

SUBSTRATE & MACROINVERTEBRATES
IN THE
EAST FORK BLACK RIVER, MISSOURI

A Thesis

presented to

the Faculty of the Graduate School
at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

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MAY 2023

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IN THE
EAST FORK BLACK RIVER, MISSOURI

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ACKNOWLEDGEMENTS

I owe the completion of this project to a great many people. First and foremost, I would like to thank my advisor, Dr. Alba Argerich, who provided guidance, support, and patience well before being accepted into the University of Missouri and through the entirety of my time as a student. Furthermore, I would like to express gratitude to Dr. Debra Finn, Dr. Susannah Erwin, Dr. Elizabeth King, Dr. Craig Paukert for serving on my committee, sharing their specialized knowledge on various topics within this work, providing constructive criticism on study and experiment designs, thinking on results, and coordinating between so many people with busy schedules. Though not an official committee member, Brett Landwer served at least as much in all of these roles, introduced me to the East Fork, assisted with field work, and deserves similar gratitude.

I would further like to thank the Missouri Department of Conservation (MDC) for their funding of this project. Within MDC, I would like to thank Nicole Farless, Bill Mabee, and Seth Lanning for their tireless assistance with many aspects of field work and macroinvertebrate sample processing. Ameren Missouri and Kenneth Lee further provided access to the river through their land and fine conversation about the history of the land. Without their aid, data collection and all subsequent components of this work would have been impossible.

I am deeply indebted to multiple people within the University of Missouri: of the Limnology Lab, Nicholas Trusso, Eli Bunting, Kiah Wright, Jesse Olburg, and Tabitha Gatts worked hard to help process macroinvertebrate samples and conduct field work; no analysis would have been possible without them. Erin Petty, Jessi Wilson, Jacob Cianci-Gaskill, and Emily Kinzinger provided invaluable friendship, field and lab assistance, and general support; I similarly could not have finished this work without them. Dan Obrecht, Tony Thorpe, and Carol Pollard provided invaluable guidance in lab, field, and general matters. Finally, the faculty of the University too deserve my thanks for helping to challenge and hone my ways of thinking in ways that subtly but invaluablely permeate this project.

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ABSTRACT

Freshwater streams and their associated riparian and floodplain environments are crucial components to global biodiversity. Aquatic macroinvertebrates within streams are often adapted to and dependent on multiple physicochemical characteristics of these systems, but anthropogenic actions may disrupt these conditions and thus macroinvertebrates and other organisms. Stream impoundment, dam construction, fragment streams longitudinally; the effects of such may take effect through the stream's flow regime, thermal regime, and substrate composition and transport. These and other consequences may then further impact biological metrics such as macroinvertebrate diversity; changes to substrate size and distribution pose immediate challenges to physical habitat conditions. With an increasing awareness of the ecological harm impoundment may cause, mitigation efforts are similarly increasingly considered. Substrate augmentation is one such method which aims to restore pre-impoundment substrate conditions downstream of a dam. Here, we sought to further understand the influence of a dam on downstream substrate conditions and to determine what substrate and other physicochemical conditions yielded relatively high macroinvertebrate biodiversity. We utilized multiple lines of evidence to assess the macroinvertebrate community among various substrate and other habitat conditions in Missouri's East Fork Black River, an impounded Ozark Stream. The downstream waters of this river are unique in their flow

and thermal regimes being relatively unaffected due to the presence of the Lower Taum Sauk Dam, leaving substrate as the sole primary stream parameter impacted. Ultimately, we determined how macroinvertebrate diversity was related to the various substrate conditions found in five sites downstream of the dam across four sampling events spanning three years. As expected, we observed that median substrate size decreased with downstream distance of the dam, while substrate size distribution increased. On average, median particle size ranged from 112.5 mm in our most-upstream site (R1) to 36.2 mm in our most-downstream site (R5), and the coefficient of variation of particle size ranged from 70.3% to 101.5% across the same longitudinal gradient. Substrate itself was not enough to predict macroinvertebrate abundance or diversity, however, as our most-downstream site was substantially deeper and slower-flowing than the sites upstream (R2, R3, R4); corresponding mean abundance and diversity in R1 were, respectively, 139.4 and 1.39, while those values in R5 were, respectively, 57.18 and 1.4. Looking to R4 instead, we observed a macroinvertebrate community more in line with expectations, with mean abundance of 268.6 and mean diversity of 1.92, showing improvement with downstream distance from the dam. Partial Mantel tests indicated that substrate size and size distribution were important drivers of difference in macroinvertebrate abundance and Shannon diversity between sites, but so too were seasonal and hydrologic parameters, such as water temperature, dissolved oxygen concentration, conductivity, and turbidity. Analyzing heatmaps, which allowed for utilizing both substrate parameters as input variables against macroinvertebrate metrics, we concluded that median abundance and Shannon diversity were maximized around median substrate particle sizes of 45 mm to 64 mm and size coefficients of variation of 65% to 75%, underscoring the importance of

a heterogeneous mixture of substrate centered about gravels and cobbles. Future substrate augmentation projects in naturally gravelly streams may consider utilizing mixtures of these conditions to benefit macroinvertebrate communities.

1. INTRODUCTION

1.1 Impoundment of streams

Stream systems and their associated terrestrial environments, including riparian areas, floodplains, and wetlands, are crucial to global biodiversity as they host some of Earth's most productive ecosystems and unique communities (Naiman et al., 1993; J. Ward et al., 1999). Few stream and river systems, however, flow freely without regulation. In the United States, upward of 1 million kilometers, nearly 30%, of all stream mileage is affected by dams (Poff et al., 1997). Construction purposes vary from power generation to flood control, drinking water supply, navigation, and recreation (Pohl, 2003). Over 45,000 large dams are found across the world, with one found on nearly half of all river systems (Duflo & Pande, 2007). This number is still growing: as of 2014, an additional 3,700 large hydropower dams were either in planning or construction phases (Zarfl et al., 2015). In 2020, the National Inventory of Dams had catalogued over 5,300 dams in Missouri that were at least 10 feet tall, almost all of which held at least 10 acre-feet of water under normal conditions.

Dams are points of fragmentation within a stream and between a stream and its surrounding environment (Bednarek, 2001; J. V. Ward & Stanford, 1995; J. V. Ward & Stanford, 1983). This fragmentation may be dispersed through multiple physicochemical aspects, such as the flow regime, thermal regime, and substrate composition, and through biological metrics such as macroinvertebrate and fish abundance and diversity.

The flow regime is tightly linked to the structure and integrity of a stream ecosystem and among the primary components of such a system to be affected by a dam's impoundment (Poff et al., 1997). In many instances of impoundment, streamflow may become homogenized or actually increase in variability, high and low flows may shift in timing, the duration of high and low flows may be lengthened, and shifts in flow may occur more rapidly in ways that favor human wants and needs (Bednarek, 2001; Poff et al., 1997). By affecting streamflow patterns, other broad physicochemical patterns related to hydrology may be affected, such as the concentration of dissolved and suspended material. Changes

more specific to flow may disrupt still other physicochemical and biological components of the stream system. Such effects may include changes to sediment transport, connection of the channel to its floodplain, detritus and other resource availability, habitat availability, and various cues and further aspects of organisms' life histories (Bednarek, 2001; Kondolf, 1997; Poff et al., 1997).

The thermal regime is similarly important to the biological community. The life cycles of plants and animals, let alone the community able to live in a particular section of stream, may be impacted by changes to this regime (Bednarek, 2001; Jacobsen et al., 1997; Tonkin, 2014). While mean annual temperatures of an impounded stream may be relatively unaffected by the presence of a dam, the variation over the course of a year and both the timing and magnitude of diurnal and seasonal temperature minima and maxima may be affected quite dramatically (J. V Ward & Stanford, 1979). Reservoirs often release cold water from the hypolimnion, which, depending on the time of year, may cause tailwaters to be cooler or warmer than their unimpounded counterparts (Bednarek, 2001). Moreover, these waters are often low in dissolved oxygen, further reducing the capacity to support certain organisms (J. V Ward & Stanford, 1979).

More than just water, streams act upon and transport sediment. As such, a dam impounds not just water flow but so too that of sediment, leading to accumulation upstream of the dam and sediment starvation downstream (Kondolf, 1997). This downstream reduction in sediment may cause changes in channel morphology: meandering patterns may shift, and the channel may incise deeper and migrate laterally (Kondolf, 1997). At a finer scale, the composition of the channel substrate may too shift: along mid-order streams, smaller sizes of sediment are transported downstream without replacement, leading to an armoring effect where only larger sizes remain, which additionally increases homogeneity among substrate sizes (Ellis & Jones, 2013; Kondolf, 1997; J. V Ward & Stanford, 1983). The particular composition of sediment sizes in a streambed may directly affect habitat and aspects of organisms' life histories directly, but so too does it influence them indirectly through its effect on detritus, with gravel and small pebbles retaining more fine particulate organic matter than larger substrates (Culp et al., 1983; Mackay, 1992).

1.2 Aquatic Macroinvertebrates

1.2.1 Taxonomy, Function

Taxonomically and functionally diverse, macroinvertebrates often represent much of in-stream biodiversity and greatly contribute to the normal functioning of a stream system. Macroinvertebrates sit at intermediate levels of stream food webs and act as bidirectional conduits for the transfer of matter and energy: they may act as scrapers, grazing on algae and biofilms; shredders of coarse organic matter; gatherers and filterers of fine organic material; piercers of vegetation; and predators of zooplankton, other macroinvertebrates, and, in rarer cases, small vertebrates (Wallace & Webster, 1996). As intermediaries, they depend upon in-stream primary productivity, allochthonous inputs such as riparian leaves and large woody debris, and the waste and other byproducts of consumption of other macroinvertebrates (Wallace & Webster, 1996). Alterations to the quantity and quality of these resources may be observed in changes to, or by the disappearance of, the dependent macroinvertebrates and organisms of higher trophic levels (Wallace et al., 1997). Many fish, reptiles, amphibians, other macroinvertebrates, and terrestrial predators may utilize macroinvertebrates through different life stages as important food resources (Power et al., 2004). Different life histories, feeding strategies, mobilities, and preferences/tolerances for in-stream conditions result in different taxonomic groups responding differently to the same changes in environment (Wang et al., 2020).

Stream macroinvertebrates may be used as important indicators of water quality. One might find advantage in using them as bioindicators in their short lifespans, high numbers, widespread distribution, and relatively sedentary lives (Wallace & Webster, 1996; Wang et al., 2020). Perhaps most useful is their sensitivity to various physicochemical aspects of a stream, including the flow regime, thermal regime, and sediment composition.

1.2.2 Impacts of Impoundment

Changes to the streamflow magnitude and frequency, timing, duration, and rate of change may flush or strand organisms (including food resources), disrupt timing cues used for

emergence and spawning, affect vegetation growth and therefore macroinvertebrates' habitat, disrupt connections to floodplains, and alter or remove certain types of physical habitat (Dewson et al., 2007; Poff et al., 1997). These changes may impact macroinvertebrate abundance and diversity. (Dewson et al., 2007) highlighted the complexity of specifying particular responses given the web of pathways through which these impacts may be realized. For example, though taxonomic richness, if altered, always decreased from reduced streamflow, macroinvertebrate density was more variable and may increase, decrease, or exhibit no change. While the Ephemeroptera, Plecoptera, and Trichoptera (EPT) tend to be sensitive to altered conditions, for example, tolerant taxa like the Chironomidae may increase in density within tailwaters (Bednarek & Hart, 2005). Global trends indicate that, between sites upstream and downstream of impoundments, dams decrease macroinvertebrate richness yet increase abundance (Wang et al., 2020). Restoration and management strategies may need to make clear specific goals for end community structure or stream function as a single macroinvertebrate metric alone may not indicate a favorable post-restoration community, and the intent of the strategy may favor certain community structures over others.

Alterations to the thermal regime may affect organisms while in the affected stream or, in the case of most adult macroinvertebrates, as they leave it; temperature contributes to macroinvertebrate phenology and provides cues for when juveniles transition and leave streams as adults (Woods et al., 2022). Temperature may broadly stand in for seasonality, through which plant and animal communities wax and wane. As algae and vegetation increases, that which grows accessible to macroinvertebrates may be fed upon; come autumn, allochthonous leaf inputs serve to further fuel the stream food web (Vannote et al., 1980; Wallace et al., 1997). Decoupling the stream's thermal regime from natural seasonal patterns may affect in-stream organisms through the availability of certain food resources, growth rates, mobility, and adult emergence timing (J. V Ward & Stanford, 1979). Should adult insects emerge from waters whose temperatures are out of sync with those of the above air, the temperature difference may be lethal, and they may be without sufficient food or mates. More directly, the water temperature itself may be unsuitable for

the original community of organisms, with some taxa only able to tolerate narrow thermal niches (Bednarek, 2001).

Modified substrate characteristics downstream of a dam may affect organisms directly and indirectly. Inorganic substrate, ranging in size from silt to boulders and exposed bedrock, provides much of the streambed habitat. Increased particle size reduces the ability for the substrate to be used as habitat and refuge by reducing interstitial surface area, and certain taxa that require small or fine particles for spawning, burrowing, or case construction may be unable to utilize the coarser substrate of tailwaters (Mackay, 1992). Channel incision may change groundwater interactions and affect the riparian plant community that supports in-stream biota (Bednarek, 2001; J. V Ward & Stanford, 1979). Coarser substrate may favor the growth of algae and microbial mats, but is less capable of retaining more fine food resources, affecting macroinvertebrates who graze, collect, and filter, those who prey upon them, and higher trophic levels (Mackay, 1992; Parker, 1989; Wallace & Webster, 1996; J. V Ward & Stanford, 1979). The lower substrate heterogeneity causes a similar reduction in habitat diversity, which has been associated with lessened diversity of organisms in such environments (Ellis & Jones, 2013). In their global synthesis, Wang et al. (2020) found changes in substrate composition to be responsible for reduction of macroinvertebrate richness downstream of dams and suggested that a reversal of such changes may in turn restore macroinvertebrate richness.

1.3 Mitigation

Efforts to mitigate the effects of dams on streams have increased in response to an increasing awareness of their associated ecological detriments (Bednarek, 2001; Harvey et al., 2005; Merz & Chan, 2005). These efforts may take place at the dam itself or at further downstream sections of the stream, depending on the characteristics prioritized for improvement. Depending on the style and specifics of the dam, desired benefit, and other constraints, there exist multiple mitigation techniques to choose from: fish passes allow the movement of organisms otherwise blocked from migration; surface-water releases address alterations to the thermal regime by discharging water more similar in temperature to the non-impounded stream; and substrate augmentation projects restore

natural substrate composition, which may benefit aquatic biota reliant upon them for habitat, spawning, and other purposes (Harvey et al., 2005; McManamay et al., 2010; Merz & Setka, 2004).

Substrate augmentation is a method in which substrate is added into a stream. This may be done directly, with material deposited onto the existing streambed, or indirectly, with material deposited alongside the channel to be mobilized and redistributed via future high flow events. Much variation in methodology still remains, with aspects of exact placement, timing, volume, composition, frequency, etc. dependent on specific project goals (Harvey et al., 2005). Substrate augmentation projects are, further, still a developing management technique, and with most targeting fish communities, there are numerous knowledge gaps regarding those targeting macroinvertebrate communities (Harvey et al., 2005; McManamay et al., 2013; Merz & Chan, 2005). Specific conditions in substrate composition, food availability, water velocity, flow regime, water quality, habitat structure, and colonization mechanisms all effect how macroinvertebrates utilize substrate in a stream (Harvey et al., 2005); this is to say nothing of the specifics of the substrate augmentation itself, including the composition, volume, and location, timing, and distribution of substrate, and improvements to any one of the latter specifications may result in more efficient and targeted substrate augmentation projects.

Physicochemical parameters of a stream other than those directly related to substrate may still overshadow or confound the effects of substrate augmentation (McManamay et al., 2013; Merz & Chan, 2005).

The East Fork Black River (EFBR) in southeastern Missouri presents a fine opportunity to utilize substrate augmentation as a restoration tool for macroinvertebrates. For a mid-order stream with a sizable dam, we may expect some of the aforementioned impacts to the stream and its associated biological communities. However, there have been three notable practices enacted which have lessened the effects of the dam on the EFBR: flow-matching, the deliberate releasing of water from a reservoir to mimic incoming discharge conditions, the releasing of water from upper layers more closely aligned with ambient thermal conditions, and trial substrate augmentations.

With most substrate augmentation projects having taken place in streams containing salmonids, such projects in the United States have typically been conducted in the West, where the streams, geology, and climate substantially differ from conditions found in Missouri and its broader region within the country (McManamay et al., 2013). Further, despite the crucial importance of macroinvertebrates toward the diets of fish and other vertebrate predators, substrate augmentation projects are rarely conducted to primarily target macroinvertebrates. The EFBR lends itself well to considering substrate, fish, and macroinvertebrates collectively. A study considering substrate and fish was conducted on the river; our work may complement this and broader community-level considerations of organisms and their relationships to substrate (Brant, 2020). Such fish are widespread across the Ozark Plateau and much of the southeastern United States, allowing further coordination between efforts toward macroinvertebrate and fish communities in similar systems.

Given the emphasized impact of the EFBR's dam on the substrate over impacts to streamflow and thermal regime, the dearth of substrate augmentations conducted targeting macroinvertebrates, the reliance of fish and other vertebrates on macroinvertebrates as food resources, the lack of substrate augmentations conducted in the central United States, and the previous work done relating substrate conditions to fish that may benefit from substrate augmentation in the EFBR, we here have a prime opportunity to further illuminate relationships between substrate and macroinvertebrates in a relatively novel context. Results from such work may further aid augmentations targeting macroinvertebrates and those conducted in the central United States, and given the increasing construction of dams, understanding how to mitigate the effects of existing and future dams on the physicochemical and biological components of the impounded stream system are of similarly increasing importance.

1.4 Purpose

This project aimed to determine substrate composition and other habitat conditions that yield relatively high biodiversity in the EFBR's macroinvertebrate community. The EFBR experiences channel armoring and varying substrate conditions along a

longitudinal gradient, from tailwaters containing a heterogeneous mixture of particle sizes dominated by large rocks to a more homogenous mixture rich in the gravel characteristic of the Ozark region. Conditions that most closely match those of free-flowing Ozark streams should yield a macroinvertebrate community most closely resembling those of the same streams. Interactions among substrate and other habitat conditions may complicate this relationship, however. To address this complexity in responses, we have conducted multiple samplings of habitat conditions and macroinvertebrate communities along multiple sites on the EFBR, taken across multiple seasons. A more refined analysis of the influence of substrate composition and other habitat conditions on macroinvertebrate community structure in the EFBR may allow for more efficient and targeted substrate augmentation projects for macroinvertebrates and fish alike and, more specifically, an improved plan for upcoming substrate augmentation projects on the EFBR.

In this thesis we aim to understand and describe the effects of the Lower Taum Sauk Dam on the East Fork Black River tailwaters, particularly on bed substrate, and how macroinvertebrate community structure is related to substrate and other measured parameters. We aim to do this by characterizing the physicochemical environment and macroinvertebrate community of riffles along a roughly four-kilometer stretch of the East Fork Black River as they appeared across four sampling events, then relating structural patterns and differences within the macroinvertebrate community to those found within the physicochemical environment.

2. METHODOLOGY

2.1 Study location

2.1.1 General characteristics

The East Fork Black River (EFBR) is a fifth-order stream within the Missouri Ozarks' St. Francois Mountains, part of the Ozark Highlands Level III ecoregion, flowing for 32 km before entering the Black River 1 km south of Lesterville. The stream flows through the St. Francois Knobs and Basins Level IV ecoregion until its confluence with the Black

River, at which point the system transitions to the Current River Hills ecoregion. Draining approximately 250 km², the EFBR holds headwaters near Elephant Rocks State Park and flows approximately 32 km, passing through Johnson's Shut-Ins State Park and the Ameren Union Electric Company's Lower Taum Sauk Reservoir, before joining the Black River, itself a tributary to the White River in Arkansas.

2.1.2 Geology of the East Fork Black River

Ozark streams are typically characterized by relatively steep gradients and coarse substrates; the karst geology, flashy flow regime, and myriad springs contribute to low turbidity and high biodiversity. The EFBR largely resembles these streams, though it contains portions which flow through Precambrian rhyolite and more strongly resist erosion than the Cambrian dolomite and shale of the remainder of the stream (Cieslewicz, 2004). Despite flowing through largely karst terrain, none of the EFBR reaches in our study are losing according to the Missouri Department of Natural Resources.

2.1.3 Land use

The EFBR's watershed is predominantly covered by deciduous forest with minimal human occupation or development; oak-hickory forests, igneous glades, and grasslands dot the landscape (Cieslewicz, 2004). The watershed of the upper Black River is not unfamiliar to anthropogenic disturbance. Historic land use includes timber harvesting to the point of discontinuing due to no remaining timber. This resulted in a conversion to shrubs and bushes replacing forest as dominant ground vegetation. Coupled with widespread deliberate burnings to reduce the abundance/spread of those shrubs and bushes, this led to heavy surface erosion, depositing excess amounts of soil and gravel into streams (Martin, 2005). The St. Francois Mountains are furthermore host to the largest amount of lead ore deposits in the United States leading to the region's strong mining industry and "Lead Belt" moniker, though no active lead mines persist in the EFBR's watershed.

2.1.4 Climate

Like much of Missouri, the EFBR resides within the Köppen-Geiger humid subtropical climate zone. 2001-2021 records from a weather station in the nearby town of Farmington, located approximately 50 km to the northeast of the study site, indicate that the EFBR receives over 1100 mm of precipitation over the course of each year and that mean daily temperature ranges from 0.6 °C in January to approximately 25 °C in July and August (Figure 2). Maximum precipitation tends to fall in May, averaging over 101.6 mm. On April 30, 2017, Farmington received its 2001-2021 maximum precipitation event with a recorded 121 mm of rain. This corresponded to the EFBR's 2008-2021 maximum discharge event, recorded by USGS stream gage 07061290 as over 270 m³/s, evidence of the reservoir's flow-matching practice. Discharge data from this gage is provided in the results.

The study was carried out along a 3800 m reach of the EFBR between the Lower Taum Sauk Dam and Lesterville, Missouri. The Lower Taum Sauk Reservoir is operated as a pumped-storage hydroelectric power system, in which water is regularly cycled between the Lower Taum Sauk Reservoir and an upper reservoir located approximately 250 m high on the nearby Proffit Mountain (Rogers et al., 2010). This cycling has little effect on discharge patterns of the stream: outflows from the lower reservoir closely match inflows from the EFBR and a small stream emptying into the lower reservoir, Taum Sauk Creek. A sediment trap, designed to limit sediment accumulation within the lower reservoir, resides just upstream of the reservoir. This trap accumulates sediments and thus must be periodically excavated. This trap has been the source of augmented substrate in the EFBR.

On December 14, 2005, the upper reservoir suffered a catastrophic failure in which early 4,000,000,000 L of water flowed down and scoured Proffit Mountain to bedrock, severely damaging both the EFBR upstream of the lower reservoir and neighboring terrestrial environments. Peaking at an estimated discharge of over 8100 m³/s down the mountain and 2690 m³/s in the EFBR, the flood affected an estimated 180 acres of timber and deposited an estimated 220,000 m³ of sediment into Johnson's Shut-Ins State Park

and the EFBR (Rydland Jr., 2006). The Missouri Departments of Natural Resources and Conservation (MDNR, MDC) began sampling the EFBR on December 19, 2005, in order to assess the impacts of the reservoir failure and monitor physical and biological changes within the stream. While the lower reservoir contained most of the impacts, large amounts of fine sediment made their way into the downstream reaches of the EFBR, though these were washed out of the study area by 2007 (Brant, 2020). The upper reservoir was restored in 2010, and regular operations resumed soon after.

2.2 Sampling design

We selected five sampling locations downstream of the dam to characterize the macroinvertebrate community and habitat circa 2017. All sites were historically riffles; in the time since their demarking, however, one has relocated approximately 100 m downstream and appeared to be transitioning away from being a riffle to being a run, and another is in the process of its own relocation. These two relocating riffles were sampled where they were found in the times of each sampling event, not in their historical locations. MDC personnel denoted them Riffles 1, 3, 6, 9, and 12 for their own purposes; for clarity, these are henceforth denoted Riffles (R) 1-5, and give their most recent locations at approximately 59 m, 569 m, 1260 m, 2130 m, and 3890 m below the dam, respectively (Figure 1). Two of these riffles, R3 and R4, are accessed through private property whereas the other three riffles are on public property. Between the lower reservoir and R5, there are minor intermittent/ephemeral headwater tributaries and at least one minor spring.

Four sampling events and two gravel augmentations were conducted by project personnel and MDC between September 2017 and August 2019 (Table 1). The sampling events are henceforth respectively denoted by season (i.e., Fall and Spring samplings) and year as F17, S18, F18, and S19.

2.3 Hydrology

To characterize streamflow regime and streamflow variation between sampling dates, we analyzed discharge data between January 1st 2009 to December 31st, 2020 from the USGS

gage 07061290 positioned approximately 200 m downstream of the dam (coordinates 37°29'37" N, 90°50'18" W). A count of records per year yielded a minimum of 34,285 records in 2009 to a maximum of 35,085 in 2012, a relative difference of 2.3%. These data are collected every 15 minutes and represent a near-continuous record of flow conditions. We assumed that variation in discharge at this station may be taken as approximately valid for each riffle downstream of the dam given minimal tributary inputs and, due to its lack of classification as a losing stream, assumed insignificant amounts lost to the karst terrain.

2.4 Physicochemical environment

On each sampling date, we characterized habitat conditions at each riffle by measuring water temperature, dissolved oxygen concentration, specific conductivity, turbidity, water velocity, water depth, and substrate particle size.

Water temperature, dissolved oxygen concentrations, specific conductivity, and turbidity were measured at the approximate center of each riffle using a YSI ProDSS multiparameter water quality meter. Water velocity and water depth were measured at ten random locations in each riffle using, respectively, a Hach FH950 portable velocity meter and a ruler marked with centimeter increments. We determined sampling locations by dividing each riffle's length and width into tenths. Using a uniformly random number generator, we then determined ten sampling locations within the riffle: for numbers 00-99, the tens digit denoted how many tenths along the riffle's length to travel, the one's digit how many tenths across the width.

At each of the ten locations per riffle, we randomly sampled ten substrate particles, thus sampling 100 particles per riffle. We classified particles into fifteen size classes using a gravelometer (in mm: 2, 2.8, 4, 5.6, 8, 11, 16, 22.6, 32, 45, 64, 90, 128, 180, and > 180). The largest size class, for data analysis purpose, is henceforth set as 250 mm. We used a near-identical process to selecting a sampling location within a riffle to characterize substrate. In this process, the tens digits were grouped into 1-2, 3-4, 5-6, and 7-8 (with 0 and 9 being discarded and another number produced), and each group was consistently paired with the same side, relative to stream flow, of a 0.5 m x 0.5 m frame centered

about the sampling location. The ones digit represented how many tenths along the frame's side to travel from a corner, again consistently aligned with flow. At each determined point, we selected the first-touched substrate particle and measured or, if too large to move, noted its size. We chose particles without replacement.

Per riffle per sampling event, then, we collected ten macroinvertebrate samples; 100 particles of substrate; ten water velocities and depths; and one water temperature, dissolved oxygen content, conductivity, and turbidity measurement (Table 2).

2.5 Macroinvertebrate collection and identification

Following substrate characterization, we collected macroinvertebrates at each riffle's ten randomly selected sites using a D-net. We disturbed substrate within an area approximately equal in dimensions to the frame, 0.5 m x 0.5m, or an 0.25 m² area, and scrubbed substrate too large to move to dislodge clinging organisms. We again scrubbed large collected material within the net, and all remaining material was transferred to a holding jar. We then washed all contents remaining in the net into a sorting tray and transferred all macroinvertebrates therein to the holding jar to be preserved in formalin of at least 10% concentration. We associated internal and external labels with each jar.

We transported the samples to MDC's regional office in Columbia, where we transferred them to 80% ethanol. We separated all discovered macroinvertebrates from substrate and detritus using a dissecting microscope. Next, we counted all macroinvertebrates in each sample and identified them to the lowest possible or useful taxonomic level, usually family, using the keys found within Merritt and Cummins (2008), Stewart and Stark (2002), and Wiggins (1996).

Finally, we grouped each family into their primary functional feeding groups (FFGs; shredders, collector-gatherers, collector-filterers, scrapers, herbivorous piercers, and predators) using Merritt and Cummins (2008) (Table 4). We evenly divided chironomid abundances between collector-gatherers and predators given their taxonomic diversity, two common general feeding habits, and our leaving their identifications at the family level.

Each FFG was paired with the environmental data in the same way as the taxa in the original framing of the data. This allowed us to run the analyses described here in essentially identical fashions.

2.6 Data Analysis

All analyses were performed using R Statistical Software (v4.0.5; R Core Team 2021). Data importing, tidying, and basic manipulation were aided by the readxl, tidyverse, and dplyr R packages; Mantel tests and Shannon diversity calculations were conducted with vegan and geosphere; hydrologic analyses were conducted with the aid of dataRetrieval, lubridate, Rwater, fasstr, and xts; and plot generation was performed with use of ggplot2 and akima.

2.6.1 Hydrology

To put the flows observed during the study period in context we conducted a hydrologic analysis of the EFBR using historical USGS gage data from January 01, 2009 to December 31, 2021. In addition to reporting basic ranges and summary statistics, we created a flow duration curve, assessed system flashiness, conducted a flood frequency analysis, and assessed seasonal and inter-annual flow variability.

We created the flow duration curve by calculating the percentage of time each recorded flow value was equaled or exceeded during the period of record. We assessed system flashiness similarly; in this case, our values of interest were changes in flow across each 15-minute gage recording. For this, we calculated the proportion each change in flow constituted of the whole, allowing us to calculate percentage thresholds, e.g., 95% of all changes in flow were at most of a certain value across a 15-minute period. The flood frequency analysis was conducted based on Adnan Rajib's online tutorial.

Seasonal and inter-annual flow variability was assessed by plotting the period of record's median flow against each month. For finer detail, we then calculated each day of the year's 25th, 50th, and 75th percentile flow values.

2.6.2 Physicochemical environment

We calculated median substrate particle size and the particle size coefficient of variation per riffle per sampling event. We did not collect dissolved oxygen or conductivity data in the S18 sampling event because of sensor malfunction; missing data were replaced by the median value per riffle outside that event.

To explore differences in the physicochemical environment across riffles and sampling events, we used a Welch's ANOVA and an analysis of the coefficient of variation. For the Welch's ANOVAs, we utilized the "oneway.test" function, part of base R, with equal variances declared false. We calculated the coefficient of variation for each parameter per riffle per sampling event as the $100 \times (\text{standard deviation}) / (\text{mean})$. We used the Shapiro and Bartlett's tests to check data normality and homogeneity of variances.

To better visualize and understand relationships between the physicochemical variables, we ran a Principal Components Analysis (PCA). The physicochemical data were scaled, i.e., each parameter's mean and standard deviation was normalized. We selected the first three principal component axes by the amount of variation they described and drew meaning from the loadings of the physicochemical parameters upon each axis.

2.6.3 Macroinvertebrate community structure

By the end of the project, we had 200 macroinvertebrate samples classified into 49 taxa and six functional feeding groups. A total of 138 out of 200 rows in the final data set contained at least one NA macroinvertebrate value, indicating no quantitative value could be assigned; of all 9,800 possible records (200 macroinvertebrate samples times 49 taxonomic groups), 3,096 were NA. As an NA anywhere within a row of data prohibits certain calculations, particularly those of diversity metrics, to be run, we assigned the median value of each respective taxon from its respective sampling event and riffle grouping to NA entries.

For each sample, we calculated total abundance, richness and EPT richness values as the number of different macroinvertebrate and EPT-specific taxa, Shannon diversity, and the

Missouri biotic index (MOBI) score (Table 3), generating a matrix of 200 rows by 74 columns.

2.6.4 Macroinvertebrates in relation to their physicochemical habitat

After we completed data collection and macroinvertebrate processing, we had a derived data frame containing all environmental and macroinvertebrate data in addition to (EPT) richness values, diversity indices, and other biological indices.

To understand the influence of the physicochemical parameters on the macroinvertebrate community, we used a few methods: basic plots of macroinvertebrate metrics against physicochemical parameters, heatmaps which allowed for plotting a macroinvertebrate response variable against two physicochemical parameters, and partial Mantel tests.

These last tests were the most involved. As our data are spatially correlated, we sought to eliminate some of this correlation. For ease of spatial analysis, we first assumed the EFBR was a straight north-south-flowing channel. Later analysis utilized spatial coordinates, which the sinuous nature of a stream and the along-channel distance do not comfortably pair with. We recorded the latitude and longitude of the Taum Sauk Lower Dam approximately corresponding to where water is released from the reservoir. Next, assuming a spherical Earth with radius 6378137 m per `distHaversine` in the `geosphere` R package, we converted distances downstream from the lower reservoir into degrees latitude south of the dam while fixing the longitude. Each calculated latitude placed each respective riffle within 0.01 m of the downstream distance measurements.

We then ran partial Mantel tests, which, in this study's context, are correlation tests between two dissimilarity/distance matrices, that of the macroinvertebrate community and that of either a single physicochemical variable or the total set of those variables, while accounting for a third matrix, that of our downstream distances. These tests tell a user how dissimilar the macroinvertebrate community became in response to increasing dissimilarity in the physicochemical environment while accounting for the spatial distance between sampling locations. When testing against the environment as a whole, we scaled environmental variables, while the values of those in the individual cases we

kept as originally measured. Depending on which distance matrix we were constructing, we used a specific distance metric: Bray-Curtis dissimilarity for macroinvertebrates, Euclidean distance for environmental variables, and Haversine distance, or that traveled along the curved surface of Earth, for downstream distances along the straightened stream. To prepare the data further, we constructed three distance matrices. We then ran multiple partial Mantel tests: first a set of tests, one per physicochemical variable, then one for the environment as a whole. Finally, we adjusted our p-values using the Benamini & Hochberg method.

3. RESULTS

3.1 Environment

3.1.1 Hydrology of the East Fork Black River

Flow magnitude

During the study period, streamflow ranged from 0.1 m³/s and 133.4 m³/s (Figure 3). Median flow was 1.0 m³/s. Generally, discharge quantiles were lower for the study period compared to the historical record, although minimum and maximum flows were within the bounds set by the historical record, i.e. the study period's minimum flow was well above the historical minimum. Minimum, median, and maximum flows during the historical record were, respectively, 0.008 m³/s, 1.11 m³/s, and 739.1 m³/s.

A flow duration curve is presented in Figure 4. This plot indicates that flows of at least 0.21 m³/s are present just under 90% of the time, that the median flow value, i.e. that present/absent 50% of the time, is approximately 1.1 m³/s, and that a discharge of at least 7.4 m³/s occurs just 10% of the time. More specific details are presented in the table accompanying the plot in Figure 4. These data reinforce the impression of relative stability of the EFBR's hydrologic regime: while its largest flow event, reaching approximately 739 m³/s, exceeded 650 times median flow, such events are expected to be fairly rare given the 5% occurrence threshold value.

System flashiness

Though change in absolute flow in the EFBR is fairly gradual, over 95% of all changes in discharge within a 15-minute timespan for both the historical and study periods were within absolute magnitude of $0.3 \text{ m}^3/\text{s}$, approximately one-third of median flow. The greatest change in discharge over such a timespan was approximately $127 \text{ m}^3/\text{s}$, itself part of a swelling of the EFBR by approximately $350 \text{ m}^3/\text{s}$ over a four-hour period on April 29, 2017. April and October had the greatest 15-minute changes in discharge and the most 15-minute differences in discharge of at least approximately $30 \text{ m}^3/\text{s}$; all months but March and June had 15-minute differences in discharge of such a magnitude.

Flood frequency analysis

We present a flood frequency analysis plot in Figure 5. This figure relates the magnitude of a given flow event to its expected return period, given in years, i.e. the number of years expected between floods of a specified size. The line of best fit, in black, was created using the method of moments; the red and blue lines denote 95% confidence limits. A flow event of $227 \text{ m}^3/\text{s}$ may be expected approximately once every other year. The largest flow reported during the historical record of 2009-2021 measured approximately $739 \text{ m}^3/\text{s}$.

Over the course of the study period, the EFBR experienced at least 18 distinct high-flow events of at least $28 \text{ m}^3/\text{s}$, or approximately 1000 cfs, totaling 40 distinct days with elevated water levels. This exceeded the expected quantity given a return interval of slightly over 1.1 years for that rate of discharge. Historical data up to the point of our study suggested these flows may be expected to occur slightly over once per year. Out of these high-flow events, 10 were at least $56 \text{ m}^3/\text{s}$ in magnitude, more uncommon as predicted yet still more frequent than their recurrence interval of approximately 1.3 years.

Seasonality

Historically, minimum flows at the EFBR occur during July and August and maximum flows during March and April (Figures 6, 7). Over the course of the historic dataset, July and August were near-equal in minimum median flow at approximately $0.28 \text{ m}^3/\text{s}$ to 0.37

m^3/s , while March and April were near-equal in maximum median flow at approximately $3.68 \text{ m}^3/\text{s}$ to $3.82 \text{ m}^3/\text{s}$. The exact timing of these minima and maxima was variable, however, with abnormally dry and wet months occurring well outside normal times in some years on record.

To similarly present and consider flow data during the sampling period, we calculated each day's 10th, 50th, and 90th percentile flow values, here presented in Figure 6. This shows a distinct wet season, from approximately mid-September through early May, although the “dry” season is still prone to a small number of high flow events and vice versa. In this figure, variability over the course of the hydrologic year is readily apparent, while Figure 6, calculated from the same data but on a coarser time scale, highlights broader behavior over time.

As an assessment of variability in the flow regime, we calculated the coefficient of variation for discharge per month; a greater value represented more heterogeneous flow conditions. This analysis yielded October, August, and July as months with the most heterogeneous flow with respective coefficients of variation of approximately 803%, 545%, and 544%. March, May, and February were least heterogeneous with respective coefficients of approximately 141%, 157%, and 173%.

Inter-annual variation

Although the EFBR experienced more high-flow events during our study period than a flood frequency analysis suggested it might, the study was conducted in a slightly dry period as median and maximum flows were lower, respectively slightly and greatly, than those on historical record.

Over the historical record, EFBR mean and median annual flows were $3.68 \text{ m}^3/\text{s}$ and $1.1 \text{ m}^3/\text{s}$, respectively. Mean annual flow ranged from approximately $1.95 \text{ m}^3/\text{s}$ in 2012 to $5.89 \text{ m}^3/\text{s}$ in 2009, while median annual flow ranged from approximately $0.54 \text{ m}^3/\text{s}$ in 2017 to $1.61 \text{ m}^3/\text{s}$ in 2020. Mean flows in each year of our study, 2017 through 2019, were, respectively $3.80 \text{ m}^3/\text{s}$, $3.40 \text{ m}^3/\text{s}$, and $4.20 \text{ m}^3/\text{s}$, while median flows for each respective year were $0.54 \text{ m}^3/\text{s}$, $0.82 \text{ m}^3/\text{s}$, and $1.29 \text{ m}^3/\text{s}$.

2017 stood out as a year with particularly low median annual flow but also the highest discharge on record. This is reflected in 2017's discharge having the greatest coefficient of variation (635%), well above 2011's next-highest of 356% and 2009's 334%. 2010, 2013, and 2014 were the least heterogeneous years by flow, with coefficients of variation of, respectively, 179%, 180%, and 183%. Since 2017, flows have generally been increasing; 2018-2021 saw successive increases in median annual discharge. Though 2022 was not yet complete upon most-recent data retrieval, year-to-date data further indicate relatively higher flows than previous years on record. That said, maximum annual flows present a different picture: over the course of available data, the EFBR has experienced two multiyear swings in which maximum flows were well above 170 m³/s before dropping to be below. In addition to having the highest flow on record, 2017 marked the end of the most recent higher-flow period; maximum flow in 2018 was approximately 100 m³/s. Maximum flow continued to rise until 2021, a local minimum, and has resumed a slight increase within this lower-flow period. These data help provide a broader perspective on the lower-than-usual flows experienced during the study period compared to the historical record.

These data are further presented in Figure 7 where monthly median flow values were calculated for each year and plotted. Though of more coarse resolution, this figure highlights how each year within the river may come with its own flow regime.

3.1.2 Collected physicochemical data

Across our sampling events, we calculated mean (\pm standard deviation) water temperature to be 14.63 ± 5.89 °C and dissolved oxygen concentrations of 8.85 ± 1.46 mg/L. Mean specific conductivity was 137.65 ± 37.61 μ s/cm, and turbidity was 9.70 ± 8.63 NTU. Water flow velocity was, on average, 0.31 ± 0.24 m/s, while water depth was 28.68 ± 13.99 cm. The calculated average of the median particle size recorded was 67.41 ± 32.67 μ m, while the substrate CV came out to be $77.91 \pm 15.89\%$.

More specifically, water temperature below the dam ranged from 7.3 °C to 24.6 °C; these values were recorded in, respectively, F18 and F17, both in R1. The EFBR typically held well oxygenated water (DO above 6.5 mg/L, 90% of measurements above 7.5 mg/L),

specific conductivity values from 76.0 $\mu\text{s}/\text{cm}$ to 179.0 $\mu\text{s}/\text{cm}$, and turbidity values between 0.0 and 30.8 NTU. Random sampling measures recorded standstill flow velocities, measured in R1, and values up to 1.16 m/s; water depth ranged from 5.0 cm to 70.0 cm. Raw substrate particle sizes measured ranged from the minimum measurable by our gravelometer of 2 mm up to sizes greater than the maximum measurable size of 180, defined to be 250 mm; median substrate sizes per riffle ranged from 22.6 mm to 180 mm. The substrate CV ranged from approximately 50% to 110%.

The abiotic environment of the East Fork Black River displayed longitudinal and temporal trends across most variables (Tables 5 & 6). Water temperature, dissolved oxygen, and conductivity displayed little longitudinal variation but were significantly different across sampling events (Table 6). Turbidity also varied both longitudinally and temporally but did so significantly in both cases. In contrast, temperature, DO, conductivity, and turbidity strongly and significantly varied across space; flow velocity, depth, median particle size, and the substrate size CV were all weakly but significantly varying across sampling events.

Our PCA indicated a number of relationships between our measured parameters. The first and second axes of PCA accounted for 30.8% and 22.3%, respectively, of the variance associated to the environmental parameters (Figure 14). The first axis (PC1) was positively correlated with downstream distance, substrate size CV and water depth and inversely aligned with median particle size and flow velocity. The second axis (PC2) was positively correlated with turbidity and negatively correlated with the percentage DO and specific conductivity. We interpret the first axis as a longitudinal gradient realized through substrate as, in absolute terms, substrate size CV, median particle size, water depth, and flow velocity were most associated with it. Conductivity, turbidity, DO, and median particle size most strongly associated with the second axis, interpreted as predominantly seasonal or hydrologic effects.

Longitudinal patterns

Water flow velocity, depth, the median particle size, and the substrate CV were all, on average, more spatially heterogeneous than temporal. Table 5 lists the results of the

following Welch's ANOVAs, while Table 9 lists the measured coefficients of variation of our physicochemical parameters. In Figure 15, we present our physicochemical parameters as functions of downstream distance, separated out by sampling event.

Mean water temperature ranged from 13.8 °C in R1 to 14.6 °C in R5. In aggregate, water temperature increased with downstream distance up to R4, with a mean of 15.5 °C, although not enough to create statistical differences across riffles. This relationship between downstream distance and water temperature was furthermore inconsistent across sampling events; S19, for example, monotonically increased in temperature with distance up to R4, while F17 decreased from R1 to R3, rose slightly in R4, and dropped in R5 (Table 5). Even more similar in temperatures, S18 and F18, were roughly inverse along the longitudinal gradient. The reach below the dam was well oxygenated throughout all the riffles with mean DO concentrations ranging between 8.65 mg/L at riffle 1 and 9.34 mg/L at R5 (Table 6). Although it appears that there is a longitudinal gradient, the differences between riffles were not statistically significant.

Specific conductivity displayed a minute longitudinal trend, with mean values strictly increasing from 134 $\mu\text{s}/\text{cm}$ in R1 to 140 $\mu\text{s}/\text{cm}$ in R5, but spatial differences were not significant. Differences between mean turbidity across riffles were significant (Welch's ANOVA, $F_{4, 94.889} = 5.6902$, $p < 0.001$), and there was another clear separation between the two more-upstream riffles and those more downstream, with upstream riffles more turbid on average.

Mean flow velocity was lower in R1 than further-downstream riffles at just 0.22 m/s, but R5 stood out as a near nonflow site with a mean velocity of 0.10 m/s, this despite no pool being present. Intermediate riffles' mean velocity was more homogenous, ranging from 0.39 m/s in R3 to 0.42 m/s in R4. Though Welch's ANOVA indicated mean flow velocity was significantly different across riffles ($F_{4, 89.068} = 46.606$, $p < 0.001$), this was only true if R1 and R5 data were included; intermediate riffles' mean flow velocities were not significantly different from each other. Mean water depth strictly decreased with downstream distance until arriving to R5, which was approaching twice as deep as R1.

Differences in mean water depth were significant across riffles regardless of the inclusion of either end riffle (with all riffles, Welch's ANOVA, $F_{4, 96.74} = 53.952$, $p < 0.001$).

The mean of median substrate size generally decreased with downstream distance from the dam. This relationship failed from R3 to R4; this mean in R3 was 52.1 mm but 59.2 mm in R4 before declining further to 36.2 mm in R5. Maximum median size was found in R1 at 112.5 mm. The differences between mean particle size across riffles were significant (Welch's ANOVA, $F_{4, 95.419} = 89.793$, $p < 0.001$). Differences between mean substrate CV were also significantly different across riffles (Welch's ANOVA, $F_{4, 97.154} = 130.45$, $p < 0.001$). The mean substrate size CV ranged from 62.6% in R2 to 101.5% in R5. Absolute minimum and maximum values were, respectively, calculated from R2 and R5 substrate data, with values of 50.7% and 110.1%. Of particular note is that while the coefficient of variation between R1 and R2 decreased for all samplings but F17, S18 stood out as the sampling with unusual substrate conditions: while R1 was comparable in its coefficient of variation to F18 and S19, the CV plunged to just over 50% in R2, over 10 percentage points lower than the next-lowest, but skyrocketed to nearly 100% in R3, over 20 percentage points higher than the next-highest. Though it declined to R4 and rose in R5 like all other sampling events, it remained well higher in R4 than the other riffles, still over 15 percentage points greater than the next-highest.

Temporal variability

Water temperature, DO concentration, conductivity, and turbidity were all, on average, more temporally heterogeneous than they were spatially. Table 6 lists the results of the following Welch's ANOVAs, while Table 9 lists the measured coefficients of variation of our physicochemical parameters. In Figure 15, we present our physicochemical parameters as functions of downstream distance, with each sampling event plotted individually.

Water temperature below the dam ranged from 7.3 °C to 24.6 °C; these values were recorded in, respectively, F18 and F17, both in R1. Differences in temperature across sampling events were significant (Welch's ANOVA $F_{3, 106.4} = 1537$, $p < 0.001$). DO concentrations showed significant differences across sampling events (Welch's ANOVA

$F_{3, 90.019} = 1692, p < 0.001$). Measured values ranged from 6.57 mg/L in F17 to 11.34 mg/L in F18, while mean values per sampling event ranged from approximately 7.5 mg/L to 11.2 mg/L in the same respective seasons. DO concentrations varied differently across riffles in each sampling event. F17, notably, had a decrease in concentration of 1.52 mg/L from R1 to R2, while in all other events the concentration increased between the same riffles, however whether that was followed by another increase or a slight decrease was not generalizable.

Temporal differences in specific conductivity were significant (Welch's ANOVA, $F_{3, 105.89} = 6898, p < 0.001$) and held some seasonal aspect: mean values were highest and comparable in value in F17 and F18 (170 $\mu\text{s/cm}$ and 174 $\mu\text{s/cm}$, respectively), and lower in S18 and S19 (124 $\mu\text{s/cm}$ and 83 $\mu\text{s/cm}$, respectively). These spring sampling events were not comparably similar in values as earlier events' were; S18 stood more as intermediate between a high-valued pair of events and a lone low-valued event. This temporal trend was somewhat reversed with turbidity. S18 and S19 were high-valued, at 20.7 NTU and 12.5 NTU, respectively, while F17 and F18 were low at, respectively, 3.6 NTU and 2.0 NTU. Differences between these means were significant under Welch's ANOVA ($F_{3, 82.353} = 469, p < 0.001$).

Mean flow velocities were significantly different across sampling events (Welch's ANOVA, $F_{3, 107.1} = 9.272, p < 0.0005$). F17 held notably reduced velocity compared to the other sampling events, and significant differences between riffles were not maintained without that sampling event. Mean flow velocities ranged from approximately 0.23 m/s in F17 to 0.47 m/s in S18. Mean water depth was significantly different across sampling events (Welch's ANOVA, $F_{3, 86.574} = 6.8784, p < 0.001$). Minimum mean depth was in F17 at 20.08 cm, and mean depth increased in F18 and further into S19 at 23.35 cm, but S18 stood out as an unusually deep sampling event with mean depth of 30.88 cm; if this sampling event's mean depth was excluded, Welch's ANOVA indicated no significant difference between mean depths across sampling events.

The differences between mean median particle size across sampling events was significant under Welch's ANOVA ($F_{3, 105.13} = 6.01, p < 0.05$). F17 held the largest mean

median size at approximately 81.5 mm, while S19 held the lowest mean median size at 57.1 mm. S18 was comparably low at 60.4 mm. Differences between mean substrate CV were significant (Welch's ANOVA, $F_{3, 104.98} = 3.081$, $p < 0.05$). The range of substrate size CV was 50.7% to 110%, both in S18, but mean CV was more constrained, ranging from 74.4% in F18 to 83.9% in S18.

Spatiotemporal analysis/interactions

To gauge whether physicochemical parameters were more variable across riffles or across sampling events, we calculated each parameter's mean coefficient of variation across the two groupings. Thus when grouped by sampling event, calculated values reflect across-riffle variation, and when grouped by riffle, values reflect variation across sampling events. Sampling event coefficients of variation were greater for water temperature, dissolved oxygen content, conductivity, and turbidity; across-riffle coefficients of variation were greater for water flow velocity, water depth, median substrate particle size, and the particle size coefficient of variation. Values were somewhat comparable for water depth (sampling events: 41.7%, riffles: 48.5%), flow velocity (sampling events: 60.4%, riffles: 76.9%), and particle size coefficient of variation (sampling events: 12.6%, riffles: 18.9%).

3.2 Macroinvertebrate community

3.2.1 Community structure

We collected a total of 33371 individuals representing 49 taxa, most family-level, over the duration of the study. The ten most-abundant taxa were, in decreasing order, heptageniids, isonychiids, chironomids, elmids, hydropterygids, philopotamids, caenids, perlids, leptohyphids, and baetids.

The majority of all taxa were poorly represented, with only twelve reaching over 1% of the total abundance. Just five taxa constituted over two thirds of all identified macroinvertebrates: heptageniid mayflies (22.4%), isonychiid mayflies (11.8%), chironomid flies (11.7%), elmid beetles (11.4%), and hydropterygid caddisflies (11.2%). Mayflies (Ephemeroptera) accounted for nearly half of all individuals, with caddisflies

(Trichoptera), beetles (Coleoptera), and true flies (Diptera) constituting most of the remaining individuals but for a small remainder split between stoneflies (Plecoptera) and other taxa, insect and non-insect alike (e.g. Megaloptera, Platyhelminthes).

Longitudinal patterns

Macroinvertebrates generally increased in abundance with downstream distance up until R5 (Table 7, Figure 8). In total, riffles 3 and 4 held over half of all identified macroinvertebrates, with nearly 18,800 individuals against the other riffles' 14,600. F17 had a roughly even distribution across riffles, and approximately 40% of all macroinvertebrates, largely due to a relatively heavy presence of chironomid midges in R1. S19 had pronouncedly fewer macroinvertebrates in riffles 2 and 3 in comparison to riffles 1 and 4 and in comparison to abundance counts riffles 2 and 3 had in earlier sampling events, this in part due to a paltry number of mayflies. When all sampling events and riffles are considered in aggregate, we observed an increase in mean richness with increasing distance until R5 (Table 7).

Mean taxa richness increased from 9.4 in R1 to 13.0 in R4 before declining to 7.6 in R5. The maximum richness of a sample was reported in R3 with 21 identified taxa. EPT richness increased from 5.1 in R1 to 8.1 in R4 before too declining to 4.5 in R5 (Table 7). Shannon diversity was maximized in R4 and minimized in R1 and R5, both at 1.40. When individual sampling events were considered, it is especially apparent that no intermediate riffle shone as most diverse, but rather that R1 and R5 stood out as least-diverse: these two paled against R2-4, which had Shannon diversity indices of, respectively, 1.85, 1.81, and 1.92. The mean Missouri biotic index score varied slightly from riffle to riffle, with a minimum of 4.58 in R4 and a maximum of 4.80 in R5.

While Ephemeroptera collectively increased in abundance with downstream distance (Figure 8), this trend was driven primarily by heptageniids, caenids, and isonychiids. Leptohiphids were more consistent across riffles save for a notable decrease in R3. R1 was poorer in baetids who were most abundant on average in R2. Leptophlebiids consistently diminished with distance until a resurgence in R5. Ephemerids generally

increased in abundance with distance, but their relative abundance was not meaningfully influential on overall trends, similar to the remaining mayfly taxa.

Perlids were essentially the only plecopteran family identified with negligible occurrences of nemourids, taeniopterygids, perlodids, and chloroperlids; while generally heavily reduced in number in R1 and R5, there were seasonal differences in their exact distribution with a notable spike in abundance in R2 during the F18 sampling. In that sampling event, stoneflies reversed their otherwise general trend of increasing with downstream distance until R5.

Trichoptera, ignoring their near absence in R5, were most abundant in R3 and over 1.5 as abundant in the two downstream riffles as they were in the two upstream. This distribution too was influenced by relatively few taxa within the group; both philopotamids and hydropsychids were generally more abundant downstream than upstream. Philopotamids were nearly absent from R1 and had more consistent numbers downstream; hydropsychids most abundant in R3 and more equally abundant between the remaining sites. Hydroptilids were nearly absent from R2, polycentropodids entirely absent from R3, heliocopsychids almost exclusively located in R4 and absent from R2, and leptocerids predominantly found in R1.

True flies, the dipterans, were dominated by chironomid midges; R1 hosted nearly half the total count and R2 less than 10%, beyond which their numbers gradually increased with distance until sharply falling in R5. The ratio of chironomids to other taxa is shown in Figure 11. Elmids were the most abundant beetles and were more common in R3 and R4 than elsewhere, and while their numbers in R5 were lower than other riffles, they were comparable to those of R1 and R2. Water pennies, the psephenids, in contrast increased in abundance with increased distance from R1 to R4; R5 had slightly more on average than R1 did. Coenagrionid damselflies, an abundant predator, were heavily reduced in R1, most abundant in R2, and declined in abundance with further downstream distance. Megaloptera, represented by corydalids, were in contrast over 2.5 times more abundant in R2 and R3 than they were in R1, R4, and R5.

Seasonal patterns

In aggregate, macroinvertebrates were over twice as abundant in fall sampling events than spring events with a total of 13,300 and 9,900 individuals in F17 and F18, respectively, and 5,600 and 4,600 in S18 and S19 (Table 8, Figure 9). In contrast, total macroinvertebrate richness decreased with each sampling event; in temporal order, we identified 42 (F17), 33 (S18), 31 (F18), and 27 (S19) unique taxa. Mean richness was still greater in fall than spring samples, however, with respective values of approximately 12.2 and 8.8. Shannon diversity followed suit, with mean respective values of approximately 1.8 and 1.6. Mean MOBI scores were marginally lower in spring samples than they were fall, with respective values of approximately 4.67 and 4.69.

Of our ten most-abundant taxa, all were most abundant in our fall sampling events, typically with at least twice the abundance as the respective taxa had in spring sampling events. One exception was elmids and perlids, which were closest in ratio between the two seasons, while caenids and baetids were over seven times as abundant in fall samples than spring. F17 was the most-common sampling event to yield the greatest abundance for our most-abundant taxa. Isonychiids, hydropsychids, philopotamids, and perlids were exceptions being most abundant in F18; none of the top ten were most abundant in either spring sampling event. Though exact distributions varied, the abundance of these taxa from R1 to R5 was generally similar across sampling events. The chironomids, hydropsychids, and caenids were notable exceptions; the caenids were massively most abundant in our F17 sampling and displayed clear increase in numbers with downstream distance. Chironomids owed much of their total abundance to a particularly fruitful F17 sampling in R1. Hydropsychids similarly were much more abundant in R3 during the F18 sampling than elsewhere or during other events.

Spatiotemporal interactions

By comparing coefficients of variation for each taxon when first grouped by riffle and then by sampling event, as we did with physical variables, we assessed whether each taxon was more variable spatially or temporally. Taxa with under 50 total identified individuals were not included as the calculation used returned equal variation slightly

below this threshold. Overall, twice as many taxa were more variable across riffles than sampling events. The five taxa most variable across riffles were, in order, Gomphidae, Hydrachnidia, Leptoceridae, Baetidae, and Leptoheptageniidae, ranging in CV from approximately 137% to 73%. Those most variable across sampling events were, in order, Helicopsychidae, Philopotamidae, Corydalidae, Hydropsychidae, and Isonychiidae, ranging in CV from approximately 113% to 63%. Heptageniidae, Caenidae, Hydroptilidae, and Elmidae each had a difference of CVs with absolute value of at most 10%, indicating relatively even variability across riffles and sampling events. Richness, EPT richness, and MOBI scores were all somewhat more variable across sampling events than across riffles; Shannon diversity was slightly more variable across riffles than across sampling events.

3.2.2 Functional feeding groups

Insect abundance was dominated by collector-filterers (33%), collector-gatherers (26%), and scrapers (25%); predators constituted an additional 15% of the total, while piercers (<0.5%, represented only by the hydroptilid caddisflies) and shredders (<0.1%) completed the community.

Longitudinal patterns

Shredders were found only in R1 and R3 while all other FFGs were dispersed across all riffles. Collector-gatherers were more abundant in R1 and R4 than the other riffles. Piercers were nearly absent from R2 and R5 but somewhat equally dispersed through the other riffles. Collector-filterers increased with downstream distance in approximately linear fashion from R2 to R4; R1 had less than a third the total abundance than R2, and the FFG was nearly absent in R5. Scrapers slightly declined from R1 to R2 but generally increased in abundance with increased downstream distance before dropping to their lowest numbers in R5. Predators had the most linear relationship with distance, also strictly increasing with downstream distance until crashing in number in R5. These data are presented in Figure 10.

Again grouping by riffle, then by sampling event, and calculating coefficients of variation for each FFG when grouped by one of the two variables, we considered spatial versus temporal variation within FFGs. Shredders, scrapers, and piercers were each more variable across riffles, while collector-gatherers, collector-filterers, and predators were more variable across sampling events. Only shredders (net CV of 190%) and collector-filterers (net CV of -32%) were more than 10 percentage points different in this spatiotemporal comparison of variability.

Seasonal patterns

Shredders and piercers were absent from S19 samples, but the remaining FFGs were represented in all sampling events (Figure 10). Collector-gatherers and -filterers were more abundant in fall sampling events than spring, while scrapers and predators were more evenly distributed across events. While collector-gatherers were dramatically more abundant in F17 than F18, collector-filterers displayed the opposite pattern. Scrapers also had a notable reduction in numbers from S18 to S19, with just half the number found in the latter sampling.

3.3 Macroinvertebrate community response to the environment

3.3.1 Influence of individual variables (taxonomic community)

Recorded water temperatures were roughly equally split between those cooler (approximately 7 °C to 10 °C) and those warmer (approximately 15 °C to 25 °C), as were total macroinvertebrate counts between those temperature groups. Mean Shannon diversity was higher in warmer waters (Shannon index = 1.85) than cooler (Shannon index = 1.52). Most individual taxa, however, displayed temperature preferences. Most mayflies, except the heptageniids and isonychiids, were dramatically more abundant in warmer waters. Heptageniids were slightly more abundant in cooler waters, while isonychiids were over twice so. Stoneflies were approximately equally split between the temperature groups, although the largest total count of stoneflies occurred in cooler waters. Hydropsychid and helicopsychid caddisflies were substantially more abundant in cooler waters, philopotamids and hydroptilids slightly so, and both leptocerids and

polycentripodids were more abundant in warmer waters. Chironomids were over twice as abundant in warmer waters than cooler and had a much greater relative proportion to the remaining community in such conditions. Elmids beetles were approximately equally divided between water temperatures, and psephenids were more abundant in warmer waters, as were all Odonates and megalopterans. Mean richness, EPT richness, and Shannon diversity were all greater in warmer temperatures than cooler. The mean MOBI score was slightly lower in water cooler than 15 °C.

Dissolved oxygen was similar, with high abundance noted at our lowest recorded values, slightly below median values, and around maximum values, with low abundances noted around mean DO concentrations. There was seasonal variation in exactly how total abundance responded to each of these parameters; for example, there was little change in abundance with increasing DO in F17 but a sharp increase of abundance with a lesser increase of DO in F18. In many cases, conditions across sampling events only slightly, if at all, overlapped; flow velocity, water depth, and, to lesser extents, both substrate metrics had considerable overlap, allowing similarities/differences across sampling events to be more apparent.

Macroinvertebrate abundance, richness, and Shannon diversity each increased with increasing conductivities, and the same metrics generally decreased with increasing turbidity. Maximum concentrations of macroinvertebrates and the maximum richness and diversity measures were found among conductivities ranging from approximately 160 to 180 $\mu\text{s}/\text{cm}$ and turbidities ranging from 0 NTU to approximately 15 NTU.

Macroinvertebrate abundance by taxon varied considerably across flow velocity conditions. In general, mayflies were most abundant up to flow velocities of approximately 0.5 m/s; stoneflies were most abundant among conditions ranging from approximately 0.4 to 0.6 m/s but had considerable numbers outside this range; caddisflies generally increased with increasing flow velocities and had a massive spike of identified individuals from 0.7 m/s conditions. Flies generally decreased with increasing flow velocities and had their own massive spike of representatives around 0.1 m/s; a smaller local maximum occurred at 0.7 m/s. Beetle abundance was primarily maximized at

approximately 0.2 m/s and secondarily at 0.5 m/s; between and outside of these maxima, abundance generally decreased. Overall macroinvertebrate abundance increased with increasing flow velocities until an approximate mark of 0.6 m/s, after which point abundance declined. Richness and EPT richness were both, on average, greater in flow velocities below the median of 0.31 m/s, with a greater difference in richness values among all macroinvertebrates than just EPT taxa; Shannon diversity values were even closer across the median flow value but greater in faster waters than slower. EPT richness was maximized around 0.9 m/s flow velocities, overall richness around 0.4 m/s, and Shannon diversity slightly under 0.2 m/s. The mean MOBI score was maximized around a flow velocity of 0.5 m/s, but minimum values were recorded across a range of just over 0.0 m/s to approximately 0.5 m/s.

Macroinvertebrates were generally most abundant among conditions with median particle sizes ranging from 45 mm to 64 mm; abundance increased with increasing median particle size to this range, beyond which it decreased with the exception of particularly high abundance at 200 mm due to the particularly high abundance in R1 during the F17 event (Figure 12). Notable exceptions include the Chironomidae, Hydroptilidae, Leptoceridae, Leptohiphidae, Leptophlebiidae, and Pyralidae, for whom the data indicated substantial pluralities or otherwise large proportions of total abundance clustered around median particle sizes of 180 mm. Ephemerid mayflies and hydroptilid caddisflies were unique in being most abundant where median particle sizes were smallest. Specific responses to increasing median particle size differed by taxon. Focusing on the 45 mm to 64 mm range, there were two dominant patterns of abundance: twelve taxa strictly increased with increasing median particle size, and ten decreased from 45 mm to 54.5 mm before well increasing at 64 mm, forming a lopsided U when plotted. The Gammaridae were nearly exclusively found where median substrate size was 90 mm, and the Gomphidae around 64 mm. General macroinvertebrate and EPT richnesses were greatest at a median substrate size of 54.5 mm. Shannon diversity was maximized at median substrate size of 54.5 mm and was slightly lower at 64 mm. Mean richness, EPT richness, and Shannon diversity were lowest at a median substrate size of 32 mm. The mean MOBI score was lowest at 32 mm and highest at 22.6 mm. Like with

total abundance, the relationships between these macroinvertebrate metrics may be viewed in Figure 12.

When considering the coefficient of variation of substrate particle size, we again noticed general trends most macroinvertebrate abundances followed. Coefficients from about 65% to 75% captured most of all identified macroinvertebrates, and specific relationships were more individual than with median particle size. In some cases, as they were with the baetids and psephenids, almost all individuals were associated with an even tighter subset of that range; many others, including most mayflies and the hydropsychids, philopotamids, and polycentripodids, were more distributed across all observed coefficients of variation but with distinct concentrations within the 65% to 75% range; others yet had distinct pluralities at values around 50%, 90%, and 100%. Notable others include the heptageniids, which were generally most abundant around 70% to 75% and tailed off with substantial numbers toward extreme coefficients; the chironomids, with a large plurality around 57%; the elmids, well evenly dispersed from extreme to extreme; the hydroptilids, nearly absent entirely from the range of coefficients capturing most of most other taxa, seemingly preferring CVs of approximately 60%, 90%, and 100%; and the pyralids, who, despite being low in abundance, were nearly exclusively found around a CV of approximately 60%. Richness and EPT richness were maximized and somewhat clustered around 75%; interestingly, the lowest values for both metrics were nearly minimized at approximately 70%, but the true minima were at 110%. Shannon diversity was similarly reaching its near-lowest and highest values within the narrow range of approximately 70% to 75%. The maximum mean MOBI scores were found among a range of CV values from about 65% to 100%, with most restricted to the 65% to 75% subrange; minimum mean scores were shifted slightly lower in range. Absolute minimum and maximum MOBI scores were recorded at CVs of, respectively about 58% and 75%. Keeping in mind the relationships observed with median particle size, Figure 13 offers heatmaps which display our macroinvertebrate metrics as functions of median particle size and the particle size CV.

3.3.2 Influence of individual variables (FFGs)

Functional feeding groups were split by water flow velocity. By mean abundance, collector-gatherers, scrapers, and predators were generally most abundant around low flow velocities under 0.4 m/s and decreased with increasing velocity. Collector-filterers were abundant at higher velocities and only declined when flows began exceeding 0.6 m/s. Most of their numbers were concentrated at flow velocities below 0.6 m/s, but other FFGs did not display such relatively large numbers around small velocity ranges. Piercers vaguely increased in abundance to 0.4 m/s and decreased beyond, but they were in total confoundingly low in abundance. Shredders were approximately equally split between near-0 flow velocities and those around 0.6 m/s, but their numbers were even more poor.

All functional feeding groups were most abundant around median particle sizes of 45 mm to 64 mm except for shredders. The abundances of collector-gatherers, collector-filterers, scrapers, and predators furthermore had clear pluralities at the 64 mm size class, though the collector-gatherers and predators also had large spikes in numbers at 180 mm. Shredders and piercers were both most common at that size class, while collector-filterers and scrapers were heavily reduced in number at that size range.

Similar to macroinvertebrate taxonomic groupings, there were distinct increases in abundance around an intermediate subset of substrate CV values, here approximately 65% to 75%. Collector-filterers, collector-gatherers, predators, and scrapers, to a lesser extent, all displayed this concentration, although they too were commonly found among other conditions. Collector-gatherers had a distinct plurality around the same values, though they were otherwise well dispersed into values to approximately 90%. Piercers were most common at approximately 90%.

3.3.3 Individual variables and sampling events

We examined the abundances of our ten most-abundant taxa against our physicochemical parameters, split by sampling event; for example, we examined how heptageniid abundance related to water temperature in F17, S18, F18, and S19 to see what, if any, further influence the conditions of a particular sampling event may have had on the

taxon's abundance as it related to a particular parameter. This analysis yielded few notable additional details.

Temperature, serving as a pseudo-proxy for sampling event, yielded few remarkable relationships, although S18 and F18 occurring within approximately the same temperature range allowed us to make some comparisons: Median heptageniid abundance decreased with temperatures beyond 8.7 °C in F18 but increased with temperatures above the same value in S18; elmids, displayed a similar yet less pronounced relationship. Relationships with dissolved oxygen were similarly entangled with sampling events, but F18 stood separate from the other events with much greater concentrations, and F17 was somewhat similarly isolated with a particularly low recorded value. Responses to increasing DO concentrations were similar between S18 and S19 sampling events, while F17 median abundances appeared to follow the same general pattern but shifted toward the lower end of the DO scale: in most events, maximum median abundance was not with the highest DO concentrations of a given season but among those values found in R3.

Specific conductivity in F17 and F18 overlapped in range, and relationships between taxa abundance and conductivity were similar for most of the ten most-abundant taxa between those two events. Caenids were the notable exception, where abundance sharply increased with increases in conductivity in the F17 sampling event. Abundances showed no clear additional relationships with turbidity when sampling event was considered.

Abundance as it related to water flow velocity showed no particular distinction between sampling events, as did it for water depth. Some samples stood out in abundance compared to others but did not appear specifically attributable to either parameter or sampling event.

Abundance responded to changes in median particle size similarly across sampling events for most of our most-abundant taxa. F17 held the lone riffle with a median particle size of 180 mm and was thus more difficult to consider among the range otherwise constrained between 45 mm and 90 mm. Median heptageniid and elmids abundances notably increased with increasing particle size in S19 while decreasing in other sampling events; perlids behaved similarly in F18. The other taxa otherwise did not display notable

differences in their responses. Substrate CV had overlap from approximately 62% to 75%, and macroinvertebrate abundance varied in relation to the parameter between sampling events. Heptageniids were generally similar in relation, while caenids again stood out in their F17 sampling's abundance. Perlids, across the 62%-75% range, increased in F17 and S18 with increasing CV, while they displayed unimodal relationships in F18 and S19.

3.3.4 *Mantel tests*

Partial Mantel tests between environmental variables and macroinvertebrate abundances indicated often significant yet moderately weak positive relationships (Table 10); i.e. more-similar macroinvertebrate communities also possessed more similar in-stream conditions. In both cases running these tests against macroinvertebrates in their standard community and those grouped by FFG, the environment as a whole was most-strongly serving to drive difference in the macroinvertebrate community, but we may examine individual physicochemical parameters for their particular influences in these and a third test.

Specifically, the results indicated that difference in macroinvertebrate communities was most strongly related, out of individual environmental variables, to conductivity (Mantel statistic of 0.18), the median substrate particle size (0.17), and the substrate size CV (0.16). Stream flow velocity was most weakly related to macroinvertebrate community structure (Mantel statistic 0.06), followed by DO concentration (0.10), water temperature (0.11), and depth (0.12). All parameters were significant at $p < 0.05$.

Running partial Mantel tests on the same variables against richness yielded a different set of results: water depth was the primary driver with a Mantel statistic of 0.18, exceeding that of the environment as a whole (0.08), and was followed by water temperature (0.12) and turbidity (0.11). In contrast to the previous tests, median particle size was not a significant driver, nor was flow velocity or DO concentration; all variables save the three listed drivers were below 0.06 in their Mantel statistics. The substrate size CV and conductivity were the weakest significant drivers with Mantel statistics of, respectively, 0.06 and 0.05.

The same tests, when run on the same macroinvertebrate community but grouped by Shannon diversity, yielded a different set of relationships. Water temperature was the primary driver (Mantel statistic 0.11), followed by median substrate particle size (0.09) and water depth (0.08). These were significant drivers, and while their effects were weak, they were stronger than that of the environment as a whole (0.04). The substrate size CV was not a significant driver.

Finally, when grouping the macroinvertebrates by FFG and running the same tests, we yielded a fourth and again distinct set of results. The conductivity (Mantel statistic 0.15), DO concentration (0.14), and depth (0.13) were the primary individual drivers of functional differences; however, the environment as a whole (0.20) was again a stronger influence. All variables were significant drivers, and here we saw the substrate size CV exceed the median particle size in influence (respective statistics of 0.12 and 0.09).

4. DISCUSSION

4.1.1 Overview

We analyzed the hydrology, physicochemical environment, and macroinvertebrate community of the East Fork Black River downstream of the Lower Taum Sauk Reservoir. Hydrologic data was collected by USGS gage 07061290 positioned immediately downstream of the reservoir; physicochemical and macroinvertebrate data were collected from five riffles each in four sampling events. We observed hydrology consistent with regional expectations, a physicochemical environment that displayed both longitudinal and temporal trends and differences, and a macroinvertebrate community that responded to its environment.

4.1.2 Hydrology

We observed that the EFBR held a fairly normal flow regime during our study period compared to data recorded over a 13-year historical record. Our median study flow was approximately equal to median historical flow, and minimum and maximum study flows were strictly within the range of minimum and maximum historical flows; maximum

study flows were especially less comparable to historical maximums. Median flows from 2017 to 2019 were less variable and closer to median historical flow than individual years prior. While occasionally flashy, our study period and the historic record pointed to more gradual change to flow as the norm. There were noted dry and wet seasons with most minimum flows occurring in August and most maximum flows occurring in March and April. Compared to conclusions from our flood frequency analysis, our study period experienced more higher-flow events than expected. Considering median, minimum, and maximum flows of the study period against historical data, we may conclude that the EFBR held less overall flow and concentrated flow into more frequent high-flow events.

(Leasure et al., 2016) determined most streams of the St. Francois Mountains and broader Ozark Highlands to be of a similar hydrologic profile, with drainage basins between 11 km² and 3237 km² (the EFBR drains 250 km²) and mean daily flows from 0.12 m³/s to 25.63 m³/s, while the EFBR experienced mean daily flows typically well below 25.63 m³/s despite minimum and maximum mean daily flows a factor of approximately 10 less and greater than, respectively, those values. In total, the USGS gage's historical data well places the EFBR within the bounds of the "groundwater flashy" hydrologic regime this team identified: mean daily flows ranging from 4.3 cfs to 905 cfs, perennial but occasionally severely reduced flow compared to daily means, and watersheds ranging from 11 km² to 3237 km².

We would benefit from a longer historical data set and flow data collected from multiple points downstream of the dam in order to better understand longitudinal trends in flow, to better record those trends over time, and to be able to relate such trends to our physicochemical and macroinvertebrate data in the absence of discrete flow measurements. We operate under the assumption that discharge downstream of R1, which is approximately collocated with the USGS gage on the EFBR, is insignificantly different from discharge at R1/the gage. We do not have, however, complete transect width or depth data at any of our sampled riffles, and our assumption contains implicit assumptions about the negligibility of intermittent or ephemeral tributary inputs and about losses/gains in flow due to hyporheic waters or groundwater. There is at least one spring noted in proximity to our study riffles, and the karst bedrock underlying the EFBR

downstream of the dam allows for greater subsurface influence than the igneous bedrock further upstream. We would furthermore benefit from multiple and/or more complete assessments of Ozark stream hydrologic regimes. Given the diversity in topography, bedrock, and subsequent interaction between surface and subsurface waters, there is a diversity in flow regimes as noted by Leasure and collaborators; while their categorization of Ozark and Ouachita flow regimes was useful and thorough, a more nuanced view at the natures of streams within their categories would allow for a more nuanced contextualization of the EFBR within its broader region. This would aid in a larger effort to understand how substrate particles are transported through the EFBR and provide a better understanding with which to conduct substrate augmentations. Finally, we hesitate to be too conclusive with the results of our hydrologic analysis given the limited amount of data considered. While in comparison to the data collected by in-stream probes during our study's sampling in event the USGS gage provided an extraordinary amount of data and our only discharge data, 13 years is not a long-enough period of time to be free of long-term patterns in precipitation and hydrology, especially in an increasingly shifting climate. While recognizing we might always request more data, we would feel more confident in our conclusions about the degree to which our study period and utilized historical record were truly normal for the EFBR.

4.1.3 Physicochemical environment

Dams and their associated reservoirs are multiple-scale environmental stressors that alter a sweeping range of environmental conditions in streams, most notably through direct modifications to flow and sediment transport regimes (Bednarek, 2001; Duflo & Pande, 2007; Grant et al., 2013; Kondolf, 1997; McManamay et al., 2010; Poff et al., 1997; Poole & Berman, 2001; Weisberg et al., 1990). These modifications diminish with increasing downstream distance from the dam, indicating recovery of in-stream environmental conditions (Ellis & Jones, 2013; J. V. Ward & Stanford, 1995; J. V. Ward & Stanford, 1983), and may be hastened by the influence of tributaries (Munn & Brusven, 1991).

We observed that water quality conditions were overall outside deleterious ranges. Measured temperatures and DO concentrations were seasonally appropriate and expressed greater seasonal variability and significant seasonal differences without notable longitudinal trends.

Both water temperature and dissolved oxygen were not expected to substantially vary across riffles due to the meso-limnetic release strategy of the Taum Sauk Reservoir; water temperature should be more reflective of broader daily or seasonal conditions in addition to any vegetative and groundwater influences. That said, we did observe in all samplings but S18 a 2 °C – 4 °C temperature difference between R1 and R2; water temperature decreased between R1 and R2 in F17 but increased between the two in S19 and F18, indicating that the relationship was not strictly driven by the overall water temperatures, i.e. a seasonal mismatch in reservoir and river water temperature as is observed in many impounded systems (J. V Ward & Stanford, 1983). The effect of the dam on water temperature, regardless, quickly attenuated, with no great differences in temperature between riffles beyond R2, slightly over 500 meters downstream of the dam.

Conductivity was similarly much more variable across sampling events than across riffles and recorded values were consistently fairly low. Conductivity is known to be related to hydrologic conditions, human behavioral responses to seasonal weather patterns (i.e., road salt applications), and watershed land use and geology (Barr, 2009; Morgan II et al., 2012). Examining discharge and conductivity data from the USGS gage downstream of the dam, over the course of our study, there is some relationship between discharge and conductivity in which higher flows generally come with lower conductivity readings. That said, the highest flows recorded do not correspond with the lowest conductivity readings, and while the lowest flows do correspond with the highest conductivity readings, there are comparably high values recorded for relatively high flows, indicating hydrology alone does not account for the variability in conductivity. Our low measured values could be reflecting the geology and land use of the river's watershed upstream of our sites. In the EFBR's watershed upstream of our sites, human impacts are low; there is minimal urbanization or row-crop agriculture, and most land is dominated by forest, leading to conductivity in our sites more likely influenced by local geology. The EFBR

drains an area dominated by carbonate rock like dolomite, shale, and with pockets of igneous rock. The sedimentary rocks easily erode and dissolve in water, elevating conductivity and placing the EFBR within normal range for Ozark streams (Barr, 2009; Cieslewicz, 2004; Griffith, 2014).

Turbidity was similarly more variable across sampling events, though both spatial and temporal differences were significant. Measurements indicated consistently clear waters, themselves consistent with Ozark streams (Barr, 2009). The clear separation in turbidity up- and downstream of the R2-R3 boundary was only so apparent in aggregate; though turbidity was always lower in R3 than R2 which we attribute to possible dilution by minor tributaries. Differences in turbidity across sampling events may be derived from the specific hydrologic conditions under which we sampled; we note, for example, that S18 was also when four of our five-highest turbidity values were recorded, likely reflecting increased surface runoff and in-stream erosion leading up to the sampling event, already known to have higher flows than other sampling events.

Water flow velocity was more variable across riffles than it was across sampling events, and both spatial and temporal differences in measurements were significant. The EFBR was fairly slow in all measurements, but velocities were particularly reduced in R1 and R5, with respective mean velocities of about two-thirds and one-third the overall mean flow velocity. These reductions in flow velocity compared to intermediate riffles' velocities was responsible for significant differences across riffles, indicating little overall variability in channel depth and/or width further downstream, assuming near-constant discharge. The particular longitudinal changes in flow velocity, both in magnitude but so too direction, were inconsistent across samplings, indicating the particular interaction between hydrology and channel morphology was changing across time. Flow velocity was positively aligned with median substrate particle size and inversely aligned with substrate size CV, water depth, and downstream distance from the dam. The relationships with median particle size, the substrate size CV, and water depth were expected from faster waters by hydrologic principles relating stream power and sediment transport dynamics. That flow velocity decreased with increasing distance from

the dam may suggest a decreasing slope to the streambed, and we indeed observed flow velocity decreasing with depths beyond approximately 20 cm.

Water depth was more variable across riffles than across sampling events, and both spatial and temporal differences in measurements were significant. Despite the range in depth measurements, the EFBR was, overall, fairly shallow, with mean depth near half the maximum. Our depth measurements were biased by virtue of in-stream sampling requiring depths safe for sampling. Both the overall mean and individual sampling events' depths declined with distance save for S18, which slightly deepened from R3 to R4, before sharply increasing in R5. The S18 sampling event was further responsible for significant differences between depth across samplings; the difference may be the result of higher flows, the increased depth potentially from tributary input or high-flow-related morphological changes to the channel. We cannot discount the influence of R5 on our broader understanding of flow velocity and water depth, as it was dramatically slower and deeper than the four upstream sites.

The median substrate particle size was more variable across riffles than it was across sampling events, the most-so of all measured parameters, and exhibited significant differences across both riffles and sampling events. Both in the raw substrate data and per-sampling, per-riffle median data, the median substrate size was 64 mm, among the boundary between coarse gravel and small cobble. In aggregate, median particle size decreased through successive riffles except from R3 to R4; this pattern was driven by and observed in F17 and S19 samplings, whereas the two riffles possessed equal median sizes in our remaining samplings. The increase in median size from R3 to R4 was notable and may be attributed to the heavily localized substrate conditions, a slight armoring effect from a minor tributary, or some interplay between hydrology, channel morphology, sediment transport abilities of the hydrologic conditions, and the influence of the dam on these factors. Again, our PCA pointed toward expected relationships between median substrate size, water depth, the substrate size CV, water flow velocity, and downstream distance of the dam. Distance is here of more note as it appears to reflect the recovery from impacts to substrate by the dam; for example, the armoring effect observed in R1 quickly diminished with increasing distance.

Further suggesting some complex interaction of hydrology, sediment transport, and the dam were multiple particular aspects of the substrate CV. The CV was more variable spatially than temporally, and both spatial and temporal differences were significant. With a mean CV of approximately 80%, our sites were fairly heterogeneous, but the particular substrate conditions for a given riffle and/or sampling event were variable, with no consistent longitudinal trend in CV across sampling events beyond the CV in R5 exceeding that in R1. This further suggests an active streambed in which bed conditions were frequently altered by shifting hydrologic and channel-morphologic conditions. Both mean values and individual sampling events' values suggested complexities: the riffles differentiated, albeit with only slight differences, into two or three groups, one of R1 and R2, and one of R3, R4, and, loosely, R5. The more downstream of these groups had higher CVs than the more upstream, but the more upstream riffle of each group had a higher CV than the more downstream. As with turbidity, this may be due to influences by minor tributaries or channel morphology if not some complex interplay of instream conditions. The particularly unusual CV measurements in S18 we attribute to substantially higher flows reported during that sampling event; under such conditions, the bed would be more easily mobilized and actively reorganizing substrate conditions, underlining the influence of the flow regime on physical conditions within a stream. Similar to median substrate size, the PCA aligning the CV and downstream distance suggests some recovery of substrate conditions from the effects of the dam.

The serial discontinuity concept (SDC) predicted that, while substrate size would generally decrease with increasing stream order, damming mid-order streams would increase substrate size for a relatively minute downstream distance before it resumed its decreasing trends, quickly returning to non-impounded conditions (J. V Ward & Stanford, 1983). Ellis & Jones noted broad literature agreement with Ward & Stanford's predictions and further considered previous work on stream-lake networks applicable to surface-release reservoirs like the Lower Taum Sauk Reservoir; such work highlighted the sediment-starved nature of the released water, its proneness to erode vertically and laterally, and increased channel armoring through the loss of gravels (Ellis & Jones, 2013; Jones, 2010; Kondolf, 1997). Our data were largely in line with these predictions

and observations. Maximum median substrate size, and indeed raw substrate size data, generally decreased with downstream distance from the dam, although we observed some deviation from this prediction in that riffle-to-riffle changes were not always strictly decreasing, again perhaps due to localized conditions or interactions with hydrologic conditions of the time leading up to and during sampling. That we do not find a strictly decreasing trend in substrate size with downstream distance from the dam is perhaps unsurprising, however, given that substantial recovery of substrate conditions may take anywhere from 1 km to at least 20 km beyond the impoundment (Ellis & Jones, 2013). Current Ozark stream substrate conditions are largely composed of gravels due to intense historical logging and agricultural practices (Martin, 2005); our results were not quite in line with this, with the slight majority of all measured substrate particles measuring at least 64 mm, or as at least small cobbles, in size, but this is likely due to the armoring effect observed in our sites. Similarly, while fine sediments often accumulate downstream of reservoirs due to water releases being too weak to flush them, flow-matching practices at the Taum Sauk Reservoir allow high-flow events to still occur downstream of the reservoir. Substrate armoring might intuitively lower the particle size CV, i.e. decrease bed heterogeneity, yet we observed our lowest CV values in R2, both on individual sampling events and when samplings were averaged per riffle. In S19, R1 even had the highest CV of all riffles. This may be due to the large substrate in R1 effectively trapping smaller particles in interstices, preserving a more diverse substrate than more normal sediment transport dynamics based on flow and particle sizes might predict, in addition to the simple fact of the high number of large particles. Furthermore, the SDC predicting increased substrate size as a result from impounding mid-order streams would shift scouring downstream from the impoundment, establishing a new equilibrium (Ellis & Jones, 2013); we might be observing some effect of that in R2. As other parameters have been apparently only slightly affected by the presence of the dam due to surface-release and flow-matching practices, we might anticipate similarly relatively light impacts to substrate downstream of the impoundment; indeed, while R1 and R2 are generally possessing larger substrates and R2 generally has the lowest, or close to lowest, substrate CV of each riffle per sampling event, conditions in R3 and R4

are usually more similar to each other and sometimes quite similar to R2, indicating little distance across which sediment appears to be impacted.

Our assessment of physicochemical conditions within multiple riffles across four sampling events help to broadly characterize the EFBR in ways conducive to augmenting the stream with substrate. Substrate augmentation is a practice still rife with uncertainties (Harvey et al., 2005). Macroinvertebrates aside, understanding some of the hydrologic and physical conditions within the stream will aid efforts to augment the stream with substrate in a way conducive to restoring more natural physical conditions.

The scope of this project should be sufficient to provide some improved guidance on substrate augmentation in the EFBR; however, there are always practices wished done in retrospect and improvements to be made for future iterations of similar work. Our data collection was limited: we sampled a small number of sites within relatively close proximity to the dam, and what might have been taken to be a reference site about 1700 m further downstream from R4 was substantially deeper and slower-moving, i.e. not a riffle, at least of more comparable type to those found upstream. This left us with little capturing of the variability of conditions that might be experienced over time and space, both important factors in deciding how to conduct a substrate augmentation. The lack of explicit discharge data was furthermore unfortunate; while some assumptions may be made about channel width to allow calculations of approximate discharge per riffle for each sampling event, or assumptions about tributary/spring/groundwater inputs being negligible may allow some use of the lower gaging station's discharge data, considering the broad influence of the flow regime on physicochemical characteristics of streams, the extra data collection would have been preferred so more detailed considerations of flow may have been made. That said, work has been done to show, among other substrate characteristics, that the velocity/depth ratio is a useful tool for differentiating riffles, runs, and pools (Jowett, 1993); this could perhaps allow for a similar sort of gradation of riffles to further or otherwise characterize habitat. Our method for characterizing substrate may not be most reflective of true substrate conditions: particles were selected and measured on a "first-touch" basis, and particles were selected without replacement, i.e. they were relocated so as to not be selected twice. This may affect our representation of large

particles especially; for a given patch of stream bed, a large particle is more likely to be selected by surface area, so allowing a large particle only up to one chance of being represented in the characterization might not reflect its representation in surface area or volumetric considerations. Alternative methods to more accurately reflect surface area representations, let alone volumetric ones, however, may be diminishingly time-consuming. There is, furthermore, multiple studies' worth of data-collection and analysis done regarding more nuanced substrate parameters, such as roughness and area/volume representation, and sediment transport dynamics that could shed even more useful light on the conditions and behavior of substrate in the river. As substrate is naturally transported, better understanding the dynamics of such might be of great aid for augmentation projects. The influence of any tributaries along the study length of stream might be useful to at least basically quantify as well.

Analytically, we may have selected more descriptive tools and explored more nuanced subsets/combinations of samplings, riffles, and parameters. For example, we used temperature as a sort of proxy for true seasonality, but less was done to flesh out how physicochemical conditions varied across thermal/seasonal regimes, and less still for similar sort of regimes for other parameters. The amount of potential paths to explore quickly blows up, however, with the right insight into which paths might be more worth exploring, more useful results might be gleaned, even with the amount of total data collected. Analyses were further hampered by incomplete data: in S18, DO was not collected due to probe malfunction, so median DO per riffle, as calculated from all other samplings, was used in place. Overall, this likely had minimal influence on drawn conclusions, yet a complete record would have been preferred.

In future related work, we believe in, overall, more data collection: a longer-term and/or finer-resolution study encompassing, depending on specific goals of such work, a larger sampled distance downstream of the dam, at least to include a reference riffle, might be the most basic improvement. A more specific sampling of flow, and perhaps of substrate transport under certain flow conditions, as some MDC staff have done or are in the process of doing, would further benefit a more nuanced understanding of how best to augment substrate under existing physicochemical and hydrogeomorphological

conditions. A more structured approach to exploring relationships between and within various combinations of parameters might also yield a more effective approach to understanding more complicated relationships that might exist.

In summary, much of our measured data is in line with regional expectations and with expectations for impounded streams, both with the further consideration of the relatively low impact of the dam and reservoir on our parameters. Water temperature and DO appeared only slightly affected by surface-release practices, and effects quickly diminished with distance. Conductivity and turbidity appeared to reflect low-intensity human land use in the watershed and local geology, coupled with variation in hydrology during sampling events. Flow velocity and depth appear reflective of a lessened slope and perhaps minor tributary input with downstream distance from the dam. Our substrate metrics, finally, demonstrate that there is some amount of channel armoring present, as per expectations in many impounded systems, but that these effects too appear to diminish in magnitude with downstream distance. There is room for improvement, restoration, of substrate conditions in the EFBR, and through the lens of desired biological outcomes, we hope that our examination of physicochemical conditions in the stream, coupled with biological data, might provide guidance on augmentation projects.

4.1.4 Macroinvertebrates

Overview

Aquatic macroinvertebrates are a major component of stream biodiversity and are useful as bioindicators (Wallace & Webster, 1996). They may be sensitive to any given or multiple environmental variables and changes to such, most notably alterations to the flow regime, thermal regime, and bed substrate composition (Beisel et al., 1998; McManamay et al., 2010, 2013; Poff & Zimmerman, 2010). Due to management practices in place on the Lower Taum Sauk Reservoir, we anticipated alterations to the flow and thermal regimes to be minor in comparison to those of the bed substrate. While we did not compare conditions upstream of the dam, we did observe more pronounced gradients in our substrate metrics than we did other metrics. These gradients, then, might be expected to affect the benthic macroinvertebrates. The relationships we observed

between the macroinvertebrate community and substrate, among other environmental variables, may then be used to help inform substrate augmentation projects aimed at providing more sufficient and beneficial substrate conditions for aquatic biota.

We recorded 49 unique taxa, although representation among identified individuals was not equally divided: just five taxa represented approximately two-thirds of all identified individuals, ephemeropterans themselves nearly half, and just twelve taxa reached over 1% representation of the total. Our most-common taxa were, in decreasing order of relative abundance, heptageniid mayflies, isonychiid mayflies, chironomid midges, hydropsychid caddisflies, elmids beetles, philopotamid caddisflies, caenid mayflies, perlid stoneflies, leptohyphid mayflies, and clams.

Insects were initially intended to be identified to genus if not species. Most insect families, however, were identified as being represented by a single genus (e.g. *Caenis* (Caenidae, Ephemeroptera), *Trichorythodes* (Leptohyphidae, Ephemeroptera), *Ceraclea* (Leptoceridae, Trichoptera), *Chimarra* (Philopotamidae, Trichoptera)). There is continued debate (Bowman & Bailey, 1997; Chessman et al., 2007; Ferraro & Cole, 1990; Resh & Unzicker, 1975) on how fine a taxonomic resolution might be sufficient if not necessary when utilizing macroinvertebrates in ecological studies. We left macroinvertebrate identification at family-level in this study due to apparent low generic richness; damaged or absent identifying features due to collection, storage, or juvenile individuals; and interests of time and that of training assistants.

In addition to taxonomic groupings, we grouped macroinvertebrates by their functional feeding groups. Our methodology was not totally inclusive of all identified taxa, but we identified members of all major FFGs. These, too, were quite unevenly represented, with approximately 75% of all individual macroinvertebrates falling into three groups, the collector-filterers, collector-gatherers, and scrapers. Predators were less represented but much more so than the remaining piercers and shredders, both holding less than 1% of all macroinvertebrates.

We examined our macroinvertebrates via abundance, richness, EPT richness, Shannon diversity, and the Missouri biotic index.

Taxonomic metrics: sheer abundance, richness, EPT richness, Shannon diversity, Missouri biotic index

We observed some clear relationships/partitionings of our broader macroinvertebrate metrics against our measured parameters.

Overall, macroinvertebrate abundance increased with downstream distance from the dam. Mean values per riffle ranged from approximately 60 individuals in R5 to 270 individuals in R4. There was variation in exactly how this trend played out across sampling events, but most notable was S19's decrease in abundance in R2 and R3 relative to R1 and R4; the other sampling events were monotonically increasing with distance (again with the qualifier against R5) or with less notable decrease between successive riffles. There were notable outliers in samplings, as well; a sample from R3 in F18, for example, with over 1200 individuals, over half of which were of one taxon. Richness and EPT richness also increased with downstream distance from the dam. There were further sampling-specific variations for both metrics, with F17 appearing distinctly more rich than other sampling events, while S18 was more variable across riffles than others. Shannon diversity was notably low in R1 but fairly stable across downstream riffles until R5; it too had sampling-specific variation like the richness metrics. The MOBI scores, on average, varied extremely little from a value of 5, but there was considerable variation across sampling events, with no clear longitudinal or seasonal pattern.

Macroinvertebrates increased in abundance with increasing temperature, reflecting conditions more generally favorable to their life histories, including an increase in autochthonous and allochthonous food resources and overall available energy.

Differences in exact response to increasing water temperature across sampling events is likely an artifact of different samplings within a single event taking place at different times of day and capturing variation in other physicochemical parameters found at the time of sampling rather than an influence of temperature itself. This is similarly likely why responses to DO concentrations are so variable across sampling events. Total and EPT richness generally increased with increasing water temperature. This, like

abundance, is likely reflective of multiple aspects of life history, perhaps even more so given the timings of emergences that are often temperature-influenced (Woods et al., 2022, Merritt & Cummins 2008), allowing for a total missing of a taxon if sampling occurred just too late in the season, or sampling it in egg form. The specific phenologies various macroinvertebrates may be related to the relatively strong influence of temperature on macroinvertebrate richness in our Mantel tests. Given too some potential attribution of hydrologic patterns to those of turbidity, we may make a similar case for turbidity and perhaps water depth, as hydrologic conditions, including reduced flows that could be reflected in reduced water levels, may influence macroinvertebrate phenology (Giberson & Garnett, 1996; Harper & Peckarsky, 2006). Lower richness values in cooler waters are further perhaps reflective of macroinvertebrates retreating into the substrate for seasonal refuge, as the hyporheic zone may host a large amount of macroinvertebrate biomass (Dorff & Finn, 2020; Stanford, Jack A.; Ward, 1993). Given our method of sampling macroinvertebrates, we expected to stir up surface levels of substrate but may miss taxa deeper beyond. Shannon diversity similarly increased with increasing water temperature, although the relative increase was lesser than for our richness metrics. The reduction in relative increase we might attribute to the accounting for abundance present in Shannon diversity but absent in richness metrics: disproportionate representation of our identified taxa may lead to diversity measures painting a different picture than richness measures. The relatively weak influence of this variable overall in our Mantel tests, however, is perhaps reflective of some stability in our community makeup despite differences in abundance, where seasonal difference were more pronounced.

Relationships between dissolved oxygen concentrations and our macroinvertebrate metrics were less clear, again in part likely due to the interplay between time of year and time of day on oxygen levels. We observed greater macroinvertebrate abundance in our lower and higher extremities of DO values and minimum mean abundance around the 9 mg/L concentration, normally a desirable level. While macroinvertebrate abundance is not totally reflective of overall community health, we will qualify the minimum mean occurring at such a concentration by noting that such concentrations were found only in our most-downstream sampling site, R5, which was substantially deeper and slower-

moving than our other riffles. We thus attribute the apparent relationship between DO and abundance to other factors. This general relationship, higher macroinvertebrate metrics on the tail ends than toward the median DO values, further held for richness, EPT richness, and Shannon diversity. For the MOBI score, the highest and lowest values overall were recorded at an intermediate concentration, but its mean score was higher and lower at other concentrations. For all cases, we do not see DO concentrations guiding the macroinvertebrates but rather other conditions present alongside observed DO concentrations.

The conductivity of our water was among the top drivers of differences in our community when examined by taxonomic abundance and by FFGs. We did not identify a clear reason for such; our study system is not expected to receive much seasonal influence from road salting or other contributors to increased conductivity more common in urban areas, nor does the geology appear to differ enough to drive such an influence. Conductivity here may be related to or coincidental with broader seasonal and hydrologic patterns in which runoff and erosion may interact with flow conditions to concentrate or dilute dissolved materials. Our measured values, and differences between such, were always low and slight, whereas conductivity is more commonly observed as problematic or otherwise more influential in higher concentrations.

Macroinvertebrate abundance generally increased with increased flow velocity up to approximately 0.6 m/s before slightly decreasing beyond such. This may suggest some macroinvertebrates were in drift; increased flow velocities impose higher shear stresses on macroinvertebrates directly and through mobilization of substrate (Gibbins et al., 2007). That abundance peaked around 0.6 m/s, about twice the mean velocity, may suggest preference for more active components to riffles than more pooled or otherwise slow habitat caught in our riffles' sampled areas. It may otherwise suggest a more general incongruity between such velocities and the favored conditions of the macroinvertebrates of the stream: while the taxa most abundant at flow velocities exceeding 0.8 m/s were mostly, by Merritt & Cummins, taxa more associated with erosional habitat, a few, notably the caenid mayflies, were more associated with depositional habitats, inconsistent with these higher flow velocities. (Quinn et al., 1996) reported an increase in

macroinvertebrate abundance, richness, and Shannon diversity with decreasing upstream roughness, which they showed to relate to increasing flow velocities in a system with similar substrate sizes. They noted, however, the confounding influence of other parameters on end macroinvertebrate metrics. While Shannon diversity displayed a more clear, if slight, increase with flow velocity, our richness metrics showed little clear relationship with velocity; MOBI was similar beyond the initial 0.15 m/s increase, with which it noticeably dropped but then held constant. We hesitate to draw too much conclusion from our flow velocity data, however, as riffles in aggregate and separated out by sampling event hold considerable overlap in recorded measurements, which may be a contributing factor to its absence as a primary driver in any of our Mantel tests.

With increasing depth came decreasing macroinvertebrate abundance. This is consistent with long-held conclusions distinguishing between riffles and deeper habitats (Logan & Brooker, 1983) and was particularly apparent comparing data from R5, perhaps better classified as a run, against that from our other riffles. Richness, EPT richness, and Shannon diversity each declined with increasing water depth as well. Depth alone could not explain the macroinvertebrates, as there was some considerable overlap in measured depths in R5 and those in the other riffles, and the relationship between water depth and flow velocity, with other hydrologic parameters held constant, lends credence to such. Along a gradient of increasing depth, we observed a maximum flow velocity at approximately 20 cm, below and above which velocities decreased, pointing to lower-flow conditions and a likely change in channel morphology, e.g. moving from a greater bed slope to a lesser one, consistent with an observed relationship between water depth and median particle size. This is all to say that, in isolation, water depth might not suggest much about our macroinvertebrate community but rather, like flow velocity, may be more broadly tied to stream discharge, channel morphology, and substrate. We issue, however, the same caution interpreting too deeply with the data we hold as depth conditions, while significantly different across riffles, did too considerably overlap.

Macroinvertebrates increased in abundance with increasing median substrate particle size from 22.6 mm to 64 mm, declined with a further increase to 90 mm, and sharply rose at a median size of 180 mm. Excluding the 180 mm median size for the moment, these results

were in line with general acceptance of the gravel-cobble size range of substrates being optimal for total macroinvertebrate abundance (Jowett & Richardson, 1990; J. Ward, 1975). Mid-sized substrate is known to offer benefits to macroinvertebrates over both smaller and larger substrates: over smaller substrate, mid-sized substrate offers increased substrate stability and greater amount of available surface habitat (Khalaf & Tachet, 1980; Richards & Bacon, 1994; Shaw & Minshall, 1980; Williams & Mundie, 1978). Over larger substrate, mid-sized substrate offers increased habitat heterogeneity and a greater ability to trap food resources over larger substrates (Parker, 1989; Wise & Molles Jr., 1978). The influence of this variable was further evident in its primary driving of structural differences in the macroinvertebrate community in our Mantel test leaving taxa grouped as such and its similar influence on driving differences in macroinvertebrate richness.

Only the F17 sampling event in R1 had such a large median particle size, and over half of the total number of macroinvertebrates identified from that sampling event were chironomids; if just these chironomids were absent, mean macroinvertebrate abundance at a median particle size of 180 mm would be only marginally larger than the same measure at a median particle size of 90 mm, pointing to a particularly chironomid-heavy sampling event as a primary cause for the edge-case relationship between substrate size and macroinvertebrate abundance.

Richness, and especially EPT richness, however, are much less influenced by the heavy presence of chironomids in that particular sampling event, and they too both suggested that a median substrate size of 180 mm was providing some substantial benefit to macroinvertebrates. Both richness metrics were at their second-highest values at the 180 mm size, but the overall pattern of values maximized around 54.5 mm to 60 mm and decreasing away from those median substrate sizes still held. The (EPT-) rich macroinvertebrate community observed in R1 during the F17 sampling event, synonymous with the 180 mm median substrate size condition, is itself instead part of the broader rich sampling event as a whole, where R1 was instead the least-rich of all true riffles sampled. The same phenomenon contributed to the high Shannon diversity value.

We noted the interesting presence of *Ceraclea* (Leptoceridae) caddisflies among median particle sizes of 180 mm. This was of at least initial note given the sand-based cases some species, including *C. flava*, of the genus constructs, apparently in some disagreement with the substrate it was recorded as living among: most specimens came from R1 in F17, which held the largest median substrate size. (Resh, 1976) notes, however, how immatures of this genus fasten themselves to larger substrate than that their cases are built upon. This leaves some conclusion, still, though, that due to the apparently sufficient supply of sand with which to build cases, and the near-absence of sand in collected substrate data from that sampling, that our methodology for characterizing substrate was not totally adequate.

The influence of substrate heterogeneity has been long recognized as an important influence on macroinvertebrates, although precise determinations of such have been difficult if not outright contradictory (Erman & Erman, 1984); as the substrate size CV is closely related to median particle size in that, for a system with bounded substrate sizes and finitely many distinct median sizes, an increase in CV must come with a greater representation of substrate sizes

The greater mean abundances were found at CVs of approximately 65%, 70%, and 90%, or about mid-range for our observed CV range of 50% to 110%. These values represent moderately to highly heterogenous substrates which had median particle sizes ranging from 45 mm to 90 mm, with most measuring around 65 mm. Erman & Erman (1984) might suggest placing much greater weight on the size of the substrate itself rather than the heterogeneity, as they found that, for a constant median particle size, heterogeneity was not an important driver of macroinvertebrate community structure; we, however, do not have quite the sample size to meaningfully discuss such an examination. That said, richness, EPT richness, Shannon diversity, and the MOBI scores each displayed relatively little variation across our observed gradient of substrate CV. Furthermore, each metric save for the MOBI score has its value maximized around the same 65% to 75% range. This may suggest that while the heterogeneity in our substrate did significantly differ across both riffles and sampling events, other factors were more influential in shaping specific aspects of the macroinvertebrate community or that we largely found

macroinvertebrates in environments where the substrate heterogeneity was less variable than it was across the entire sampled environment. This is further perhaps suggestive of some relative unimportance of substrate heterogeneity, so long as size preferences are met, as these CV values correspond to median substrate values ranging from 45 mm to 90 mm, or from a coarse gravel to small cobble. That the CV was a top driver of macroinvertebrate community structure, however, and exceeded median particle size in influencing FFG structure, does keep some note of its importance present.

We noted a decrease in macroinvertebrate richness across successive sampling events. This appeared to be at least in part due to increased chance of collecting relatively rare taxa with a greater number of individual macroinvertebrates. The ratio between total abundance and richness for each season is then, approximately and from oldest to most recent sampling event, 315, 170, 320, and 170. This shows that greatest richness was paired with greatest abundance, and lowest richness with lowest abundance. In the F17 sampling event, for example, a total of 22 taxa, each with at most 10 individuals, contributed 76 specimens, inflating richness with negligible abundance and highlighting how very few taxa made up the majority of all collected macroinvertebrates. The relationship is not perfect, however, as S18 and F18 do not match ranked abundance with ranked richness.

Functional feeding groups

All functional feeding groups exhibited at least a slight decline in abundance with increasing water depth. The depth at which the FFG exhibited a noticeable inflection point in this relationship, however, varied, perhaps reflecting the different needs and behaviors of the macroinvertebrates of each group and the food resources they prioritize. Collector-gatherers exhibited no relationship between depth and abundance until approximately 30 cm, at which point their numbers declined. This may be some reflection of the relationship between downstream distance from the dam and water depth; particles the CGs feed upon might not be carried across the dam in full or adequate amount compared to upstream quantities, and so downstream distance from the dam may

provide additional distance and time for sufficiently sized particles for CGs to utilize them as food resources. Collector-filterers, for similar reasons, may have little clear relationship with depth until about 20 cm, at which point their numbers declined. The difference in depths may be attributable to the difference in size of food resource. CGs feed on material of up to approximately 1 mm in diameter while CFs prioritize particles up to approximately 0.05 mm (Wallace & Webster, 1996); the smaller particles may be more easily passes across the dam and more readily accessible to macroinvertebrates. On top of this, the shallower depths are not found until further downstream reaches, which provides more opportunity for fine material to be refined to sizes sufficient for CFs. While these FFGs' numbers decline proportionally slightly, those of scrapers are reduced by almost half starting at a depth of just over 20 cm. As consumers of algae and periphyton, this shift may reflect suitable conditions for such organisms to grow as well, though decline in absolute numbers is slight. Predators, finally, are the least apparently affected by depth, perhaps due to flexibility in diet, life history, and mobility. As before, all considerations of depth should also include those outside of the parameter itself, as the conditions present among each depth measurement may hold greater influence over the macroinvertebrate or FFG.

Flow velocity, though qualified under similar terms, held different relationships with our FFGs. While CGs did not display much relationship with flow velocity, CFs increased with increasing velocity. This appeared to be reflective of the shifting of more favorable filtering conditions for different taxa: isonychiids increase with flow velocity up until approximately 0.4 m/s, while hydropsychids and philopotamids, our remaining dominant CFs, continue increasing up until approximately 0.6 m/s; the mayflies rely more on a swimming behavior, perhaps easier in slower flows, while the caddisflies rely on nets which benefit from faster flows (Merritt & Cummins 2008). Like the community as a whole, CF abundance began to decline with flows greater than 0.6 m/s, likely for similar reasons. Scrapers, on the other hand, declined with velocities above 0.3 m/s, again likely reflecting conditions becoming unfavorable for growing algae and periphyton. Finally, predators increased with velocity as prey could be found with generally increasing abundance in the same fashion.

We again note the perplexing apparent influence of conductivity on FFG structure, as issued by our Mantel test. We do not propose strong reason to believe such an effect is meaningful given slight differences in the variable across riffles, while differences across sampling events were more substantial and significant. Instead, we interpret this result as reflective of broader seasonal and hydrological conditions. This is further evidenced by DO concentration and water depth following conductivity as the top drivers of FFG structure; these are both variables reasonably expected to vary by seasonality and hydrologic patterns.

Median substrate size exhibited clear relationships with FFGs. Collector-gatherers increased with substrate until a median size of 54.5 mm, but resurged at 180 mm. This may be to say R1 held particularly favorable conditions that may reflect more than just substrate; the large surfaces of the rocks, for example, may have provided ample room for algae and periphyton growth, which themselves may have aided in trapping and retaining suitably-sized particles for CGs. A substantial contributor to the result, however, is the dividing of chironomid abundances evenly between this FFG and that of predators. In the smaller ranges of substrate size, the CGs may have been affected by various substrate sizes' varying abilities to trap and retain coarse particulate organic matter (CPOM), though the particular size range of maximum CG abundances is slightly larger than may have been predicted (Parker, 1989; Wise & Molles Jr., 1978). Their numbers, especially compared to those of the CGs, were quite low at the 180 mm size, demonstrating the differences in desired food resources and methods of obtaining such. Scrapers too were quite abundant at 54.5 mm for perhaps the same reason as CGs; though their feeding methods differ, the same substrate sizes might be favorable for accumulation of coarse material to promote periphyton growth. As previously mentioned, the substrate in R1 is particularly large and holds massive amounts of continuous surface area; though smaller substrates fill a space better and in aggregate possess greater surface area, their discontinuity and decreased stability under flow is detrimental to the growth of algae and periphyton, thus the substrate in R1 is and was noticed to be conducive to surface growth. That the scrapers were abundant there supports such, but their abundance being greater at smaller sizes points to more favorable conditions overall being present further

downstream. Finally, the predators were distributed similarly to how the other FFGs were, but their numbers among the 180 mm median size were greatest. This again appears largely due to the assigning half of all chironomids to PR, which may not have accurately reflected true FFG distribution of the taxon.

We have forgone commenting here on relationships between our environment and the piercers and shredders as they were nearly entirely absent from our samples. This itself, however, is worth comment. The river continuum concept predicted a community in a mid-order stream like the EFBR to be primarily and equally composed of collectors (then comprising collector-gatherers and -filterers) and grazers, with shredders and predators comprising the remaining 10-20% of the community (Vannote et al., 1980). This concept, however, was based on free-flowing streams; the serial discontinuity concept was created as an analogue for impounded waters. This concept did not so explicitly address feeding group makeup but did predict a slightly decreased CPOM/FPOM ratio, increased predictability of the stream, and decreased nutrient levels (J. V Ward & Stanford, 1983). These effects, though without an unimpounded and otherwise comparable stream against which to compare, may serve to influence the makeup of our feeding groups by affecting available particulate organic material and algal growth, which influence, scrapers, and piercers directly. While shredders are not a group predicted to be well supported in our system, piercers would seem presumed present were adequate plant material itself present. Our sampling for macroinvertebrates, however, was done in riffle areas where macrophytes and other plants were less likely to be found, despite any abundance in the stream as a whole.

Our study comes with multiple limitations, and we risk drawing or considering conclusions that may be beyond the ability of our data to assert. Our data collection was limited: similar to physicochemical data collection, we sampled a small numbers of sites relatively close to the dam, including one site which was later dropped from consideration entirely and another, R5, which was unlike the remaining four riffles. Like depth and flow velocity, however, we benefitted from collecting ten macroinvertebrate samples per riffle per sampling event.

That said, sample management was lacking, with a substantial number of samples either lost or going unused due to their ethanol evaporating prior to identification, missing labels, or duplicate labels between samples. Our method of interpolation may not have been best-suited for the data we had, but it allowed us to more evenly compare sites and sampling events than we otherwise would have been able. We left macroinvertebrate identification at a level more coarse than often preferred, and we may have missed finer nuances of particular organisms' responses to environmental conditions as a result. Furthermore, a finer taxonomic resolution would have aided in a more accurate characterization of functional feeding groups, as different genera within a family may belong to different groups, which notably led to our splitting of the chironomids between two groups. What's more, we did not assign all taxa to an FFG, which contributed to an imperfect assessment. Many of these results were in part consequence of relative inexperience in data management and macroinvertebrate identification, which, while improved via the progression of this study, would have been preferred better from the start.

The timing of sampling events could have been improved, as no two "seasonally equal" events were conducted in the same month or in similar temperatures: F18 and S18 were nearly equal in mean water temperature, while F17 and S19 differed by approximately 5 °C, each at least 10 °C warmer than the cold-watered sampling events. This, coupled with broader time-of-year sampling considerations, may have affected development and emergence patterns in the macroinvertebrates, leading to a higher proportion of early-instar and/or fewer individuals overall. We noted many early-instar specimens, and their stage of development may have been too early to have developed more pronounced features to aid in identification.

Analytically, and similar to our physicochemical examinations, we may have selected more appropriate or powerful tools and explored more nuanced subsets and combinations of our data. Little was done, for example, to explore the influence of substrate metrics on macroinvertebrates across different water temperatures, which may have provided some insight into the timing of planned substrate augmentations. Our method for addressing incomplete macroinvertebrate data, utilizing medians, may have been improved upon to

account for any structure within present/absent data. Performing an NMDS ordination, a common method in ecological studies, may have shed insight onto relationships between our macroinvertebrates and environment. We furthermore could have retained and used our original substrate, flow velocity, and depth data instead of reducing these measures to medians and, in the case of substrate, the coefficient of variation; given their relationship to discharge, we may have done well to further calculate and utilize the CVs for flow velocity and discharge.

This study was not without its strengths, however. Perhaps the greatest of these was the ability to more readily isolate the effects of substrate on macroinvertebrates from those of other variables affected by dams, again due to the management strategies of the Lower Taum Sauk Dam and Reservoir and otherwise lower variability in other environmental variables. This allowed for a more direct analysis on the effects of median substrate particle size and the substrate size CV on macroinvertebrates without the need of additional experimental setup.

In future related work, we believe in more data collection and better data management: a longer-term study capturing more and more consistent seasonal variation in the environment and macroinvertebrates both, a better retention of samples, and a finer identification of macroinvertebrates would benefit this project were it redone. The increased data could encourage and support a stronger analysis of more precise interactions between environmental variables and our targeted organisms.

We would be remiss to not additionally mention the potential collection of organic material among sampling substrate. Given the difficulty in separating the degree to which substrate directly affects macroinvertebrates versus indirectly affecting them through the retention of organic material, this may open up an improved, if somewhat tangent, study. We ourselves twice prepared an experiment aimed in part at disentangling these related influences and providing some additional insight into substrate size preferences and rates of colonization by macroinvertebrates. These experiments, however, were lost in high flow events and unable to be completed.

Further directions of future work may also include some examination of relationships between sediment transport and flow dynamics: we did not directly measure or measure enough to calculate stream flow, and the substrate in the East Fork Black River is actively redistributed. This may open room to examine how substrate in the river is transported under various flow conditions, how shifting distributions of substrate affect flow, and how macroinvertebrates may respond to this interplay and colonize fresh deposits of material.

Comparing Against Context

The Missouri Department of Natural Resources conducted a biological assessment (bioassessment) of the East Fork Black River in September 2017 and March 2018, closely coinciding with our F17 sampling and overlapping with our S18 sampling. This bioassessment was conducted to assess water quality based on, in part, macroinvertebrate communities in the EFBR. Two of the sites MDNR sampled closely aligned with our study sites: EFBR #3 with R1, and EFBR #2 with R5. Whereas our sites were numbered moving downstream from the Taum Sauk Reservoir, MDNR's sites moved upstream from a highway crossing downstream of R5. In each site, as conditions allowed, flowing water over coarse substrate, non-flowing water over depositional substrate, and rootmat substrate were sampled; the first habitat type most closely coincides with our sampled riffles.

Our results are largely in line with those of MDNR. We noted many similar dominant taxa: while MDNR typically identified taxa to genus or species, our most-dominant taxa both included members of Elmidae, Caenidae, Chironomidae, Heptageniidae, Philopotamidae, Isonychiidae, Hydropsychidae, and Leptoheptageniidae. Both sets of samplings agreed in that the proportion of Trichoptera was greater in R1 than R5 in both sampling events and that they were of low overall proportional abundance; similarly, our samplings were consistent in that the proportion of Plecoptera was greater in R5 than R1 in both sampling events and that stoneflies were very low in relative abundance. Further, they noted nearly all stoneflies from R1 and R5 to be perlids,

a dominance shared in our own samples. Our samples notably did not coincide, however, when it came to the Ephemeropterans: while both sets had large proportions of mayflies, MDNR reported percent Ephemeroptera as higher in R1 during both sampling events. In our F17 sampling, R5 held over twice as many mayflies as R1, and though S18 was about even in relative abundance, all of our sampling events came to hold over 1.5 times as many mayflies in R5 than in R1. This we might attribute to more diverse habitats sampled by MDNR and a combination of our lack of and interpolation of data for R5: of the samples retained, most had mayfly specimens, and many had little, if anything, else, leading to a bias in the resultant numbers to favor the order.

Given spatiotemporal overlap and similarity in sampling techniques, and despite differences in tools and other methodology used in identification, including microscope and taxonomic resolutions, we should expect the macroinvertebrate communities reported in this study and that of MDNR to be similar, which appears to be the case. Differences might be attributable to exact differences in sampling methodology, MDNR's inclusion of non-riffle habitat in sampling, differences in identifiers' experience/tools, data interpolation a la mayflies in R5, and natural fluctuations. MDNR, for example, did not report any pyralid caterpillars in these sampling events, while we reported at least one from every riffle/sampling event save for R5 and S19.

5. CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

In this work we have covered three primary subjects, those of the East Fork Black River's hydrology, physicochemical environment, and macroinvertebrate community. We have further striven to draw connections between these subjects and conclusions from such.

We observed that the EFBR experienced flows consistent with its recent historic norms. Such flows may be somewhat affected by the presence of the Lower Taum Sauk Dam, yet the regime we observed was still similar to that of many free-flowing streams of the St. Francois Mountains and Ozark Highlands.

The physicochemical environment predominantly stayed outside of concerning conditions. All measured parameters significantly differed in at least one of spatial and

temporal measurements, and such differences fell largely along expected lines. Substrate in particular decreased in median size and increased in heterogeneity with downstream distance of the dam, reducing an armoring effect observed near Riffle 1 and becoming more represented by small cobbles, gravels, and sand.

We collected and identified nearly 30,000 individual macroinvertebrates. These organisms represented 49 taxa, and representation with such was heavily skewed toward Ephemeroptera, at nearly half of all total individuals, in particular the Heptageniidae, who were nearly half of all mayflies. In general, macroinvertebrate abundance, richness, and Shannon diversity increased with downstream distance from the dam, with the exception of Riffle 5. We noted this site severely differed in multiple physicochemical aspects from the upstream sites and attribute the low abundance, richness, and Shannon diversity present to such. Seasonality too was strikingly evident in our final results, with over twice the total abundance of macroinvertebrates, greater richness, and greater Shannon diversity in Fall sampling events than Spring. Macroinvertebrates were noted to be greatest in abundance, richness, and Shannon diversity in conditions where the median substrate particle sizes ranged from 45 mm to 64 mm while the substrate size CV ranged from 65% to 75%.

The maximized macroinvertebrate metrics among those substrate conditions offer a constrained yet flexible target for conditions subsequent to substrate augmentation. Riffles 3 and 4 were most in possession of such conditions, and Riffle 4, as most-downstream of the dam, may be expected to be least affected of the standard riffles, further suggesting it may serve as a model for desired post-augmentation conditions. The time of year an augmentation, or a reaching of desired conditions, may be striven for, however, must too be taken into consideration. We noted clear changes to the hydrologic regime over the course of a normal year, significant temporal differences in much of the physicochemical environment, and large differences in the macroinvertebrate community depending on the time of year.

The interplay between hydrology, the physicochemical environment, and the life histories of organisms is complex, and though we have but scratched the surface at some of such

relationships, we present information that may aid in disentangling some of this web of interaction.

With the continued existence of stream regulation in all its forms and further development of dams on these globally crucial waters, we must ever strive to lessen their impacts not just to the abiotic system we seek to manage, but so too to the organisms which have relied upon their free-flowing waters and natural conditions. Substrate augmentation is one such method to lessen one of a multitude of pathways through which the impacts of a dam on its stream system and organisms may flow. Given the critical roles macroinvertebrates play within streams, we hope an increasing amount of substrate augmentation projects are conducted and further refined, and we hope an increasing subset of those are carried out with direct benefit to macroinvertebrates, not just higher organisms, in mind. Finally, we hope that this work here will contribute to such efforts and ultimately serve to improve environmental conditions with the East Fork Black River for the sake of the river itself and the organisms who call it home.

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7. TABLES

Table 1: Timeline of sampling events and substrate augmentation efforts. Additional information includes the location of samplings and augmentations, augmentation substrate recipes, and discharge as reported by USGS gage 07061290 during each sampling and augmentation.

Timeline of sampling events and substrate augmentation efforts						
	F17	Augmentation 1	S18	F18	S19	Augmentation 2
date	September 12 – 14, 2017	November 08, 2017	March 20 – 22, 2018	November 16 – 18, 2018	June 13 – 15, 2019	August 2019
location	R1 - R5	between R1 and Lower Dam	R1 - R5	R1 - R5	R1 - R5	between R1 and Lower Dam
recipe	NA	24 yd ³ : 15% < 0.16", 30% < 0.5" 45% < 1.0", 10% < 1.5"	NA	NA	NA	Unknown
flow mean ± SD (cubic meters/second)	0.29 ± 0.03	0.38	1.79 ± 0.15	0.89 ± 0.03	0.85 ± 0.18	Unknown

Table 2: List of physicochemical and macroinvertebrate variables measured during sampling events, their units, the number of measurements per riffle per sampling event, and where the measurements were taken.

List of physicochemical and macroinvertebrate variables measured during sampling events		
Parameter	Units	Number of measurements per riffle per sampling event
water temperature	°C	one measurement at the center of the riffle
dissolved oxygen concentration	mg O ₂ /L	one measurement at the center of the riffle
specific conductivity	µS/cm	one measurement at the center of the riffle
turbidity	NA	one measurement at the center of the riffle
water velocity	m/s	one measurement at ten random locations per riffle
water depth	cm	one measurement at ten random locations per riffle
substrate particle size	mm	ten particles at each of the 10 random locations per riffle
macroinvertebrate community	-	one sample at each of the ten random locations per riffle

Table 3: List of macroinvertebrate community metrics calculated and the metric sources.

List of macroinvertebrate community metrics calculated and metric sources	
Metric name	Reference
Taxa richness	NA
EPT richness	NA
Shannon-Wiener diversity index	Shannon & Weaver, 1948
Missouri biotic index	MDNR Semi-quantitative macroinvertebrate stream bioassessment project procedure

Table 4: Macroinvertebrate functional feeding groups attribution.

Functional feeding groups attribution					
Shredder	Collector-Gatherer	Collector-Filterer	Scraper	Piercer	Predator
Neumoridae	Leptohyphidae	Isonychiidae	Heptageniidae	Hydroptilidae	Perlidae
Taeniopterygidae	Baetidae	Philopotamidae	Helicopsychidae		Perlodidae
Tipulidae	Leptphlebiidae	Hydropsychidae	Psephenidae		Chloroperlidae
Dryopidae	Ephemeridae	Polycentropodidae	Pyralidae		Chironomidae ¹
	Baetiscidae	Simuliidae			Ceratopogonidae
	Ephemerellidae				Tabanidae
	Siphonuridae				Sciomyzidae
	Leptoceridae				Corydalidae
	Chironomidae ¹				Coenagrionidae
	Elmidae				Gomphidae
	Dixidae				Aeshnidae
					Cordulegastridae
					Veliidae
					Gerridae
					Corixidae

¹ Chironomidae abundance evenly divided between collector-gatherers and predators

Table 5: Mean \pm standard deviation and the results of Welch's ANOVA on physicochemical conditions per riffle, grouped by sampling event.

Physicochemical conditions across the riffles: mean \pm standard deviation, Welch's ANOVA							
Parameter (units)	R1	R2	R3	R4	R5	F statistic	<i>p</i>
water temperature (°C)	13.8 \pm 6.9	14.6 \pm 5.8	14.7 \pm 5.9	15.5 \pm 6.2	14.6 \pm 4.6	$F_{4, 96.945} = 0.316$	< 1
DO (mg/L)	8.7 \pm 1.4	8.7 \pm 1.7	8.8 \pm 1.5	8.9 \pm 1.4	9.3 \pm 1.3	$F_{4, 97.309} = 1.754$	< 0.5
specific conductivity (μ s/cm)	134.5 \pm 37.5	135.0 \pm 38.8	138.8 \pm 37.0	139.8 \pm 39.7	140.3 \pm 36.4	$F_{4, 97.476} = 0.201$	< 1
turbidity (NTU)	11.8 \pm 9.8	14.3 \pm 10.8	7.2 \pm 4.8	8.5 \pm 8.8	6.8 \pm 5.0	$F_{4, 94.889} = 5.690$	< 0.0005
flow velocity (m/s)	0.22 \pm 0.14	0.42 \pm 0.27	0.39 \pm 0.22	0.42 \pm 0.25	0.08 \pm 0.07	$F_{4, 89.068} = 46.606$	< 0.0005
water depth (cm)	29.0 \pm 10.6	28.6 \pm 14.0	21.0 \pm 8.2	18.8 \pm 8.0	46.0 \pm 9.7	$F_{4, 96.740} = 53.952$	< 0.0005
median particle size (mm)	112.5 \pm 39.5	77.0 \pm 13.2	55.1 \pm 8.0	59.5 \pm 8.3	36.2 \pm 9.6	$F_{4, 95.419} = 89.793$	< 0.0005
particle size CV (%)	70.3 \pm 8.6	62.6 \pm 7.6	79.9 \pm 11.2	75.4 \pm 9.1	101.5 \pm 8.2	$F_{4, 97.154} = 130.450$	< 0.0005

Table 6: Mean \pm standard deviation and the results of Welch's ANOVA on physicochemical conditions per sampling event, grouped by riffle.

Physicochemical conditions across the sampling events: mean \pm standard deviation, Welch's ANOVA						
Parameter (units)	F17	S18	F18	S19	F statistic	p
water temperature (°C)	22.44 \pm 1.29	9.29 \pm 0.96	8.96 \pm 0.92	17.78 \pm 1.84	$F_{3, 106.400} = 1536.800$	< 0.0005
DO (mg/L)	7.54 \pm 0.52	N/A	11.2 \pm 0.12	8.27 \pm 0.57	$F_{3, 90.019} = 1691.800$	< 0.0005
specific conductivity (μ s/cm)	170.4 \pm 3.92	N/A	173.6 \pm 2.83	82.6 \pm 4.41	$F_{3, 105.890} = 6897.500$	< 0.0005
turbidity (NTU)	3.64 \pm 3	20.68 \pm 7.7	2.02 \pm 0.2	12.54 \pm 2.21	$F_{3, 82.353} = 468.980$	< 0.0005
flow velocity (m/s)	0.19 \pm 0.18	0.41 \pm 0.29	0.33 \pm 0.19	0.31 \pm 0.26	$F_{3, 107.100} = 9.272$	< 0.0005
water depth (cm)	26 \pm 16.01	33.96 \pm 12.27	28.02 \pm 13.9	26.72 \pm 12.43	$F_{3, 108.410} = 3.903$	< 0.05
median particle size (mm)	81.5 \pm 50.26	60.4 \pm 24.88	70.6 \pm 17.47	57.12 \pm 22.65	$F_{3, 105.130} = 6.010$	< 0.005
particle size CV (%)	75.09 \pm 12.37	83.85 \pm 21.3	74.44 \pm 9.47	78.26 \pm 16.49	$F_{3, 104.980} = 3.081$	< 0.005

Table 7: Mean \pm standard deviation on macroinvertebrate metrics per riffle, grouped by sampling event.

Macroinvertebrate community metrics across riffles: mean \pm standard deviation					
Metric	R1	R2	R3	R4	R5
abundance	139.4 \pm 144.11	168.08 \pm 149.11	201.03 \pm 216.02	268.6 \pm 146.07	57.18 \pm 35.02
richness	9.43 \pm 3.69	10.53 \pm 2.51	11.98 \pm 3.19	12.95 \pm 2.19	7.55 \pm 2.73
EPT richness	5.08 \pm 2.44	6.1 \pm 1.89	6.55 \pm 1.83	8.1 \pm 1.55	4.45 \pm 2.05
Shannon-Wiener diversity index	1.39 \pm 0.31	1.85 \pm 0.24	1.81 \pm 0.34	1.92 \pm 0.24	1.4 \pm 0.39
Missouri biotic index	4.74 \pm 0.46	4.66 \pm 0.29	4.62 \pm 0.35	4.58 \pm 0.23	4.8 \pm 0.62

Table 8: Mean \pm standard deviation on macroinvertebrate metrics per sampling event, grouped by riffle.

Macroinvertebrate community metrics across sampling events: mean \pm standard deviation				
Metric	F17	S18	F18	S19
abundance	266.18 \pm 170.24	112.32 \pm 96.04	197.52 \pm 213.04	91.4 \pm 75.52
richness	14.06 \pm 2.85	8.1 \pm 3.16	10.24 \pm 2.6	9.54 \pm 1.97
EPT richness	8.32 \pm 1.54	4.74 \pm 2.36	6.02 \pm 1.87	5.14 \pm 1.64
Shannon-Wiener diversity index	1.93 \pm 0.34	1.43 \pm 0.4	1.62 \pm 0.37	1.72 \pm 0.23
Missouri biotic index	4.67 \pm 0.26	4.44 \pm 0.43	4.71 \pm 0.43	4.89 \pm 0.4

Table 9: Coefficients of variation per physicochemical parameter per riffle, grouped by sampling event, and per sampling event, grouped by riffle.

Parameters' coefficients of variations across riffles and sampling events		
Parameter	Riffle CV (%)	Sampling event CV (%)
temperature	9.16	40.34
dissolved oxygen	4.94	16.43
conductivity	2.78	27.54
turbidity	36.75	80.35
flow velocity	76.89	65.70
depth	48.46	37.54
median substrate particle size	41.81	21.61
substrate size CV	18.92	11.70

Table 10: The Mantel statistic and p value per partial Mantel test run on macroinvertebrate metrics with each physicochemical as an input.

Partial Mantel test results			
Taxonomic abundance	Richness	Shannon diversity	FFG abundance
environment	depth	temperature	environment
$r_M = 0.249, p < 0.0001$	$r_M = 0.176, p < 0.0005$	$r_M = 0.117, p < 0.005$	$r_M = 0.201, p < 0.005$
conductivity	temperature	median substrate particle size	conductivity
$r_M = 0.182, p < 0.0001$	$r_M = 0.123, p < 0.0005$	$r_M = 0.087, p < 0.05$	$r_M = 0.148, p < 0.0005$
median substrate particle size	turbidity	depth	dissolved oxygen
$r_M = 0.160, p < 0.0001$	$r_M = 0.110, p < 0.005$	$r_M = 0.078, p < 0.005$	$r_M = 0.137, p < 0.0005$
substrate size CV	environment	dissolved oxygen	depth
$r_M = 0.160, p < 0.0001$	$r_M = 0.076, p < 0.05$	$r_M = 0.048, p < 0.5$	$r_M = 0.133, p < 0.0005$
turbidity	substrate size CV	environment	substrate size CV
$r_M = 0.135, p < 0.0001$	$r_M = 0.057, p < 0.05$	$r_M = 0.041, p < 0.5$	$r_M = 0.119, p < 0.0005$
depth	median substrate particle size	substrate size CV	temperature
$r_M = 0.118, p < 0.0001$	$r_M = 0.056, p < 0.5$	$r_M = 0.040, p < 0.5$	$r_M = 0.087, p < 0.0005$
temperature	conductivity	turbidity	median substrate particle size
$r_M = 0.113, p < 0.0001$	$r_M = 0.048, p < 0.05$	$r_M = 0.030, p < 0.5$	$r_M = 0.086, p < 0.005$
dissolved oxygen	dissolved oxygen	conductivity	turbidity
$r_M = 0.101, p < 0.0001$	$r_M = 0.023, p < 0.5$	$r_M = -0.023, p = 1$	$r_M = 0.084, p < 0.005$
flow velocity	flow velocity	flow velocity	flow velocity
$r_M = 0.059, p < 0.05$	$r_M = -0.078, p < 1$	$r_M = -0.087, p = 1$	$r_M = 0.065, p < 0.05$

8. FIGURES

Figure 1: Site map displaying Missouri, Reynolds County, the East Fork Black River, the Upper and Lower Taum Sauk Reservoirs, and the study riffles.

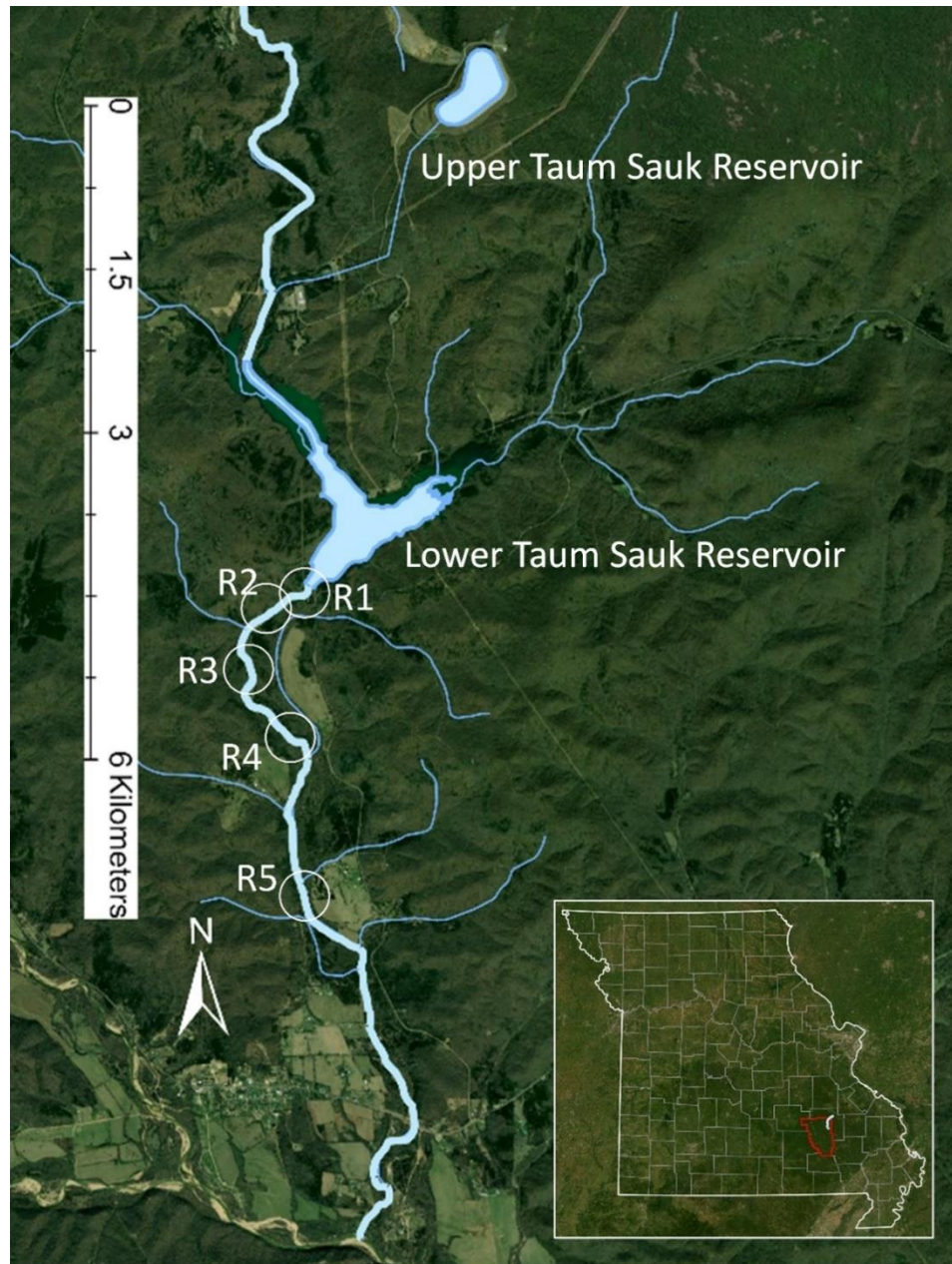


Figure 2: Monthly mean air temperature and precipitation for Farmington, Missouri, a community near the East Fork Black River.

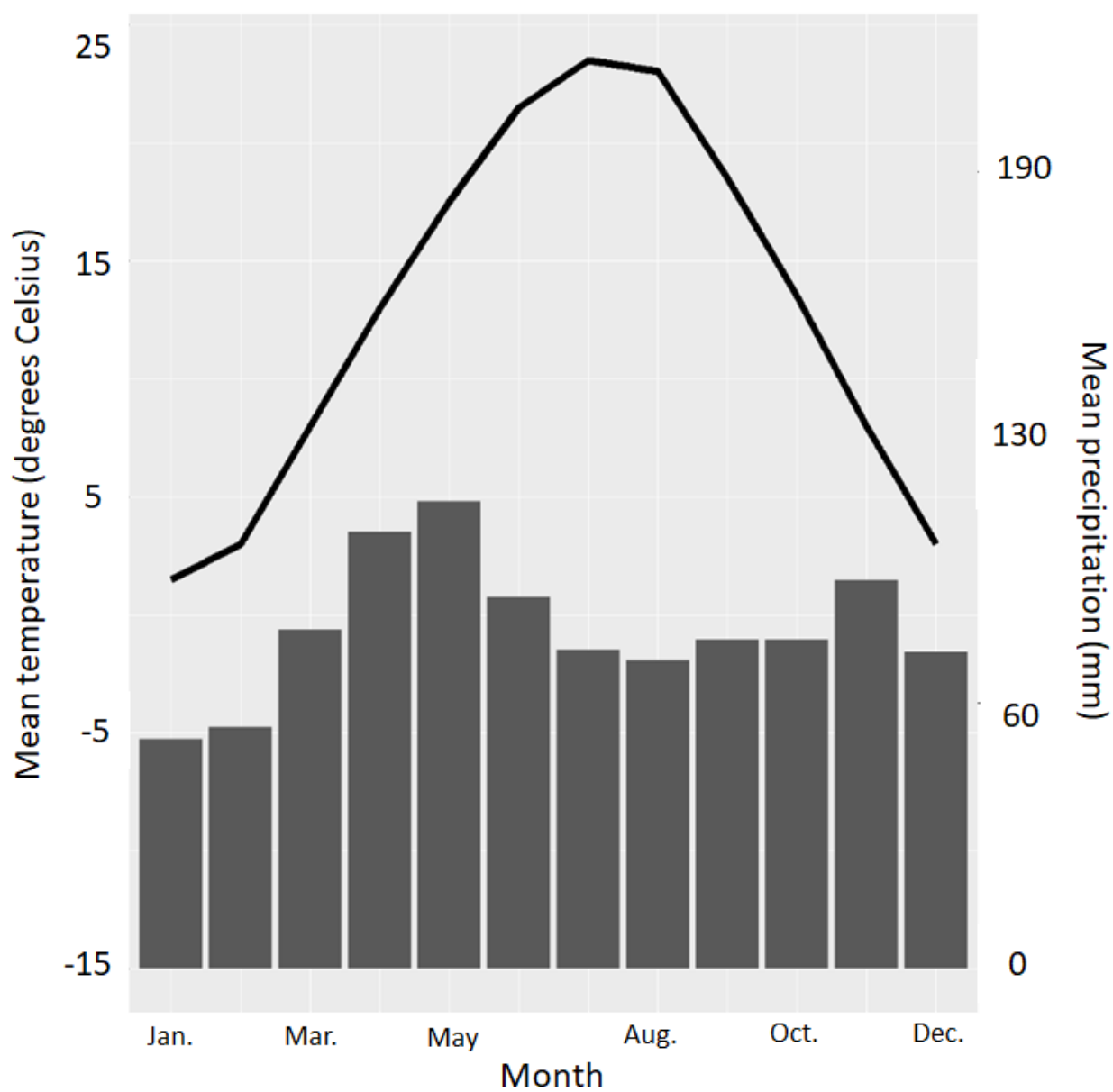


Figure 3: Discharge as measured by USGS gage 07061290 across the historical period of January 01, 2009 through December 31, 2021, with the study period highlighted in red.

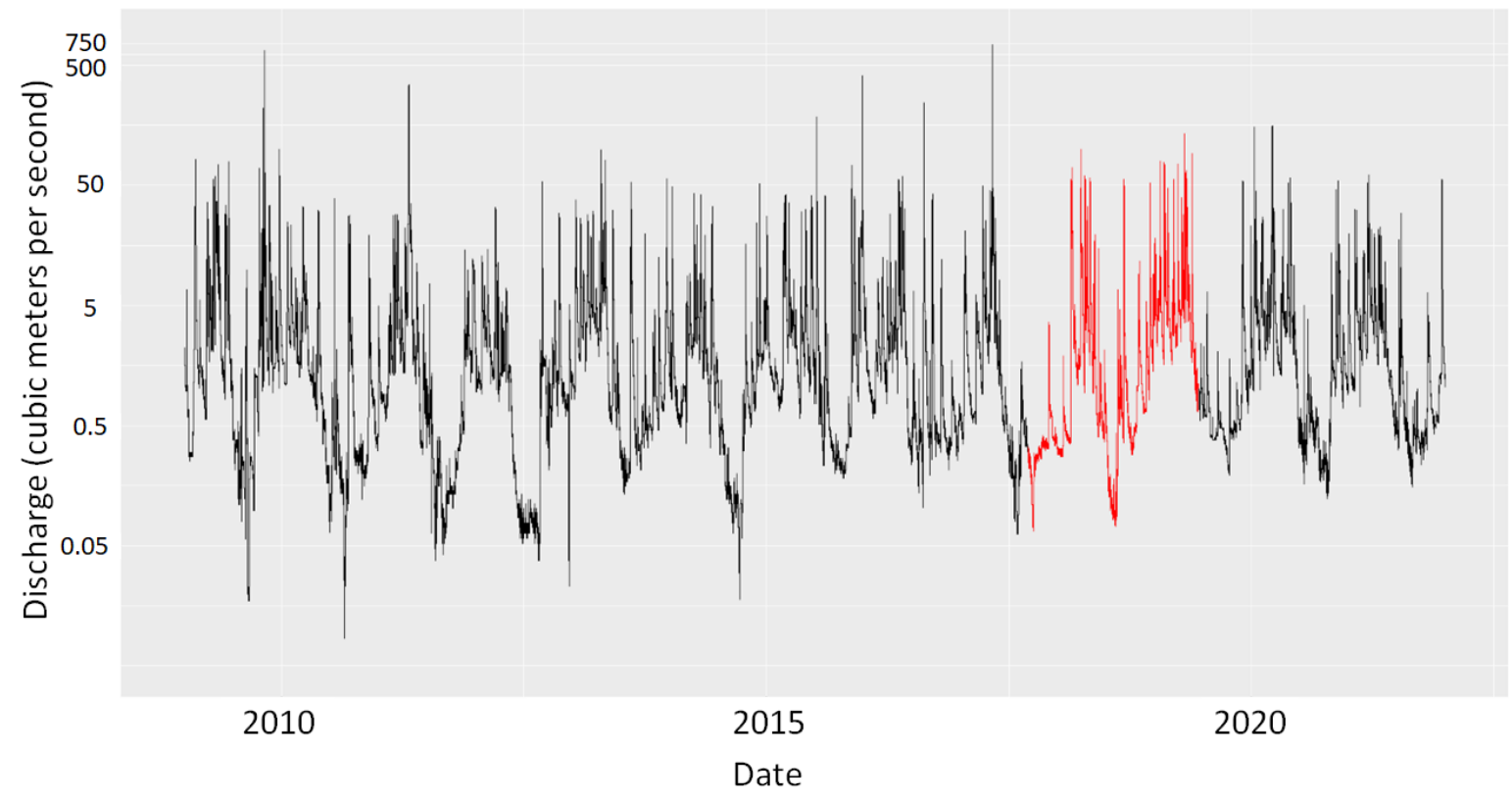


Figure 4: A flow duration curve constructed from discharge data as reported by USGS gage 07061290 across the historical period of January 01, 2009 through December 31, 2021, displaying graphically and in text the percentage of time flow is equaled or less than a given rate.

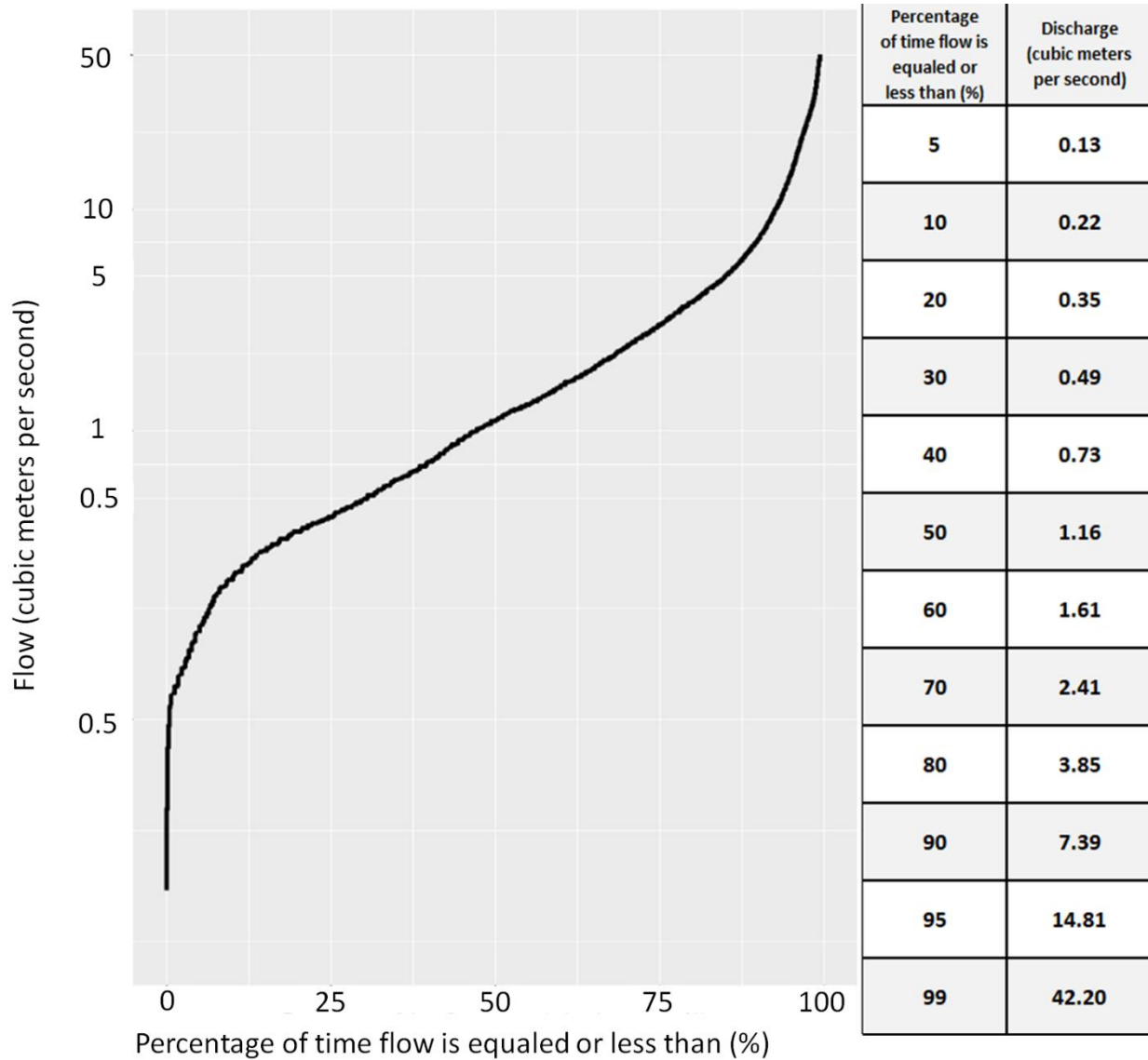


Figure 5: Median (black), 90th percentile (blue), and 10th percentile (red) flow for a given time across the year, constructed from the record reported by USGS gage 07061290 from January 01, 2009 through December 31, 2021.

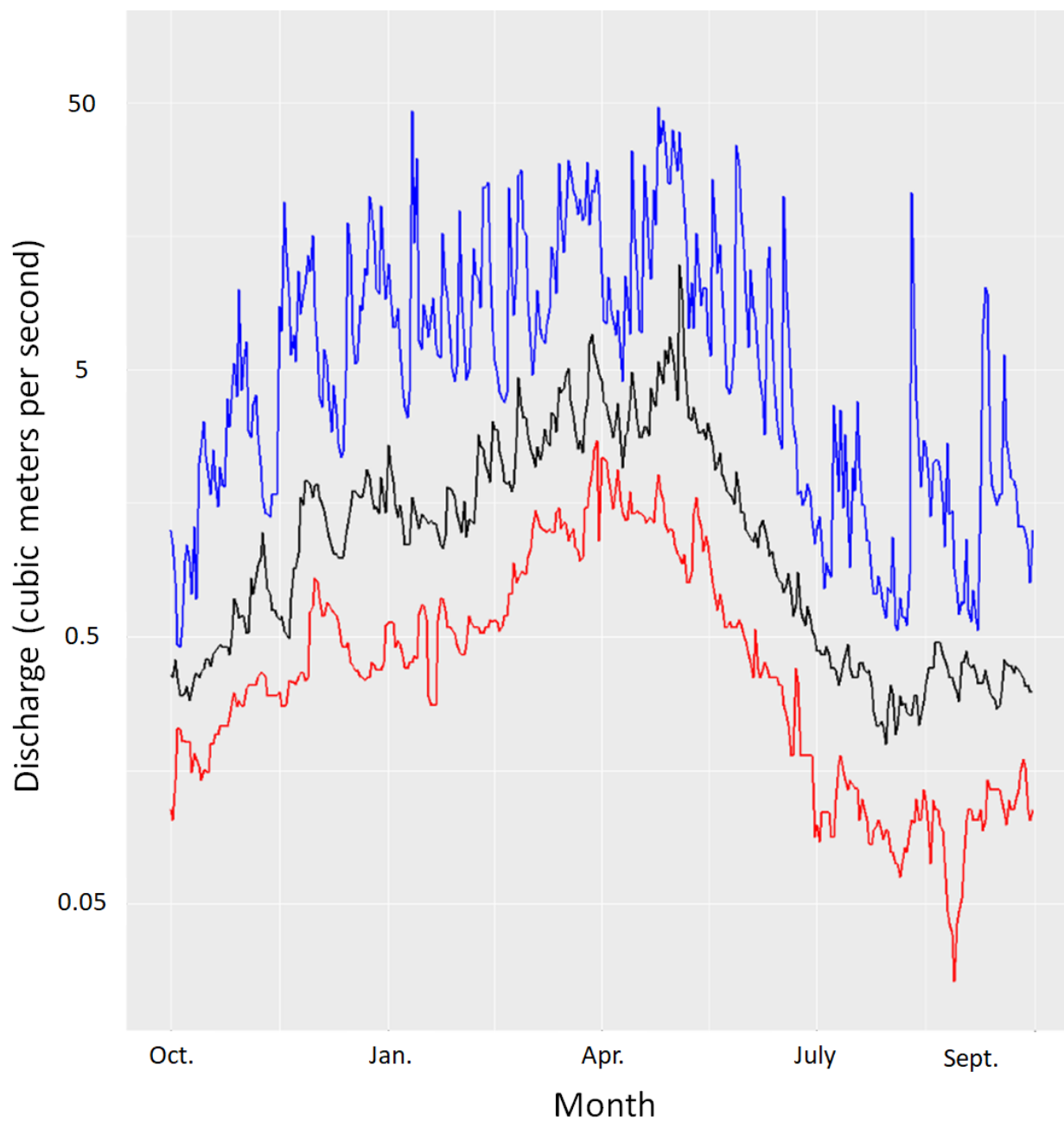


Figure 6: Monthly discharge data across the year, constructed from the record reported by USGS gage 07061290 from January 01, 2009 through December 31, 2021. Reported are the median, first (Q1) and third (Q3) quartiles, and, as whiskers, $Q1 - 1.5(Q3-Q1)$ and $Q3 + 1.5(Q3-Q1)$.

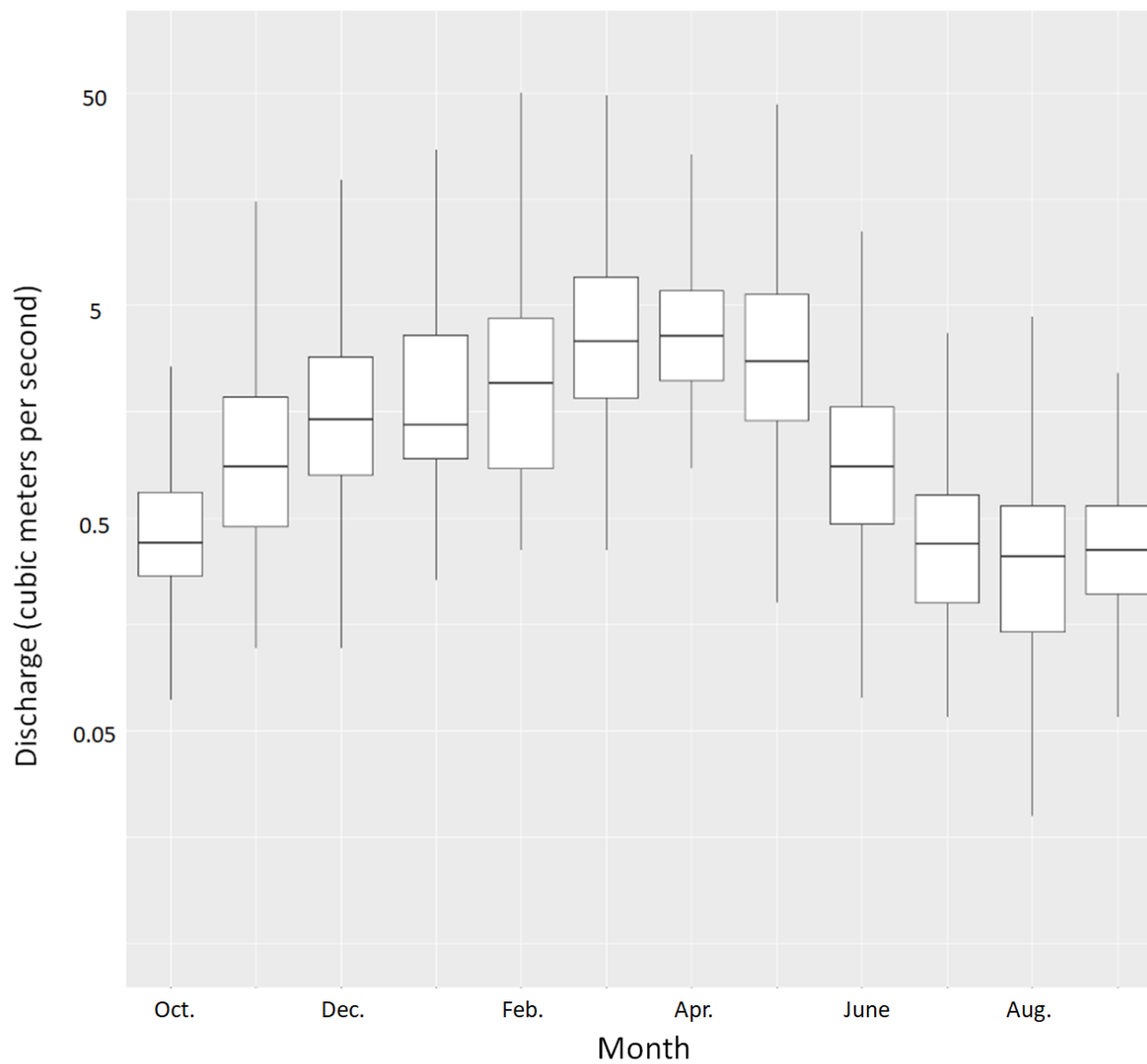


Figure 7: A flood frequency plot for the East Fork Black River, constructed by the discharge record reported by USGS gage 07061290 from January 01, 2009 through December 31, 2021.

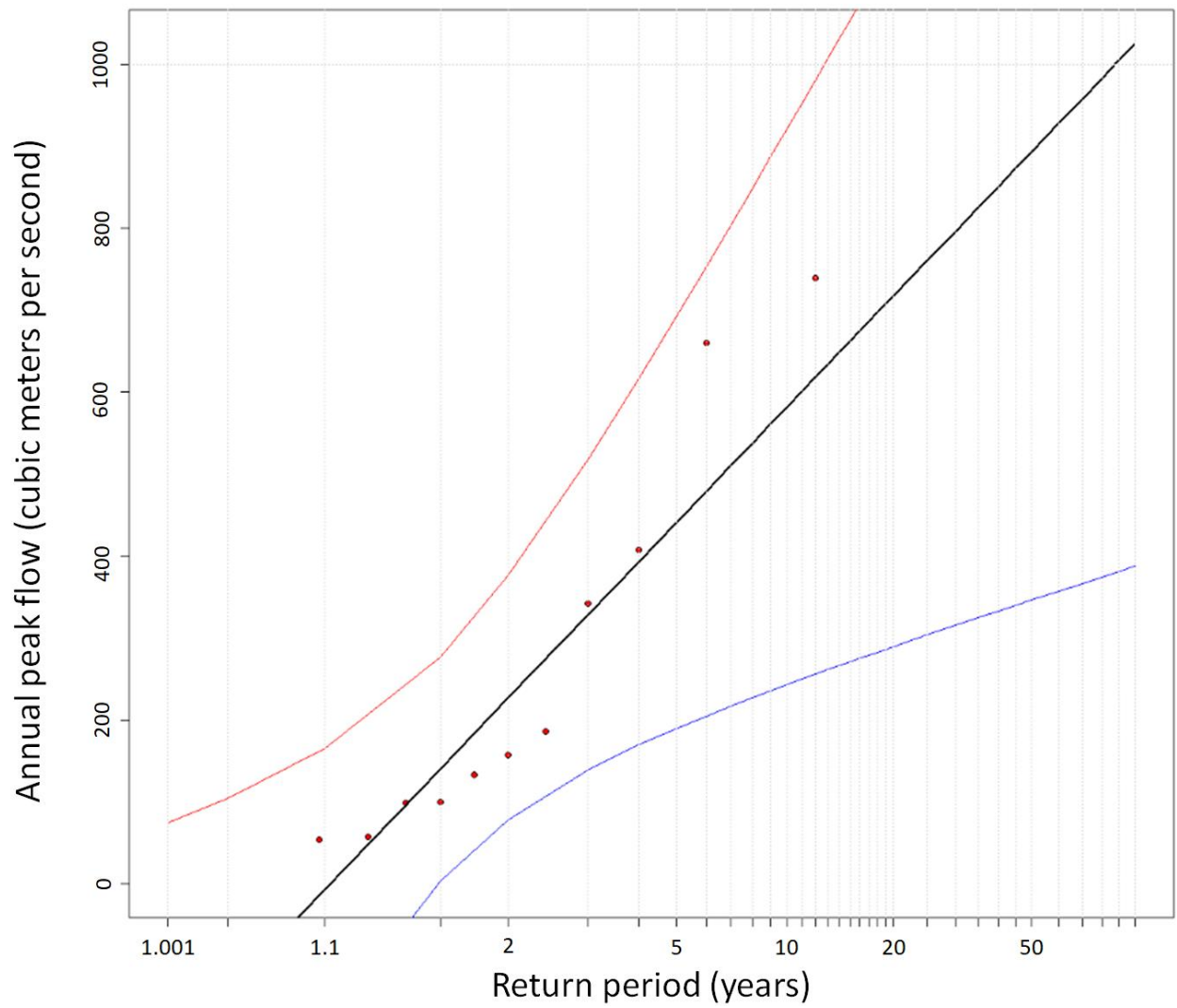


Figure 8: Macroinvertebrate abundance per riffle, split into EPT and non-EPT taxa for clarity. EPT taxa are further divided into orders by black bars.

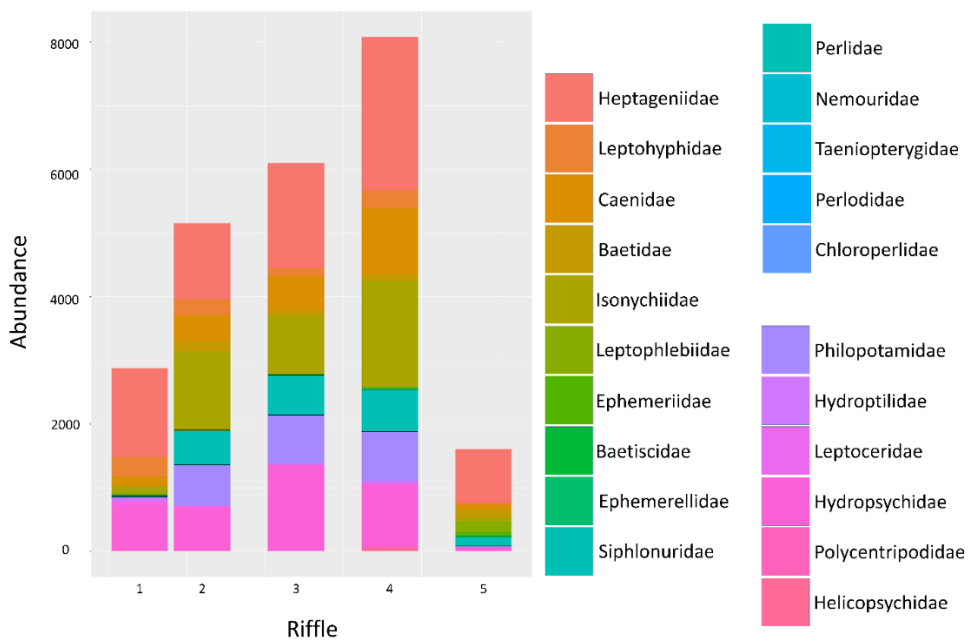
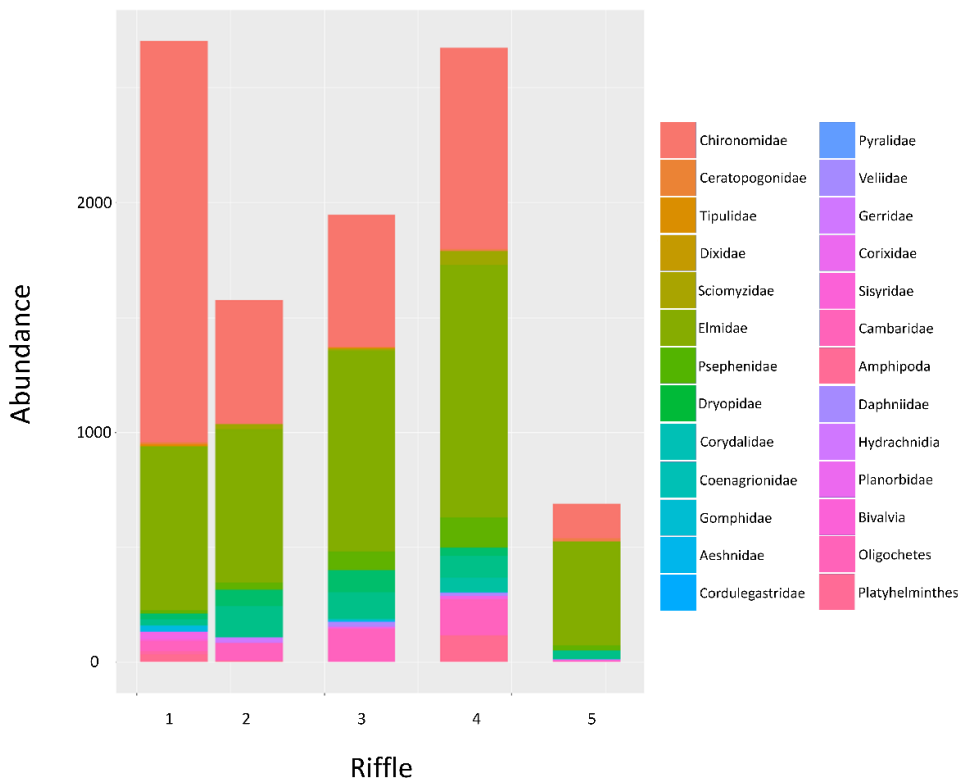
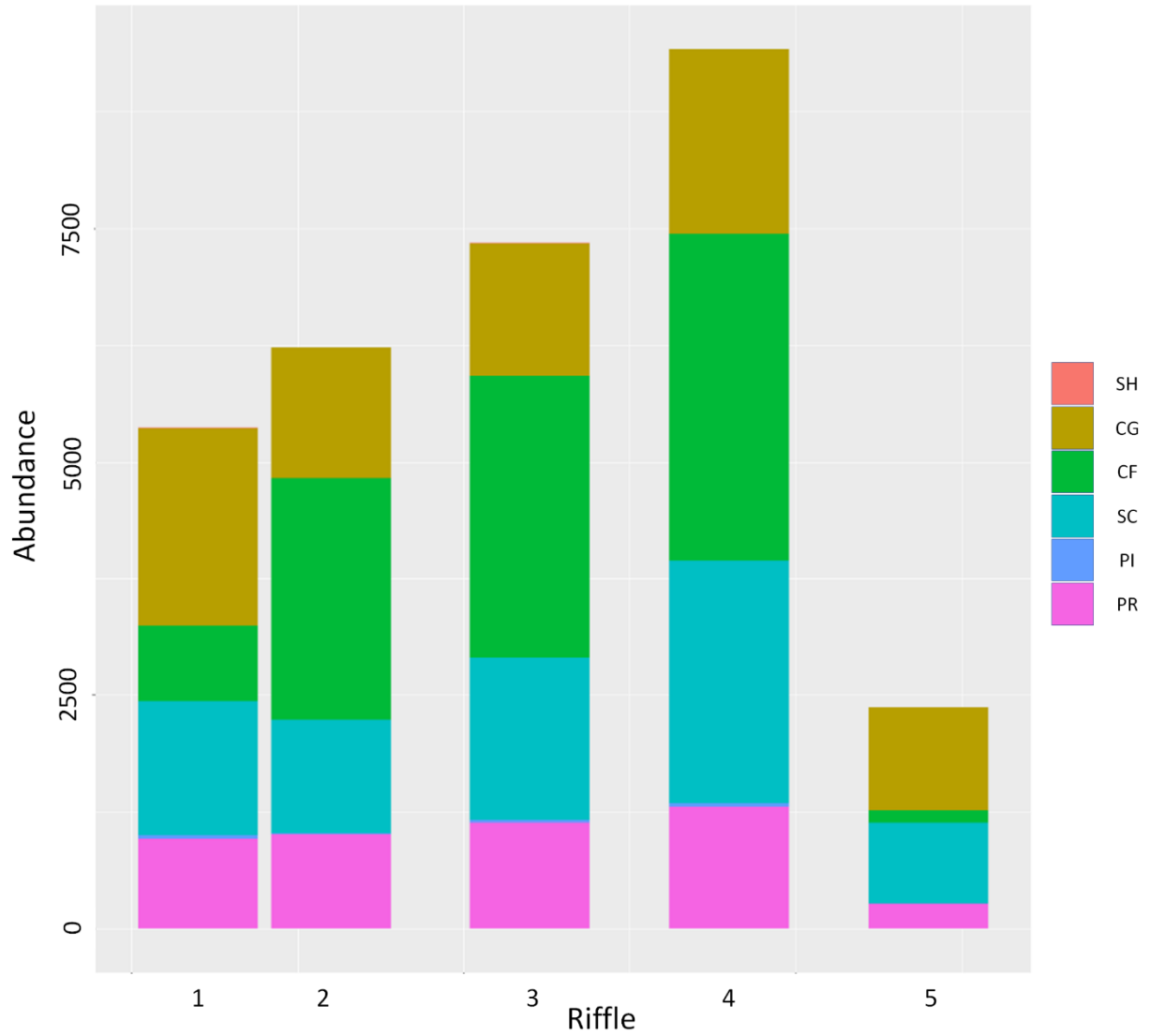


Figure 10: Macroinvertebrate FFG abundance per riffle and macroinvertebrate FFG abundance per sampling.



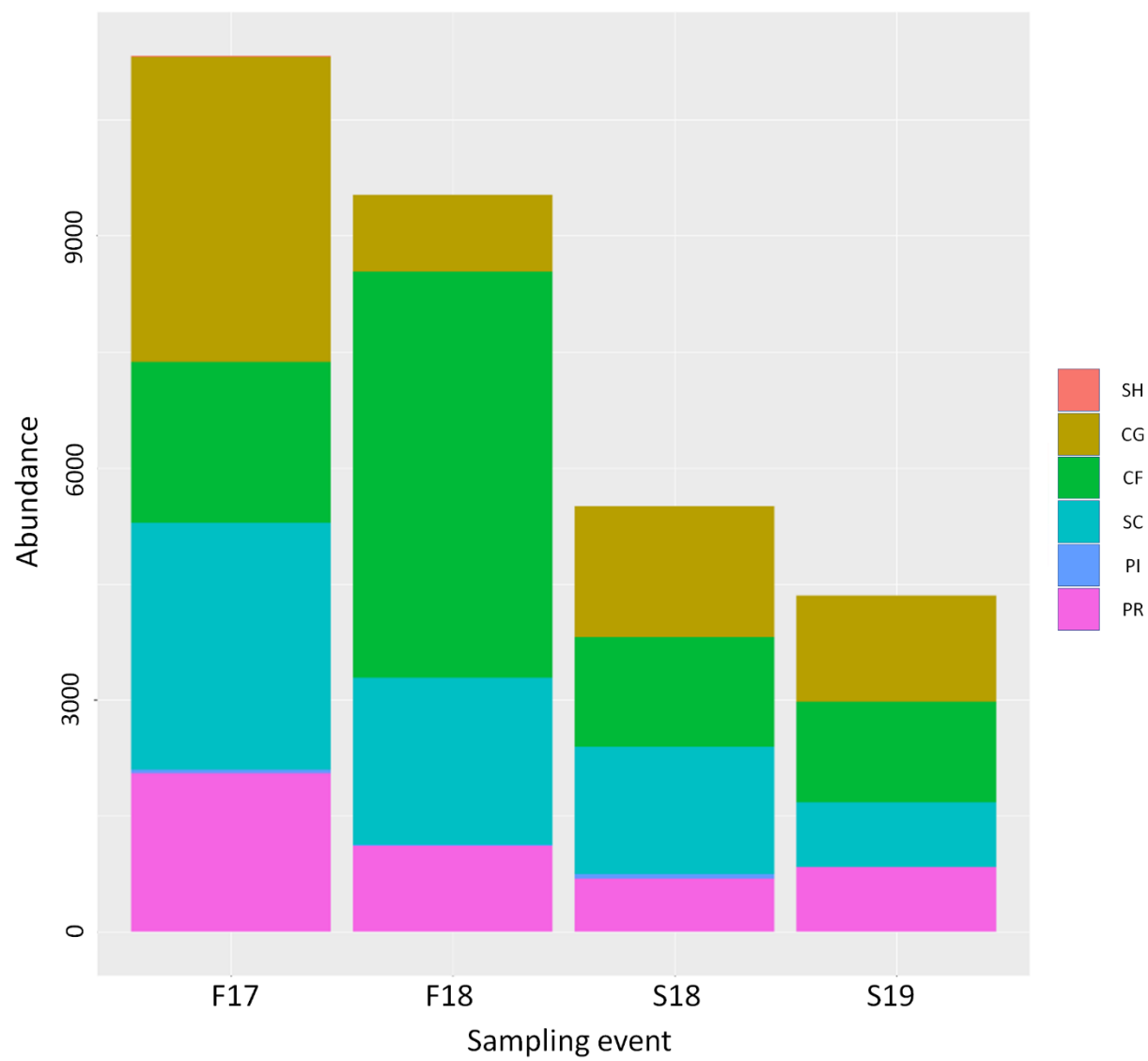
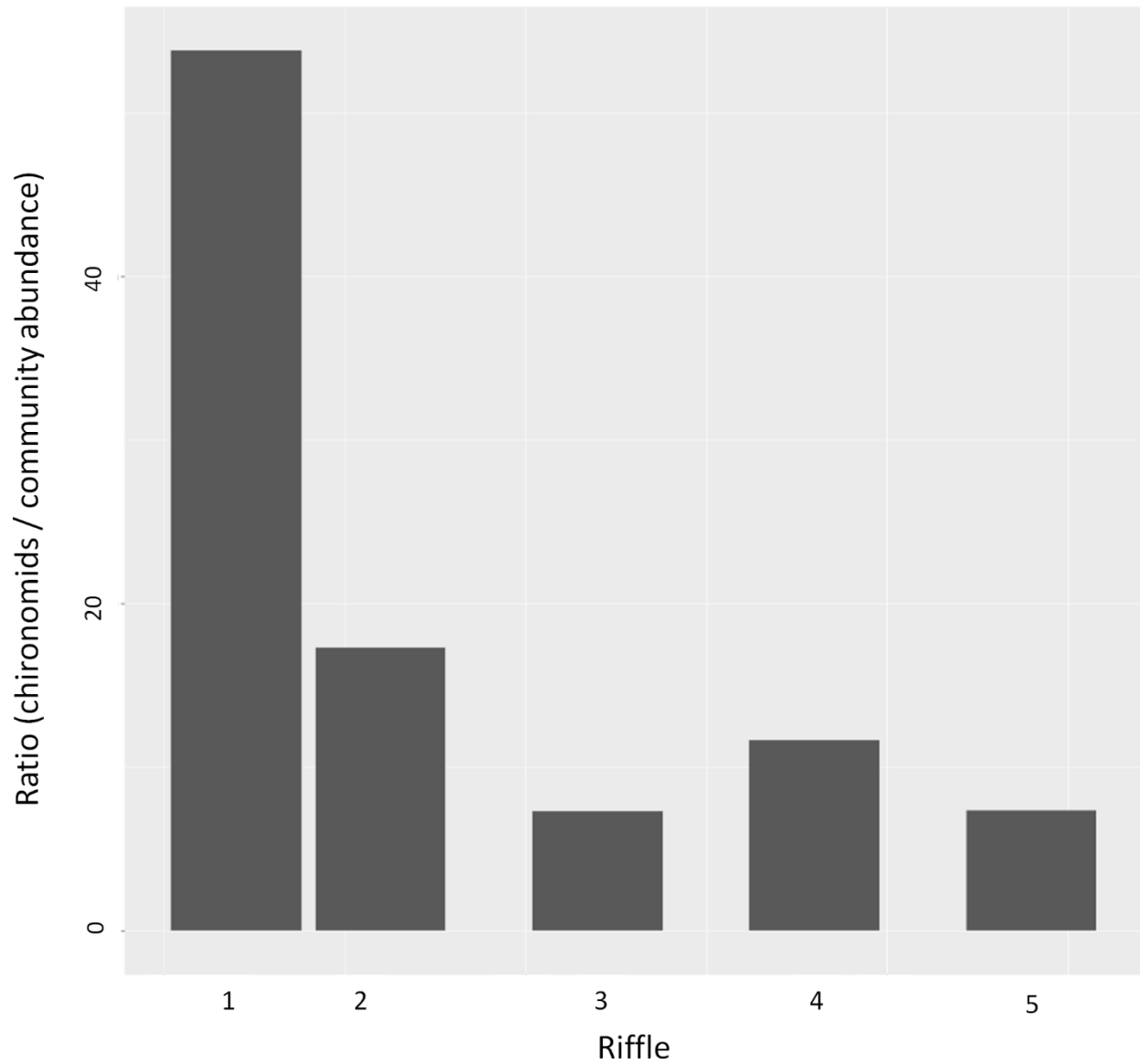


Figure 11: The ratio of chironomid abundance to community abundance per riffle and the ratio of chironomid abundance to community abundance per sampling event.



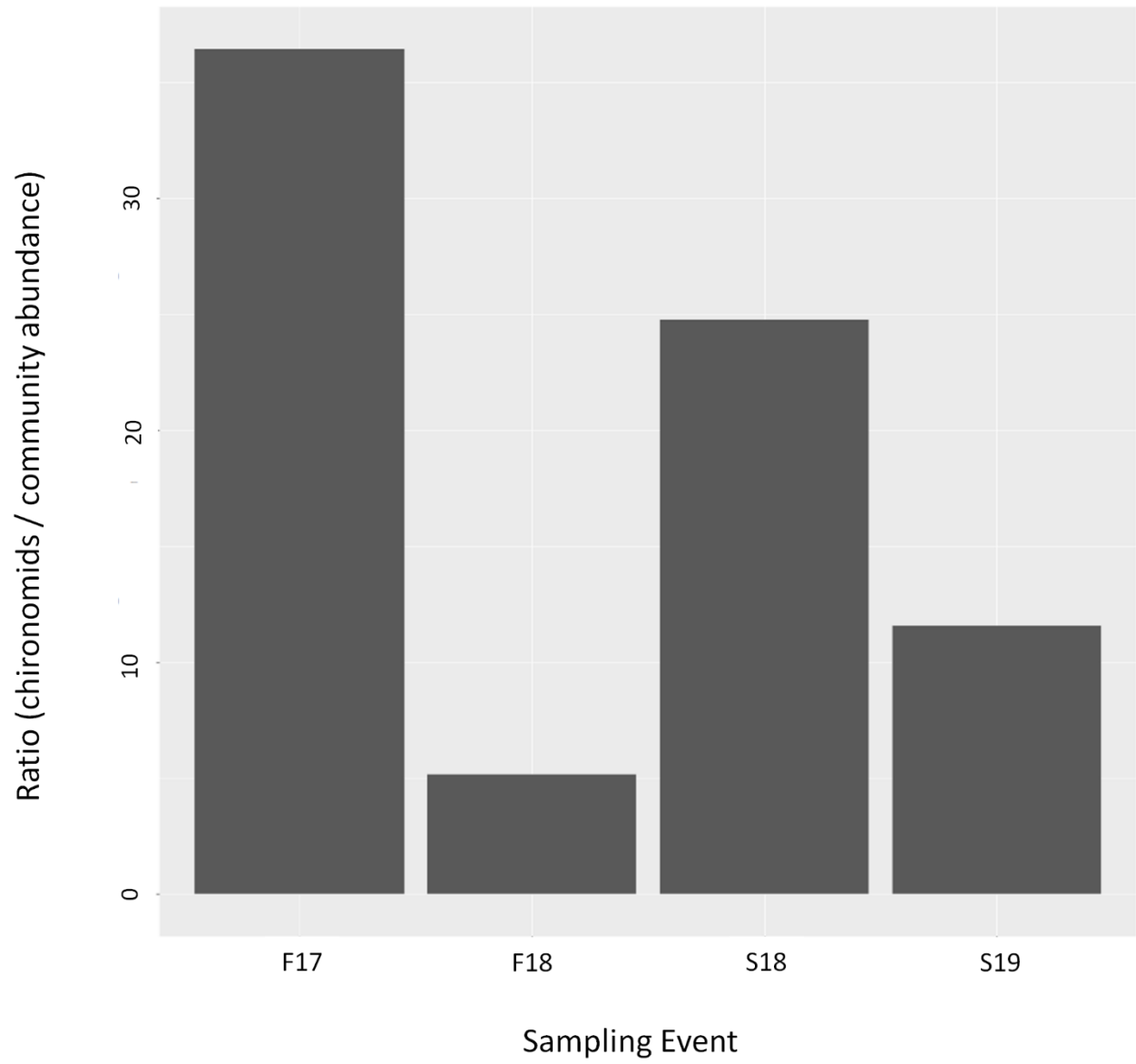
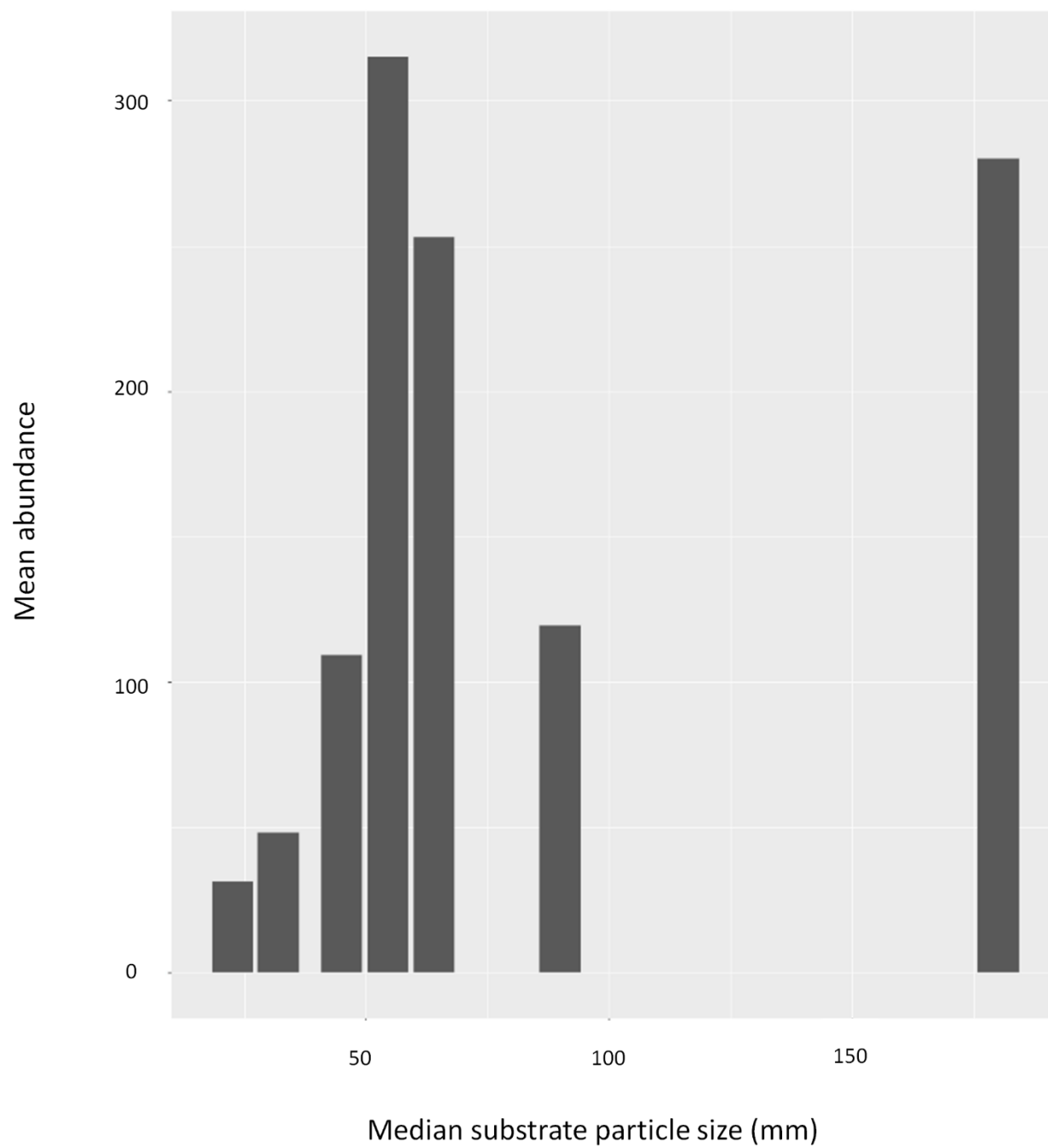
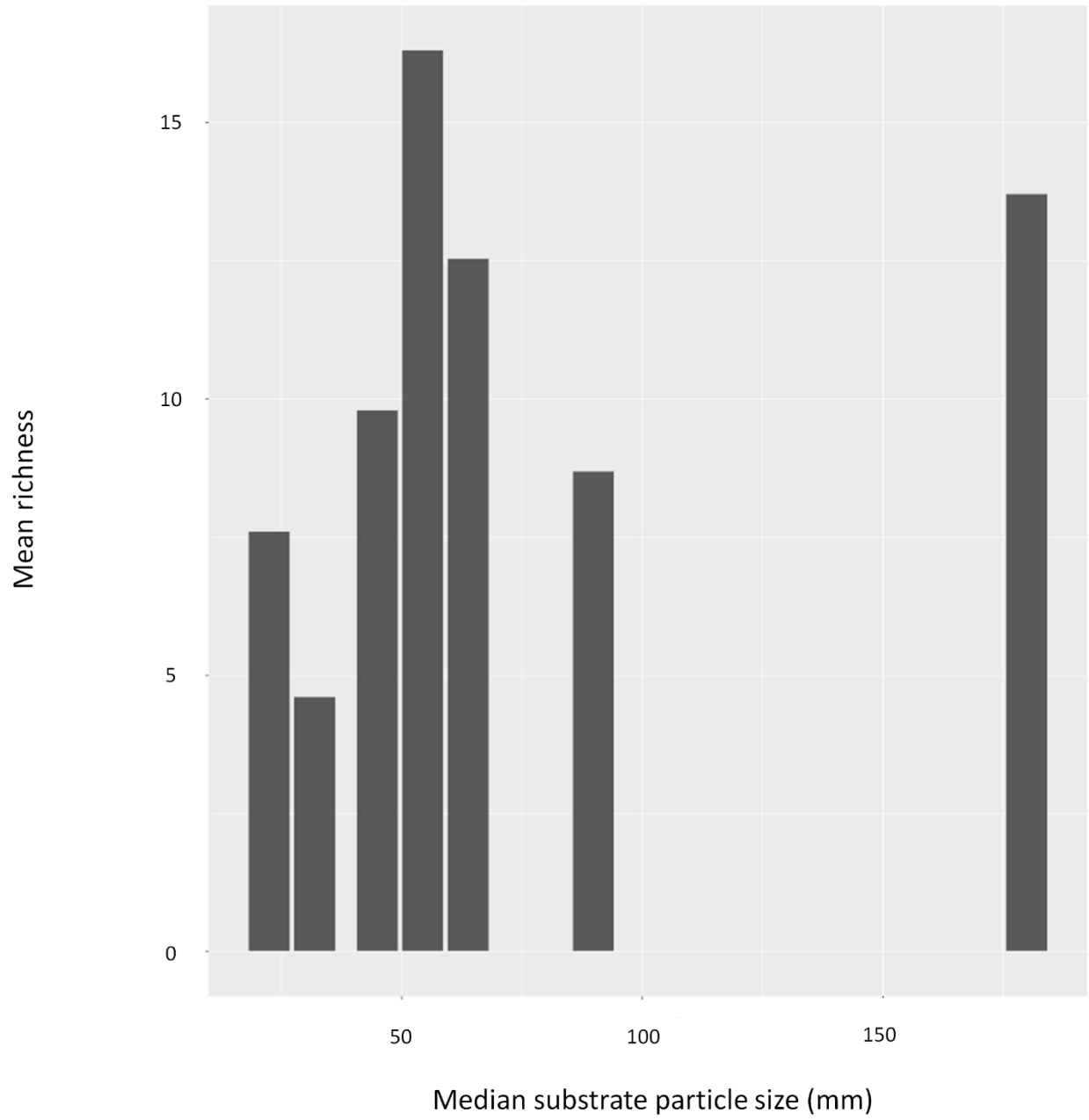
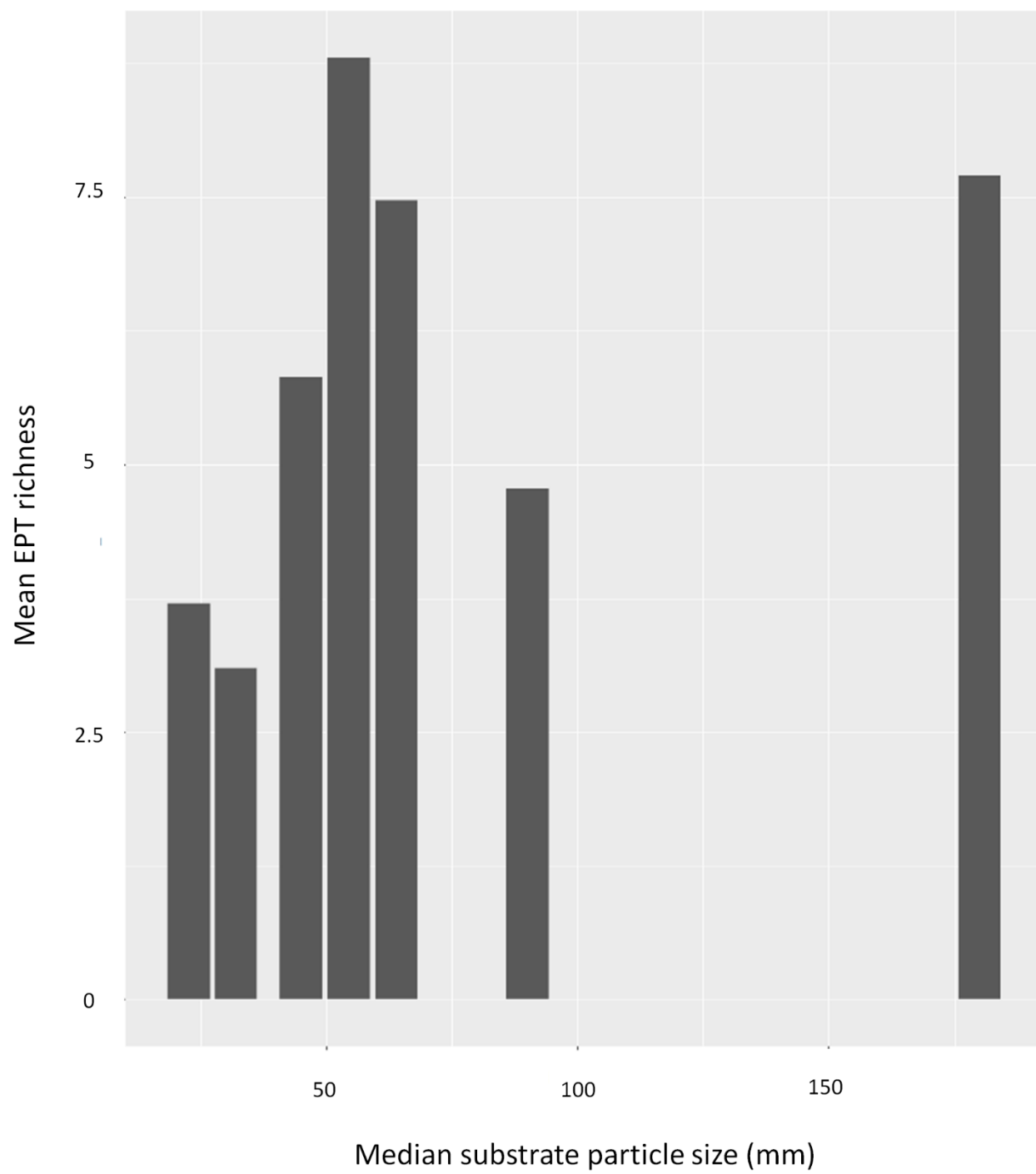
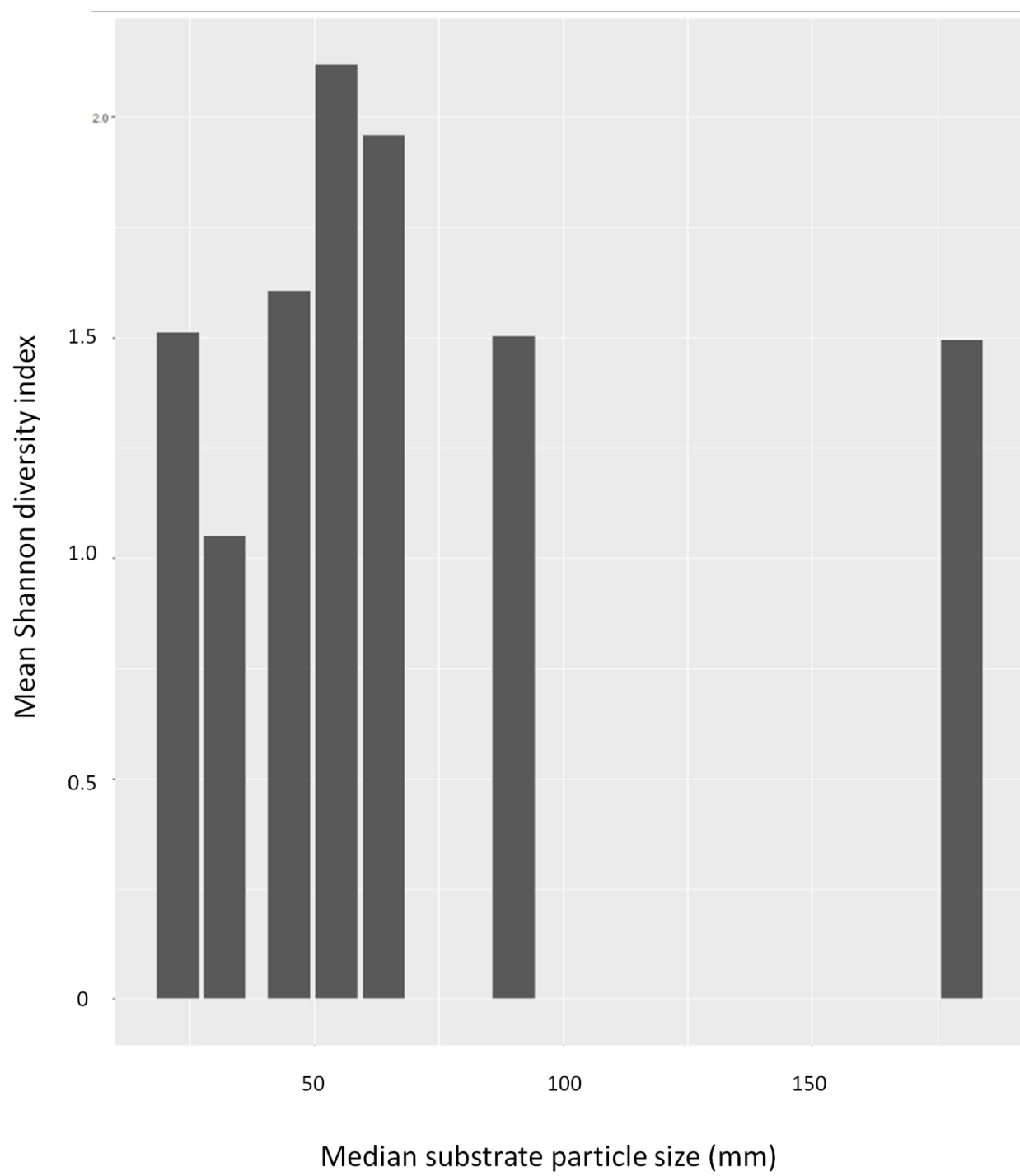


Figure 12: Mean macroinvertebrate abundance, richness, EPT richness, Shannon diversity index, and Missouri biotic index plotted against median substrate particle size.









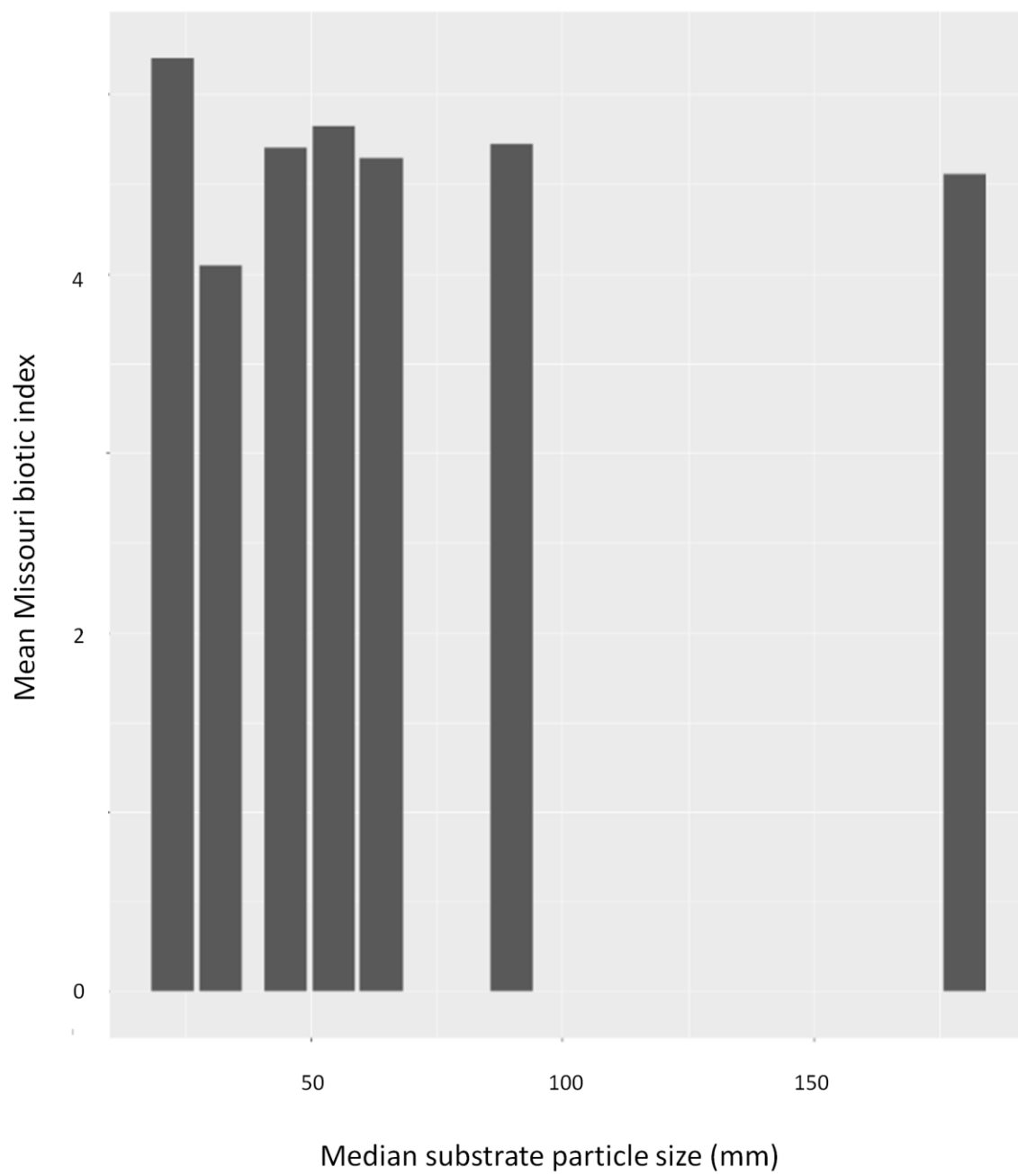
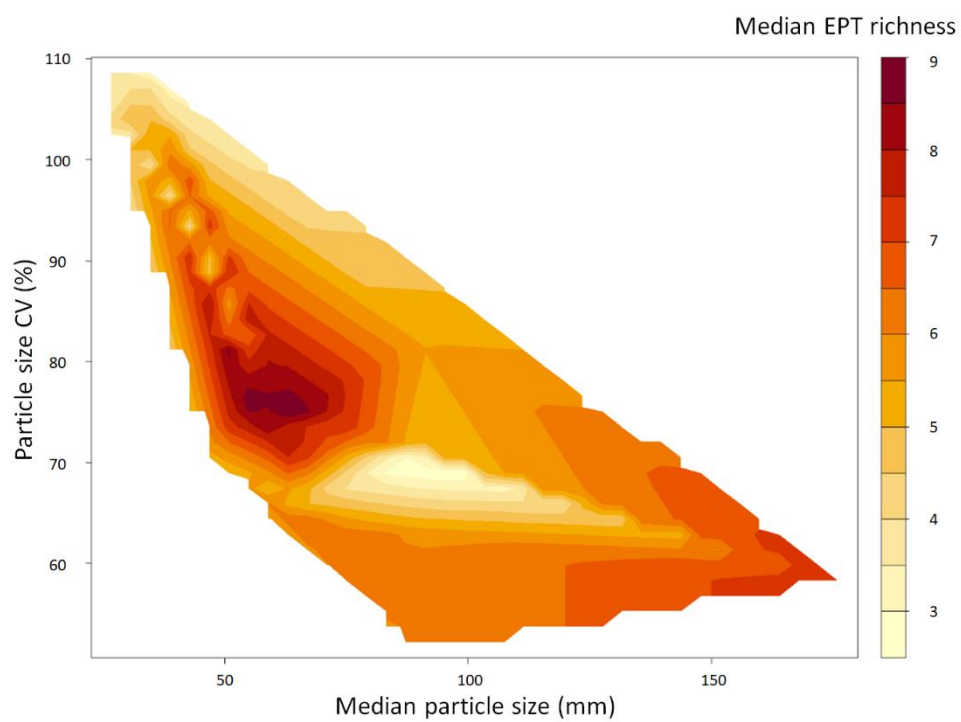
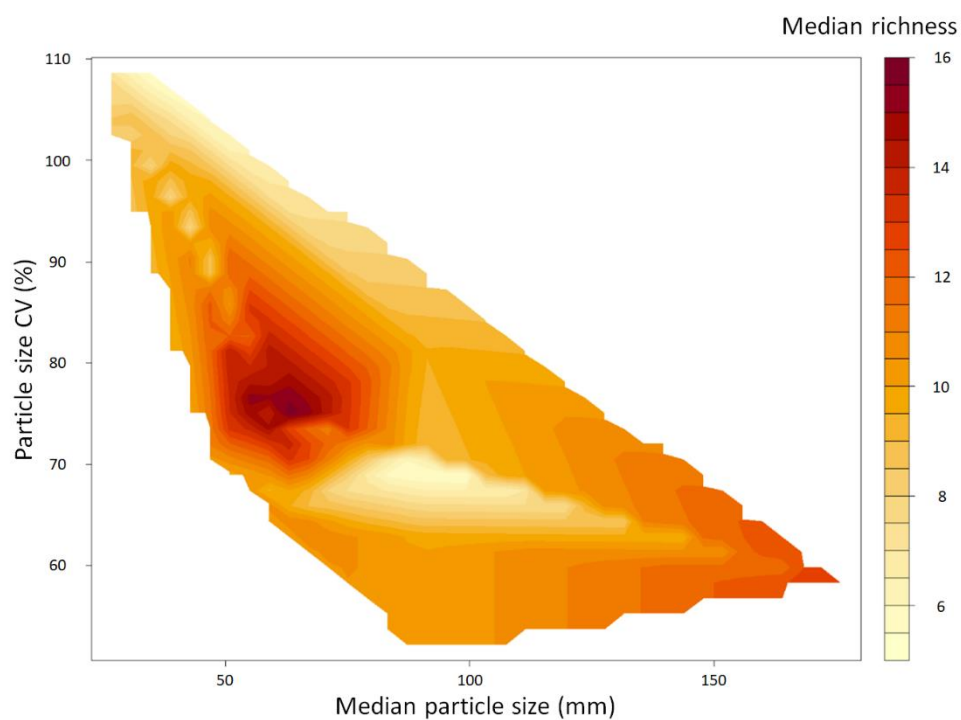
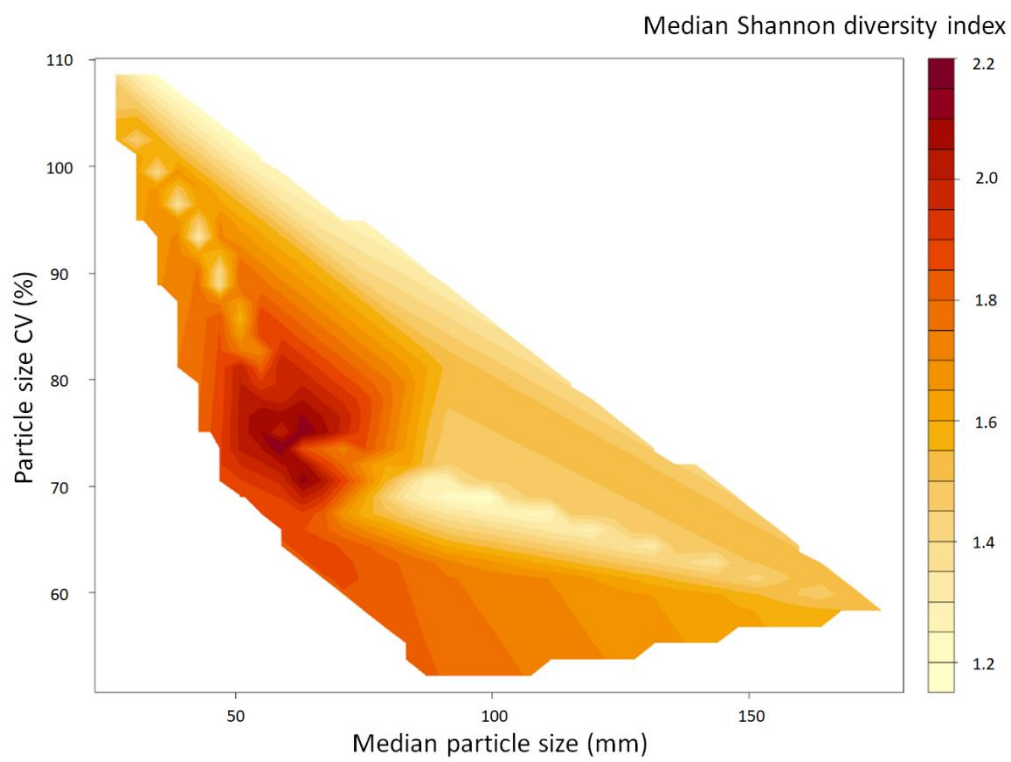
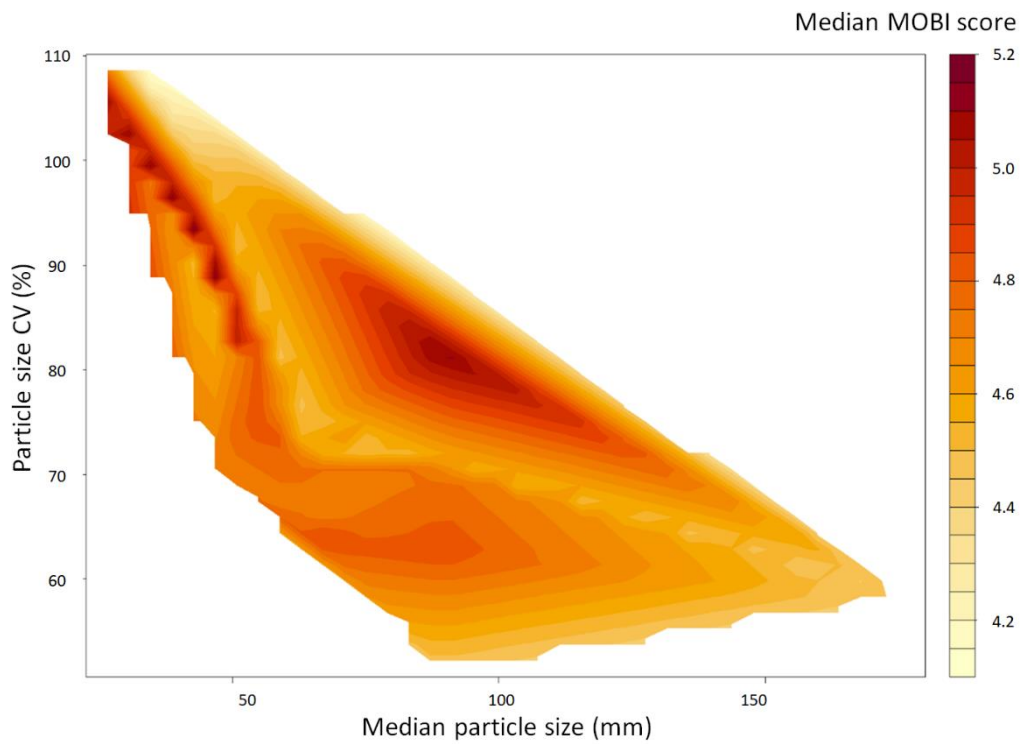


Figure 13: Heatmaps of median macroinvertebrate, richness, EPT richness, Shannon diversity index, Missouri biotic index, and abundance plotted against median substrate particle size and particle size coefficient of variation.





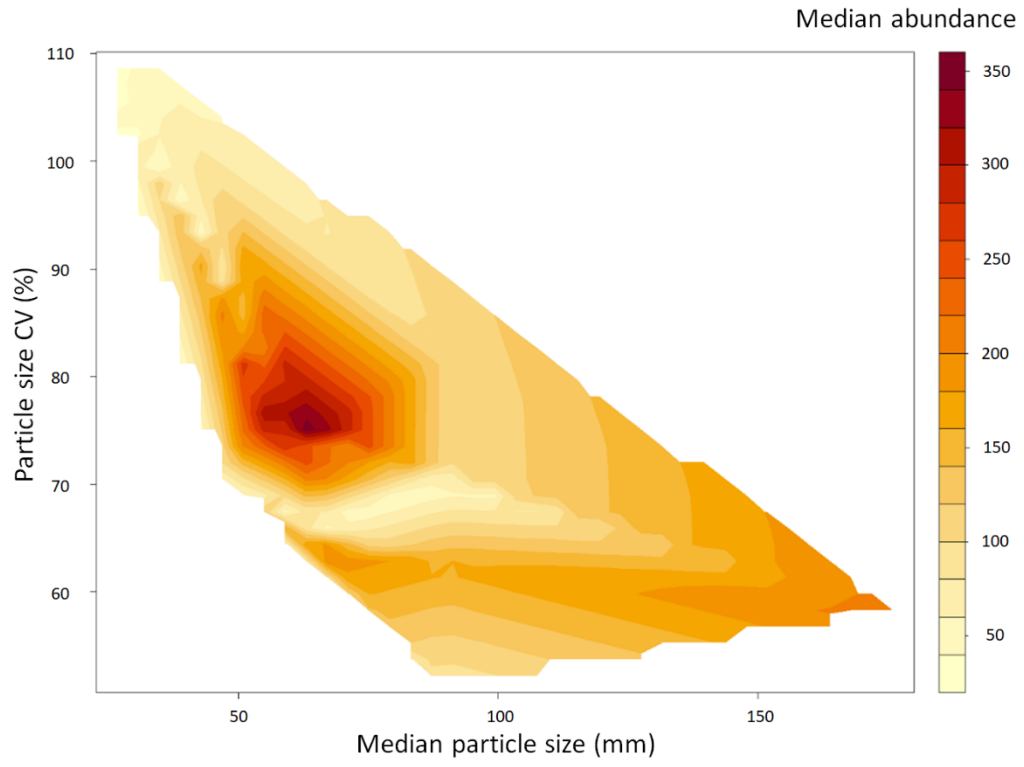


Figure 14: Principal components analysis plot of measured and calculated physicochemical parameters, including principal components 1 and 2.

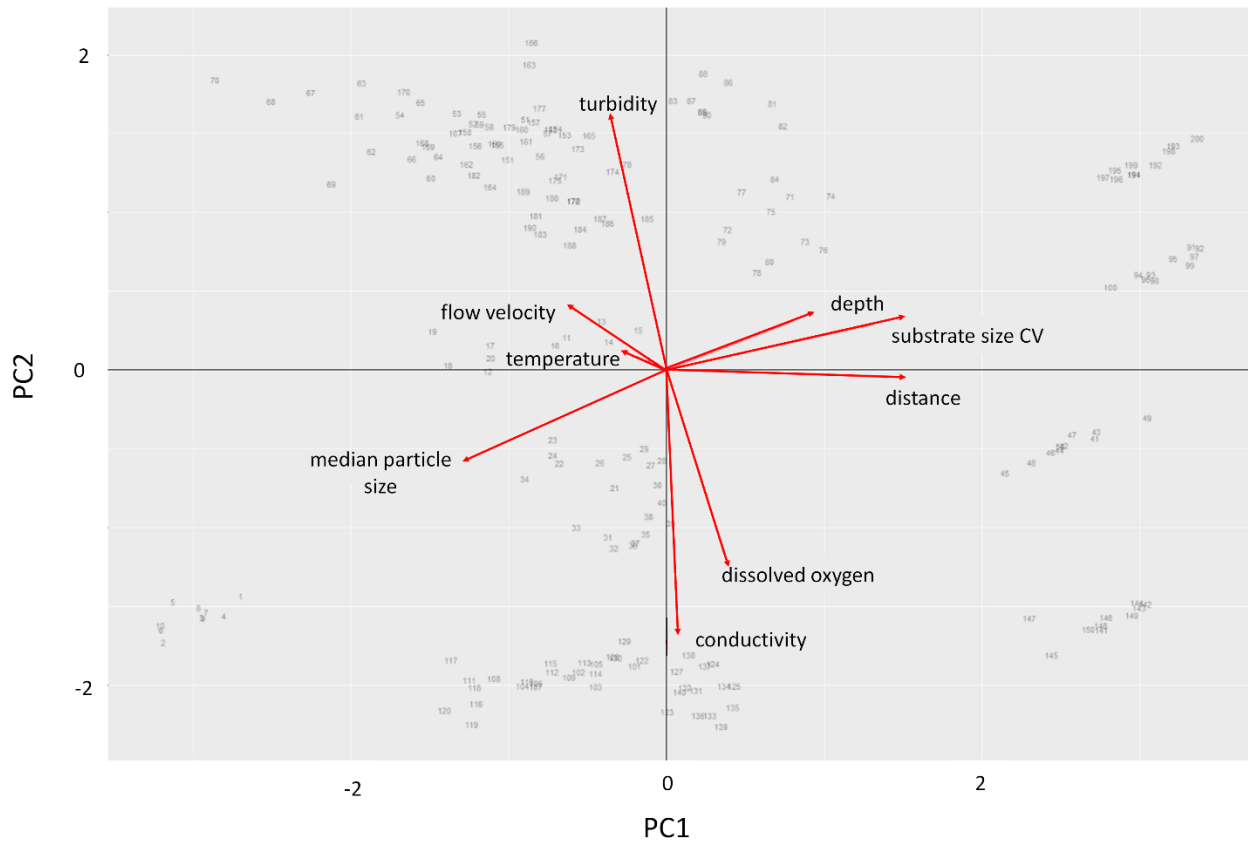
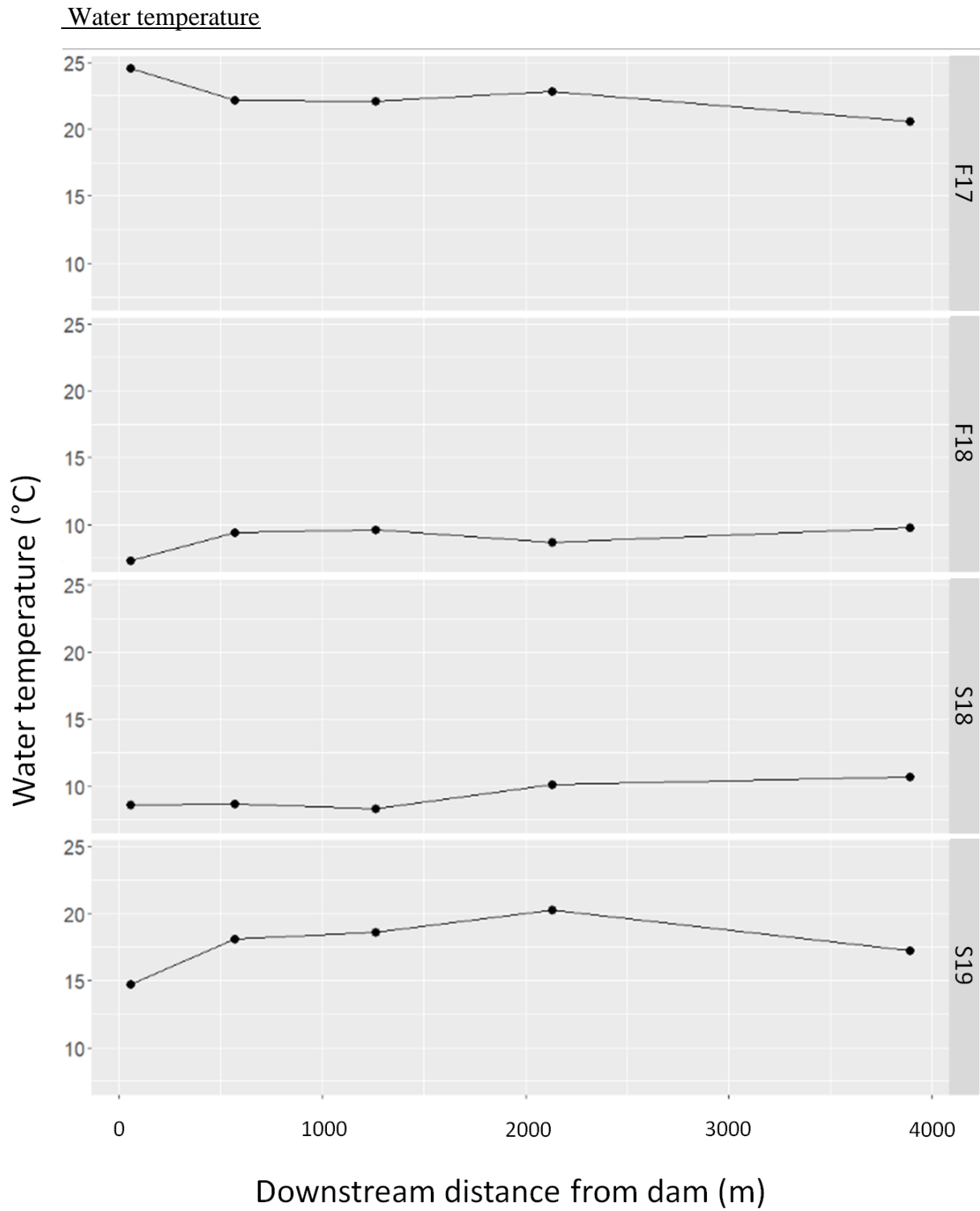
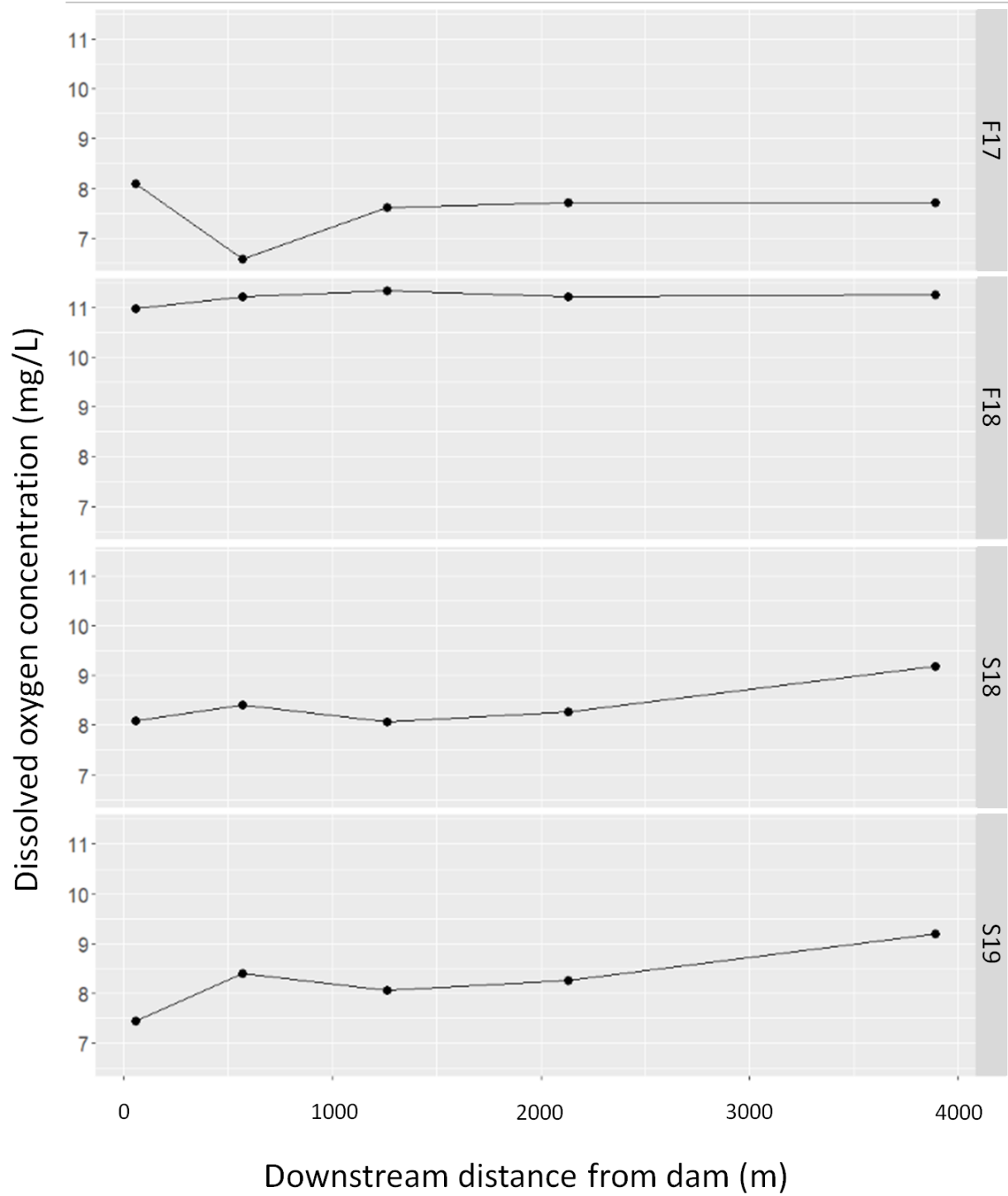
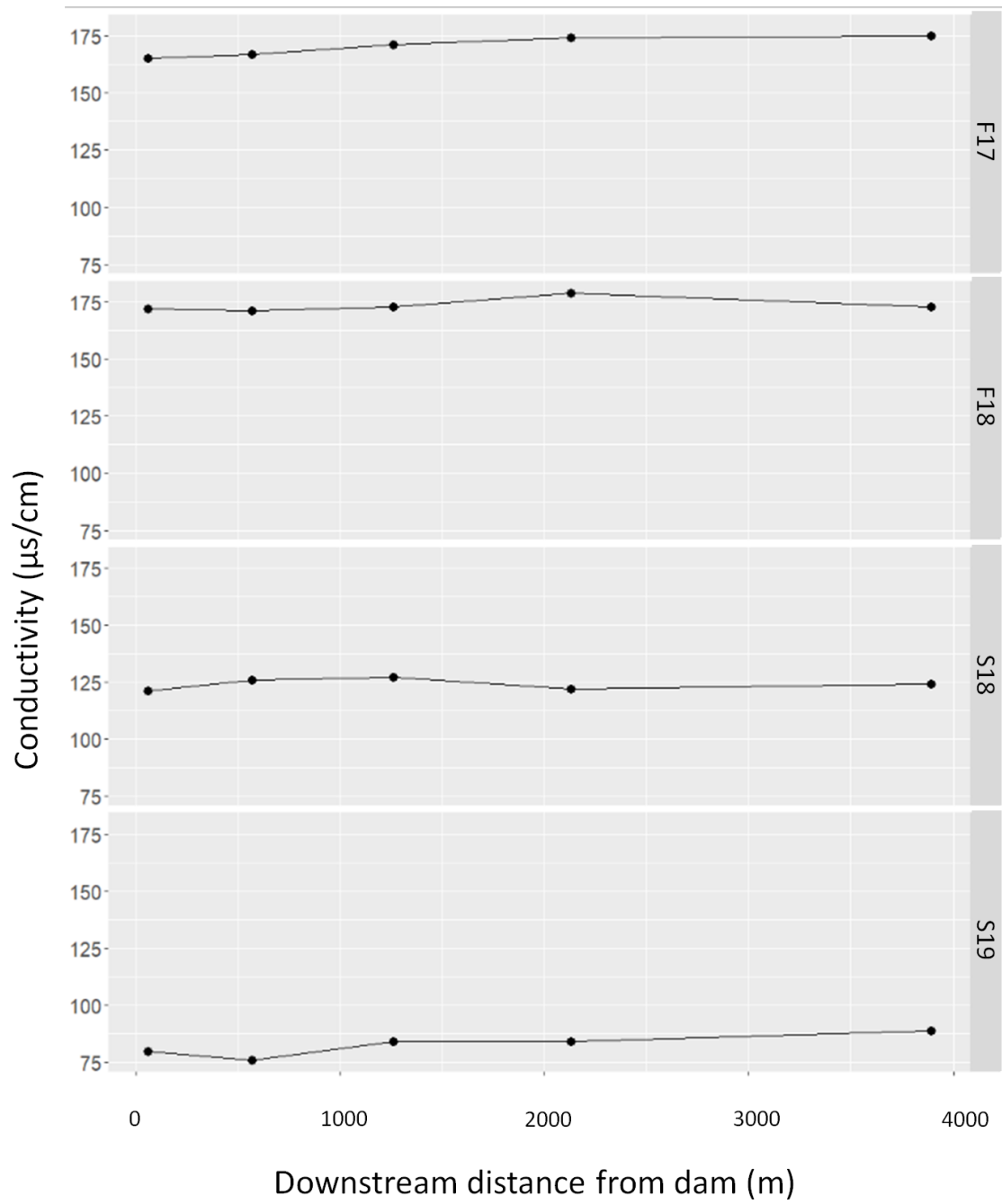
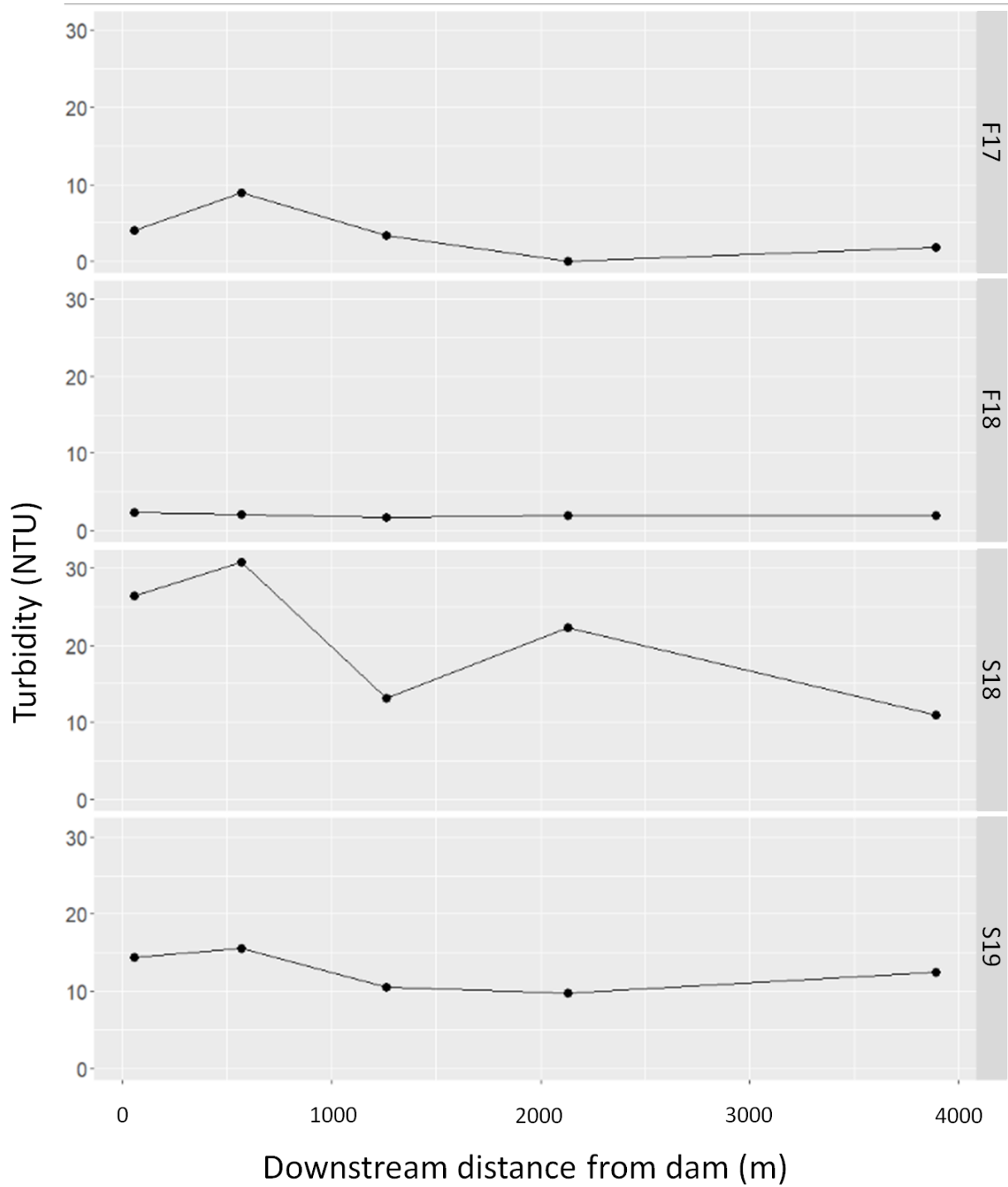


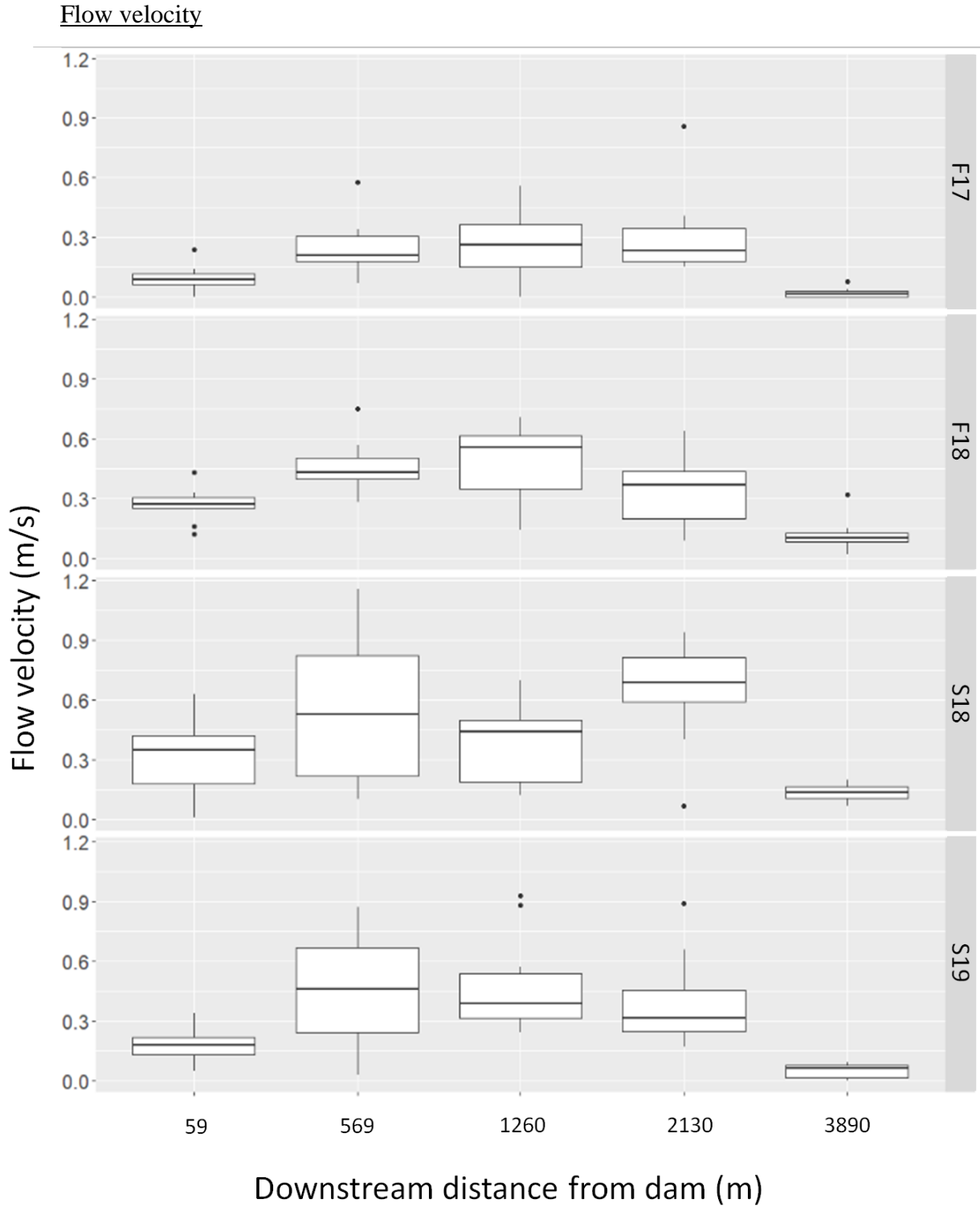
Figure 15: Physicochemical parameters plotted as a function of downstream distance of the dam, separated by sampling event.

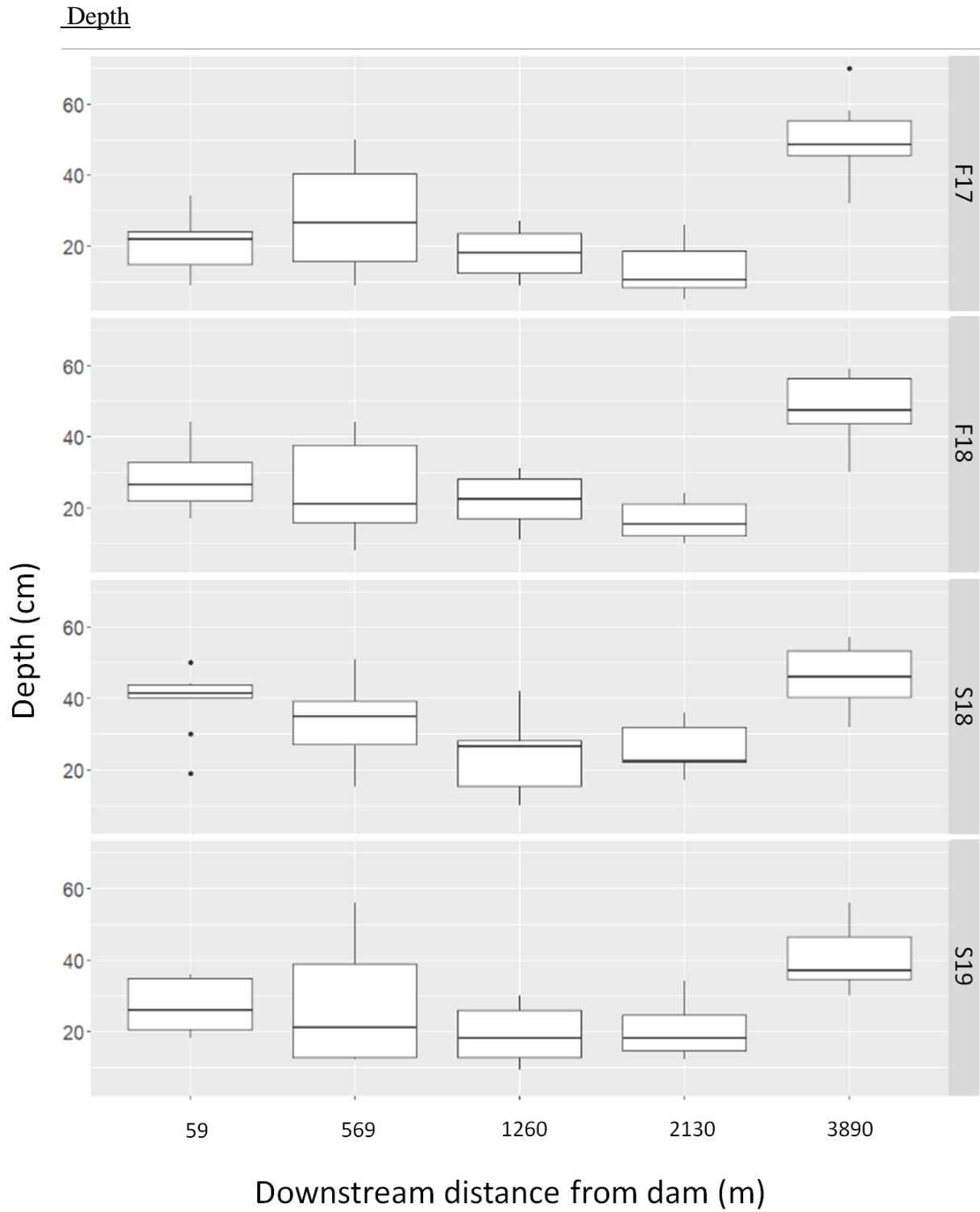


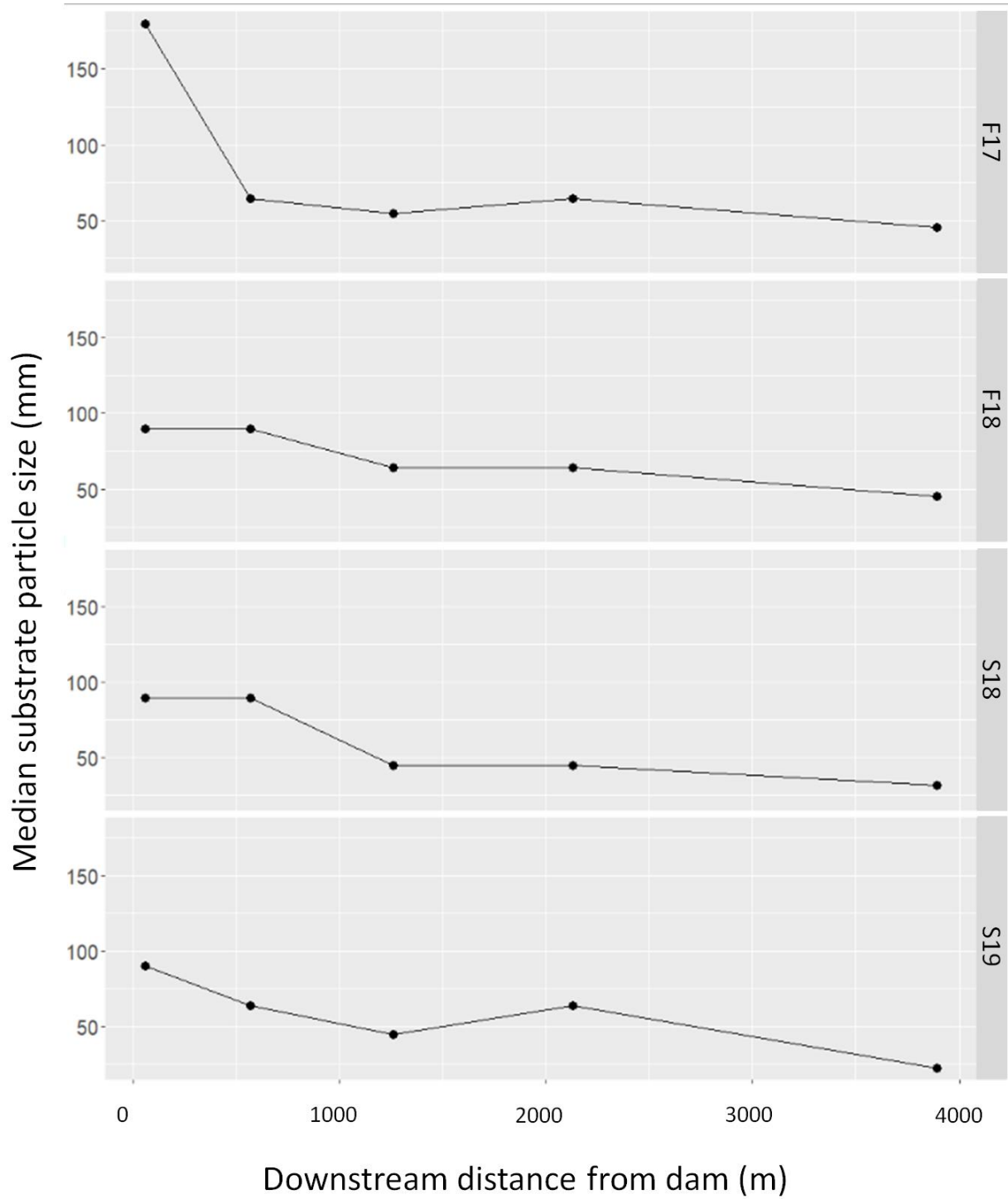
Dissolved oxygen concentration

Conductivity

Turbidity





Median particle size

Substrate size CV