on every sampling date (n = 4) using a SonTek FlowTracker Handheld-ADV (YSI Inc., San Diego, CA). A measuring tape was suspended across the stream perpendicular to the current and affixed to rebar inserted into the bank. The flowtracker measured water velocity and depth at regularly spaced predetermined points (minimum of eight) along the measuring tape. The velocity and depth measurements were used to compute the total volume of water flowing through each subsection and added together to get total discharge (m^3/s).

Basin attributes and land-use categories for each tributary were mapped and calculated with ArcGIS Pro 2.9 (ESRI, Redlands, CA, USA). The basin boundaries and land-use shape files were obtained from Montana State Library's geographic database.

To quantify suspended sediment at each sampling location on every sampling date, two 1-liter Nalgenes were submerged mid depth at the thalweg and filled. Samples were frozen within 12 hours and stored for later processing. After being thawed, samples were passed through a pre-ashed, pre-weighed GF/F 47 mm filter and placed in a muffle furnace at 500°C for

1.5 hours. Once cooled, the filters were weighed and then subtracted from the original filter weight to measure the inorganic matter per liter.

To measure biofilm at each sampling location on every sampling date, three rocks of approximately 30-50 mm diameter were selected at random and a 4 X 4 cm square stencil was used to identify the area that was scrubbed with a brush into a 63 µm sieve. The biofilm slurry was placed into a Whirl-Pak and frozen within 12 hours and stored. The slurry was thawed in the lab and the volume was recorded with a graduated cylinder. Samples were then passed through a pre-ashed GF/F 47 mm filter and placed in a muffle furnace at 500 °C for 1.5 hours. The response per cm^2 was scaled and is reported as ash-free dry mass (AFDM) m^{-2}.

Benthic organic matter (OM) was quantified from the Surber samples also used for benthic macroinvertebrates. At each sampling location on each sampling date an oversized Surber sampler (0.75 m x 0.75 m; 250 µm mesh) was randomly placed in the benthos in triplicate and the substrate was scrubbed with a brush and washed into the 250 µm Surber net. Each of the three samples were placed into a Whirl-Pak with 90% ethanol and stored at 4°C within 12 hours. In the lab, sieves were used to separate coarse (>1 mm) from fine (<1 mm) particulate organic matter, and a Folsom Plankton Splitter (Wildlife Supply Company) was used to subsample down to at least 100 macroinvertebrates. Macroinvertebrates were separated from the samples (see next section) and the remaining organic and inorganic material was placed into aluminum tins and dried at 55°C. After approximately 48 hours, tins were weighed and subsequently burned in a muffle furnace at 500 °C for 1.5 hours. The AFDM was calculated by subtracting dried weight from the post muffle furnace weight and multiplied by a constant to account for subsampling fraction to report AFDM m^{-2}.

Macroinvertebrate Sampling

Benthic macroinvertebrates were collected at each sampling location on each sampling date using an oversized Surber sampler (0.75 m^2; 250 µm mesh). The sampler was randomly placed in the benthos in triplicate (n = 12) and the substrate was scrubbed with a brush. Rocks with a diameter larger than approximately 5 cm were flipped over and scrubbed within the opening of the sampler while the macroinvertebrates/organic matter were encouraged to flow into the net. Samples were placed into Whirl-Paks with 90% ethanol and stored at 4°C within 12 hours. Using nested sieves, each macroinvertebrate sample was separated into coarse (>1 mm) and fine (<1 mm), and a Folsom Plankton Splitter was then used to subsample until at least and close to 100 individuals per sample were enumerated. Macroinvertebrates were identified to genus or lowest practical taxonomic level (Merritt and Cummins, 1999). Members of the family Chironomidae were identified down to Tanypodinae or Non-Tanypodinae. Density was calculated by multiplying the number of individuals found in each subsample by the reciprocal of the fraction it was split down to and then multiplied again by a constant to scale from the sample area to density (individuals m^{-2}). To estimate biomass, length of individuals was measured to the nearest mm using a dissection scope (Leica M165 C, 120 x). Individual biomass for each mm size class and taxon was calculated by the length-mass equation:

$$M = aL^b$$

where M is organism mass (mg), L is linear dimension, and *a* and *b* are genus specific coefficients (Benke et al., 1999). Biomass of each sample was estimated by multiplying the density in each size class by the individual biomass and summing across size category and taxon. This quantity was then multiplied by the reciprocal of the fraction and scaled to biomass as mg AFDM m^{-2}.

Macroinvertebrates in the drift were collected at each sampling location on each sampling date using drift nets (45 cm x 25 cm x 100 cm; 500 µm mesh). The drift nets were deployed in pairs (n = 8) at each location by driving rebar through eyelets in the net frame and into the substrate. The height of the driftnets in the water column was secured at least five cm off the substrate to reduce crawling macroinvertebrates. Nets were left for approximately 30 minutes, and clogging was minimized by shaking down the nets every 10 minutes. Water velocity was measured at the beginning and end of the sample period at the center of the net using a Marsh-McBirney Flowmeter (Hach Company, Loveland, Colorado). The two velocity readings were then averaged and used to calculate the volume of water that was filtered during the time the nets were submerged. When nets were only partially submerged, water volume was calculated based on the cross-sectional area of the net that was submerged. Samples were placed into Whirl-Paks with 90% ethanol and stored at 4°C within 12 hours. In the lab, a Folsom Plankton Splitter was used to subsample until at least 100 individuals per subsample were enumerated. All macroinvertebrates were then placed into aluminum tins and dried at 55°C. Tins were then weighed and subsequently burned in a muffle furnace at 500 °C for 1.5 hours. Drift density was calculated by multiplying the number of individuals in each subsample by the reciprocal of the fraction of the subsample and then scaled to biomass (individuals per m^{-3}). Mean per capita weight (ind. weight (mg) $sample^{-1}$) was calculated by subtracting post muffle furnace weight from the dried weight, dividing by the number of individuals in each subsample, and multiplying by the reciprocal of the subsampling fraction.

Statistical Analysis

Statistical analyses were performed in R version 4.2.2 (R Core Team, 2022). For each environmental response variable (biofilm, suspended sediment, benthic organic matter, MWMT, discharge), a mixed effects model was used to assess differences between tributaries using the lme4 package (Bates et al., 2015). A fixed effect of tributary (Jack, Odell, Indian, Ruby, or Standard) and a random effect of sampling date (July 2020, November 2020, April 2021, July 2021) was included in each model. If the tributary effect in the mixed model was significant, then a post-hoc comparison was performed to determine differences between tributaries using the glht function from the multcomp package in R (Torsten, Bretz, & Westfall, 2008). The significance threshold was set at 0.1, with strong evidence assigned to values less than 0.05 and moderate evidence assigned to values between 0.05 - 0.1.

To investigate which environmental response variables drive differences among tributaries, principal components analysis (PCA) was performed on data recorded on each sampling event using the stats package. PCA scores were also used for additional analysis to investigate relationships between environmental drivers and biotic response variables. To isolate the effects of tributaries on invertebrate communities downstream of the confluence and compare the magnitude of this effect across different tributaries, a log-response ratio (LRR) was used to calculate the proportional change in density, biomass, and richness between sites that were upstream and downstream of the confluences for each tributary: $LRR = ln\left(\frac{Down}{Up}\right)$. LRRs were calculated using the Metafor package (Viechtbauer, 2010). Linear regressions were used to test relationships between LRR of density, biomass, and richness and the PCA score previously estimated from the environmental characteristics of the tributaries. Data from all sampling dates were included in the analysis to capture seasonal variation.

Community structure of benthic macroinvertebrates was compared among tributaries and sites using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities among all sampling dates using the vegan package (Oksanen et al., 2022). We tested for differences in benthic macroinvertebrate community assemblages based on densities and biomasses among tributaries and sites. We also used a mixed effects permutational multivariate analysis of variance (PERMANOVA) to assess pair-wise comparisons. Homogeneity of dispersions between tributaries and sites was tested using a permutation test of multivariate homogeneity of group dispersions (PERMDISP). PERMDISP tested the validity of the PERMANOVA differences due to the multivariate position of samples rather than to heterogeneous dispersion among tributaries and sites. Post-hoc multilevel pairwise comparisons were calculated using the pairwiseAdonis package (Martinez, 2017).

To investigate patterns of beta-diversity within the Madison River watershed, we categorized benthic invertebrate response variables into a mainstem category (that included all mainstem sites) and compared community structure there to community structure in each of the tributaries using an NMDS based on Bray-Curtis dissimilarities of both density and biomass by sequentially adding tributaries and calculating the increase in area.

Results

Environmental Characteristics

The environmental characteristics of the five tributaries of the Madison River were distinctly different and we expect those differences to potentially drive the magnitude of any influence of the tributary on the mainstem (Table 1). Biofilm biomass varied among tributaries (ANOVA; $F_{4,50} = 7.56$, p = 0.001), with Jack Creek having higher amounts than Indian Creek (p

= 0.0065), Odell Creek ($p < 0.001$), and Ruby Creek ($p < 0.001$). Standard Creek had higher biofilm biomass than Odell Creek ($p = 0.049$). MWMT was different amongst the tributaries (ANOVA; $F_{4,50} = 65.46$, $p < 0.0001$), with strong evidence that Standard Creek was the coolest, and that differences occurred between all tributary pairs except for Ruby Creek and Indian Creek ($p = 0.27$). Suspended sediment also varied among the five tributaries (ANOVA; $F_{4,50} = 7.78$, $p = 0.001$) with Indian Creek and Standard Creek having lower amounts than the others: Jack Creek ($p = 0.01$ and $p = 0.008$), Odell Creek ($p = 0.009$ and $p = 0.007$), and Ruby Creek ($p < 0.001$ and $p < 0.001$). There was strong evidence that benthic organic matter (OM) differed among the tributaries (ANOVA; $F_{4,50} = 3.364$, $p = 0.01$). Standard Creek had higher quantities than all others: Indian Creek ($p = 0.03$), Jack Creek ($p = 0.02$), Odell Creek ($p = 0.03$), and Ruby Creek ($p = 0.03$). Discharge varied greatly between the five tributaries (ANOVA; $F_{4,50} = 387.22$, $p < 0.0001$), with Odell Creek having a 433% higher discharge than the next highest, Jack Creek ($p < 0.001$). There was also strong evidence for Jack Creek having a higher discharge than both Ruby Creek ($p < 0.001$) and Standard Creek ($p = 0.008$). Within the five chosen tributaries for this study, Standard Creek had the coolest temperature regime, the highest amounts of benthic organic matter, flowed through the largest proportion of National Forest, received the highest riparian assessment score, and exists the farthest upstream in the watershed.

Principal components analysis indicated that the tributaries varied substantially in their environmental conditions. The first two PCA axes explained 77% of all variation in the environmental characteristics (Figure 3). The first axis represented a gradient of neighboring land use, temperature, discharge, and stream elevation and explained 62.86 % of variation. The second axis represented a gradient of riparian assessment score and in-stream characteristics of biofilm and suspended sediment and explained 14.61 % of variation. We identified two distinct groupings of tributaries based on PCA 1. Jack and Odell Creeks cluster to the left of PCA 1 and the other three, Indian, Ruby, and Standard Creeks, cluster to the right. Jack and Odell Creeks were characterized by a higher proportion of agricultural land use (60 - 70%), warmer temperature regimes (18.97 - 20.17°C MWMT), higher discharges (0.73 – 3.87 m^3/s), and a smaller magnitude of elevation loss (69 – 564 m). Indian, Ruby, and Standard Creek were characterized by a higher proportion of United States Forest Service land (79 – 92%), cooler temperature regimes (13.45 – 17.41°C MWMT), smaller discharges (0.24 – 0.45 m^3/s), and a larger magnitude of elevation loss (818 – 1,007 m).

Macroinvertebrates

There was strong evidence that benthic macroinvertebrate densities differed among the five tributaries (ANOVA; $F_{4,50} = 7.12$, $p = 0.0001$; Figure 4a). Standard Creek drove this difference, with a higher density than Indian Creek ($p <0.0001$), Jack Creek ($p = 0.002$), Odell Creek ($p < 0.0001$), and Ruby Creek ($p = 0.002$). Strong evidence was also found for biomass, which differed among the five tributaries (ANOVA; $F_{4,50} = 6.09$, $p = 0.0004$; Figure 4b). Odell Creek had a higher mean biomass than Indian Creek ($p = 0.001$), Jack Creek ($p = 0.007$), and Ruby Creek ($p =0.003$). Standard Creek also had a higher mean biomass than Indian Creek ($p = 0.03$). Mean species richness was highest at Standard Creek (S = 32), which had ten more unique taxa than the lowest representative, Jack Creek (S = 22).

There was moderate evidence that drifting per capita weight differed among the five tributaries (ANOVA; $F_{4,13} = 2.55$, $p = 0.08$; Figure 5a). Odell Creek had the highest mean per capita weight, but only differed from Ruby Creek ($p = 0.028$). Drifting macroinvertebrate density strongly differed among the five tributaries (ANOVA; $F_{4,13} = 7.18$, $p = 0.0028$; Figure 5b). Jack Creek contained the highest mean level of drifting macroinvertebrate density (ind./m^3 = 8), which was found to be higher than Odell Creek ($n = 3$; $p < 0.0001$) and Standard Creek (ind./m^3 = 3; $p < 0.0001$).

Macroinvertebrate community analysis for both biomass and density revealed distinct macroinvertebrate community structure between tributaries and sites. Because patterns for biomass and density responses generally supported one another, biomass responses are reported here (see Figure A1:2 for density responses). There was strong evidence that macroinvertebrate community structure based on Bray-Curtis dissimilarities of biomass differed by tributary (PERMANOVA; $F_{(4, 176)} = 6.221$ $p < 0.001$; Figure 6a) and site ($F_{(2, 176)} = 9.202$, $p < 0.001$; Figure 6b), with an interaction effect ($F_{(8,176)} = 3.256$, $p < 0.001$). Post-hoc pairwise comparisons showed macroinvertebrate communities in all tributaries to differ from one another except for Indian Creek and Ruby Creek ($r^2 = 0.0057$, $p = 0.86$), Indian Creek and Standard Creek ($r^2 = 0.042$, $p = 0.26$), and Ruby Creek and Standard Creek ($r^2 = 0.031$, $p = 0.57$). This analysis of biomass revealed two distinct groupings of tributaries: (1) Standard, Indian, and Ruby Creeks; and (2) Jack and Odell Creeks. Post-hoc pairwise comparisons between sites provided strong evidence that macroinvertebrate community biomass in the Upstream and Downstream sites differed from the Tributary site ($r^2 = 0.166$, $p = 0.003$ and $r^2 = 0.14$, $p = 0.001$ respectively), and moderate evidence that Upstream and Downstream sites differed from each other ($r^2 = 0.02$, $p = 0.087$). There was strong evidence that multivariate dispersions of macroinvertebrate community biomass differed by site (PERMDISP; $F_{(2, 176)} = 4.2781$, $p = 0.015$), with higher dispersion among communities from Tributary sites compared with Upstream and Downstream. There was moderate evidence that dispersions differed by tributary ($F_{(4, 176)} = 2.1489$, $p = 0.0768$), with higher dispersion among communities from Odell Creek. These results indicate that differences among sites could have resulted from differences in dispersion and not necessarily differences in the multivariate position of samples, but when viewed alongside NMDS plots differences in the multivariate position of samples are apparent (Figure 6a, 6b). The second result indicates that differences among tributaries resulted from the multivariate position of samples and not from differences in dispersion. Tributaries also increased the functional β-diversity of the watershed compared to mainstem sites (Figure 7), but not all tributaries had similar influence; Odell and Jack partially overlapped with mainstem sites, while Indian, Ruby, and Standard all existed separately, likely indicating their stronger influence on β-diversity. This is a similar pair of tributary groupings found within the environmental PCA and the community composition PERMANOVA, indicating an association between habitat characteristics, macroinvertebrate community composition, and β-diversity between samples within the watershed.

Strong evidence was found for differing magnitudes of tributary influence on macroinvertebrate community metrics near confluences. Macroinvertebrate richness was found to increase downstream overall, with Standard and Ruby Creeks having the greatest influence on the model (Figure 8a), while mean per capita weight of drifting macroinvertebrates was found to decrease downstream overall, with Odell Creek having the greatest influence on the model (Figure 8d). There was strong evidence for the macroinvertebrate drift density effect size to increase downstream of Jack Creek specifically (Figure 8c), while effect sizes for macroinvertebrate biomass were found to increase downstream of Odell Creek and decrease downstream of Indian Creek (Figure 8b). Although no evidence was found to suggest that macroinvertebrate density changed between upstream and downstream sites for any tributary across all sampling dates (Figure A3), there was strong evidence of a downstream effect within individual seasons and the magnitude and direction of this effect was tributary-specific (Figure A4). These inter-seasonal variations were not the focus of this study; however, it is of note that all tributaries had a downstream effect during spring and summer. To further elucidate if density results could be attributed to tributary confluences and not to the natural variability of communities in the mainstem, the coefficient of variation (CV) of density compared between up and downstream sites was consistent across tributaries, and showed no longitudinal pattern along the river network, providing additional evidence that tributary confluences are likely driving any differences in habitat and invertebrates observed at the downstream sites (Figure A5).

To determine if heterogeneity in environmental conditions arising from different tributaries drove patterns in macroinvertebrate benthic density, benthic biomass, benthic richness, drift density, and drift per capita weight, we used linear regression to relate PCA scores to the LRR effect sizes of macroinvertebrates responses. A positive relationship indicates that larger responses (e.g., the magnitude of the tributary influence is larger) occur downstream of tributaries that are characterized by lower agricultural land use, cooler temperatures, lower discharge, and higher confluence elevation. We found strong evidence for a negative relationship between PCA axis 1 and LRR density ($R^2 = 0.27$, $p = 0.02$; Figure 9a). We also found moderate evidence for a negative relationship between PCA axis 1 and LRR biomass ($R^2 = 0.17$, $p = 0.075$; Figure 9b), strong evidence for a positive relationship with LRR richness ($R^2 = 0.33$, $p = 0.009$; Figure 9c), and LRR drift per capita weight ($R^2 = 0.33$, $p = 0.01$; Figure 9d). There was no evidence for a relationship between PCA axis 1 and LRR drift density ($R^2 = 0.03$, $p = 0.567$).

The influence of tributaries on macroinvertebrate communities downstream of confluences was related to land use and abiotic factors within the tributary. Richness in the benthos and mean per capita weight in the drift downstream of confluences increased with increasing proportions of USFS land use, cooler temperature regimes, and lower discharges while density decreased along this gradient.

Discussion

The role of tributaries in creating heterogeneity in physical habitat and food resources for fishes could be critical, yet little is known about how variable conditions in different tributaries or how restoration in tributary locations in regulated river systems will influence the mainstem. These tributary restoration efforts are often targeted to habitat improvement and take place outside of the river's mainstem because smaller tributaries are more easily accessible and projects therein more financially feasible, with the hopes of ultimately having a beneficial influence on mainstem conditions or providing refuge (Wang et al. 2020). We found that tributaries in the same network and within relatively short distances of 60km varied greatly in their environmental conditions, macroinvertebrate densities, and community structure. Additionally, while tributaries running through a larger proportion of USFS land with cooler temperature regimes supported higher benthic richness and larger drift body size downstream of confluences, tributaries that encompass a larger proportion of agricultural land with warmer temperature regimes supported high benthic density and biomass downstream of confluences. This second finding may be the result of the substantial restoration that has been performed on Jack and Odell Creeks, which were both impacted by agricultural land use practices. Along with mainstem remediation efforts such as environmental flows, restoration in tributaries is a valuable management tool. However, our results highlight the complicated nature of potential outcomes for macroinvertebrate communities because of underlying and context specific conditions in different tributaries, and spatial sampling may be needed to capture the range of conditions that regulate food availability for fish consumers.

Our study found that many environmental conditions were largely dependent upon tributary identity, which can have underlying differences as well as different levels of previous restoration efforts of within-tributary habitat. Jack and Odell Creeks were characterized by a higher proportion of agricultural land, warmer temperature regimes, higher discharges, and a smaller magnitude of elevation loss. Indian, Ruby, and Standard Creek were characterized by a higher proportion of United States Forest Service land, cooler temperature regimes, smaller discharges, and a larger magnitude of elevation loss. Standard Creek stood out as the least disturbed tributary with the lowest values of temperature and highest median substrate size, biofilm level, proportion of USFS land within its watershed, and riparian assessment score. These findings could have implications for the integrity of refugia and habitat for the salmonid populations that exist in the Madison River; a previous study found important sport fishes (Westslope cutthroat, Rainbow, and Brown trout) in three of our studied tributaries: Indian Creek, Ruby Creek, and Standard Creek (Sloat, 2001). A need for analyses of tributaries in regulated river systems that concentrate on the entire watershed to develop a strategy for targeted restoration has been emphasized in the literature (Benda et al. 2004), and our assessments of individual tributaries, their differing abiotic characteristics, and the varying levels of restoration performed upon them is a first step towards such targeted restoration.

The level of human impact on tributaries, assessed here using previously described riparian assessments, thermal regimes, and land use types, influenced macroinvertebrate density, biomass, and body size. Standard Creek, the least impacted stream, had the highest density and Odell Creek, the most impacted stream, had the highest mean biomass. Interestingly Odell Creek also had the lowest density sampled, suggesting it supports few but large individuals. The more stable temperature and flow regimes of Odell Creek, being spring fed, may be favorable to larger bodied taxa with warmer year-round temperatures increasing growth potential and smaller fluctuations in discharge during spring run-off decreasing habitat turnover. Rivers in agricultural watersheds with degraded riparian corridors have been related to a loss of habitat diversity, with their forested counterparts showing a higher density and richness of macroinvertebrates (Orlinksy et al. 2015, Fiero et al. 2017). One study found that regulated streams where restoration was performed that increased habitat heterogeneity contained higher levels of abundance when compared to unrestored reaches (Nakano and Nakamura, 2006). In contrast, a metanalysis of macroinvertebrate responses to habitat restoration found that increasing physical habitat heterogeneity of homogenized reaches did not produce the anticipated increase in density, but it did increase richness (Miller et al. 2010). Density of macroinvertebrates in the drift was significantly higher in Jack Creek than all other tributaries, and over 200% higher than in Standard Creek which had the lowest mean drift density. Although we have not identified a definitive mechanism, several factors might drive the high drift densities in Jack Creek. Elevated drift levels within Jack Creek could indicate increased secondary production (Siler et al. 2001), reduced predation pressure (McIntosh et al. 2002), or higher emigration due to poor stream quality (Hay et al. 2008). Because salmonids are size-selective predators with a strong preference for the largest prey items (Kreivi et al. 1999), moderate differences in per capita weight of drifting macroinvertebrates that we found could have consequences for fish growth and behavior.

Macroinvertebrate community composition revealed two groupings of tributaries that were consistent with their different habitat characteristics: 1) Standard, Indian, and Ruby Creeks, and 2) Odell and Jack Creeks. These findings are supported by previously documented findings that surrounding land use and physical habitat characteristics control macroinvertebrate communities (Townsend et al. 1997, Katano et al. 2009). This clear distinction between tributary groups was also observed when examining beta-diversity, which increased with the addition of Standard, Indian, and Ruby Creek, while communities within Jack and Odell overlapped with the mainstem. Contradictory to our prediction, no difference was found between up and down sites, and both were found to be different to the within-tributary site. However, while little evidence for a difference was found, downstream sites were intermediate between upstream and within tributary sites, suggesting there is a moderate downstream effect of tributaries on macroinvertebrate community structure. A few underlying mechanisms for the slight differences in communities between upstream and downstream sites: (1) discharges of tributaries were too small in comparison to the mainstem to pick up a signal from the tributary downstream of the confluence, and (2) mobile macroinvertebrate larvae and adults may be able to disperse upstream of the confluence (Elliott, 2003; Wilson and McTammany, 2014). Future work might include a larger sample size of tributaries that incorporates a wider range of discharges.

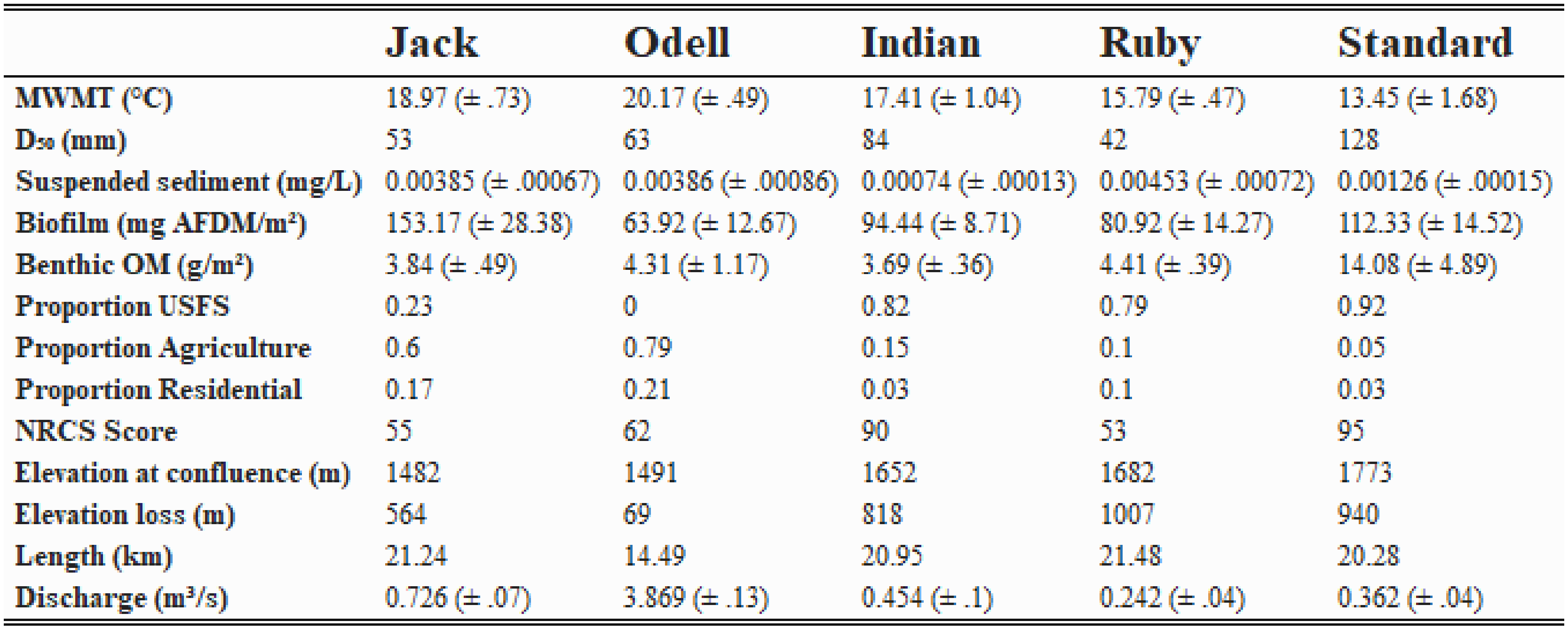
Tributary confluences influenced richness and per capita weight (both important metrics for riverine fish), but in opposite directions, suggesting that multiple metrics are likely necessary to fully elucidate the influence of tributaries on food resources for fishes. Increased biodiversity of prey items can provide a more stable food supply for stream fishes (Allan et al. 2021), and salmonids have been shown to predate upon the largest drifting macroinvertebrates available (Dunbrack and Dill, 1983). Downstream of confluences the effect size for macroinvertebrate richness increased overall and per capita weight within the drift decreased overall, suggesting that confluences may act as hotspots for biodiversity in regulated rivers in addition to introducing smaller bodied macroinvertebrates in the drift. While drift density effect size was not affected by tributary identity on average, it did significantly increase downstream of Jack Creek. One mechanism we propose for this increase is higher amounts of suspended sediment introduced from the high proportion of residential and agricultural land contained within the watershed of Jack Creek, as increasing suspended sediment levels have been linked to increases in the density of drifting macroinvertebrates (Doeg and Milledge, 1991). The amount that a tributary influenced richness in the benthos and mean per capita weight in the drift downstream of confluences was related to land use and abiotic factors within the tributary; both metrics significantly increased in magnitude downstream of confluences with corresponding increases in the percentage of USFS land, cooler temperature regimes, decreasing discharges, and increasing elevation loss. The magnitude of a tributary's influence on benthic density significantly decreased downstream of confluences along the same environmental gradient. While benthic biomass was found to decrease downstream of confluences along the same environmental gradient, the relationship was only moderately significant. These relationships suggest that smaller tributaries with less agricultural land use and cooler temperatures increasingly yield higher macroinvertebrate richness and mean per capita drift weight, with lower densities, downstream of confluences. This pattern is supported by findings from previous studies that found an increase in taxon richness and evenness downstream of confluences (Vinson, 2001; Rice et al. 2001; Kiffney et al. 2006).

Although we clearly demonstrate that tributaries are important sources of physical and biotic heterogeneity to a mainstem river, this study has several limitations that could be addressed with additional research. Restrictions from landowners and getting access to field sites necessitated a non-random selection of tributaries that exist on the Madison River. Incorporating additional or randomly selecting tributaries in future work might allow for broader inferences to be made. Because all tributaries within this study emptied into the same river, confluence sites may not be considered fully independent. One study, for example, found that metrics of tributary influence decreased with distance from the confluence at the scale of 240 – 920 meters (Kiffney et al. 2006). However, we did choose sites that had at minimum 2 km between them, which we deemed to be enough to minimize any downstream influence. Temporally, sampling was done during Summer, Fall, and Spring within one calendar year. Differences in snowpack level, timing of spring melt off, and precipitation interannually could be a significant source of variation that deserves further attention when attempting to characterize such a dynamic system.

A tributary’s influence on mainstem habitats, and consequently the biotic communities downstream of their confluence in a regulated system, is dependent upon the ecological integrity of within-tributary habitat and should not be overlooked when developing mainstem restoration projects. We find that tributaries can influence mainstem rivers and the macroinvertebrate communities that inhabit them. Restoration efforts that prioritize the protection of tributary habitat and connection to the mainstem in order to maintain the ecological integrity and biodiversity of river networks when the timing and discharge of upstream dams cannot be altered are likely a good investment. It would also be prudent to acknowledge the weight of these directed efforts when deciding where to allocate limited resources in a restoration project, as the combination of a smaller area and fewer stakeholders within a tributary sub-basin compared to the mainstem could result in a more cost-effective restoration strategy with less jurisdictional limitations. Our results offer strong insight into the function of tributaries on creating heterogeneous habitats in mainstem rivers that in turn structure macroinvertebrate communities, which could be applied and tested in other nearby systems and under other global change influences such as pollution, invasive species, and drought.

Tables and Figures

Tables



	Jack	Odell	Indian	Ruby	Standard
MWMT (°C)	18.97 (± .73)	20.17 (± .49)	17.41 (± 1.04)	15.79 (± .47)	13.45 (± 1.68)
D_{50} (mm)	53	63	84	42	128
Suspended sediment (mg/L)	0.00385 (± .00067)	0.00386 (± .00086)	0.00074 (± .00013)	0.00453 (± .00072)	0.00126 (± .00015)
Biofilm (mg AFDM/m^2)	153.17 (± 28.38)	63.92 (± 12.67)	94.44 (± 8.71)	80.92 (± 14.27)	112.33 (± 14.52)
Benthic OM (g/m^2)	3.84 (± .49)	4.31 (± 1.17)	3.69 (± .36)	4.41 (± .39)	14.08 (± 4.89)
Proportion USFS	0.23	0	0.82	0.79	0.92
Proportion Agriculture	0.6	0.79	0.15	0.1	0.05
Proportion Residential	0.17	0.21	0.03	0.1	0.03
NRCS Score	55	62	90	53	95
Elevation at confluence (m)	1482	1491	1652	1682	1773
Elevation loss (m)	564	69	818	1007	940
Length (km)	21.24	14.49	20.95	21.48	20.28
Discharge (m^3/s)	0.726 (± .07)	3.869 (± .13)	0.454 (± .1)	0.242 (± .04)	0.362 (± .04)

Table 1: Environmental characteristics of the tributaries (mean ± se). D50 = median particle size, MWMT = mean weekly max temperature, OM = organic matter, NRCS = Natural Resources Conservation Service. Non-fixed values averaged across sampling dates (July 2020, October 2020, April 2021, and July 2021).

Figures

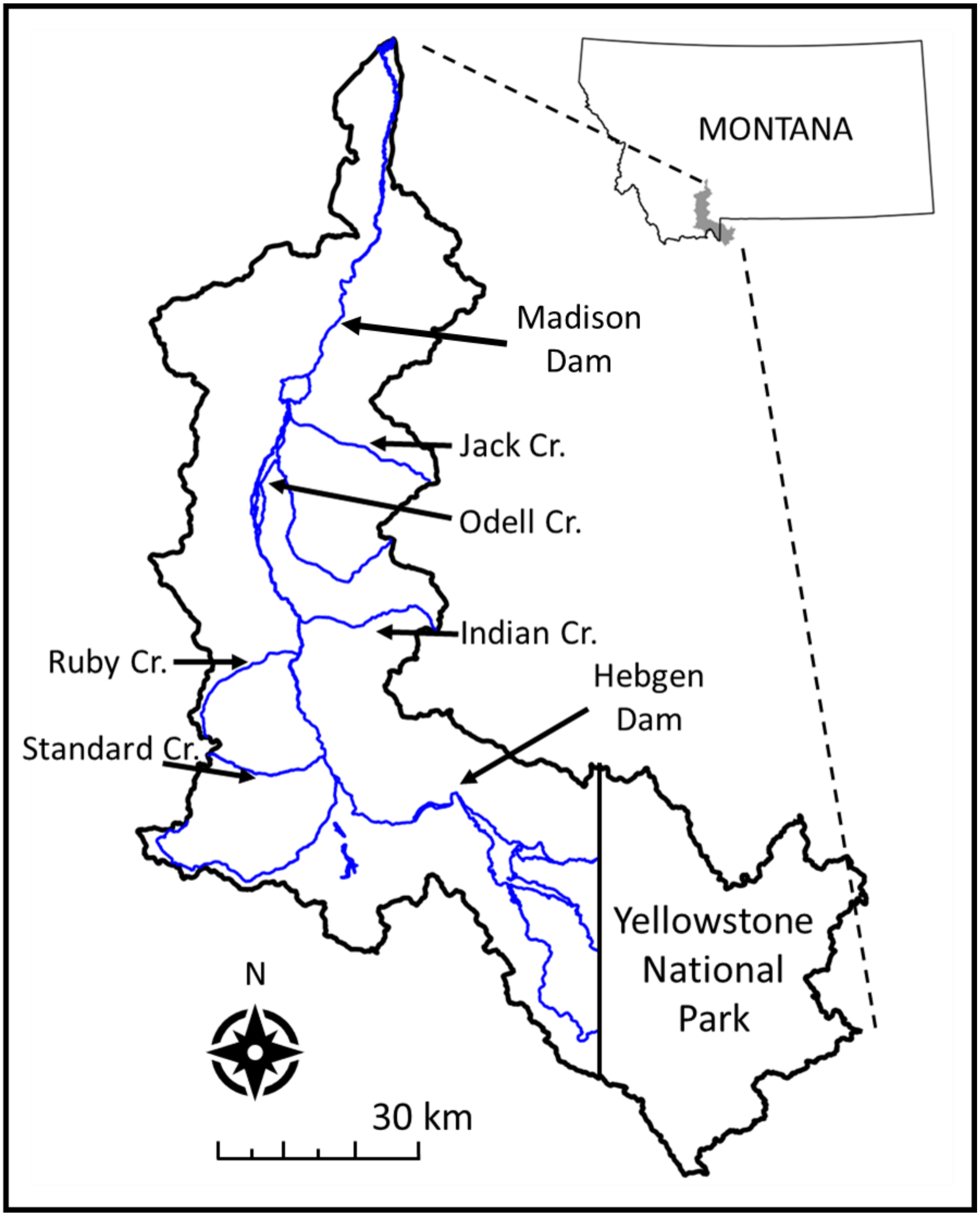


Figure 1: Map of the study area and sampled tributaries along the Madison River in southwestern Montana, USA. The dominant direction of flow is south to north.

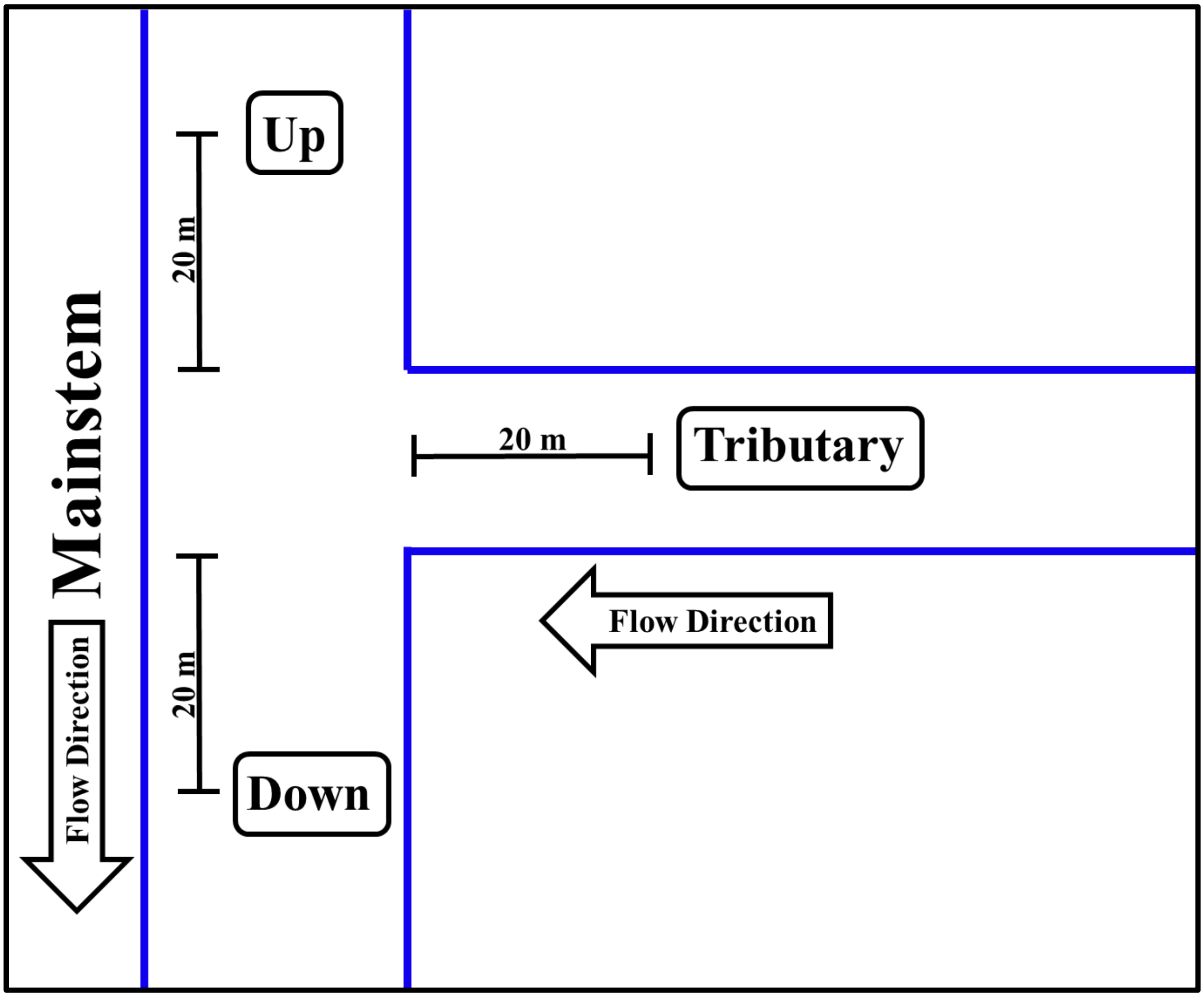


Figure 2: Schematic of three sampling locations (rectangles) at the confluence of each tributary.

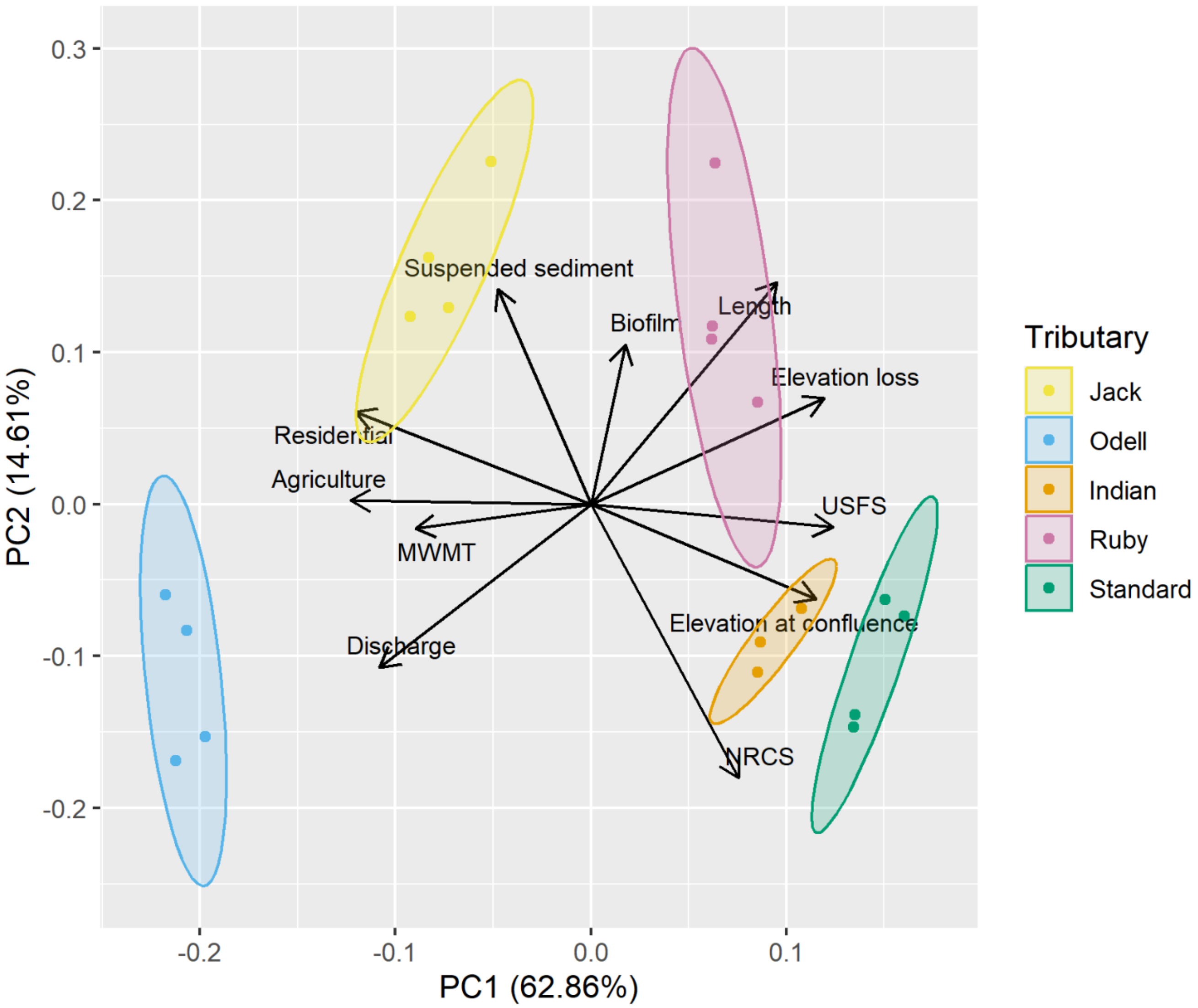


Figure 3: Principal component analysis (PCA) ordination plot of physical and environmental characteristics of tributaries. Significant explanatory variables are represented by black arrows. PCA axis 1 explains 62.86% of variance, and PCA axis 2 explains 14.61% of variance. Each point represents a sampling event.

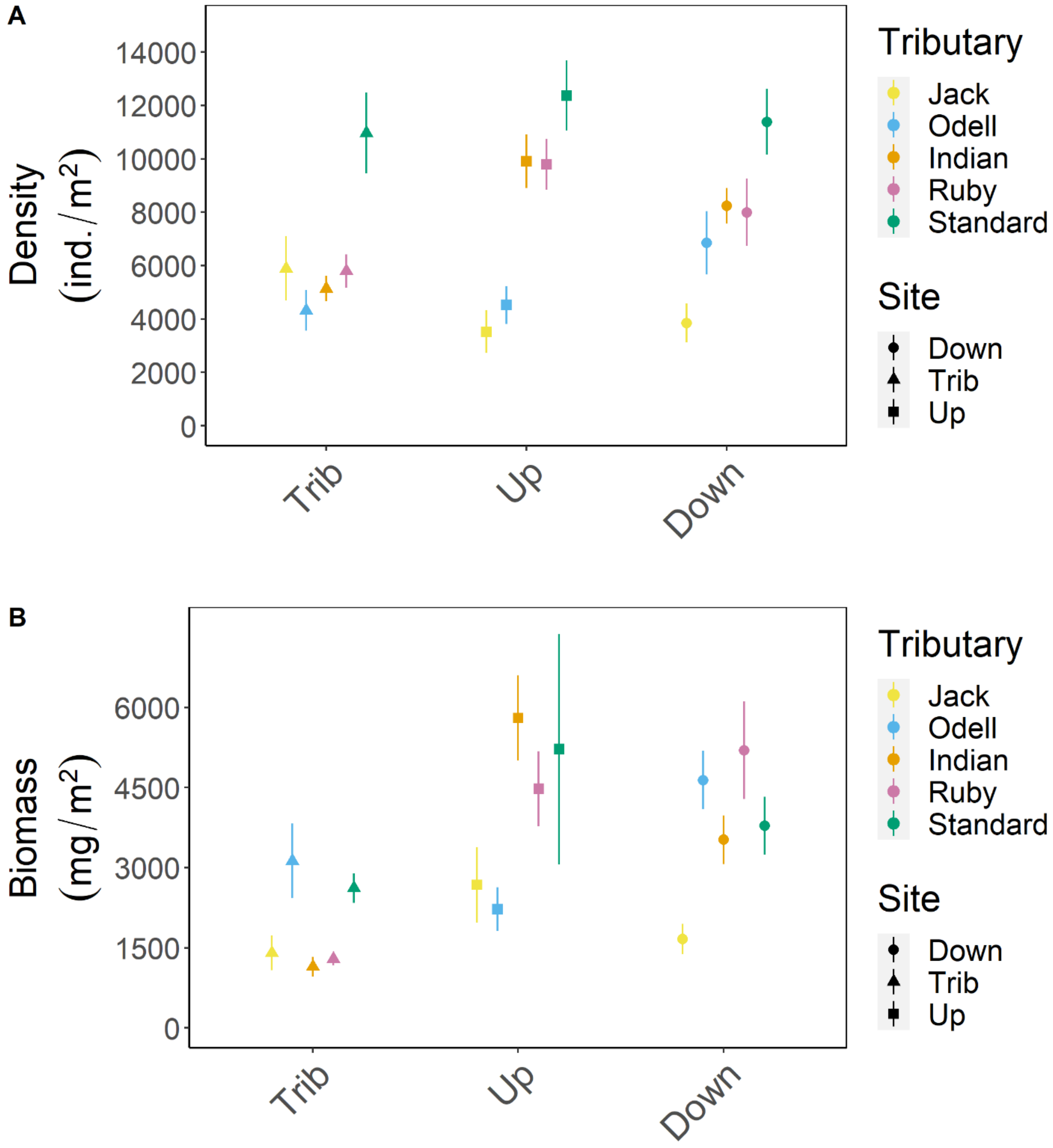


Figure 4: **A)** Benthic macroinvertebrate density (mean ± se) among tributaries and sites (n=12) **B)** Benthic macroinvertebrate dry biomass (mean ± se) among tributaries and sites (n=12). Error bars not visible are subsumed within the point.

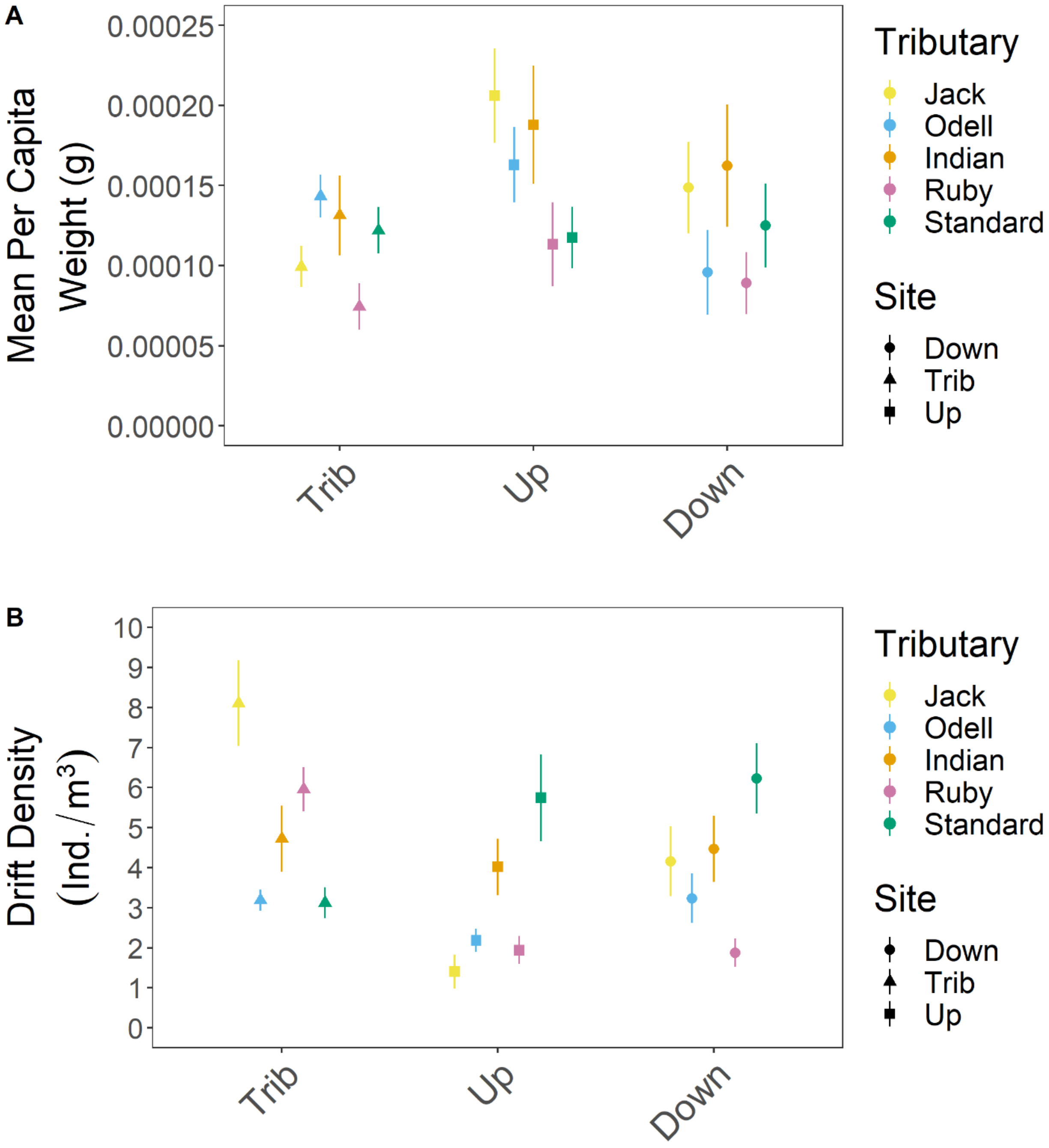


Figure 5: **A)** Per capita weight (mean ± se) of drifting macroinvertebrates among tributaries and sites (n=12). **B)** Macroinvertebrate drift density (mean ± se) among and tributaries and sites (n=12).

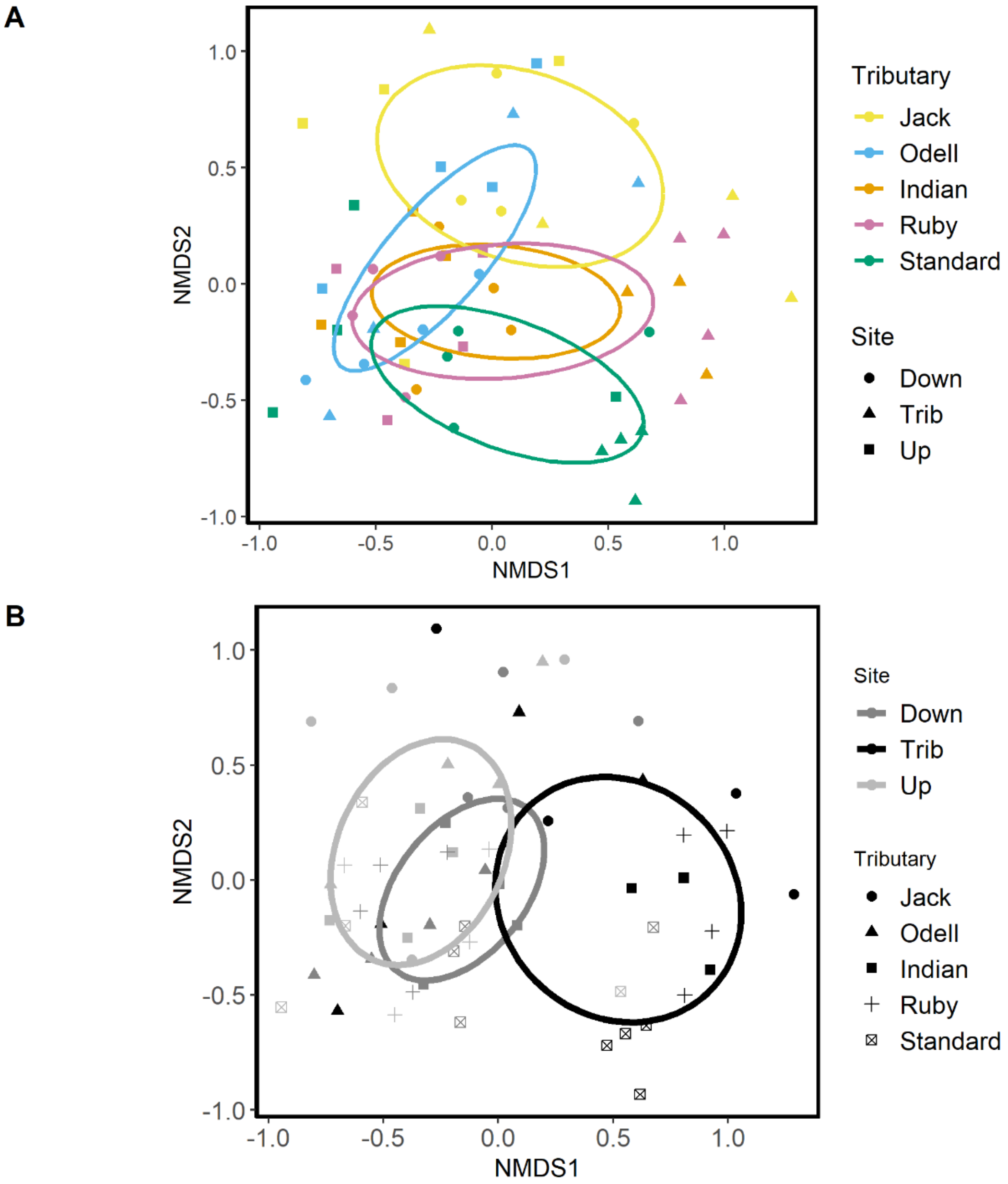


Figure 6: Nonmetric multidimensional scaling (NMDS) plots based on Bray–Curtis dissimilarities of macroinvertebrate biomass grouped by **A**) tributary (Jack, Odell, Indian, Ruby, and Standard), and **B**) site Trib (black), Up (grayscale low), and Down (greyscale medium). Ellipses show standard deviation.

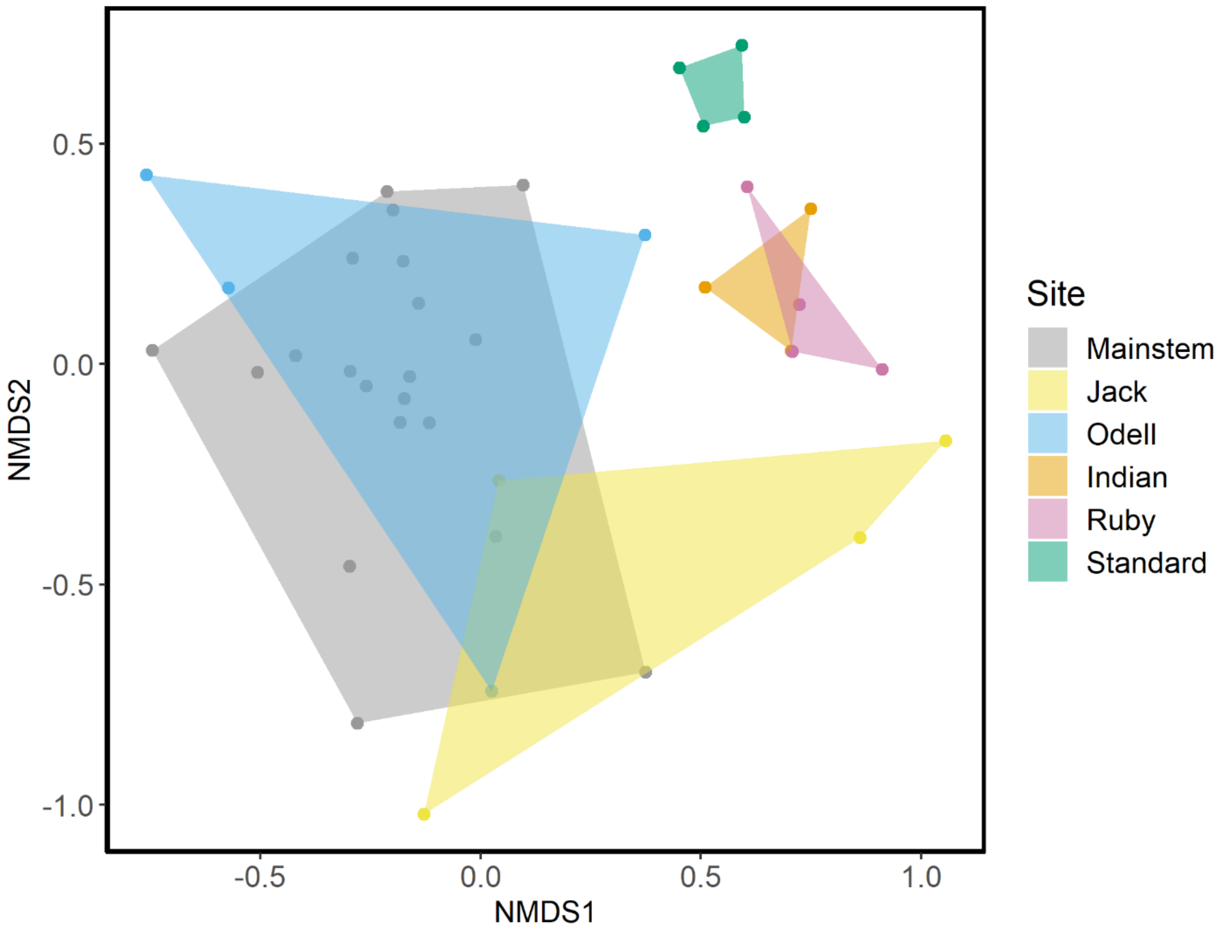


Figure 7: Patterns of functional β-diversity based on Bray–Curtis dissimilarities of macroinvertebrate biomass for each tributary and pooled for all mainstem sites (grey).

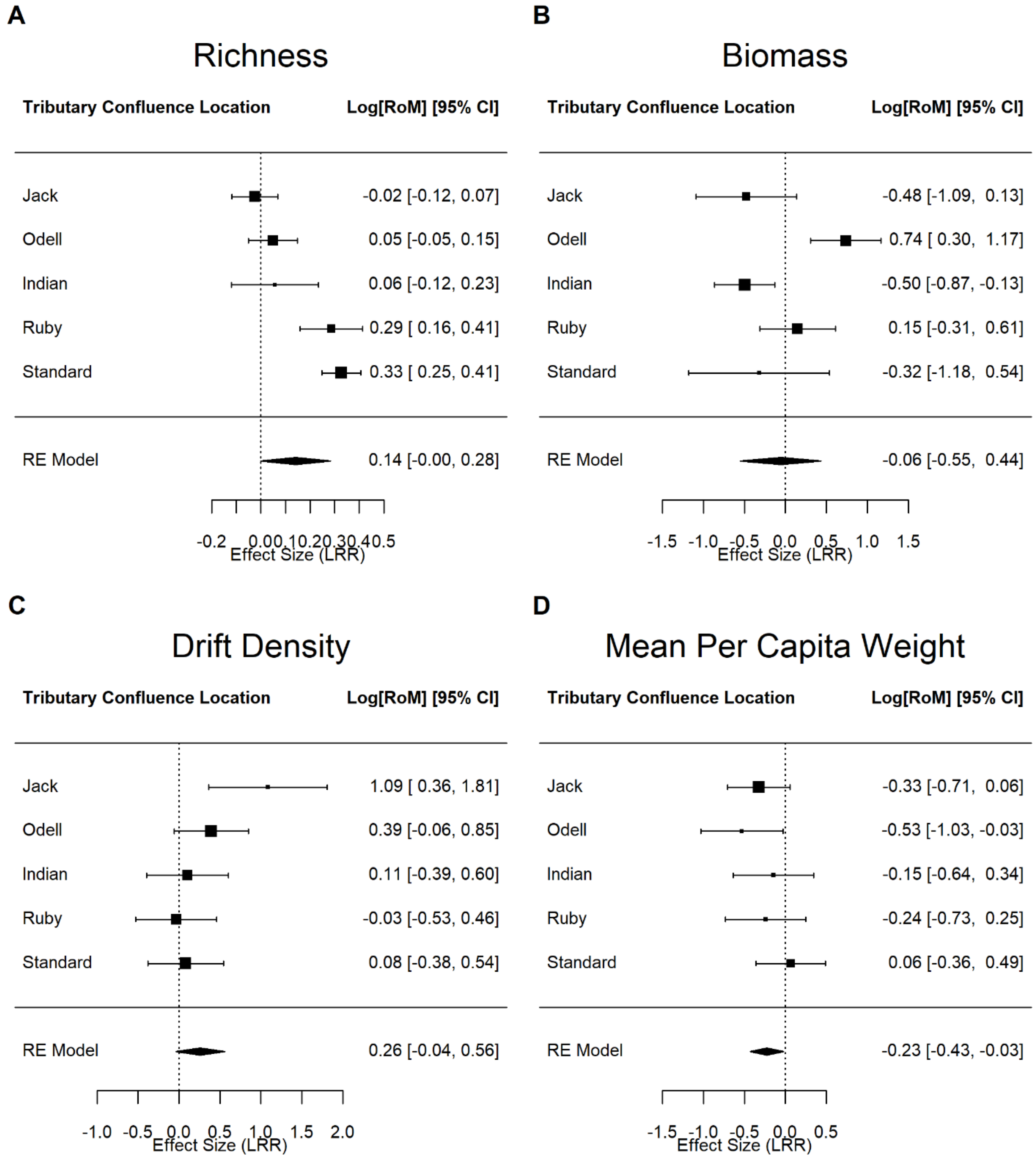


Figure 8: Effect sizes for each of the five tributaries demonstrating the magnitude of a tributaries' influence on macroinvertebrate community structure. The effect size compares a given metric downstream of a confluence to that metric upstream for the following metrics: A) benthic richness, B) benthic biomass, C) drift density, D) mean per capita weight in drift. RE Model shows the average effect size pooled across all tributaries.

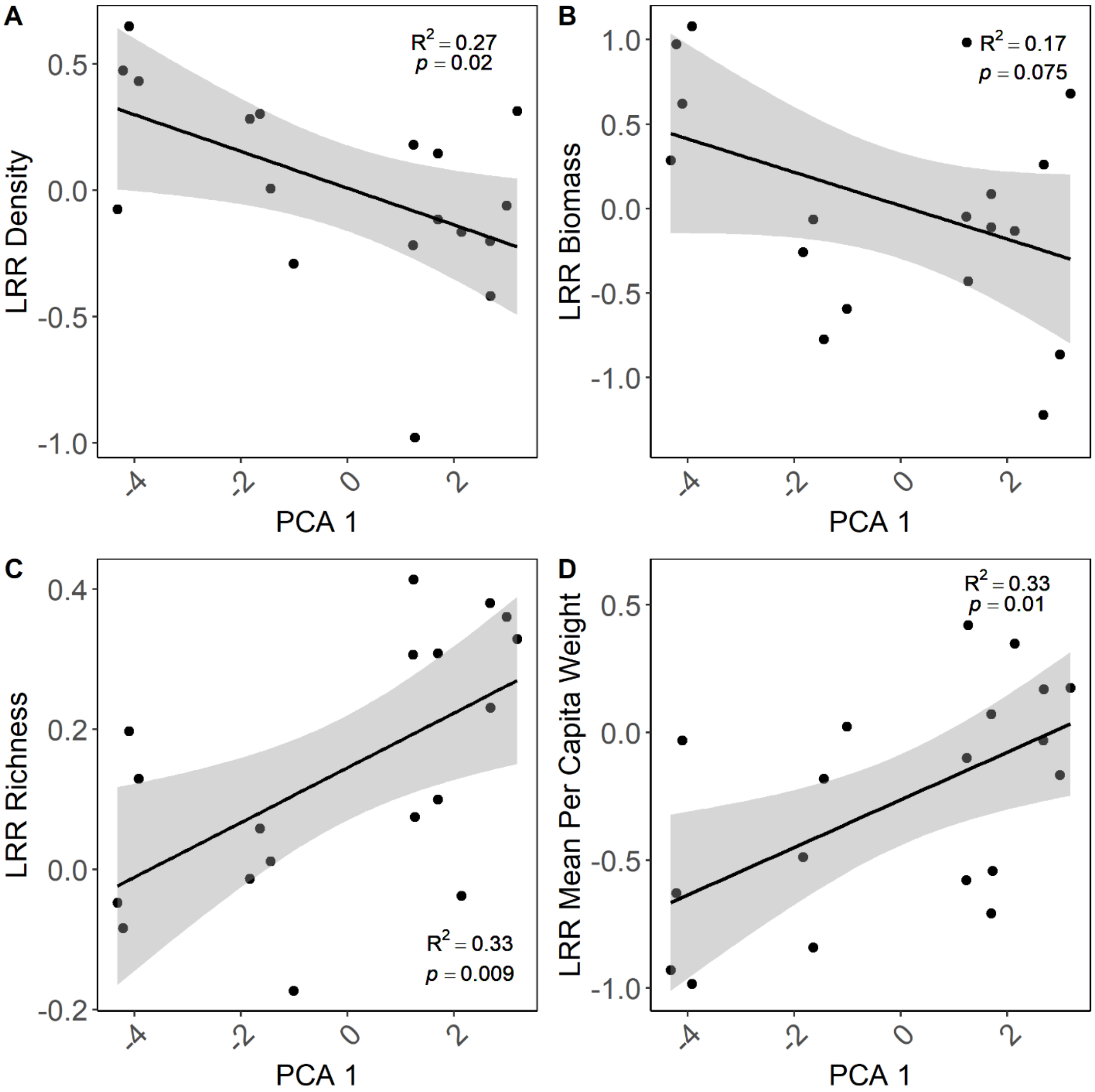


Figure 9: Patterns of environmental drivers (measured as PCA 1 scores) of A) benthic density, B) benthic biomass, C) benthic richness, and D) drifting per capita weight, measured as log response ratios. Only relationships with strong evidence for the correlation are shown; other relationships are reported in the results text.

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CHAPTER THREE

CONCLUSION

Please refer to chapter two for the conclusion.

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APPENDIX

SUPPLEMENTARY FIGURES

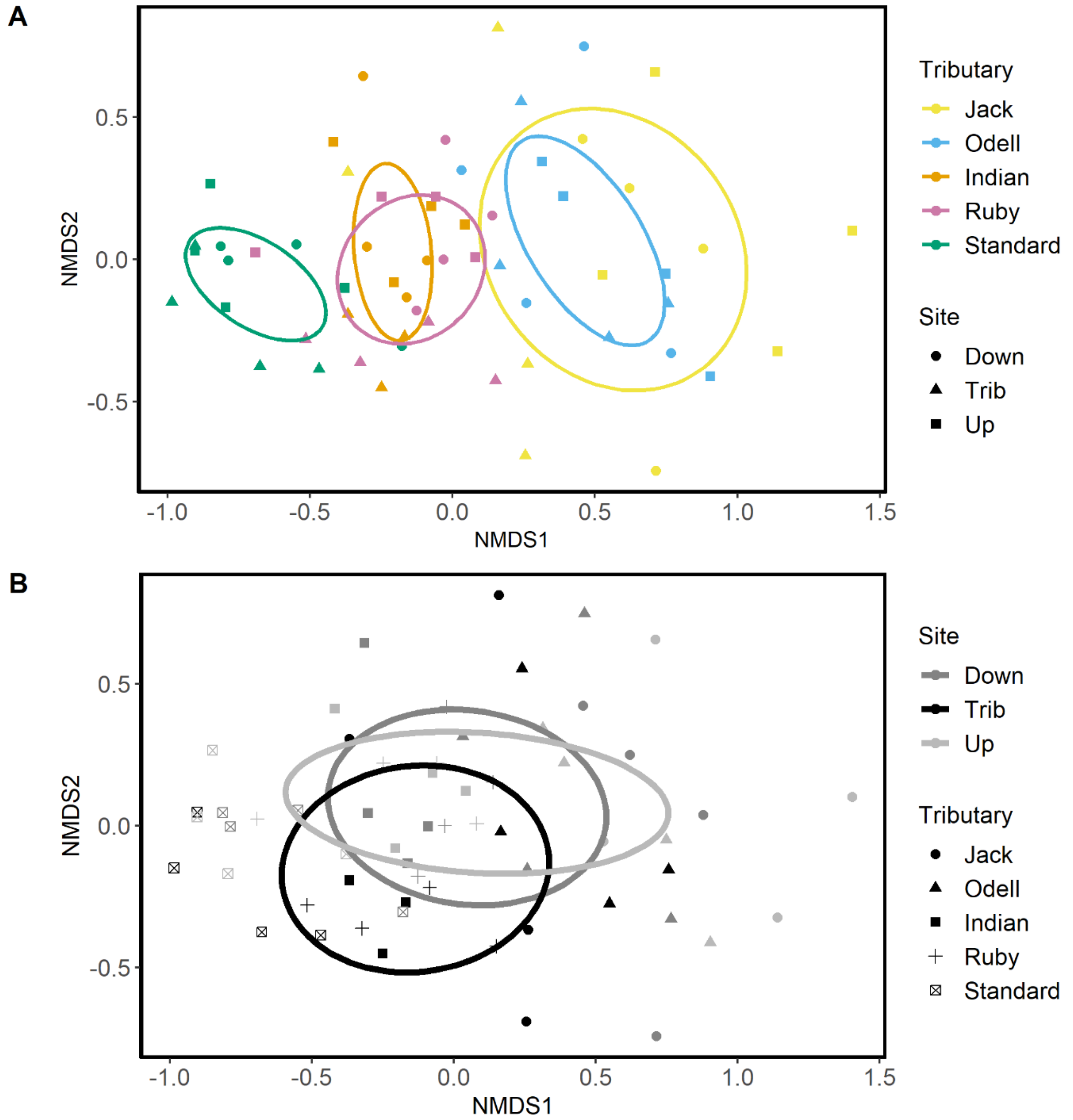

Figure A1: Nonmetric multidimensional scaling (NMDS) plots based on Bray–Curtis dissimilarities of macroinvertebrate density grouped by **A**) tributary (Jack, Odell, Indian, Ruby, and Standard), and **B**) site Trib (black), Up (grayscale low), and Down (greyscale medium). Ellipses show standard deviation.

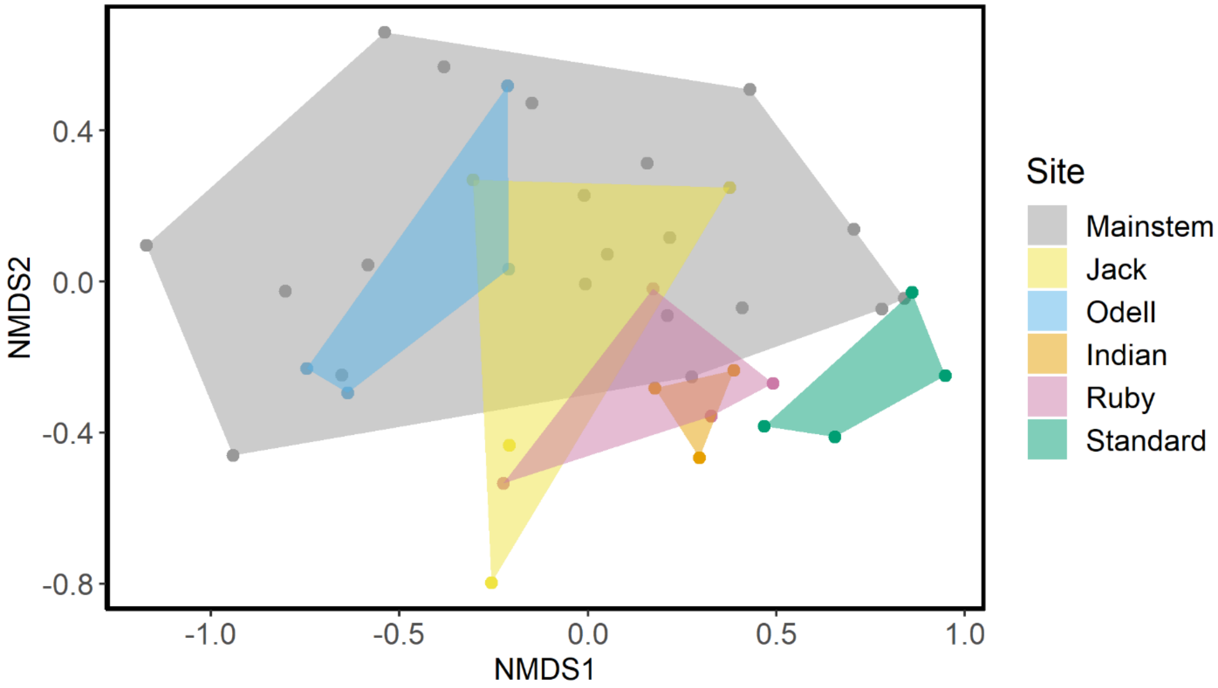

Figure A2: Patterns of functional β-diversity based on Bray–Curtis dissimilarities of macroinvertebrate density for each tributary and pooled for all mainstem sites (grey).

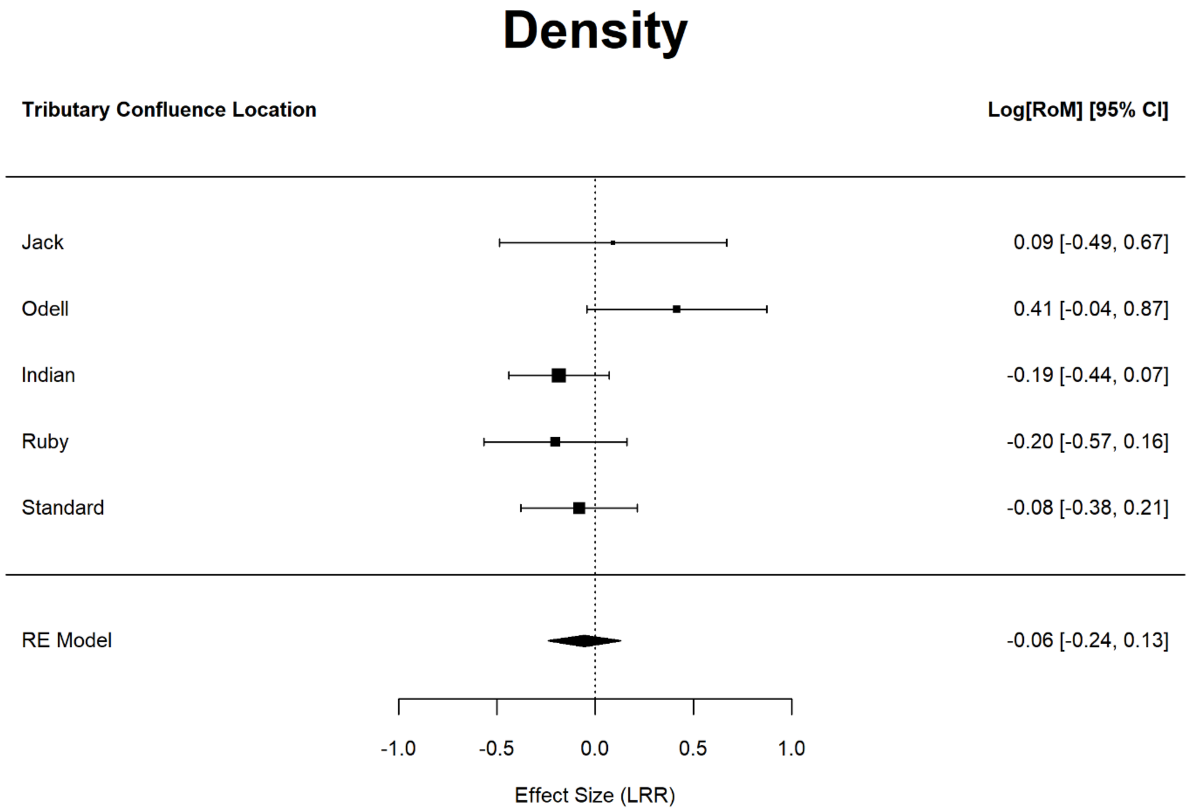

Figure A3: Effect sizes for each of the 5 tributaries demonstrating the magnitude of a tributaries' influence on macroinvertebrate benthic density. The effect size compares density downstream and upstream of a confluence across all sampling dates. RE Model shows the average effect size pooled across all tributaries.

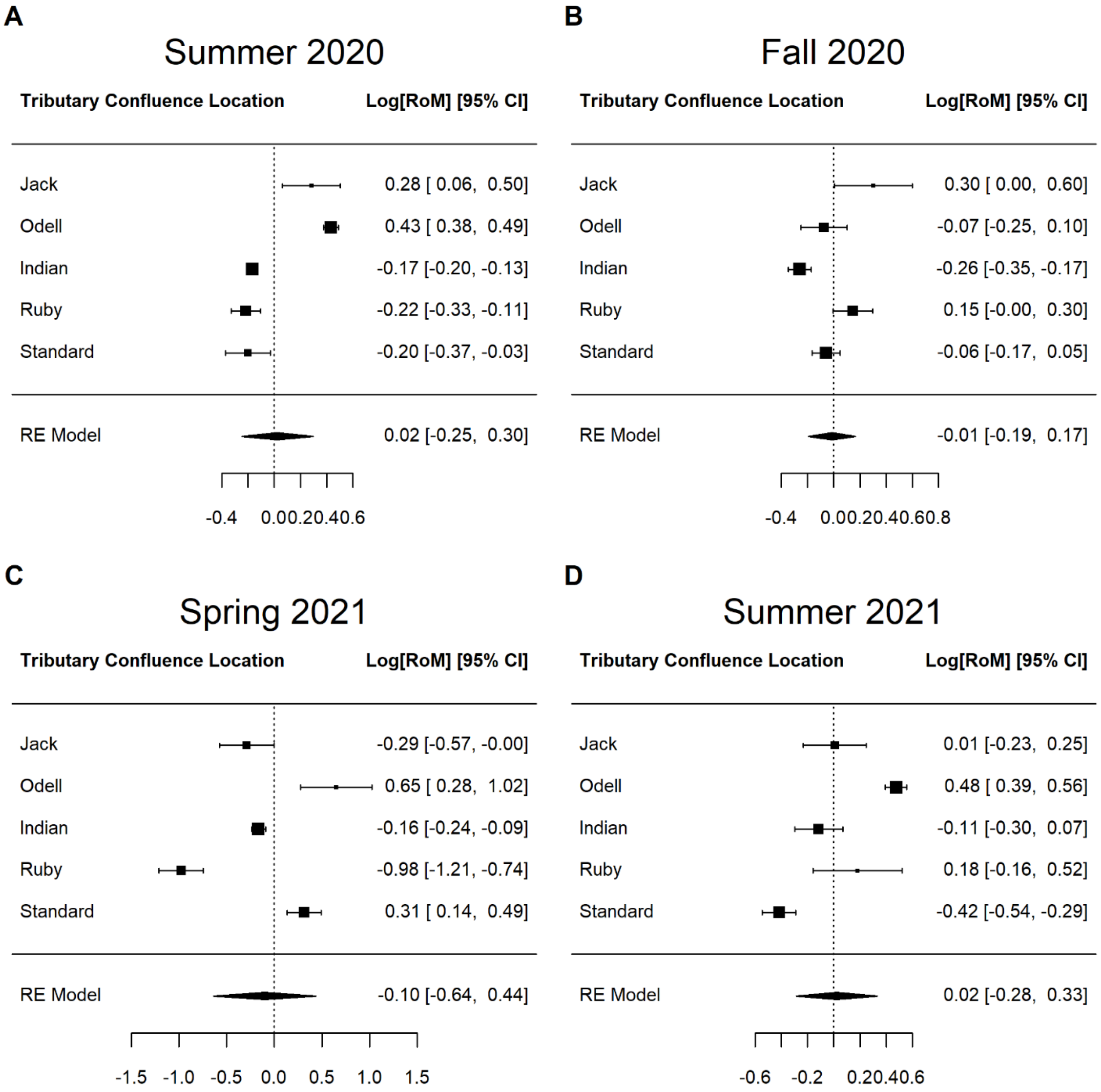

Figure A4: Effect sizes for each of the 5 tributaries demonstrating the magnitude of a tributaries' influence on macroinvertebrate benthic density. The effect size compares density downstream and upstream of a confluence for the following sampling dates: A) Summer, 2020, B) Fall, 2020, C) Spring, 2021, and D) Summer, 2021. RE Model shows the average effect size pooled across all tributaries.

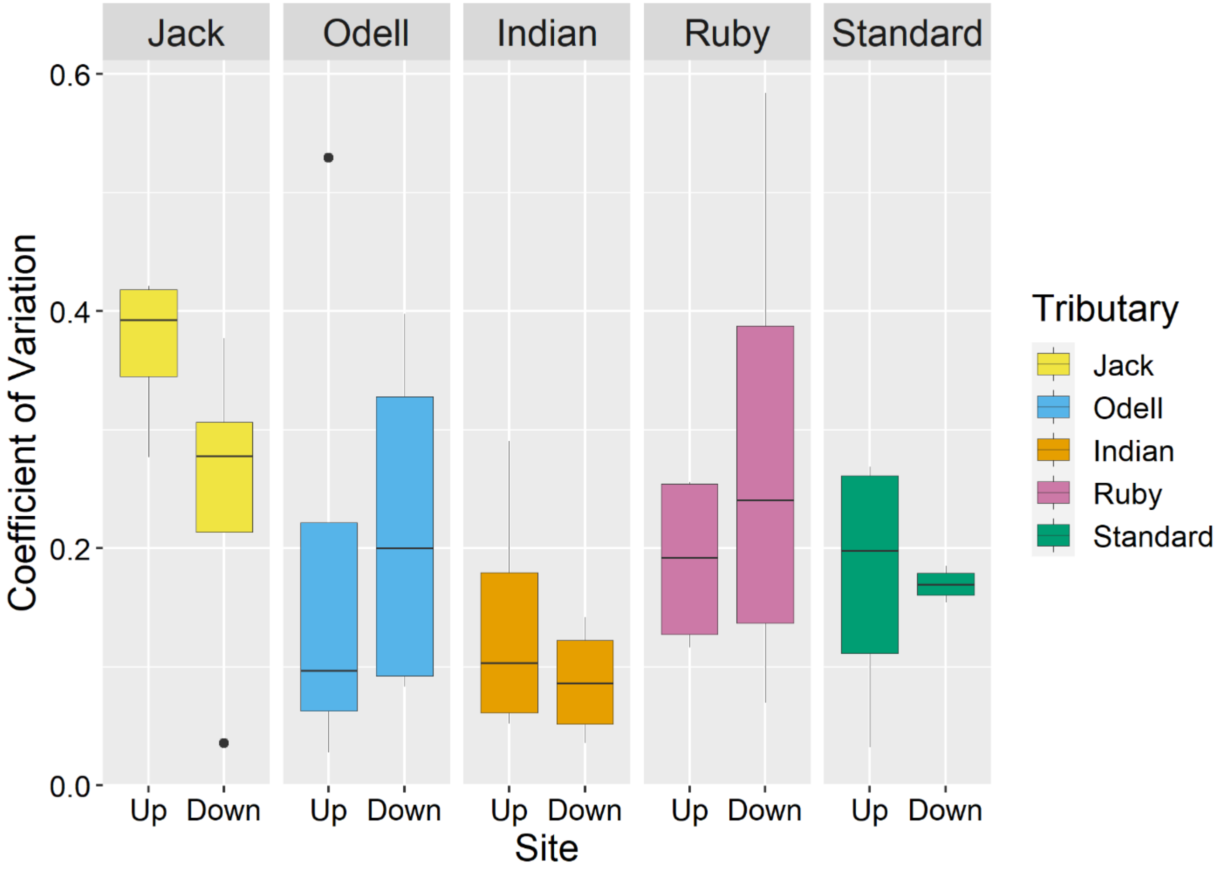

Figure A5: Macroinvertebrate density coefficient of variation (CV) for upstream (Up), downstream (Down) sites for the five tributaries, showing consistency across individual tributaries and sites and no longitudinal pattern. This comparison helps to elucidate if density results can be attributed to tributary confluences rather than to the natural variability of communities in the mainstem.