IMPACTS OF CULVERTS AND DAMS ON

AQUATIC MACROINVERTEBRATES IN OKLAHOMA

WADABLE STREAMS

By

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Abstract: Anthropogenic activities alter flowing water in many ways resulting in streams being among the most modified ecosystems worldwide. Humans cause the greatest effects to aquatic ecosystems by creating artificial structures to impede natural stream flow. The purpose of this research was to examine the impact artificial structures such as dams and culverts have on aquatic invertebrates and to test the effectiveness of homemade artificial substrate sampling devices. Dams alter discharge, temperature regimes, and disconnect upstream and downstream reaches causing changes in biological communities. Existing literature demonstrates that dams impact aquatic biota, including macroinvertebrates, but less is known about how far these impacts persist downstream of dams and whether the impact of multiple dams is cumulative. In addition, the release of hypolimnetic water from reservoirs alters downstream water quality, especially water temperatures and flow regimes, which often disrupts the natural stream community. Our first objective was to assess the effects of hypolimnetic releases and multiple dams on macroinvertebrates in a 19-km reach of the lower Mountain Fork River, a cold tailwater river, fragmented by three dams in southeastern Oklahoma. Culverts impact streams by narrowing the stream channel, preventing the movement of substrate and large woody debris, and causing erosion near the road crossing leading to subsequent sedimentation further downstream. Existing literature shows that culverts pose a threat to habitat connectivity and limit fish movement in lower order streams, but less is known about the impacts of culverts on aquatic macroinvertebrates. Therefore, our second objective was to investigate the effects of road culverts on the aquatic macroinvertebrate communities in two stream systems (Tallgrass prairie and Ozark Highlands) in eastern and northeastern Oklahoma. Finally, collecting aquatic invertebrates is an important part of monitoring ecosystem health in aquatic habitats. Artificial substrate sampling devices are constructed to attract aquatic organisms that attach to hard surfaces to hide from predators and are an established sampling method for aquatic invertebrates. Hester-Dendy devices are widely used in standard water quality monitoring programs and research; however, the devices are expensive ($22-38 each). Our third objective was to test homemade artificial substrate devices created from empty 500-milliliter plastic soda bottles.

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CHAPTER I

REVIEW OF LITERATURE

Anthropogenic (human) activities alter flowing water in many ways (Finlay 2011) resulting in

streams being among the most modified ecosystems worldwide (Allan 2004). Pollution degrades

water quality and may reduce the amount of dissolved oxygen available to aquatic organisms

(Bashir et al. 2020), while habitat alteration threatens ecosystem integrity (Dudgeon et al. 2006).

Intentional and accidental introductions alter aquatic communities and modify predator-prey

relationships (Havel et al. 2015). However, humans often cause the greatest effects to aquatic

ecosystems when they create artificial structures that impede natural stream flow (Postel &

Richter 2003).

Streams are linear ecosystems that are susceptible to fragmentation. In an unfragmented stream

that flows uninterrupted, the stream changes along a predictable gradual gradient from

headwaters to downstream. This predictive series of changes is referred to as the river continuum

concept (RCC) (Vannote et al. 1980). Humans cause significant alteration to aquatic habitat

connectivity by constructing artificial structures or barriers to stream flow (Stanford et al. 1996).

The serial discontinuity concept (SDC) describes how human alterations or fragmentation, causes

a disruption of the river continuum that begins at the point of the alteration and recovers as the

downstream distance from the alteration increases (Ward & Stanford 1983). The SDC predicts the

responses of key biotic and abiotic variables in interrupted stream systems. These variables

include temperature, flow regimes, water quality, substrate, periphyton, organic material, and

planktonic drift (Ward & Stanford 1983). Additionally, the SDC predicts reduced invertebrate diversity below the stream interruption with a gradual increase in diversity as distance from the stream interruption increases (Stanford & Ward 2001). Invertebrate reduction below stream interruptions results from a combination of changes in detrital transport, organic matter inputs, nutrient spiraling, and thermal regimes.

Artificial structures such as dams and culverts are two types of human impacts to stream continuity that change water velocity, alter stream hydrology, modify temperature regimes, and create vertical drops at outflows by increasing downstream scour (Stanford & Ward 2001; Poff & Hart 2002). These changes can alter the ecology of a stream ecosystem (Mills et al. 1993) and cause species losses and declines (Quiros 1989; Meyers 1994). However, most stream research is dedicated to analyzing the impacts of artificial structures on fish (Peter 1998; McLaughlin et al. 2006), while fewer studies have examined the effects of artificial structures on aquatic macroinvertebrates.

MACROINVERTEBRATES

Aquatic macroinvertebrates are organisms large enough to be seen without a microscope, which lack a backbone and include arthropods (aquatic insects, mites, and crustaceans), annelids (segmented worms and leeches), nematodes (roundworms) and turbellarians (flatworms) (McCafferty 1983). Macroinvertebrates are found in both lotic (running water) and lentic (still water) environments worldwide (Hauer & Resh 2006). Most natural lotic systems (streams and rivers) contain a diverse assortment of macroinvertebrates with communities consisting of numerous taxa (Helms et al. 2009). Aquatic insects are often the dominant organisms in abundance and biomass in benthic stream macroinvertebrate samples (Hauer & Resh 2006).

Aquatic macroinvertebrates found in stream systems can be benthic, associated with the bottom surfaces of the stream channel (bedrock, cobble, gravel, or sediment) or can be attached to fixed

surfaces such as trees, roots, snags and emergent or submerged vegetation, while others are free swimming or drift throughout the water column (McCafferty 1983). Most macroinvertebrates found in lotic systems are benthic organisms (Hauer & Resh 2006).

Aquatic macroinvertebrates are key components of stream ecosystems (Vaughn 2002; Hauer and Resh 2006) and are an important food source for fish (Vidotto-Magnoni & Carvalho 2009; Wallace & Webster 1996) and birds (Horváth et al. 2012). Macroinvertebrates are essential members of the food web because they include herbivores, detritivores, and predators of other invertebrates (McCafferty 1983). Macroinvertebrates play an important role in the cycling of energy and nutrients because they break down organic matter including leaf litter, and detritus and transfer nutrients within the system and to the surrounding terrestrial ecosystem (Wallace and Webster, 1996; Baxter et al. 2005; Allan & Castillo 2007; Hussain & Pandit 2012). Many benthic macroinvertebrates convert live plant and dead organic material into resources used by larger consumers in complex food webs. These organisms also release bound nutrients into solution through feeding activities, excretion, and burrowing into sediments. They also accelerate microbial processing of dead organic material, and in headwater streams, process 20-73% of terrestrial riparian leaf-litter inputs (Covich et al. 1999).

Benthic macroinvertebrates are valuable bioindicators in freshwater streams and rivers because they live most, if not all, of their lives in the water (Armitage et al. 1983). They are relatively immobile and cannot move long distances to avoid effects of stream impairment (Mitchell & Stapp 2012). Therefore, benthic macroinvertebrates respond to impacts of human disturbance and serve as a measure of the overall health of the aquatic system (Barbour 1997).

Stream macroinvertebrate communities are regulated by a variety of biotic and abiotic factors that allow different taxa to successfully occupy a range of habitats within a stream system (Minshall 1988). These macroinvertebrate communities are necessarily resilient to the natural disturbance

regimes found in streams (Robinson 2012) but can be harmed when exposed to anthropogenic stress (Novotny et al. 2005). Anthropogenic changes can alter flow patterns, sediment movement, and nutrient levels, causing physical and chemical changes in streams that impact the macroinvertebrate community (Fores & Karr, 1996). Reductions in dissolved oxygen levels from pollution or biological oxygen demand can manifest as decreases in abundance and changes in community composition (Lancaster & Belyea, 1997; Juvigny-Khenafou et al. 2021). The community composition after anthropogenic changes will often differ depending on the regional taxa composition, species' dispersal capabilities and availability of refugia (Townsend, 1989)

Aquatic macroinvertebrates move from one area of a stream to another through dispersal (Bilton et al. 2001). Dispersal is important for colonization and allows gene flow, which maintains genetic diversity among populations (Smock 2006). Aquatic macroinvertebrates use dispersal to move to more suitable habitat, find food resources, avoid predators, and escape impaired stream conditions including point-source pollution (Wallace et al 1989; Siler et al. 2000; McIntosh et al. 2002). The linear flow that occurs in natural streams is an essential mechanism for downstream dispersal and when streams are disrupted, this dispersal can be interrupted (Vaughn 2002).

DAMS

A dam is an obstruction used to block the flow of water and to create a reservoir. Reservoirs can be constructed to meet specific community needs including storage of water for drinking, industrial, cooling water supply, hydropower generation, agricultural irrigation, river regulation, flood control, navigation, and for recreation and fisheries (Richter & Thomas 2007; Schmutz & Moog 2018). Unfortunately, dams are among the most damaging human induced changes to stream ecosystems and one of the major threats to freshwater biodiversity (Nilsson et al. 2005; Chen et al. 2011; Barbarossa et al. 2020). Research shows a generalized pattern of ecological change that occurs after the impoundment of a stream (Brittain & Saltveit, 1989). The principal

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impacts associated with dams are altered flow regimes, shifts in downstream water temperature, changes in substrate transport, impaired water quality, modifications of stream channel morphology, and obstruction of the dispersal and migration of macroinvertebrates and fish communities (Satake & Ueno 2012; Castro et al. 2013; Li et al. 2017; Gebrekiros 2016; White et al. 2017).

Dams impact both upstream and downstream portions of a stream and while examined less often, the downstream impacts of dams can be more detrimental to aquatic biota (Schmutz & Moog 2018). Bredenhand and Samways (2009) compared stream segments upstream and downstream of a dam and found that macroinvertebrate species richness was 50% lower downstream of the dam. In dddition, Vaikasas et al. (2013) found that the abundance of macroinvertebrates, specifically members of the sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa were significantly reduced downstream of a hydropower dam when compared to control sites (dam-unaffected)

Flowing water is the defining feature of a stream (Allan & Castillo 2007) and dams cause hydrologic changes which alter the natural flow regime (Armanini et al. 2014). To quantify hydrological modifications generated by anthropogenic activities, including river regulation, Poff et al. (1997) defined the five facets of the natural flow regime: "magnitude," "frequency," "duration," "timing," and "rate of change." These ecologically important aspects of the flow regime are altered or eliminated when stream flow is regulated by dams and other diversions (Nilsson et al. 2005).

Dams can create channel erosion leading to a loss of channel features, bed armoring, and a reduction of geomorphic activities downstream of the dam (Dietrich et al. 1989; Johnson 1992; Ma et al. 2012). Impoundments trap sediment on the upstream side of the reservoir and the lack of sediment downstream causes loss of habitat and resources for some benthic species (Kondolf 1997). Nutrients are often associated with fine sediments and when sediments are trapped by

dams, essential nutrients such as phosphorus are unavailable downstream. For example, after construction of Three Gorges Dam on the Middle Yangtze River, suspended sediment loads decreased by 91%, and total phosphorous decreased by 77% downstream (Zhou et al. 2013). Sharma et al. (2005) found that changes in nutrient resources downstream of a dam affect both macroinvertebrate abundance and taxa richness.

Downstream segments are also subjected to other impacts related to dam operation and quality of released water. The operation of hydroelectric impoundments tends to follow demands for electricity, creating variable flow regimes (Schmutz et al. 2015). Energy demand fluctuates multiple times a day (hydropeaking), which can cause rapid changes in stream flow and depth downstream of hydropower dams (Bejarano et al. 2017). Under natural flow conditions, increases and decreases in velocity, depth, and wetted stream width develop gradually (Ashaf et al. 2017). However, changes can occur suddenly when hydropower dams release water to meet electricity demands (Postel & Richter 2003). Munn and Brusven (1991) found low taxa richness in macroinvertebrate communities in streams below hydroelectric dams, characterized by irregular flow patterns. De Jalon et al. (1994) found that smaller macroinvertebrates are underrepresented downstream of dams because they cannot tolerate the higher stream flow velocities. Controlled flows below dams can also favor an excess of undesirable taxa such as some species of the families Chironomidae and Simuliidae (De Moor 1986; Munn & Brusven 1991).

Impounded streams with modified flow regimes coupled with large shifts in the thermal regime, from hypolimnetic water releases result in cold waters that can be seasonally oxygen-deficient downstream (Crisp et al.1983; Johnson & Harp 2005). Aquatic insect larvae use degree days and day length cues to synchronize emergence (Hynes 1970; Li et al. 2011). The coldwater release of hypolimnetic waters can impact these life history processes (Johnson & Harp 2005) and cause loss of susceptible species downstream (Bunn & Arthington 2002).

Most aquatic insects have terrestrial, winged adult stages that allow for movement past barriers (Bilton et al. 2001). In contrast, fully aquatic macroinvertebrates including most crayfish, and all amphipods, and isopods cannot move past barriers (Benstead et al. 1999; Bunn & Arthington 2002). These species are important components of streams because of their direct influence on ecosystem level processes and their ability to cycle energy and nutrients (Creed 1994; Taylor et al. 1996; Covich et al. 1999).

Objective 1. Determine the effects of multiple dams on seasonal abundance and diversity of macroinvertebrates.

The Lower Mountain Fork River (LMFR) is a tailrace located below Broken Bow Dam in the Ouachita Mountain region of southeastern Oklahoma. Broken Bow Dam was completed in 1968, forming Broken Bow reservoir (Harper 1994). Prior to the construction of Broken Bow Dam, the LMFR was a warm water stream that supported a native warm water aquatic community (Eley et al. 1981). Macroinvertebrates are important to stream communities, such as the LMFR, because they serve as an essential link in the food web and provide a connection between organic matter (algae, leaf litter and detritus) and fish communities (Hauer & Resh 2006).

The objective of this chapter is to characterize the benthic macroinvertebrate communities in the Lower Mountain Fork River, below Broken Bow reservoir and examine the impacts of two hypolimnetic releases (Spillway Creek and Powerhouse), and two dam structures (Old Park dam, and Re-regulation Dam) on communities at different distances from the disruption. We structured sampling seasonally along a longitudinal gradient among successive dam structures to determine abundance, taxa richness, diversity of macroinvertebrates and compared the percent Ephemeroptera, Plecoptera and Trichoptera (EPT) among sampling sites and seasons. We also examined functional feeding groups at different distances from impoundment. We hypothesized that the sites closer to the cold-water outflow would be dominated by perennial species and would exhibit lower taxa richness, diversity, and abundance.

CULVERTS

While dams have long been recognized as a substantial contributor to habitat fragmentation and disruption of stream continuity, there is increasing concern about the effect of road stream crossings on stream continuity (Jackson 2003; Moss 2016). When roads intersect streams, a bridge can be formed above the stream, or the flowing water is directed under the road through a culvert. Culverts can be constructed out of a variety of materials, including precast or cast-in-place concrete, steel, galvanized steel, aluminum, and thermoplastic (PVC). Additionally, culverts come in many sizes and shapes including rectangular, round, elliptical, and arched.

While road-stream crossings are necessary for transportation, many culvert designs may cause environmental impairment (Resh 2005). Culverts must be of proper size and shape to accommodate stream flow, including after precipitation events, and they must be installed in a manner to reduce soil erosion around the culvert inlet. Ideally, culverts also should decrease sediment deposition upstream of the culvert and limit scouring downstream from the culvert (CODOT 2019). Because culverts are often harder that the surrounding stream substrate, and because water is constrained to flow into culvert openings, both pooling upstream and scouring downstream often result. These alterations affect stream flow, sediment transport, the passage of aquatic organisms, and the movement of woody debris (Jackson 2003). It is widely recognized that culverts impact aquatic habitats and associated biota by disrupting stream connectivity and altering stream velocity and hydraulics near the culvert (Riley et al. 2020). These alterations are recognizable by changes in stream flow, channel form, and streambed texture (Medej et al. 2009). However, little existing research has examined potential differences in macroinvertebrate communities in adjacent stream areas that do not appear impacted.

Changes in stream dynamics at the site of the culvert are also likely to impact areas farther away although physical alterations may be less obvious. Low water crossing and non-functioning

culverts create a hydrologic disturbance because the road becomes a dam during high flow events and streamflow is restricted to the culvert. Higher flows associated with flooding produce high velocity discharge of water downstream that scours substrate and results in the formation of a plunge pool immediately below the culvert (Khan & Colbo 2008). Contraction scour results when the normal flow area of a stream channel is narrowed, and the natural floodplain is blocked by the roadway (Richardson & Richardson 1999). Contraction scour is particularly relevant to culverts because culverts constrict the cross-sectional stream area which increases the velocity of water exiting the culvert outlet resulting in erosion of the stream bed and banks. Thus, culverts impact channel stability, aquatic habitat, and associated biota both immediately above and below where they exist (Khan & Colbo 2008). Culverts with steep slopes can be problematic as well because steeper slopes result in higher water velocities, increasing scour potential (Abt et al. 1985).

Fine sediment (mineral and organic particles < 2 mm in size) occurs naturally in benthic stream habitats (Wood & Armitage 1997) and culverts can increase sediment loads and turbidity (Cornish 2001). An excess of suspended or deposited fine sediment causes stream impairment by reducing available space in gravel which is used by many invertebrates as refugia from predators and flow (Davies-Colley & Smith 2001). Thus, sedimentation can alter stream communities leading to a decrease in ecological diversity. These impairments can have significant impacts on macroinvertebrates (Jones et al. 2012). Alterations in macroinvertebrate density, diversity, and community composition (Quinn et al. 1992) can further affect other aquatic biota (Bilotta & Brazier 2008; Gieswein et al. 2019).

Increased deposition of fine sediment not only leads to a loss of coarse substrate (Ryan, 1991), but decreases periphyton biomass, which is an important food resource (Broekhuizen et al., 2001), and smothers rooted aquatic plants (Yamada & Nakamura 2002). When fine sediments become incorporated in epilithic (stone-surface) biofilms, the organic content and nutritional value of the biofilm as a food source for grazing macroinvertebrates is reduced (Graham 1990).

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Reduction of the organic content of the biofilm matrix, through sediment contamination, also reduces growth rates of macroinvertebrate grazers and negatively impacts invertebrate density, biomass, and taxonomic richness (Mattingly et al.1981; Collier 2002).

An increase of fine sediment causes clogging of interstitial spaces (Rehg et al. 2005). Many early instar macroinvertebrate larvae use interstitial spaces as habitat and other similar taxa use interstitial spaces as refuge during flood events (Sedell et al. 1990). Clogged interstitial spaces can also decrease connectivity between benthic and hyporheic zones resulting in reduced oxygenation of these zones (Ryan 1991; Wood & Armitage 1997). Taxa that prefer coarse substrates, grazers, and taxa utilizing interstitial spaces for protection are often replaced by more tolerant taxa such as chironomid species, oligochaetes, and bivalves (Jowett et al. 1991; Wood & Armitage, 1997; Kaller & Hartman 2004). In addition, culverts are linked with reduced quantities of cobble in stream channels (Eaglin & Hubert 1993). Because culverts slow water flow upstream and increase velocity at the culvert opening downstream, sediment transport can be altered leading to differences in nutrient availability at riffles further downstream.

Larger potential effects from changes in movement of logs, stumps, branches, and other woody debris, that falls into streams are important components of aquatic ecology (Gregory et al. 1991). Woody debris increases stream stability (Hering et al. 2000), provides essential habitats for fish (Crispin et al. 1993), and allochthonous material for aquatic food webs (Trotter 1990). Culverts impact the movement of woody debris and deciduous leaves (Jones et al. 1999). When the stream channel is wider than the culvert inlet, large woody debris can rotate and lodge in the culvert inlet. In addition, ponded water in the inlet basin leads to the accumulation of an interlocking raft of smaller woody debris (Furness et al. 1998), reducing transport of these materials downstream.

Objective 2. Determine the effects of culverts on macroinvertebrate communities in two stream systems.

Many road culverts in Oklahoma were either poorly designed initially or have not been maintained. This directly impacts the streams and the organisms that live in the streams. Research on the ecological effects of culverts is limited to effects on fish or effects immediately adjacent to the culvert (Gibson et al. 2005; Kemp and Williams 2008; Evans et al. 2015; Birnie-Gauvin et al. 2019; Johnson et al. 2019). Often the habitat that is visually undisturbed becomes the reference site after being accessed from the road crossing. Thus, there is a need for more research on the potential effects of culverts on macroinvertebrates in areas away from the immediate influence of the culvert. I will investigate the effects of road culverts on aquatic macroinvertebrate communities in two stream systems in central and eastern Oklahoma. I hypothesize that upstream and downstream riffles that are adjacent to areas affected by culverts will have similar macroinvertebrate communities.

PASSIVE SAMPLING

Aquatic macroinvertebrate communities are commonly used as a bioindicators to detect human influence on stream ecosystems (Buss et al. 2015) The main goal of biomonitoring is to assess the organism, population, or community level for presence, abundance, and/or behavior to measure a stressor's effect (Niemi & McDonald 2004; Bonada et al. 2006). Sampling aquatic macroinvertebrates is a common biomonitoring activity because it is reliable, inexpensive, and easy to employ (Karr 1991; Rosenberg et al. 1986). However, different aquatic habitats cannot be sampled in the same manner and often researchers choose between active samplers including kick seines and D-frame nets and passive samplers such as drift nets and Hester-Dendy samplers.

A variety of equipment and methods exists for sampling benthic macroinvertebrates and the sampling device used depends on habitat characteristics including velocity, water depth, and substrate type (Mitchell & Stapp 2012). Sampling of natural substrate occurs when macroinvertebrates are collected from the existing stream bottom. This type of sampling is

restricted to the riffle area of a stream that is shallow (wadable), with moderate flow, and cobble or gravel substrate. The most common type of sampling device used for natural substrate is a kick screen or kick seine (McCafferty 1983).

Artificial substrate sampling devices are used as alternative sampling techniques when it is not possible to sample the stream bottom such as in deep or fast-moving water (Carter & Resh 2001). Artificial substrate samplers are attached to the stream bottom and often simulate the natural substrate. The standard method uses Hester-Dendy samplers that consist of a series of stacked plates that have different sized openings. Baskets of cobble can also be deployed. The artificial substrate is left in the stream for 4 to 6 weeks to al low enough time for macroinvertebrates to colonize (Davies 2001).

Objective 3. Compare capture rates of aquatic invertebrates using artificial substrate sampling devices created from empty recycled 0.59 Liter (20-ounce) plastic bottles to Hester-Dendy samplers

To measure the effectiveness of plastic bottle samplers, I placed soda bottle samplers alongside standard Hester-Dendy samplers at eleven stream sites in Oklahoma. Sampling devices remained in the water for approximately one month to allow sufficient time for aquatic invertebrate colonization.

After one month in the stream, I retrieved both sets of sampling devices, took them to the laboratory, and removed aquatic invertebrates for identification.

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CHAPTER II

CHARACTERIZING SEASONAL AND LONGITUDINAL VARIABILITY OF BENTHIC

MACROINVERTEBRATE COMMUNITIES BELOW SUCCESSIVE DAMS IN A COLD-

WATER TAILRACE

INTRODUCTION

Natural lotic systems are defined by a physical gradient that induces longitudinal changes in

biotic communities. Stream order and gradient impact abiotic factors including water temperature

and dissolved oxygen availability, while tree cover, water depth, and water clarity affect the

degree of autochthonous and allochthonous inputs. These patterns lead to the well-established

'river continuum concept' (Vannote et al. 1980). Natural and anthropogenic disruptions in lotic

systems affect water properties and the organisms utilizing the habitat. Dam impoundments are

among the most common and diverse water regulation structures in streams and a considerable

proportion of the watersheds around the world contain at least one dam (Nilsson et al. 2005).

Dams often alter flow patterns, downstream water temperature, hydraulic characteristics,

substrate composition, channel morphology and water quality (Kondolf & Batalla 2005; Poff &

Zimmerman 2010; Phillips et al 2015; Phillips et al. 2016). The break in continuity instigated by

dams begins at the point of the anthropogenic disturbance and gradually recovers as the

downstream distance from the structure increases, creating a biophysical gradient (Ward &

Stanford 1983). Additionally, impoundments cause substantial effects to biota, including fish and

aquatic macroinvertebrates (Principe 2010; Holt et al. 2014) at different temporal and spatial scales (Matthews et al. 1991; Voelz et al. 2000; Cowell et al. 2004; Allan & Castillo 2007). The "serial discontinuity concept" describes how interruptions, often associated with human activities, such as dams, cause a disruption of the river continuum (Ward & Stanford 1983). Large dams in temperate regions often release from the hypolimnion of the reservoir which is cold because of thermal stratification. (Marshall et al. 2006). Hypolimnetic releases alter downstream water quality, especially water temperature and flow regimes, which often disrupts the natural stream community (Pringle et al. 2000; Bunn & Arthington 2002), resulting in fauna tolerant of year-round cold temperatures (Johnson & Harp 2005). While these cold-water releases impact native organisms downstream, they necessitate development of alternative fisheries such as stocked trout (Salmonidae) (Eley et al. 1981)

Multiple dams in a single drainage are often used in stream regulation and serve specific purposes, including flood control, irrigation, recreation, and power generation, depending on the local hydrology and seasonal precipitation patterns (Principe 2010; Bellucci 2011). Successive dams, even when structures allow water to flow over (low-head dams), may disrupt the river continuum, but relatively few studies have examined multiple structures that disrupt flow over a relatively short distance. Santucci et al. (2005) found that low-head dams negatively affected fish and macroinvertebrate communities in Illinois by degrading habitat and water quality in addition to fragmenting the stream into a series of free-flowing and impounded habitats. Temporal and spatial factors that impact biotic communities merit further investigation, especially in river systems that have higher flows, steeper gradients, and rocky substrate.

Benthic macroinvertebrates are very important to stream communities, serving as an essential link in the food web and providing a connection between organic matter (algae, leaf litter and detritus) and fish communities (Hauer & Resh 2007). Additionally, benthic macroinvertebrates are commonly accepted as indicators of stream ecosystem health due to their wide range of tolerance to an array of environmental conditions (Rosenburg & Resh 1996; Bonada et al. 2006). Numerous

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metrics such as abundance, taxa richness, functional feeding group, the percentage of

Ephemeroptera, Plecoptera, and Trichoptera (%EPT), and Shannon and Simpson diversity index

utilize benthic macroinvertebrates to quantify stream health and are regularly implemented in

stream health assessments (Plafkin et al. 1989; Wallace et al. 1996).

The Lower Mountain Fork River (LMFR) in the Ouachita Mountain region of southeastern

Oklahoma is a unique system that was altered by the construction of a low-head dam in the 1930s

to create a swimming area in a state park (Caneday et al. 2010). The river was drastically

changed by the construction of Broken Bow Dam completed in 1968, forming Broken Bow

reservoir (Harper 1994). Concurrent with the construction of the Broken Bow dam, a re-

regulation dam was added approximately 17 km downstream (Figure 1). The LMFR receives

cold water from two sources associated with Broken Bow Dam. The LMFR originates from the

Broken Bow Spillway dam which discharges water from a depth of approximately 10 meters into

Spillway Creek. Discharge for power generation, originates at a depth of approximately 50 meters

and passes through the Powerhouse dam, entering the river approximately 7 km downstream from

the spillway discharge. This cold water supports a year-round trout fishery. The Oklahoma

Department of Wildlife Conservation (ODWC) began stocking rainbow trout and brown trout in

the Lower Mountain Fork River in 1989 to compensate for cold water releases displacing the

native warm water fishery (Harper 1994).

The objective of this study was to characterize the benthic macroinvertebrate communities in the

Lower Mountain Fork River, below Broken Bow reservoir and examine the impacts of two

hypolimnetic releases (spillway dam and powerhouse dam), and two other dam structures (Old

Park dam, and re-regulation Dam) on communities at different distances from the cold-water

disruption. We structured sampling seasonally along a longitudinal gradient among successive

dam structures to determine abundance, taxa richness, diversity of macroinvertebrates and

compared the percent Ephemeroptera, Plecoptera and Trichoptera (EPT) among sampled sites and

seasons. We also examined functional feeding groups at different distances from impoundment.

We hypothesized that the sites closer to the cold-water outflow would be dominated by perennial species and would exhibit lower taxa richness, diversity, and abundance.

METHODS

Lower Mountain Fork River Study Area

The study area is located downstream of Broken Bow Dam, approximately 4 kilometers (km) east of the community of Hochatown, Oklahoma in McCurtain County, southeastern Oklahoma. This area is primarily forested and is characterized by oak/shortleaf pine forests common to the Ouachita Mountains region (Woods et al. 2005). Because of the forests, geology, and challenging landscape features, much of this region is unsuitable for traditional agriculture, but this area does function as a productive forest and supports commercial harvesting of pine and hardwoods (Caneday et al. 2010). Much of the adjacent area is maintained by the United States Forest Service. Our study area is surrounded by state parks and wildlife management areas, with only minor development.

The LMFR is a tailwater stream below Broken Bow Dam (Figure 1). Broken Bow Reservoir was impounded primarily for flood control, recreation, water supply, fish, and wildlife habitat and to create hydroelectric power (OWRB 2012). This study focuses on the 19-km stretch of stream designated as the LMFR trout fishery, which originates at the Broken Bow Spillway dam. This upper section of the fishery exists within Beavers Bend State Park surrounded by U.S. Forest Service land of the Ouachita National Forest. In the 19-km designated trout fishery, water temperatures are maintained below 20°C. The LMFR trout fishery is separated into three management zones established by the ODWC (Figure 1). Zone 1 extends from the spillway dam to the Old Park dam. Zone 2 spans from the Old Park dam to the re-regulation dam and zone 3 covers from the re-regulation dam to the US-70 bridge near Eagletown, Oklahoma (Figure 1).

The spillway is 115 meters long and in conjunction with the dam and dike system is 1.2 kilometers long (Caneday et al. 2010). The spillway dam is approximately 10 meters below the normal pool elevation of Broken Bow Reservoir therefore water temperature released from the spillway dam is influenced by the pool elevation. Water released from the spillway dam is discharged into Spillway Creek and releases from the spillway dam providing water temperatures sufficiently cold for maintaining trout populations.

Broken Bow Dam is a rolled earth-fill structure, standing 65 meters above the streambed, allowing maximum depths to reach 55 meters beneath the surface of Broken Bow Lake (USACE n.d.). Well oxygenated, cold water (< 7°C) found in the hypolimnion is released downstream during hydropower production. Bubble plume diffusers lie at the bottom of Broken Bow Lake near the dam. When hypolimnetic water heats up during the summer months, air is pumped to the diffusers. Bubbles rise towards the surface and create an upwelling of colder water (ODWC 2008).

The powerhouse dam is constructed of concrete and contains eight gates. Discharge for power generation comes from the powerhouse dam, through the powerhouse and into the river approximately 7 km downstream from the spillway discharge (USACE n.d.). The powerhouse includes two power units. Based on power demands, flow from Broken Bow dam can fluctuate and the amount of change that can occur within a single day varies, creating an inconsistent flow regime below the dam. Additionally, releases from the dam are hypolimnetic, creating a year-round cold-water stream in a region normally characterized by warmwater stream ecosystems.

The oldest impoundment on Lower Mountain Fork River is Old Park dam. It consists of a concrete structure approximately 2 meters in height, built in the 1930, to create a swimming area. Water flows over the top of the dam and is influenced by upstream releases (Caneday et al. 2010).

The Army Corps of Engineers constructed a re-regulation dam in 1968 located within Mountain Fork Park to regulate water flows and reduce the rapid fluctuation of water levels caused by hydroelectric power generation. The re-regulation area is managed by the McCurtain County commissioners and the property surrounding the reregulation area is designated as part of the Ouachita National Forest. The re-regulation dam is constructed from concrete and is approximately 15 meters in height (Caneday et al.2010).

Sample Sites

Each month (May 2016 through July 2017) we sampled twelve sites within the 19- km designated cold water trout fishery, to detect spatial and temporal patterns of macroinvertebrate communities. Sites 1 through 4 were in zone 1 just downstream of the spillway dam within Beaver's Bend State Park. Sites 1 through 4 experienced base flow from the spillway outflow. Sites 5 through 8 were in zone 2, just downstream of the Old Park dam in the Ouachita Wildlife Management Area. Sites 9 and 10 were in zone 3 just downstream of the re-regulation dam within the Mountain Fork Park. Sites 11 and 12 were also located in zone three further downstream of the re-regulation dam in the Ouachita Wildlife Management Area. Sites 5 through 12 experienced stream flow from the spillway dam in addition to hydropower peaking flows from the power plant.

Macroinvertebrate Sampling

We collected macroinvertebrates monthly from May 2016 through July 2017, from twelve sites, in three zones (Figure 1). We used a rectangular frame kick seine frame, in one riffle, at each sample site. Macroinvertebrates were dislodged from the benthos by kicking or agitating submerged substrate or hand scrubbing, then removing larger substrate followed by kicking, for 1 minute, in a 1-square meter (m^2) area immediately upstream of the kick seine.

To decrease laboratory sorting time, we removed large organic debris and stones in the field and then hand-picked macroinvertebrates for either 5 minutes or until no macroinvertebrates remained. Samples were preserved in the field in 70% ethyl alcohol and transported to the lab where they were sorted and identified to lowest practical taxon. We implemented keys by Smith (2001), Merritt et al. (2008), and Wiggins (2014) to assist with aquatic invertebrate identification.

Data Analysis

We collected a total of 163 samples from 12 different sites over a 15-month study period. We did not sample sites 6, 7, 8, 9, and 10 in May of 2016, site 10 in June 2016, site 9 in July 2016, site 8 in August of 2016, Site 4 October 2016, site 11 in November 2016, and sites 6, 7, 8, 9, 10, 11, and 12 in February 2017 due to flow related inaccessibility issues. Temporal data for each site was aggregated by combining site data for the three months that comprise each season (summer: n=34, fall: n=35, winter: n=29, spring: n=36).

Benthic macroinvertebrates are commonly accepted as indicators of stream ecosystem health due to their wide range of tolerance to an array of environmental conditions (Rosenburg & Resh, 1996; Bonada et al. 2006). We calculated the metrics abundance, taxa richness, functional feeding group, the percentage of Ephemeroptera, Plecoptera, and Trichoptera (%EPT), and Shannon and Simpson diversity index, which are regularly utilized to quantify stream health (Plafkin et al. 1989; Wallace et al. 1996).

We quantified the number of individuals collected during the study and determined abundance by summing the number of individuals identified over the sample period for each site. We determined abundance by season by summing the number of individuals for each site for each season (summer, fall, winter, spring). We determined the mean abundance by site by averaging the abundance for each month over the sampling period for each site and mean abundance by

season by summing the abundance of the three months that make up each season for each site and then averaging all sites for each season.

We calculated taxa richness for each site using the lowest taxonomic level identified for each taxa group. Taxa richness is a measure of the number of different kinds of organisms or taxa in a sample and is common measure of biological diversity (Colwell 2009). We determined richness for each site by summing the number of unique taxa groups identified over the sample period for each site and richness by season by summing the number of unique taxa groups for all sites combined for each season. We determined mean richness by site by averaging the richness for each month over the sampling period for each site and mean richness by season by summing the richness of the three months that make up each season for each site and then averaging all sites for each season.

In addition, we calculated the metric % EPT which is the total number individuals within the generally more environmentally sensitive orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) divided by the total number of individuals in the entire sample. This summary metric is commonly used in macroinvertebrate studies because members of these sensitive orders are more vulnerable to habitat degradation (Lenat 1988; Merritt et al. 2008). We determined % EPT for each site by summing the number of EPT individuals for each site for each season and dividing by the total number of individuals for each site for each season. Percent EPT values greater than 50% are good and values below 25% are indicators of a poor bioassessment (Plafkin et al. 1989)

Macroinvertebrate diversity was assessed using previously calculated abundance data. We implemented two indices widely used to assess community structure: Simpson's Index (*D*) (Simpson 1949):

$$D = \Sigma[ni(ni-1)/N(N-1)]$$

where *ni* is the number of individuals of the *i* th taxon and *N* the total number of individuals

within the sample, and the Shannon-Wiener index (*H'*) (Wilhm 1972):

$$H' = -\Sigma pi * \ln pi$$

where *pi* = *ni*/*N* to estimate diversity. Simpson's Index measures the probability that two

individuals randomly selected from a sample will belong to the same taxa, ranges between one

and zero, and the closer the value is to zero the greater the sample diversity (Simpson 1949). The

Shannon-Wiener Index quantifies the uncertainty in predicting the taxa of an individual that is

taken at random from the sample, normally ranges between zero and 3.5 with values greater than

three being very good and values below 2 being poor (Wilhm 1972; Barbour et al. 1999). These

indices were calculated for each site over the entire sampling period and for winter, spring,

summer, and fall and represent classical α-diversity (Whittaker 1972).

Macroinvertebrate abundance (count data) from the 12 sampled sites were log (x + 1) transformed

and rare taxa with ≤ 5 individuals (n = 19) removed to reduce the variability in the dataset for

statistical analysis (Reece et al. 2001). The 12 sites were located downstream to three different

water regulation structures and grouped as follows: spillway (sites 1-4), Old Park dam (sites 5-8),

and re-regulation dam (sites 9-12).

Non-metric multidimensional scaling (nMDS) was used to characterize the macroinvertebrate

assemblage by taxa and functional feeding group below each water regulation structure. Both

nMDS plots were generated based on a rank order Bray-Curtis dissimilarity matrix. Pairwise

distances are used in the final ordination plots to intuitively interpret community associations

(i.e., the closer the points the more similar their community composition). Spatio-temporal

differences in community structure among grouped sites over time were tested with analysis of

similarities (ANOSIM), which uses an iterative permutation procedure (999 permutations). The

ANOSIM R statistic determines the average rank dissimilarity among groups. Ranging from -1 to

+1, the R statistic will typically be between 0 to +1, where 0 indicates random grouping and +1 indicates a difference in groups (Clarke & Green 1988, Michelland et al. 2010). Similarity percentages analysis (SIMPER) was used to determine the taxa and functional feeding group that contributed to the variation among groups over time. Analyses of ANOSIM, nMDS, and SIMPER were performed using PRIMER v7 (PRIMER-E software, Plymouth, United Kingdom; Clarke & Warwick 2001).

<div align="center">RESULTS</div>

Overall composition

From May of 2016 through July of 2017, we collected 163 samples yielding 21,019 individuals representing 54 taxa (14 orders, 33 families, and 37 genera) (Table 1). The orders Trichoptera, Diptera, Isopoda, and Ephemeroptera were the dominant components of the benthic macroinvertebrate community accounting for 89% of total individuals collected. Among these taxa, collector/filterers were the most abundant group with 12,522 individuals (Figure 2). The most dominant taxa were *Cheumatopsyche* (net-spinning caddisflies), *Chimarra* (finger-net caddisflies), and *Simulium* (black flies), which made up 87% of the total. Predators were the most diverse group with 21 unique taxa. The stonefly *Neoperla* was the most abundant predator collected in the study.

Variation among seasons

Mean macroinvertebrate abundance by season per site ranged from 327 +/- 108 to 513 +/- 98 individuals and was lowest in the spring and highest in the winter (Table 2). Taxa richness by season per site ranged from 9 to 24 (Table 2) and mean taxa richness by season ranged from 15 to 19 and was lowest in winter and highest in spring (Table 2). Simpson's index by season ranged from 0.48 to 0.11, with the lowest diversity in the summer and highest diversity in the spring (Table 2). Mean Shannon–Wiener diversity indices by season ranged from 1.18 to 2.45 and were

lowest in summer and highest in spring (Table 2). Mean percent EPT values by season were good, ranging from 56 to 94%, and were lowest in spring and highest in winter (Table 2).

R values for June 2016, December 2016, March 2017, and June 2017 were significant for seasonal differences among functional feeding groups below water regulation structures (p < 0.05) (Table 4). R values for May, 2016, June 2016, December 2016, February 2017, March 2017, April 2017, and June 2017 were significant for seasonal differences among taxa below water regulation structures (p < 0.05) (Table 5). Average dissimilarity for June 2016 was: spillway and old park (69.5%), spillway and re-regulation (69.3%), and old park and re-regulation (82.4%) with the highest contributions by *Simulium*, *Cheumatopsyche*, and *Cheumatopsyche*, respectively. Average dissimilarity for December 2016 was spillway and old park (68.1%), spillway & re-regulation (61.5%), and low water & re-regulation (51.2%) with the highest contributions by *Cheumatopsyche*, Chironomidae, and *Isonychia*, respectively. Average dissimilarity for June 2017 was spillway and old park (67.2%), spillway and re-regulation (70.9%), and old park and re-regulation (66.2%) with the highest contributions by *Neoperla*, *Cheumatopsyche*, and *Chimarra*, respectively. *Cheumatopsyche* and *Chimarra* appeared as the greatest contributors to pairwise comparisons in 11 and 7 of the 15 months sampled, respectively. Moreover, monthly SIMPER analyses determined collector/filterers as the most influential group with 25 of the 45 pairwise comparisons.

Variation among sites

Mean macroinvertebrate abundance per site per month ranged from 50 +/- 18 to 429 +/- 89 individuals and was lowest at site 7 and highest at site 1 (Figure 3). Macroinvertebrate abundance for the entire sampling period ranged from 412 to 6142 and was lowest at site 7 and highest at site 1 (Table 3). Taxa richness per site for the entire sampling period ranged from 24 to 35 and was lowest at site 8 and highest at sites 10 and 11 (Table 3). Mean taxa richness per site per month ranged from 8 +/- 1 to 13 +/- 1 and was lowest at site 7 and highest at site 12 (Figure

4). Mean Shannon–Wiener diversity indices per site were low to moderate, ranged from 1.47 to 2.11, and were lowest at site 1 highest at site 4 (Figure 5). Mean percent EPT values by site were poor to good, ranged from 14 to 87%, and were highest at site 12 and lowest at site 1 (Figure 6).

The nMDS reached a convergent solution after 29 iterations, and the 3-dimensional solution had a stress level of 0.03 (Figure 7). Collector/filterers and collector/gatherers functional feeding groups were abundant among spillway sites. Interestingly, collector/gatherers also displayed a strong association to sites just below the re-regulation dam. *Cheumatopsyche* was the most abundant taxa collected, and due to its ubiquitous presence among all sites, it does not appear in the nMDS ordination. However, taxa such as *Simulium*, *Lirceus*, and *Pseudocentroptiloides* were dominant among spillway sites, specifically site 1. The "multiple" functional feeding group was exclusively made up of Chironomidae and were mostly found at sites furthest away from the spillway. Predators like *Neoperla* and *Corydalus* were associated with the low water dam sites, whereas *Hansonoperla* was more associated with spillway sites. Scrapers were also primarily sampled from the low water dam sites with the flatheaded mayfly, *Maccaffertium*, accounting for most of the scrapers.

Differences in macroinvertebrate assemblages among sites were evident and supported by the ANOSIM analysis (global $R = 0.509$, $p = 0.001$). SIMPER identified *Simulium* as the greatest contributor to differences among sites when compared to the spillway. Average dissimilarity among the three pairwise comparisons was fairly consistent and was as follows: spillway and old park (32%), spillway and re-regulation (28.9%), and old park & re-regulation (27.7%). Within groups, the greatest average similarity was found below the old park dam (81.3%) with the highest contribution by *Cheumatopsyche* followed by *Chimarra*.

DISCUSSION

To date, few studies describe the impacts of successive dams on macroinvertebrate communities in a midwestern cold water tailrace. Additionally, there is limited data concerning

macroinvertebrate community assemblages in Oklahoma. We assessed the effects of multiple

dams and hypolimnetic releases on macroinvertebrates in a 19-km reach of the LMFR, a cold

tailwater stream fragmented by four dams in southeastern Oklahoma. Surprisingly, despite

alterations to the LMFR from hypolimnetic releases and multiple dams, the overall

macroinvertebrate fauna was similar to the unimpacted warm water Glover River in southeastern

Oklahoma (Orth et al. 1982). Despite overall similarity, macroinvertebrate assemblages collected

from the LMFR showed differences among sites and seasonally related to distance from the

coldwater outflow from the spillway dam.

Seasonal patterns

Aquatic macroinvertebrates that rely on external inputs have lifecycles that are influenced by

season, and we found differences among seasons for the calculated indices. Abundance was

highest in the winter, especially in January when larval Diptera (families Simuliidae and

Chironomidae), and Trichoptera, (family Philopotamidae) were common. These taxa decreased

until reaching the lowest numbers in the fall. Amphipoda and Ephemeroptera also occurred in the

greatest abundance in the winter. In contrast, Isopoda and Tricladida had the highest abundance

in the Spring. Isopoda had the lowest abundance in the winter and Trichoptera had the lowest

abundance in the Spring. The remaining examined orders were collected least during summer.

Interestingly, among sites, taxa responded differently across seasons. The differences were

mainly associated with collector/filterer groups which are characteristic of larger slower moving

rivers (Vannote et al. 1980). The numbers of these taxa increased with distance from

impoundment suggesting that downstream changes that slow flow allow small detritus to

increase.

Taxa richness, an important measure of diversity within streams, varied by site and season. Taxa

richness was highest in the spring, especially at sites 10 and 12 which had a high number EPT

genera and lowest in winter at sites 5, 6, and 7. The low taxa richness at sites 5, 6, and 7 was

below the Old Park dam and was unexpected. The reasons for lowered diversity have not been

determined but may be a result of slowing flows as water must pass over the top of the dam, unlike water sources at the other areas. Taxa dominance, measured by the Simpson's Index, was lowest (high diversity) in spring and highest (low diversity) in summer while the Shannon Index that measures overall taxa richness mirrored these results with the highest diversity in spring and lowest diversity in summer.

Percent EPT is an important measure of water quality in streams because these orders rely on dissolved oxygen and are sensitive to disturbance (Barbour et al. 1999). Surprisingly, percent EPT values by season were highest in winter and lowest in spring. This observation is a result of having many EPT genera found in the spring, but only a few representative species in each group (Table 1).

Longitudinal gradient

The highest observed abundance was at site 1, the site closest to the spillway dam, yet this site had the lowest diversity values (Figure 3 and Figure 5). This suggests that this site is dominated by only a few taxa. Not only was site 1 dominated by Simuliidae, Isopoda, Amphipoda, and Tricladida, but these taxa also had the greatest abundance nearest the spillway dam and lowest abundance at sites downstream. High numbers of these orders, collected near the Spillway dam, may be a result of the taxa in these orders requiring cold water temperatures which result from the hypolimnetic release. Generally, macroinvertebrates native to the southern US are not adapted to year-round cold-water streams (Johnson et al. 2007). When habitats are altered and water temperature decreases, intolerant taxa disappear from the environment, allowing tolerant taxa that inhabit cold water to increase in abundance (Hilsenhoff 1971). The high abundance of Simuliidae (order Diptera) collected at site 1 is consistent with Merritt et al. (1982), who found optimum larval growth and production of Simuliidae occurred over a limited range of cold temperatures. Isopoda, Amphipoda, and Tricladida, collected in high numbers and at site 1 are non-insect invertebrates that complete their entire lifecycle in the water and are tolerant of chronically cold water (Smith 2001). In contrast, the insect orders Trichoptera and Plecoptera had the highest

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abundance at sites furthest from the dam and moderate/low numbers throughout the rest of the river. Vannote and Sweeney (1980) found that hypolimnetic releases from dams negatively affect aquatic insects that require warm temperature fluxes for development.

We found the lowest percent EPT values at site 1, the site nearest to the spillway dam and the highest values at sites 11 and 12, the sites furthest from the dam (Figure 6). Johnson and Harp (2005) found similar low percent EPT values on their study of the Little Red River, a cold tailwater in Arkansas. They concluded that this metric was not suitable for use in tailrace systems because most genera used to calculate this metric cannot complete their lifecycles in chronically cold streams. Most members of the orders Ephemeroptera, Plecoptera, and Trichoptera require temperature fluctuations to end diapause, stimulate egg hatching, and emerge into adults (Lehmkuhl 1979). Hence, the high percent EPT values documented at sites 11 and 12 may be a sign that this section of stream is far enough from the dam to not be impacted by the cold-water discharge. Additionally, taxa richness values were highest at sites 11 and 12 the two sites furthest from the spillway dam.

We determined that the functional feeding group collector/filterers were the dominant feeding group throughout our study area primarily due to the high numbers of filter feeding Trichoptera in the family Hydropsychidae, Ephemeroptera in the family Isonychiidae, and Diptera in the family Simuliidae. In a study on the Savanna River, Gordan and Wallace (1975) discovered that invertebrate assemblages immediately below dams were dominated by a few tolerant filter feeding taxa, such as caddisflies in the genera *Cheumatopsyche* and *Hydropsyche*. They surmised that these taxa were flourishing due to the abundant amount of algae and other suspended matter released from impoundments. While we did not measure turbidity or the amount of algae present in our study, high numbers of filter feeding taxa such as *Cheumatopsyche* throughout our study sites may also be due to high amounts of algae and other suspended matter released from the impoundments in our study area. The number of Simuliidae was greatest at site 1, below the spillway dam, and Simuliidae abundance decreased at sites further downstream. Another study of

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invertebrate feeding habits in the Grand Canyon (Wellard- Kelly et al. 2013), demonstrated that the quality of suspended organic matter consumed by filter feeding Simuliidae declines with distance downstream. It is possible the similar Simuliidae abundance pattern found longitudinally in our study is also due to the gradient of suspended organic matter. While others have found the settling of organic and inorganic particles in reservoirs results in a reduction of particulate organic matter below dams (Donnelly 1993), we determined due to the abundance of collector/filterers in the LMFR, that this system is receiving an adequate amount of suspended material from Broken Bow Reservoir.

CONCLUSIONS

In conclusion, we found that aquatic macroinvertebrate taxa distribution within the LMFR is determined by tolerance or intolerance to year-round cold-water temperatures. While we did not look at other variables such as hypoxic conditions associated with a stratified upstream lake, allochthonous input close to the dam, high flow rates, and fluctuating water levels, all could likely contribute to observed patterns seen in this study. Future studies are needed to determine what if any roles these variables play in the spatial and temporal patterns seen in the LMFR. The knowledge of spatial and temporal patterns of macroinvertebrate communities, inhabiting the LMFR, is central to understanding aquatic ecosystem function and gauging ecosystem health for this stream.

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Table 2.1. Total macroinvertebrate taxa collected with kick seines from the Lower Mountain Fork River during surveys in May 2016 - July 2017. Sampling sites are displayed on Figure 1.

Order	Family/Super family	Genus	Site 1	2	3	4	5	6	7	8	9	10	11	12
Tricladida	Dugesiidae		393	37	16	5	22	12	7	7	23	24	19	20
Haplotaxida			1	17	21	19	4	27	2	3	4	8	17	11
Arhynchobdellida	Erpobdellidae		2	9	6	3	2	13	6	13	14	16	2	11
Isopoda	Asellidae	*Lirceus*	1713	211	76	185	62	29	26	120	142	118	116	99
Amphipoda	Hyalellidae	*Hyalella*	416	61	21	110	54	13	23	50	57	29	8	8
Trombidiformes	Hygrobatoidea					2			1		4	2		
Ephemeroptera	Baetidae	*Acentrella*	49	6	15	1	3	1	1		1	6	10	13
		Pseudocentroptiloides	76	63	2	4	1	1		2	2	5	1	1
	Isonychiidae	*Isonychia*	258	172	56	20	19	13	19	5	35	42	276	218
	Heptageniidae	*Leucrocuta*		1		1			1	5			1	
		Maccaffertium		4		24	6	13	13	5	9	25	35	18
		Stenacron	6	3	3	77	34	10	40	55	25	29	10	54
		Sternonema	1	15	17	101	59	39	29	36	143	44	10	11
	Leptophlebidae	*Neochoroterpes*									2		1	
Odonata	Aeshnidae					1								
	Calopterygidae					12								
	Coenagrionidae												1	2	
	Gomphidae					1		1						1
Plecoptera	Chloroperlidae		1						1					1
	Perlidae	*Acroneuria*		1		2	1	1	1	1		1	1	12
		Claassenia				3						2	13	13
		Hansonoperla	1		5							3		1
		Neoperla	4	2	1	39	23	52	70	20	18	23	299	428
		Perlesta	3	18	11	3	2	2			1	4	3	7
Neuroptera	Corydalidae	*Corydalus*	1	1		5	11	10	5	7	5	10	27	20

Order	Family	Genus												
	Sisyridae					1								
Trichoptera	Helicopsychidae	*Helicopsyche*				1					1			
	Hydropsychidae	*Cheumatopsyche*	79	261	150	260	233	487	173	209	358	973	531	278
		Hydropsyche	24	4	49	23	116	7	1	23	26	206	5	3
		Macrostemum										2	1	
	Hydroptilidae	*Hydroptila*	27	1	1	18	5			3	38	32	7	6
	Lepidostomatidae	*Lepidostoma*	9	7	9						9	21	19	19
	Leptoceridae		3											2
		Ceraclea									1			
		Oecetis	7								1	4	1	
		Trianodes									1			
	Philopotamidae	*Chimarra*	347	561	144	106	215	141	67	121	125	265	1236	421
	Polycentropodidae	*Neureclipsis*										4		
		Polycentropus	2	1	1	9	4	2	2	2	14	4		1
Lepidoptera	Crambidae	*Petrophila*	1				2		1		1	4	3	4
Coleoptera	Dryopidae	*Helichus*	3									1		
	Elmidae	*Stenelmis*	1	1	2	14	3	18	8	23	14	27	22	32
	Gyrinidae	*Dineutus*			1	1								
		Gyrinus				1								
	Psephenidae	*Psephenus*		1						1				3
Diptera	Athericidae		2	1		2	1							
	Ceratopogonidae										17			
	Chironomidae		186	166	56	52	63	60	31	39	132	183	495	82
		Rheotanytarsus	22	18	5	7	2	1			5	15	6	3
	Simuliidae	*Simulium*	2781	51	192	2	3	1	3	3	2	283	24	1
	Tipulidae	*Tipula*			1									1
Total			**6419**	**1694**	**861**	**1114**	**951**	**954**	**532**	**754**	**1227**	**2417**	**3202**	**1802**

Table 2.2. Macroinvertebrate values for 12 sample sites by season on the Lower Mountain Fork River, May 2016–July 2017.

						Site							
	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Abundance													
Summer	1055	270	340	337	204	106	53	49	412	1016	482	423	396
Fall	1267	501	188	279	188	279	169	312	156	780	55	309	374
Winter	2413	546	125	164	208	262	84	201	349	307	1186	314	513
Spring	1222	170	100	174	167	120	73	105	197	302	988	305	327
Mean	1489	372	188	239	192	192	95	167	279	601	678	338	
Taxa Richness													
Summer	15	14	15	23	21	16	13	11	18	24	21	18	17.4
Fall	15	16	13	11	16	16	15	18	17	21	9	18	15.4
Winter	15	14	16	15	11	13	12	15	18	18	20	17	15.3
Spring	19	15	14	21	17	17	19	14	22	23	20	24	18.8
Mean	16	15	15	18	16	16	15	15	19	22	18	19	
Simpson's Index (D)													
Summer	0.37	0.42	0.31	0.20	0.20	0.32	0.12	0.17	0.17	0.39	0.31	0.29	0.27
Fall	0.47	0.18	0.29	0.18	0.21	0.35	0.28	0.18	0.14	0.17	0.18	0.25	0.24
Winter	0.38	0.48	0.11	0.18	0.19	0.45	0.22	0.17	0.18	0.23	0.29	0.23	0.26
Spring	0.24	0.16	0.12	0.13	0.14	0.15	0.14	0.15	0.14	0.13	0.36	0.23	0.17
Mean	0.36	0.31	0.20	0.17	0.19	0.32	0.19	0.17	0.16	0.23	0.29	0.25	
Shannon's diversity Index (H')													
Summer	1.41	1.38	1.50	2.15	2.02	1.51	2.15	1.95	2.11	1.67	1.56	1.57	1.75
Fall	1.22	2.00	1.73	1.87	1.94	1.55	1.80	2.06	2.28	2.07	1.48	1.84	1.82
Winter	1.41	1.18	2.42	1.97	1.88	1.24	1.84	2.02	1.99	1.75	1.52	1.81	1.75
Spring	1.85	2.03	2.26	2.45	2.16	2.22	2.27	2.18	2.31	2.37	1.58	1.97	2.14
Mean	1.47	1.65	1.97	2.11	2.00	1.63	2.02	2.05	2.17	1.97	1.53	1.80	
Percent EPT													
Summer	9	70	44	67	76	89	72	65	70	81	94	85	69
Fall	14	62	68	52	86	82	82	68	53	57	93	89	67
Winter	14	77	42	87	77	89	86	65	64	75	66	82	69
Spring	19	43	56	41	81	70	60	29	54	48	75	92	56
Mean	14	63	53	62	80	82	75	57	60	65	82	87	

Table 2.3. Macroinvertebrate values for 12 sample sites on the Lower Mountain Fork River May 2016–July 2017.

	Site												
	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Abundance	6142	1582	860	1039	871	870	412	752	1220	2421	3131	1719	1752
Taxa Richness	31	29	25	35	28	27	29	24	31	35	35	34	30
Simpson's Index	0.27	0.17	0.13	0.12	0.14	0.28	0.14	0.14	0.14	0.17	0.21	0.16	0.17
Shannon's diversity Index (H')	1.76	2.16	2.37	2.51	2.31	1.94	2.37	2.30	2.42	2.25	1.98	2.25	2.22
Percent EPT	14	66	54	61	75	51	65	62	66	56	75	83	61

Table 2.4. Analysis of similarities (ANOSIM) testing for seasonal differences among functional feeding groups below water regulation structures (* = $p < 0.05$).

Date	*R*	*p*	Functional feeding group with greatest % contribution to pairwise differences		
			Spillway, Old Park	Spillway, Re-reg	Old Park, Re-reg
May-16	0.207	0.065	Collector/filterers	Collector/filterers	Collector/filterers
Jun-16	0.171	0.022*	Collector/filterers	Collector/filterers	Collector/filterers
Jul-16	0.032	0.33	Collector/filterers	Predators	Collector/filterers
Aug-16	0.069	0.219	Collector/filterers	Collector/filterers	Collector/filterers
Sep-16	-0.053	0.656	Shredders	Collector/filterers	Multiple
Oct-16	0.002	0.487	Collector/gatherers	Collector/filterers	Multiple
Nov-16	0.12	0.102	Multiple	Collector/filterers	Collector/filterers
Dec-16	0.201	0.021*	Collector/filterers	Multiple	Predators
Jan-17	.009	0.449	Collector/filterers	Collector/filterers	Multiple
Feb-17	0.282	0.089	Collector/filterers	Collector/filterers	Collector/gatherers
Mar-17	0.225	0.045*	Collector/filterers	Collector/filterers	Collector/filterers
Apr-17	0.074	0.179	Multiple	Collector/gatherers	Shredders
May-17	0.067	0.26	Collector/gatherers	Collector/filterers	Multiple
Jun-17	0.35	0.005*	Predators	Shredders	Shredders
Jul-17	0.174	0.067	Collector/filterers	Collector/gatherers	Collector/gatherers

Table 2.5. Analysis of similarities (ANOSIM) testing for seasonal differences among taxa below water regulation structures (* = $p < 0.05$).

Date	R	p	Taxa with greatest % contribution to pairwise differences		
			Spillway, Old Park	Spillway, Re-reg	Old Park, Re-reg
May-16	0.211	0.049*	*Cheumatopsyche*	*Cheumatopsyche*	*Cheumatopsyche*
Jun-16	0.218	0.014*	*Simulium*	*Cheumatopsyche*	*Cheumatopsyche*
Jul-16	0.049	0.337	*Cheumatopsyche*	*Neoperla*	*Neoperla*
Aug-16	0.120	0.093	*Simulium*	*Simulium*	*Cheumatopsyche*
Sep-16	-0.005	0.488	*Lirceus*	*Isonychia*	*Lirceus*
Oct-16	0.081	0.179	*Cheumatopsyche*	*Chimarra*	*Cheumatopsyche*
Nov-16	0.164	0.083	*Cheumatopsyche*	*Chimarra*	*Cheumatopsyche*
Dec-16	0.345	0.001*	*Cheumatopsyche*	Chironomidae	*Isonychia*
Jan-17	0.155	0.099	*Cheumatopsyche*	*Chimarra*	Chironomidae
Feb-17	0.269	0.048*	*Chimarra*	*Chimarra*	*Stenelmis*
Mar-17	0.343	0.01*	*Hyalella*	*Chimarra*	*Chimarra*
Apr-17	0.329	0.006*	*Lirceus*	*Lirceus*	*Cheumatopsyche*
May-17	0.153	0.112	*Lirceus*	*Neoperla*	*Cheumatopsyche*
Jun-17	0.398	0.007*	*Neoperla*	*Cheumatopsyche*	*Chimarra*
Jul-17	0.066	0.232	*Chimarra*	*Chimarra*	*Stenacron*
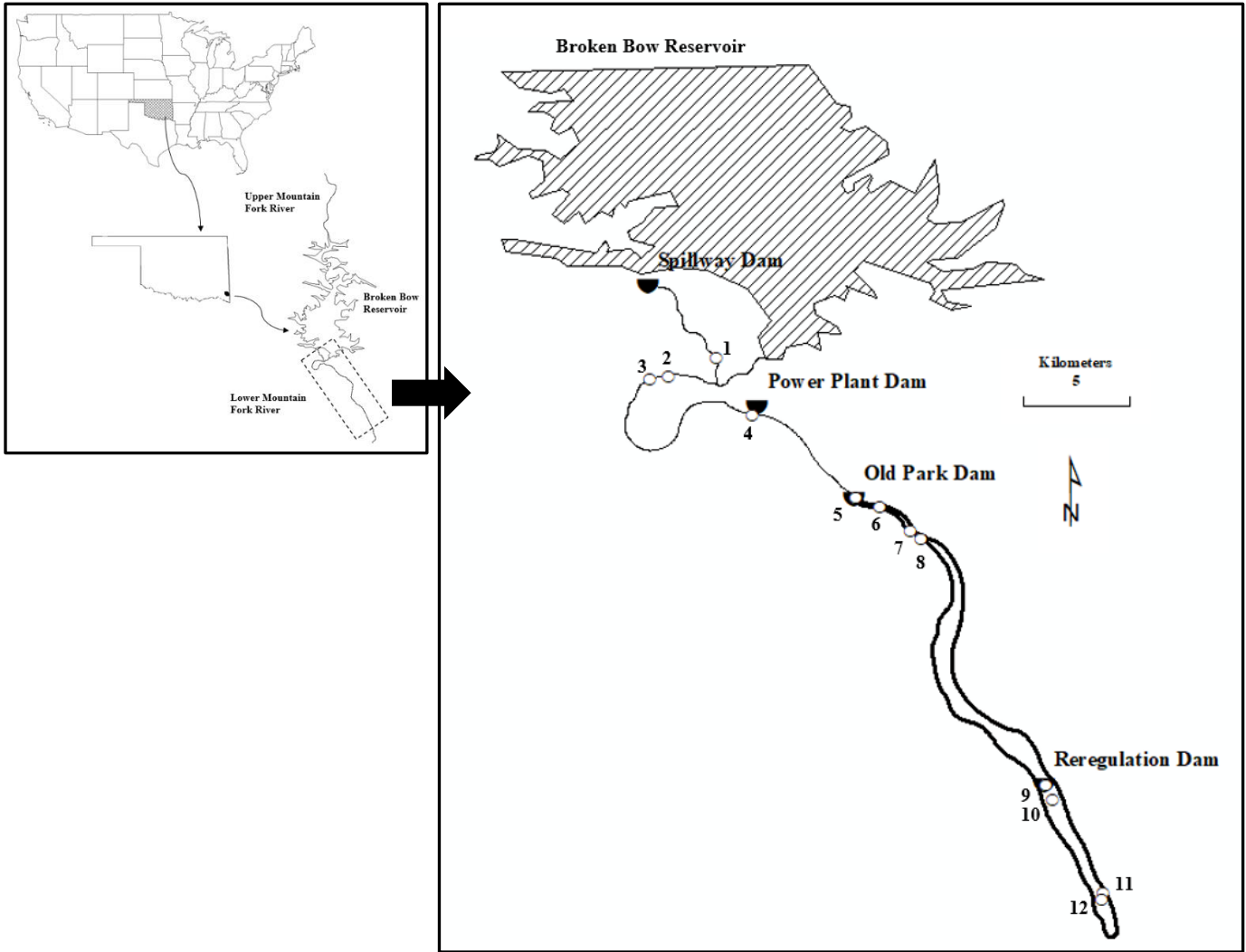
Figure 2.1. Location of twelve sample sites (May 2017 through July 2017) in the Lower Mountain Fork River below Broken Bow Dam. Dams are shown as black semi-circles and sample sites are shown as white circles.
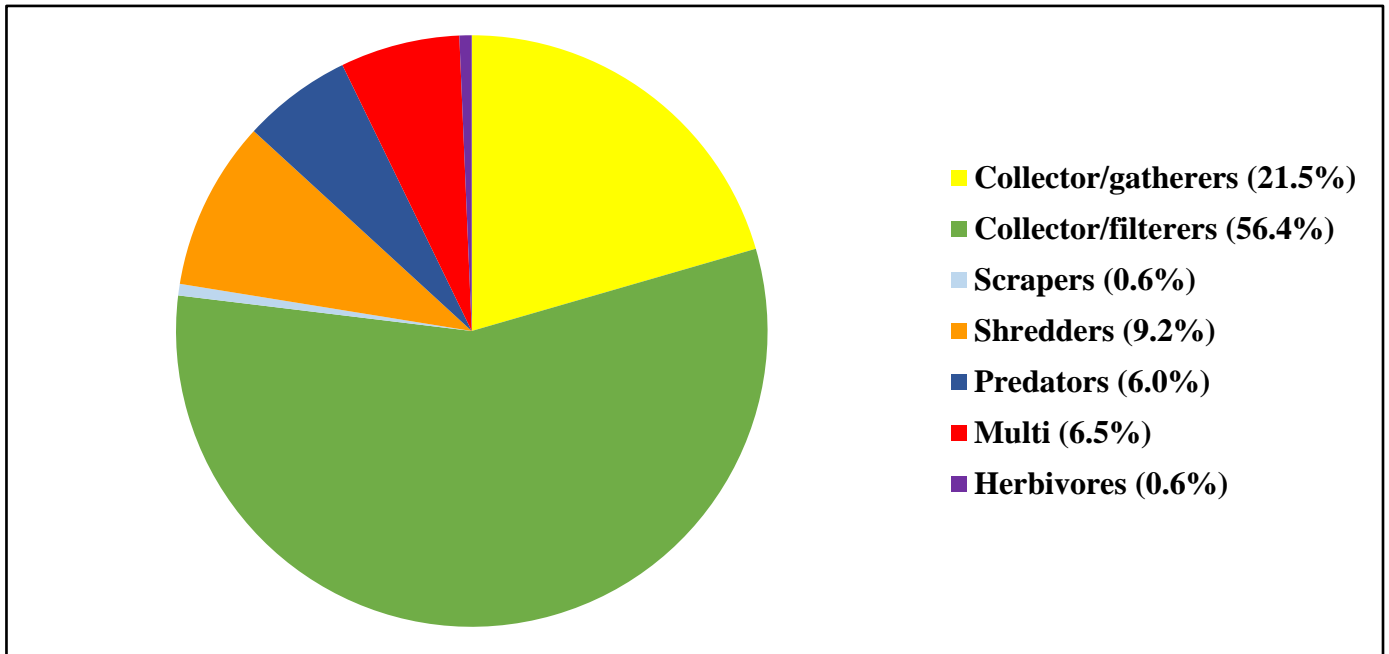
Figure 2.2. Relative abundance of macroinvertebrate functional feeding groups collected from the Lower Mountain Fork River May 2016 through July 2017.

Figure 2.3. Mean macroinvertebrate abundance (+/- SEM) by sampling site and zone for the Lower Mountain Fork River May 2016 through July 2017.

Figure 2.4. Mean taxa richness (+/- SEM) by sampling site and zone for the Lower Mountain Fork River May 2016 through July 2017.
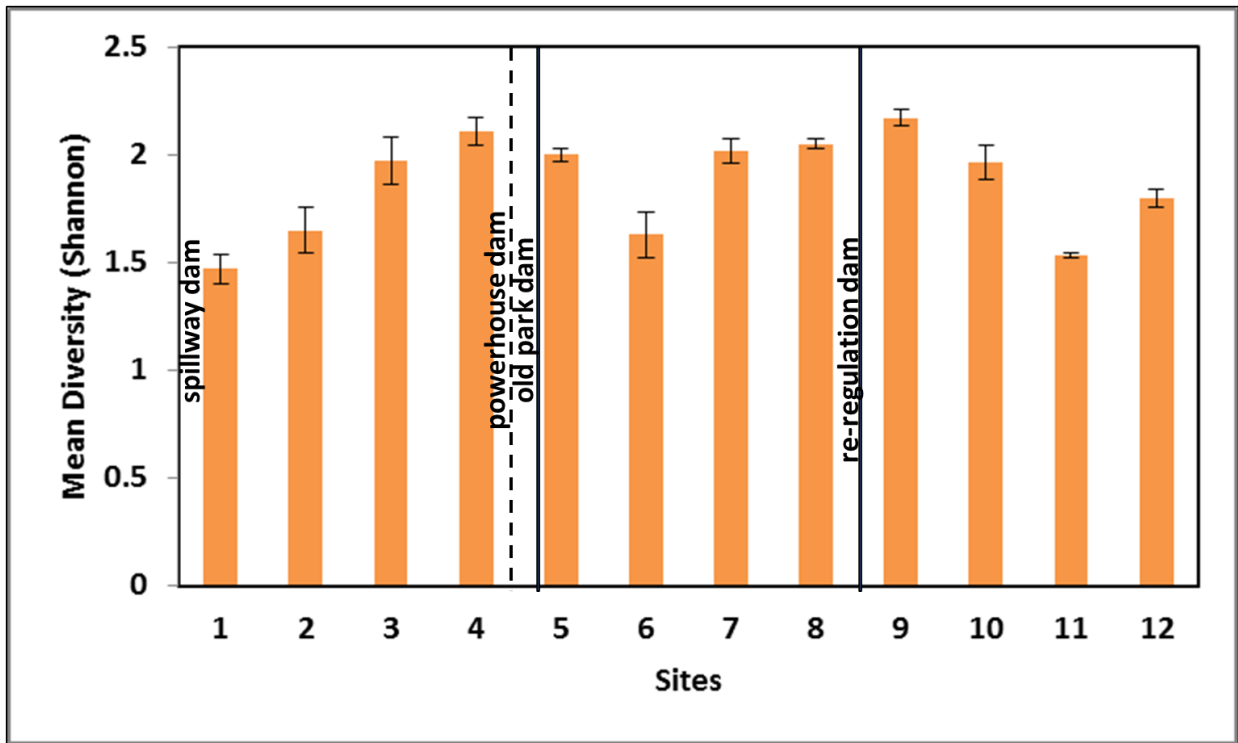
Figure 2.5. Mean Shannon diversity (+/- SEM) by sampling site and zone for the Lower Mountain Fork River May 2016 through July 2017.
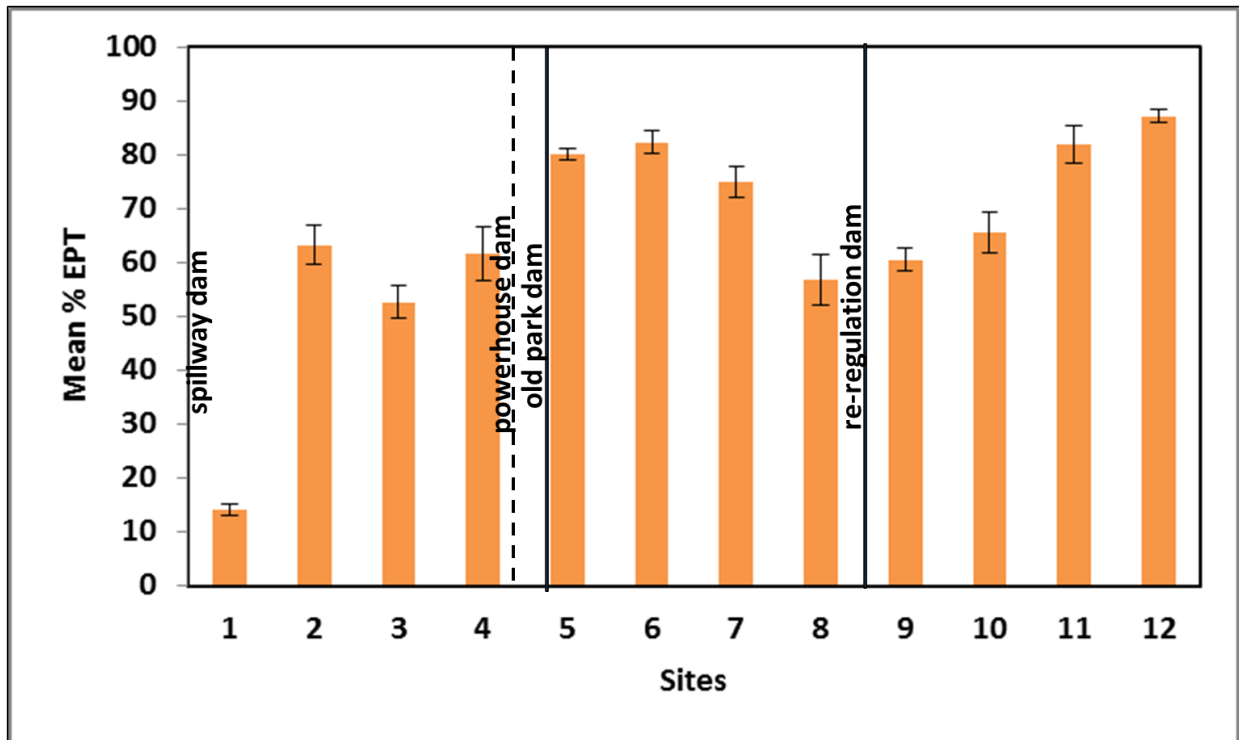
Figure 2.6. Mean % EPT (+/- SEM) by sampling site and zone for the Lower Mountain Fork River May 2016 through July 2017.

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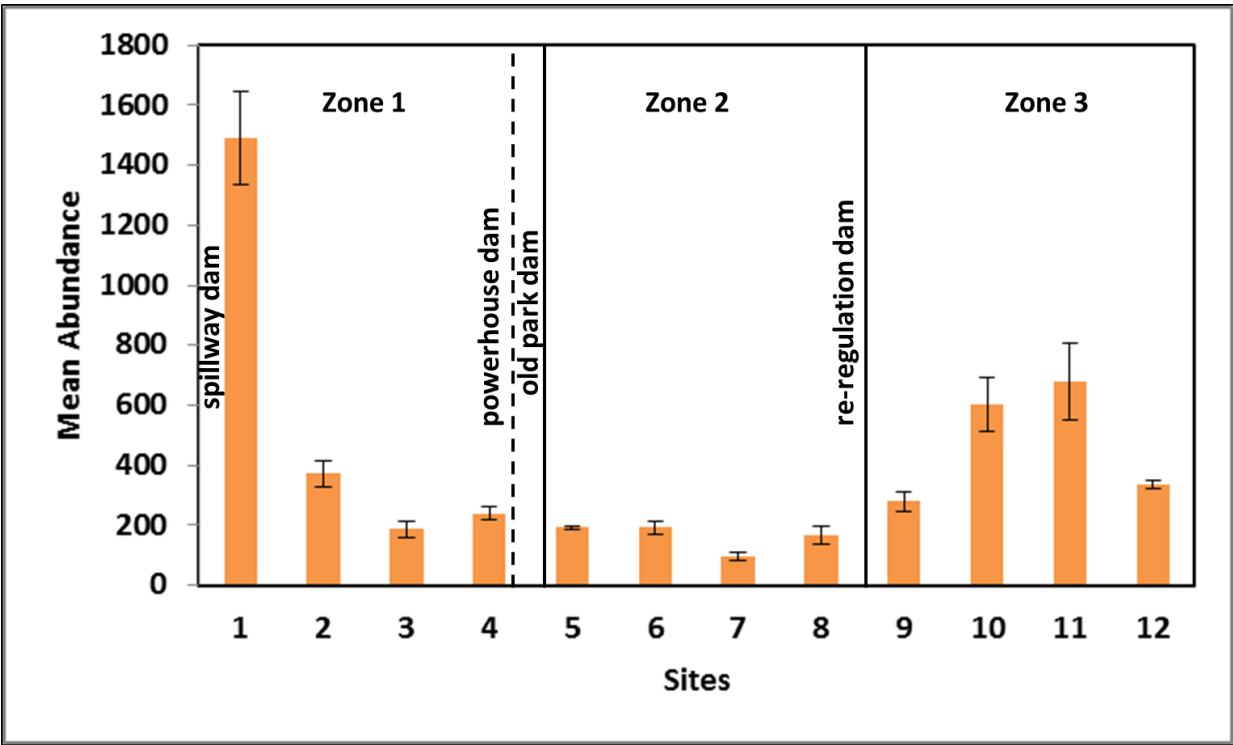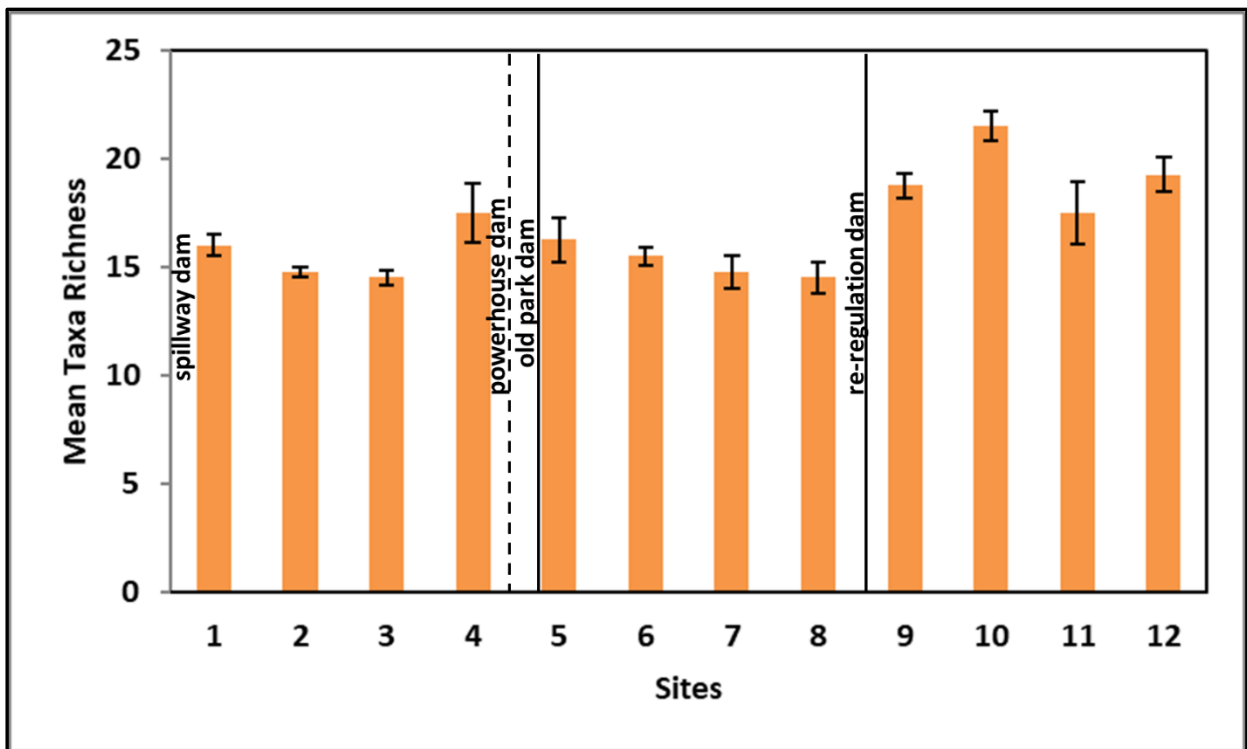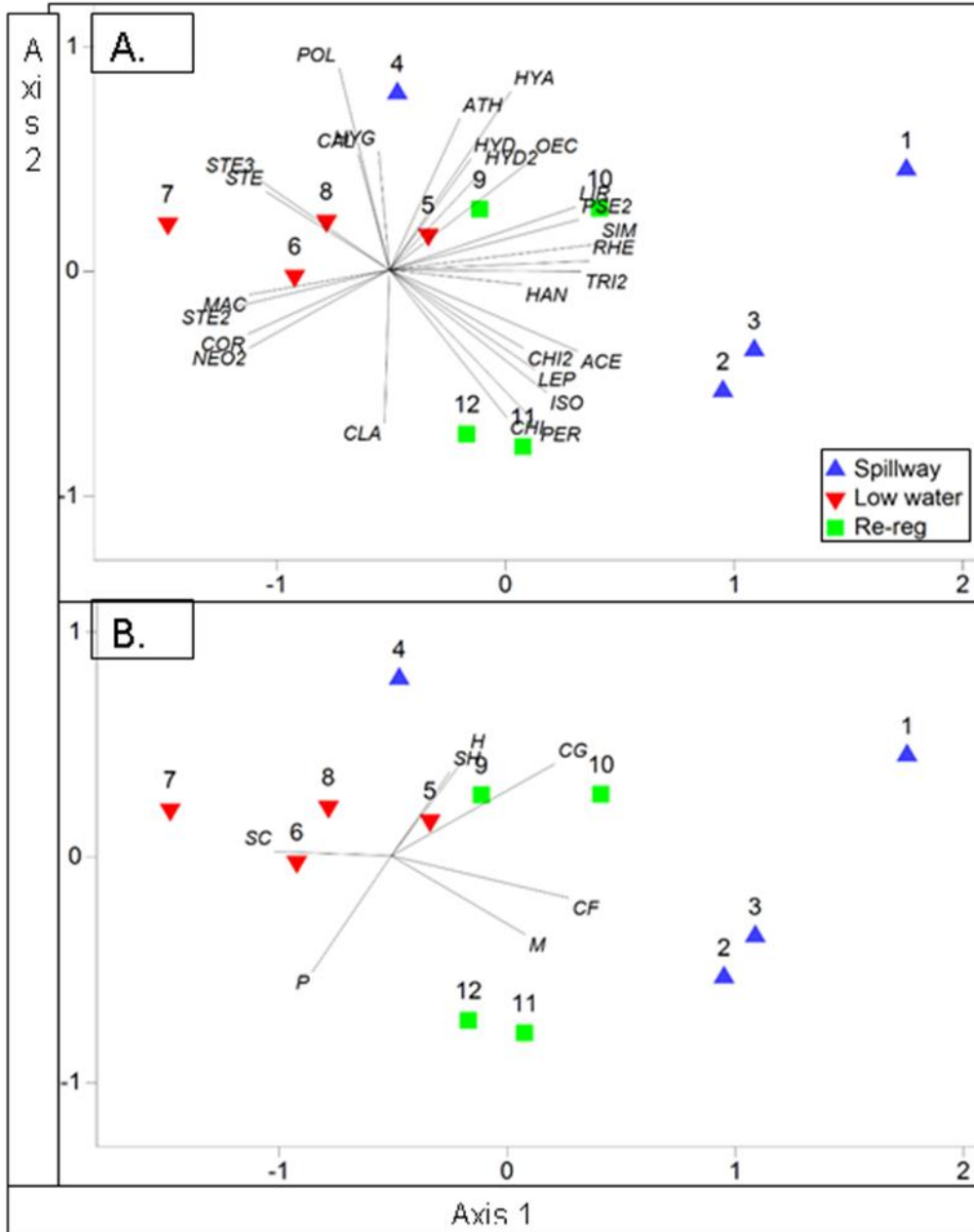Figure 2.7. nMDS ordinations of the community assemblage by taxa (A) and functional feeding group (B). Site number indicates its relative location from the spillway dam, i.e., Site 1 is the closest and Site 12 is the furthest. For clarity, taxa with <0.5 similarity were omitted and were coded as follows: *Acentrella*, ACE; Athericidae, ATH; Calopterygidae, CAL; *Cheumatopsyche*, CHE; *Chimarra*, CHI; Chironomidae, CHI2; *Claassenia*, CLA; *Corydalus*, COR; *Hansonoperla*, HAN; *Hyalella*, HYA; *Hydropsyche*, HYD; *Hydroptila*, HYD2; Hygrobatoidea, HYG; *Isonychia*, ISO; *Lepidostoma*, LEP; *Lirceus*, LIR; *Maccaffertium*, MAC; *Neoperla*, NEO2; *Oecetis*, OEC; *Perlesta*, PER; *Petrophila*, PET; *Polycentropus*, POL; *Pseudocentroptiloides*, PSE2; *Rheotanytarsus*, RHE; *Simulium* , SIM; *Stenacron*, STE; *Stenelmis*, STE2; *Sternonema*, STE3; Tricladida, TRI2. Functional feeding groups were coded as: Collector/filterers, CF; collector/gatherers, CG; shredders, SH; scrapers, SC; predators, P; multiple, M; herbivores, H.

CHAPTER III

BIOASSESSMENT OF AQUATIC MACROINVERTEBRATES UPSTREAM AND

DOWNSTREAM OF ROAD-STREAM CROSSINGS IN EASTERN AND NORTHEASTERN

OKLAHOMA

INTRODUCTION

Natural streams are linear, continuous aquatic ecosystems that are vulnerable to fragmentation

and interruption by anthropogenic structures that inhibit flow (Postel & Richter 2003). In

unfragmented, uninterrupted streams physical, chemical, and biological processes occur along a

predictable gradual gradient from headwaters to downstream catchments. This predictive series of

changes is called the river continuum concept (RCC) (Vannote et al. 1980). Humans can cause

changes to stream continuity by placing artificial structures such as culverts that become barriers

to stream flow (Stanford et al. 1996).

Culverts are commonly used to provide a road crossing for lower order streams, including those

that are ephemeral. Many types of culverts exist, and can be pipe or box shaped structures, that

are constructed of metal, concrete, or PVC pipe. Culvert construction is generally designed to

sufficiently handle streamflow from low order streams bisecting roads (VDOT, n.d.). Culverts are

usually considered minor structures and are less regulated than bridges. However, culvert design

and maintenance are important to ensure

not only the proper functioning of the roadway, but also for the stream's flow (ODOT 2014). Unfortunately, culverts can impact streams by interrupting stream connectivity, constricting the stream channel, altering stream velocity, and causing stream bed and bank erosion, especially on the downstream side (Riley et al. 2020). Most research on the ecological effects of culverts has focused on the effects on fish migration and has examined effects immediately adjacent to the culvert (Gibson et al. 2005; Birnie-Gauvin et al. 2019; Johnson et al. 2019). Less research has been conducted on the impacts of culverts on aquatic invertebrates. Aquatic invertebrate communities are frequently utilized in biomonitoring programs to detect anthropogenic impacts on stream ecosystems (Bonada et al. 2006; Buss et al. 2015). Aquatic invertebrate sampling methods are commonly used in biomonitoring activities because they are reliable, inexpensive, and easy to employ (Rosenberg et al. 1986; Karr 1991). Road stream crossings are often used as access points to sample for macroinvertebrates in monitoring projects. When the stream habitat is sampled, a riffle area is chosen that is visually undisturbed. However, changes in stream flow associated with culverts may cause nutrient or other resource differences above and below a culvert. These differences may affect the taxonomic composition upstream and downstream of the road crossing. Therefore, it is important to know if these variations translate into differences at the metric, index, or bioassessment level so that water quality assessment projects based on macroinvertebrates provides meaningful comparisons. There is also a need for more research on potential effects of culverts on macroinvertebrates in different ecoregions where stream geomorphologies differ. The objective of this study was to determine if the interpretation of water quality metrics measured by aquatic macroinvertebrate communities depends on sampling location (upstream vs downstream of culverts). We tested the hypothesis that macroinvertebrate metrics would not differ between upstream and downstream areas of culverts in two stream systems in eastern and northeastern Oklahoma.

METHODS

Study Area

To assess impacts of culverts on macroinvertebrate communities, we examined stream systems in two regions of Oklahoma, the Tallgrass Prairie and Ozark Highlands. The Tallgrass Prairie study area is in Osage county in the northeastern part of Oklahoma. It is located at the southern end of the Flint Hills and is bisected by Salt Creek and its tributaries. This region is characterized by low gradient streams. The exposed limestone formations of this rocky rolling prairie make cultivation difficult. As a result, this area is dominated by native grasses and is one of the largest remaining tracts of tallgrass prairie in the world (The Nature Conservancy 2022).

The Ozark Highlands study area is in Cherokee county in eastern Oklahoma. This study area is located in/near the Cherokee Wildlife Management Area and is bisected by Greenleaf Creek and its tributaries. This region is characterized by medium gradient streams. The area is underlain by cherty limestone and contains karst features with many cold, perennial, spring-fed streams and is dominated by oak-hickory forest and woodlands (EPA 2012).

Macroinvertebrate Sampling

In 2018, eight sites were sampled in the Ozark Highlands and seven sites were sampled in the Tallgrass Prairie in the months of June and July. In 2019, 13 sites were sampled in the Ozark Highlands and 11 sites were sampled in the Tallgrass Prairie from April through July. In 2020, 12 sites were sampled in the Ozark Highlands and 11 sites were sampled in the Tall Grass Prairie from April through July.

Streams were sampled bi-monthly at the closest riffle upstream (mean distance 10.4 meters +/- 2.8 SEM) and the closest riffle downstream (mean distance 10.9 meters +/- 1.8 SEM) of the road culvert at each sample site. Distance, in meters, was measured from the culvert to the sample site riffle at both the upstream and downstream side of the culvert (Table 1). Additionally, we

determined if a culvert was acting as a deterrent to stream flow. Some of our culvert sites were blocked with sediment or organic debris or the road had sunk down into the culvert piping crushing it. At blocked sites water was not adequately flowing through the culvert which caused water to pool on the upstream side of the culvert.

At each site, upstream and downstream riffles were sampled in three locations (upper, middle, and lower area of riffle) for one minute, in a one-square meter (m^2) area immediately upstream of a rectangular kick seine. The three samples were combined into one sample for each riffle. In the field we removed large debris and stones and then hand-picked macroinvertebrates for either 5 minutes or until no macroinvertebrates remained. Samples were preserved in the field in 70% ethyl alcohol and transported to the lab where they were sorted and identified to lowest practical taxon using keys (Merritt et al. 2008; Smith 2001; Wiggins 2014).

Data analyses

All taxa were enumerated, metrics were calculated and a bioassessment score was determined for each site. Scores of 6, 4, 2, or 0 were assigned for each metric according to the criteria in Table 2 and summed to get a total bioassessment score for each site, with a maximum of 36 points similar to methods defined by the (OCC 2020). The high-quality sites were determined for each region by identifying the site that had the highest bioassessment score and the following six metrics were used to calculate the bioassessment score:

1. Taxa richness is the total number of taxonomically different types of macroinvertebrates in the sample. The percentages used to assign scores were obtained by dividing each monitoring site metric value by the average high quality site metric value in each region.

2. Modified Hilsenhoff Biotic Index (HBI) is a measure of a macroinvertebrate sample's tolerance to organic pollution. The index ranges between 0 and 10 with 0 being the most pollution sensitive. The HBI index used for this study is based off the pollution tolerance

of invertebrates from the upper Midwest (Hilsenhoff 1987). To assign scores, the high-quality site value was divided by the monitoring site value (high quality site metric /monitoring site metric).

3. EPT Index is the number of different taxa from the orders Ephemeroptera, Plecoptera, and Trichoptera present in a sample. Most taxa within these insect orders are more sensitive to dissolved oxygen availability than other aquatic insects (Barbour et al. 1999). The percentages used to assign scores were obtained by dividing each monitoring site metric value by the average high quality site metric value in each region.

4. Percent EPT is a measure of how many individuals in the sample are members of the EPT orders. The percentages use to assign scores was based on the actual values obtained instead of being relative to the high-quality site metric.

5. Percent dominant two taxa is the percentage of the sample composed of the most common two taxa. The percentages used to assign scores were based on actual values obtained instead of being relative to the high-quality site metric.

6. Shannon-Wiener Index (H') (Wilhm 1972) is a measure of the macroinvertebrate diversity of a sample, with values ranging between zero and 3.5. This index increases as more taxa are found in the sample and as individual taxa become less dominant. The percentages used to assign scores were based on the actual values obtained instead of being relative to the high-quality site metric.

Due to the non-normality of the data, nonparametric Mann-Whitney U tests were used to explicitly compare above and below culvert bioassessment metrics calculated for each site. Using the function "wilcox_test", two-tailed tests determine if each bioassessment metric was not equal above or below a sampled culvert at each site. Samples were grouped by region to remove regionally specific variation and by year. Mann-Whitney U tests were considered significant at $p \leq 0.05$. All bioassessment metric comparisons were computed in R version 4.1.1 (R Core Team 2021). Additionally, Mann-Whitney U tests were run to ascertain if there was a significant

difference in the upstream and downstream abundance at each site and to determine if there was a significant difference in abundance at each site between the two regions.

Prior to analyses, macroinvertebrate abundance (count data) from the 26 sampled sites (14 Ozark Highlands and 12 Tallgrass Prairie) were log (x + 1) transformed. Among the 26 sites, samples were collected from 21 sites on multiple dates. Non-metric multidimensional scaling (nMDS) was used to characterize the macroinvertebrate assemblage by taxa above and below each culvert separated by region. The five most abundant families (Baetidae, Chironomidae, Heptageniidae, Hydropsychidae, and Perlidae) were selected and plotted as vectors; moreover, these families cover taxa groups essential to discerning water quality and bioassessment metrics. Both nMDS plots were generated based on a rank order Bray-Curtis dissimilarity matrix, where samples are represented points in 2-dimensional space. Pairwise distances are used in the final ordination plots to intuitively interpret community associations (i.e., the closer the points the more similar their community composition). Biplots and nMDS analyses were generated using PRIMER v7 (PRIMER-E software, Plymouth, United Kingdom; Clarke & Gorley 2015).

A series of generalized linear mixed models (GLMMs package lme4; Bates et al. 2015) were used to determine the greatest source of variance in benthic macroinvertebrate abundance sampled from road culverts. Model sets were generated for total abundance, EPT abundance, and Chironomidae abundance with a negative binomial distribution. Fixed effects included: Culvert site, Julian date, sample location (above or below culvert), and blockage (culvert blocked or free flowing). An interaction effect of culvert site * Julian date was also included. All model iterations accounted for region as a random effect. Continuous numeric variables were scaled and centered before running models. Each completely parameterized model set was subjected to an iterative model selection process that generates a subset of the most parsimonious models. Using the dredge function in package "MuMIn" (Bartoń 2020), the top models were compared and ranked

by corrected Akaike's Information Criterion (AICc) values. Normality of model residuals was evaluated using a Shapiro-Wilk test.

RESULTS

Over the three-year period of this study, we collected a total of 362 samples using kick-seine sampling at the closest riffles upstream and downstream of culvert road crossings in two regions of Oklahoma. Our sampling yielded 77,504 individuals representing 82 unique taxa (14 orders, 63 families, and 21 identified genera) (Table 3). The orders Ephemeroptera (28%), Diptera (20%), Trichoptera (18%), and Plecoptera (16%), were the dominant components of the benthic macroinvertebrate community accounting for 82% of total individuals collected (Table 3). In the Ozark Highlands region, we collected approximately twice as many individuals compared to the Tallgrass Prairie region (53,584 versus 23,920). Significantly more macroinvertebrates (abundance) were collected in the Ozark Highlands region (Greenleaf sites) than from the Tallgrass Prairie region (Sand Creek sites) (n = 180, p = <0.001). At the Ozark Highlands sites we collected 33% more individuals at upstream sites compared to downstream sites. In contrast, at the Tallgrass Prairie sites there was almost an equal distribution of macroinvertebrate abundance upstream and downstream of culvert road crossings with less than 1% difference between upstream and downstream portions of a site (Table 3). Despite a higher abundance of macroinvertebrates collected at upstream sites, no significant differences existed when all dates and sites were combined (n = 180, p = 0.114).

Two orders Lepidoptera - aquatic butterflies and Trombidiformes - aquatic mites were found exclusively in the Ozark Highlands region while the majority of the orders Tricladida - flat worms (98%), Isopoda – aquatic sow bugs (94%), Megaloptera – dobsonflies (89%) Hemiptera – true bugs (88%), Haplotaxida – aquatic worms (82%), Plecoptera – stoneflies (77%), Diptera – true flies (71%), Odonata – dragonflies and damselflies (70%), Ephemeroptera – mayflies (69%), and Trichoptera – caddisflies (67%) were collected at Ozark Highlands sites (Table 4). The order

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Collembola – springtails were found exclusively at the Tallgrass Prairie sites and the majority of the orders Arhynchobdellida – leeches (80%) and Amphipoda - scuds (82%) were collected at Tallgrass Prairie sites (Table 4). The remaining orders were collected in nearly equivalent abundances from both regions.

Collectively, metrics generated with benthic macroinvertebrate community data collected in the Ozark Highland region were not significantly different between upstream and downstream culvert samples (Table 5 and Figure 2); this included the calculated bioassessment values above and below road culverts ($Z = 0.97$, $p = 0.33$). In addition, metrics generated for each year and bioassessment scores (2018, 2019, and 2020) did not significantly differ between upstream and downstream culvert samples (Table 5 and Figure 2).

Collectively, most of the metrics generated from macroinvertebrate communities in the Tallgrass prairie region were not significantly different (Table 5 and Figure 3), although the percent EPT values and total bioassessment scores were slightly higher at upstream sites ($Z = 1.91$, $p = 0.06$; $Z = 1.90$, $p = 0.06$). Correspondingly, HBI values were statistically lower for sites upstream of culverts ($Z = -3.34$, $p < 0.01$). Metrics generated from 2018 and 2020 macroinvertebrate data did not significantly differ between upstream and downstream culvert samples, although there was a significant difference in the Shannon diversity index ($Z = -2.03$, $p = 0.04$), EPT index ($Z = 2.25$, $p = 0.03$), and the HBI values ($Z = -2.89$, $p < 0.01$) between upstream and downstream culvert samples in 2019 (Table 5 and Figure 3).

The Ozark Highlands nMDS reached a convergent solution after 36 iterations, and the 3-dimensional solution had a stress level of 0.15. The Tallgrass Prairie nMDS reached a convergent solution after 41 iterations, and the 3-dimensional solution had a stress level of 0.18. Benthic macroinvertebrate communities above and below road culverts in each region showed no distinct groups in nMDS space. Dummy coded community points (i.e., 50th, 70th, 90th percentile abundances) exhibited an abundance gradient among all samples. (Figure 4). The relative

abundance of Baetidae, Chironomidae, Heptageniidae, Hydropsychidae, and Perlidae appear to generally follow this gradient (i.e., greater counts of these abundant families are representative of a higher percentile).

GLMMs were used to evaluate the spatial and temporal sources of variation in total macroinvertebrate abundance, EPT abundance, and Chironomidae abundance above and below road culverts. The interaction term Site*Date explained significantly greater variation in the macroinvertebrate abundance than the additive terms with a lower AIC (likelihood-ratio-test LRT; $\chi2(12) = 21.2$, p = 0.04). Site was retained in the top model sets for each response variable (Table 6) and explained 24.0, 23.6, and 18.2% (adj. R^2) of the overall variation in total macroinvertebrate abundance, EPT abundance, and Chironomidae abundance data used in the global model, respectively. Date slightly improved the AIC_c of the EPT abundance model, but this was not significant (LRT; $\chi2(1) = 2.2$, p = 0.14).

Interestingly, blocked road culverts significantly impacted EPT abundance ($\beta \pm$ S.E. = 0.768 ± 0.291, Z = 2.64, p = 0.008) with greater abundance above blocked road culverts. In contrast, Chironomidae abundance was greater below blocked road culverts ($\beta \pm$ S.E. = -0.663 ± 0.344, Z = -1.93, p = 0.054). The null models for total macroinvertebrate abundance, EPT abundance, and Chironomidae abundance, containing only the random effect (region), explained 0.21, 7.9, and 2.4% (adj. R^2) of the variation, respectively.

DISCUSSION

Regions

We examined wadable stream systems in two regions in eastern and northeastern Oklahoma, the Tallgrass Prairie, and the Ozark Highlands. We collected macroinvertebrates from riffles upstream and downstream of culverts to determine if differences exist that might impact metric scores and thus, estimates of water quality. Across years and sites, we collected and identified more than 75,000 macroinvertebrates between the months of April and July. We only sampled

during these months because in the late spring/early summer in Oklahoma, there is abundant

precipitation providing consistent streamflow at sites and because many of the sites, especially in

the Tallgrass prairie become dry by late July/early August.

We collected significantly more macroinvertebrates from the Ozark Highlands region than from

the Tallgrass Prairie region. In addition to being more numerous, the Ozark Highlands also had

higher invertebrate diversity. A potential explanation for this difference is that many sites in the

Ozark Highlands are spring-fed and had modest stream flow for the entire sampling period, while

many of the sites in the Tallgrass Prairie were ephemeral and dried up before the end of the

sampling period. Correspondingly, Wilding et al. (2018) compared ephemeral and perennial

stream reaches and discovered macroinvertebrate communities in ephemeral streams were

characterized by a lower number of taxa. Alternatively, the variation in macroinvertebrates found

in each region could result from differences in stream gradient. The Ozark Highland is a medium

gradient system while the Tallgrass Prairie is a low gradient system. Studies have shown that

macroinvertebrate community composition and structure vary with different habitat features such

as stream gradient (Minshall, 1984). Additionally, the Ozark highland streams are surrounded by

deciduous trees while the Tallgrass Prairie streams are exposed to more sunlight and likely

receive less allochthonous inputs.

The riparian area of streams in the Ozark Highlands region are dominated by trees which shade

the stream, The shade from trees decreases autochthonous inputs, while the trees themselves

provide ample allochthonous inputs (leaf material). In contrast, the riparian area of streams in the

Tallgrass Prairie region are dominated by native grasses which provide limited shading to

streams. This lack of shade generates more photosynthetic activity increasing autochthonous

stream inputs. Other studies have found that grassland streams are dominated by grazer/scraper

taxa groups while forested streams are dominated by filter feeders and shredders (Canning et al.

2019; Scotti et al. 2020). Consistent with these studies, we found that shredders, including

Tipulidae and Nemouridae and filter feeders, including Isonychiidae and Simuliidae were

collected in greater abundance in the Ozark Highlands. However, we did not collect many grazer/scrapers in the Tallgrass Prairie region. A potential explanation for the low diversity of grazer/ scrapers is the seasonal nature of these streams. When the streams desiccate in late summer, it likely impacts algae and the food chains that depend upon it.

Culvert effects

Road culverts may substantially alter stream ecology and have been linked to changes in fish communities and salamander distributions because they can form barriers, downstream plunge pools, and higher flows from constriction of upstream areas (Anderson et al. 2014; Frankiewicz et al. 2021) Although less work has been conducted on macroinvertebrate response, existing studies have documented few changes, especially at distances from the culvert where the stream has visually similar unimpacted reaches. In this study, we sampled the nearest upstream and downstream riffle to assess macroinvertebrate biodiversity. We tested the upstream and downstream communities for both regions using nMDS (Figure 4) which detects the strongest pattern within a set of community data (McCune & Grace 2002). In the nMDS plot, communities in the Ozark highlands overlap strongly with stoneflies, Perlidae being more frequently encountered upstream of culverts (Figure 4A). In the Tallgrass prairie, Chironomidae separate the most and were more common downstream of culverts (Figure 4B).

The dominant feeding group collected upstream and downstream of culverts in both regions was the collector/gatherer group. Frequently collected families included Baetidae, Caenidae, Elmidae, and Heptageniidae. Culverts often cause deposition to occur upstream of the culvert because flow often slows in a pool before entering a culvert (Urban Drainage and Control District, 2001) and collector/gatherers are adapted to feed in areas of the stream where detritus has fallen out of suspension (Cummings & Klug, 1979). Previously, Peterson (2010) reported collector/ gathers to be the dominant taxa near culverts in a study of wadeable streams in Oregon.

The six metrics we calculated in this study, taxa richness, modified Hilsenhoff Biotic Index (HBI), EPT index, percent EPT, percent dominant two taxa, and Shannon-Wiener diversity index

(H'), are the same metrics used in biomonitoring programs in Oklahoma (OCC 2020). Often these programs enter streams at road crossings and sample invertebrates using the standard kick seines that we used in this study. We tested if culverts affected these metrics differentially in the closest upstream and downstream riffles. Ranked AICc tests of total abundance showed that sites were more important than the presence of culverts (Table 6) and that EPT taxa were impacted (more upstream diversity of these taxa) more at blocked culverts, although the relationship was influenced by differences in region (Table 6).

In addition to the commonly used metrics, we also scored and combined metrics to calculate a bioassessment score for both upstream and downstream areas of culverts in the two regions sampled. When all years were combined, in the Ozark Highlands we found that there was no difference between the total bioassessment score upstream and downstream of a culvert, but we did collect more individuals upstream of culverts. In the Tallgrass Prairie we found that the abundance of macroinvertebrates was nearly identical while upstream values for percent EPT and total bioassessment score were higher. Likewise, Petersen (2010) found decreased EPT taxa values at sites below culverts. At Tallgrass Prairie sites, HBI values were significantly lower (HBI values closer to zero indicate better water quality) at upstream sites, indicating better water quality upstream of the culvert. Our results were similar to other studies (Neal et al., 2007; Khan & Colbo, 2008; Kidd & Aust, 2014) that detected slight differences in macroinvertebrate-based index or metric values downstream of road stream crossings, but the differences were usually not significant. However, Gal et al. (2020) found clear differences in the taxa richness and abundance of macroinvertebrates upstream and downstream of road crossings in Hungarian lowland streams. When we looked at metric values upstream and downstream annually, we also found no significant differences in the Ozark Highlands, but interestingly we did see significant differences in three metric values, Shannon diversity, EPT index, and HBI, in 2019 in the Tallgrass Prairie region. In 2019, there was an above average amount of precipitation in Oklahoma with mean of 13.27 cm (+/- 3.55) of rainfall versus 6.53 (+/- 0.93) and 9.42 (+/- 1.29) cm in 2018 and 2020

respectively (Table 7). In addition, in late May and early June 2019, a prolonged sequence of heavy precipitation caused historic flooding. Shannon diversity and EPT index values were both higher downstream of culverts during 2019 and while HBI values were also higher downstream, a higher HBI index denotes lower water quality.

When we specifically looked at upstream and downstream differences at the blocked sites where the culvert was limiting stream flow, we found that there was less EPT abundance and greater Chironomidae abundance downstream of the culvert. This suggests a decrease in water quality, availability of nutrients, or increases in fine sediments filling interstitial spaces at sites downstream of blocked culverts. Higher numbers of Chironomidae are generally considered indicators of poor water quality because this taxa group is tolerant to impaired stream conditions and can utilize fine sediments (Serra et al. 2017) whereas members of the EPT group are intolerant to decreases in water quality or other habitat degradation (Rosenberg & Resh 1993). Ogren and Huckins (2015) compared macroinvertebrates at improperly functioning culverts in Michigan prior to and after culvert replacement. They found only slight changes (increases of less than 8% EPT diversity) in assemblages above and below impacted and replaced culverts, further supporting our conclusions that impaired and functional culverts have limited effects on these species.

CONCLUSIONS

Data obtained from this study provides important taxonomic and community macroinvertebrate information from two regions of Oklahoma. Because of differences in number of macroinvertebrates and slight differences in index values, it is best to choose a riffle upstream of the culvert, especially if the culvert is blocked in the Ozark Highlands region. While in the Tallgrass prairie, during a normal precipitation year it is best to sample riffles upstream of the culvert, but during a year with above average precipitation it is best to sample riffles downstream of the culvert.

Future research should seek to experimentally manipulate culverts to directly assess impacts on macroinvertebrate assemblages. Such research could add insights into the importance of culvert maintenance and best practices for the types of culverts installed. While we only looked at the closest riffle upstream and downstream of each culvert, differences in macroinvertebrate communities could become evident as distance from the culvert increases. Additionally, it is possible that detectable differences exist upstream and downstream of culverts in wadable streams in other regions of Oklahoma. Oklahoma encompasses twelve distinct ecoregions from the semi-arid high plains in the panhandle to cypress swamps in the southeast corner. The distinct geography in each region has the potential to affect macroinvertebrates near culverts differently than observed in our study.

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Table 3.1. Descriptions of culvert sites including culvert type, blocked (culverts with impeded stream flow), distance from culvert to riffle site in meters, and coordinates of culvert for the two regions sampled (SC denotes Sand Creek in Tallgrass Prairie region and GL denotes Greenleaf Creek in Ozark Highlands region). Number in parentheses represents the number of barrels or channels at each culvert.

Site	Culvert type	Blocked	Distance above (m)	Distance below (m)	Lat	Long
SC1	Box (3)	Yes	3.5	9.9	36.737165	-96.207861
SC2	Box (3)	Yes	4.1	8.6	36.759023	-96.314385
SC3	Metal Pipe (3)	No	12.8	19.5	36.752580	-96.343589
SC4	Low water	No	15.2	1	36.719761	-96.134289
SC5	Box (2)	Yes	2.8	3.1	36.736769	-96.282654
SC6	Arch (1)	No	5.2	11.6	36.742980	-96.180266
SC7	Box (2)	No	7.8	3.2	36.759057	-96.287745
SC8	Box (1)	No	1.5	11.3	36.798328	-96.321104
SC9	Box (1)	No	5.2	7.3	36.802412	-96.316813
SC10	Box (1)	No	1	10	36.809435	-96.448075
SC 11	Box (4)	No	24.7	28.7	36.836001	-96.446922
SC13	Box (1)	Yes	2.8	3.4	36.795368	-96.318796
GL1	Box (2)	No	1	29.9	35.784331	-95.003008
GL2	Concrete pipe (3)	Yes	5.5	7.8	35.783640	-95.016249
GL3	Metal pipe (6)	Yes	4.4	14.6	35.732353	-95.048165
GL4	Box (2)	No	11.9	11	35.720058	-95.032904
GL5	PVC pipe (1)	No	10.5	13.4	35.763978	-95.012351
GL6	Metal pipe (3)	Yes	66.5	6.7	35.769190	-95.026593
GL7	Box (4)	No	33.2	37.2	35.727741	-95.068725
GL8	Box (2)	No	1	6.7	35.706247	-95.200194
GL9	Box (2)	No	15.8	13.4	35.719548	-95.043343
GL10	Box (1)	No	1	3.7	35.721160	-95.034187
GL11	Box (2)	No	20.7	3.7	35.722227	-95.050402
GL12	Metal pipe (3)	No	1	3.7	35.751163	-95.141334
GL13	Box (1)	No	1	8.8	35.731084	-95.092972
GL14	Box (1)	No	9.4	5.2	35.752383	-95.091788

Table 3.2. Bioassessment scoring criteria used for macroinvertebrate metrics

Ozark Highlands Region Scoring Values				Tallgrass Prairie Region Scoring Values			
6	4	2	0	6	4	2	0
EPT index				EPT index			
>90	80-90	70-80	<70	>90	80-90	70-80	<70
10	9-10	8-9	8	>7	7	6-7	6
Total richness				Total richness			
>80	60-80	40-60	<40	>80	60-80	40-60	<40
18	15-18	12-14	12	14	11-14	9-11	9
HBI				HBI			
>85	70-85	50-70	<50	>85	70-85	50-70	<50
3.28	3.27-3.79	3.79-4.33	4.33	3.52	3.52-3.83	3.83-4.27	4.27
Percent EPT				Percent EPT			
>90%	80-90%	70-80%	<70%	>90%	80-90%	70-80%	<70%
% Dominant 2 Taxa				% Dominant 2 Taxa			
<20%	20-30%	30-40%	<40%	<20%	20-30%	30-40%	<40%
Shannon Diversity Index (H')				Shannon Diversity Index (H')			
>3.5	2.5-3.5	1.5-2.5	<1.5	>3.5	2.5-3.5	1.5-2.5	<1.5

Table 3.3. Total macroinvertebrate taxa collected with kick seines upstream and downstream of culverts in the Ozark Highlands and Tallgrass Prairie regions of Oklahoma 2018, 2019, and 2020.

Order	Family	Genus	Ozarks Highlands						Tall Grass Prairie						Total
			Upstream			Downstream			Upstream			Downstream			
			2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020	
Tricladida			40	34	52	2	37	6	2				2		175
Haplotaxida			22	65	23	17	34	38	12	11		2	18		242
Arhynchobdellida			5	20	13	2	32	11	12	103	50		109	58	415
Isopoda			9	1,021	722	40	1,175	249	6	95	7	1	72	12	3,409
Amphipoda			1	20	11	1	20		10	27	79	21	93	18	301
Decapoda	Cambaridae		83	71	55	43	108	58	4	124	33	14	116	39	748
Trombidiformes	Hygrobatoidea			11		3									14
Collembola										1			3		4
Ephemeroptera	Baetidae		384	1214	1,964	364	771	1,180	7	163	787	13	252	974	8,073
	Caenidae		362	824	335	1,211	666	165	22	148	88	193	356	57	4,427
	Ephemerillidae			1	1		1	2							5
	Heptigenidae	Leucrocuta		2	104		25	4							135
		Maccaffertium	268	141	203	266	68	136				22		1	1,105
		Nixe		337	530		277	251		8	1		1	18	1,423
		Stenacron	15	22	2	11	2			2	9	9	1		73
		Sternonema	109	665	37	72	332	90	104	856	693	19	824	619	4,420
	Isonychiidae	Isonychia	169	172	75	188	154	137		3	1	15			914
	Leptophlebiidae		287	211	141	87	93	118	1	143	56	64	152	28	1,381
		Neochoroterpes			2			1							3
Odonata	Aeshnidae					2	1				1	2			6
	Calopterygidae		19			1				1	1				22
	Coenagrionidae		32	38	7	13	12	5		23	48	5	17	21	221
		Argia		5			8			2					15
	Corduliidae					1				8			12		21
	Gomphidae		15	33	5	4	29	5		1	2	1		3	98

Order	Family	Genus													Total
		Arigomphus		66	50		33	17		4			1		171
	Libellulidae						1			7	1	1	7		17
Plecoptera	Chloroperlidae			559	1,270		411	539		4	1		5	1	2,790
	Nemouridae			36	318		53	151		2	19			7	586
	Perlidae	Neoperla	42	194	70	211	163	38	57	369	95	22	434	122	1817
		Perlesta	12	1,657	1,107	9	1,162	807		711	332		364	171	6,332
	Perlodidae			74	293		34	222		74	60		36	8	801
Hemiptera	Corixidae						1		1				1		3
	Gerridae		1		1	1		1							4
	Mesoveliidae		2			1				1					4
	Nepidae		1												1
	Veliidae		2		2	6		3							13
Megaloptera	Corydalidae	Corydalus	16	28	16	56	73	132	6	4	5	3	13	7	359
	Sialidae			2						3					5
Neuroptera	Sisyridae						1		1						2
Trichoptera	Brachycentridae							3					3		6
	Dipseudopsidae	Phylocentropus			1										1
	Glossomatidae				4		1	8			2				15
	Hydropsychidae	Cheumatopsyche	412	1,174	1,897	799	1,047	1,307	270	714	823	220	931	485	10,079
		Hydropsyche			3		1								4
	Helicopsyche			33	68	1	3	14			8			8	135
	Hydroptilidae			29	17	1	13	12		1	93		3	10	179
	Leptoceridae				12			3				1		2	18
	Limnephilidae				1		6	1		11			7		26
	Molannidae				1										1
	Philopotamidae	Chimarra	235	277	302	477	117	345	125	11	311	95	130	163	2,588
		Wormaldia		91	50		7	1		30	38		19	13	249
	Phryganeidae													1	1
	Polycentropodidae		19	114	34	53	54	29		7	2		1	8	321
	Rhyacophilidae							1							1
Lepidoptera	Crambidae		14	4	1	8		1							28

Order	Family	Genus													Total
Coleoptera	Curclionidae			1			1			1						3
	Gyrinidae			3	7	3	4	2	1	2	6	1	1	1	31
		Dineutus					6	1		3			3		13
	Dryopidae			1		2	7	3	21	56	36	1	24	18	169
	Dytiscidae		2	19	23	3	22	9		26	10	1	34	7	156
	Elmidae		239	814	753	361	298	355	219	977	645	121	969	577	6,328
	Hydrophilidae		6	8	6	8	10	3	6	15	39	3	20	7	131
	Lampyridae				3		1						1		5
	Psephenidae	Ectopria		8	10			1					1		20
		Psephenus	73	281	179	19	116	127				73			868
	Ptilodactylidae							1							1
	Scirtidae									3	4		5	1	13
	Staphlynidae				2					1			1		4
Diptera	Athericidae		7	12	9			1					2	4	35
	Ceratopogonidae		6	12	13		2	5	2	2	5	1	17	4	69
		Atrichopogon			1		1	3			1		6	1	13
	Chironomidae		1,041	749	2,419	948	1,157	822	136	580	654	161	1,203	433	10,303
	Culicidae										1				1
	Dixidae							3					1		4
	Empididae				1		1				11		3		16
	Ephydridae				3			1						1	5
	Simuliidae		27	1,450	582	22	1,098	308		310	237		534	56	4,624
	Stratiomyidae										4				4
	Tabanidae		23	25	35	13	15	13		10	9	2	16	11	172
	Tanyderidae								1						1
	Tipulidae		15	75	68	3	55	50	5	7	3	5	18	4	308
Total			4,015	12,703	13,915	5,333	9,819	7,799	1,031	5,661	5,315	1,092	6,841	3,980	77,504

Table 3.4. Total macroinvertebrate abundance by order for all sites combined by region during the years 2018-2020.

Order	Ozarks Highlands		Tallgrass Prairie	
	Upstream	Downstream	Upstream	Downstream
Tricladida	126	45	2	2
Haplotaxida	110	89	23	20
Arhynchobdellida	38	45	165	167
Isopoda	1752	1464	108	85
Amphipoda	32	21	116	132
Decapoda	209	209	161	169
Trombidiformes	11	3	0	0
Collembola	0	0	1	3
Ephemeroptera	8577	6672	3092	3618
Odonata	271	131	99	70
Plecoptera	5632	3800	1724	1170
Hemiptera	9	13	2	1
Megaloptera	62	261	18	23
Neuroptera	0	1	1	0
Trichoptera	4774	4304	2446	2100
Lepidoptera	19	9	0	0
Coleoptera	2438	1363	2071	1870
Diptera	6573	4521	1978	2483
Total	30633	22951	12007	11913

Table 3.5. Mann-Whitney U test results ($p < 0.05$) for each metric (Shannon diversity index, EPT index, total species richness, percent EPT, percent of the two most dominant taxa, Hilsenhoff biotic index, total bioassessment score) between the two regions sampled, Ozark Highlands (OH) and Tallgrass Prairie (TGP).

Region	Metric	2018		2019		2020		All years	
		Z	p-value	Z	p-value	Z	p-value	Z	p-value
OH	H	-0.05	0.39	-0.86	0.28	-0.69	0.31	-0.98	0.33
	EPT index	0.50	0.35	1.81	0.08	1.55	0.12	0.29	0.77
	Total richness	0.26	0.39	0.41	0.37	0.13	0.40	0.48	0.63
	% EPT	-0.91	0.26	-0.33	0.38	-0.53	0.35	-0.35	0.73
	% 2 dominant taxa	-0.22	0.39	-0.83	0.28	-0.37	0.37	1.15	0.25
	HBI	-0.05	0.40	-1.46	0.14	-0.72	0.31	-0.64	0.52
	Bioassessment score	0.91	0.26	0.70	0.31	1.70	0.09	0.97	0.33
TGP	H	-0.11	0.40	-2.03	0.04	-0.37	0.37	-1.35	0.18
	EPT index	0.74	0.30	2.25	0.03	1.57	0.12	-0.32	0.75
	Total richness	0.42	0.37	0.22	0.39	0.58	0.34	-0.40	0.69
	% EPT	-1.05	0.23	-1.80	0.08	-0.29	0.38	1.91	0.06
	% 2 dominant taxa	-0.21	0.39	-1.80	0.08	-0.48	0.36	1.12	0.27
	HBI	-1.79	0.08	-2.89	<0.01	-0.70	0.31	-3.34	<0.01
	Bioassessment score	-0.21	0.39	.029	0.38	0.55	0.35	1.90	0.06

Table 3.6. Top abundance model selection results (i.e., ranked AIC_c) assessing the relative variation from the spatial (site) and temporal (date) variables. In addition to variability observed in the upstream and downstream culvert samples and blocked culverts. Three top models with an AIC_{wt} >0.1 and the null (intercept-only) from each analysis are listed. Random effect for each model set was region of sample collection (Ozark Highlands and Tallgrass Prairie).

Model structure	Model parameters[a]			
Total abundance	AIC_c	ΔAIC_c	AIC_{wt}	-2LL
Site	4534	0.00	0.29	-2250.2
Site + U/D culvert	4535	1.52	0.22	-2249.3
Site + Blocked	4536	1.54	0.13	-2249.8
Intercept-only (null)	4575	41.14	0.00	-2284.5
EPT abundance	AIC_c	ΔAIC_c	AIC_{wt}	-2LL
Site + Blocked + Date	4167	0.00	0.17	-2064.8
Site + Blocked	4167	0.10	0.16	-2065.9
Site + Blocked + U/D culvert	4168	0.44	0.14	-2064.9
Intercept-only (null)	4107	39.85	0.00	-2100.6
Chironomidae abundance	AIC_c	ΔAIC_c	AIC_{wt}	-2LL
Site + Blocked	2852	0.00	0.29	-1408.3
Site + Blocked + Date	2853	0.67	0.21	-1407.6
Site	2854	1.52	0.14	-1410.2
Intercept-only (null)	2888	39.61	0.00	-1442.9

[a]Model parameters are listed by column: corrected Akaike's Information Criterion (AICc), change in AICc (ΔAICc), model weights (AICwt), and -2LL (−2 × log likelihood). Covariates listed in model structure: Site (location of culvert), Date (when sampling occurred), Blocked (culvert blocked or free flowing), and U/D culvert (sampled upstream or downstream culvert).

Table 3.7 Monthly precipitation totals (cm) in 2018, 2019, and 2020 in the Tallgrass Prairie region of Oklahoma (OK Mesonet 2022).

Month	2018	2019	2020
Jan	1.09	9.17	13
Feb	10.95	4.65	3.71
March	3.28	4.29	16.33
April	3.86	15.24	12.34
May	10.11	48.16	10.59
June	9.45	18.36	5.18
July	5.66	4.34	14.81
Aug	5.59	14.15	4.85
Sep	6.32	10.41	5.28
Oct	10.34	18.87	12.85
Nov	3.66	8.38	5.16
Dec	8.1	3.25	8.89
Mean	6.53	13.27	9.42
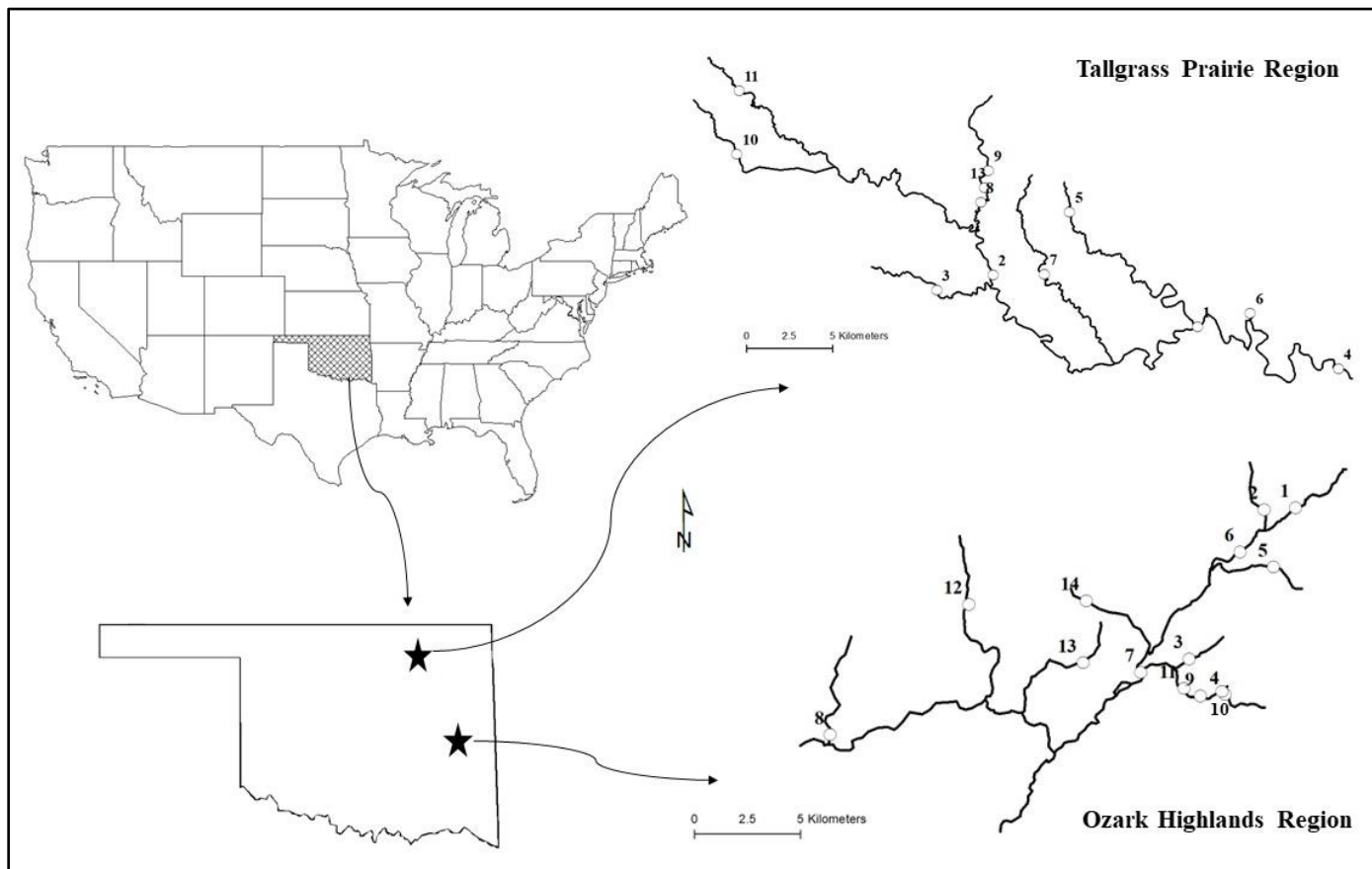
Figure 3.1. Location of culvert sample sites (2018 - 2020). Twelve sites were located in the Tallgrass prairie region (top right) and fourteen sites were located in the Ozark Highlands region (bottom right). Sites are shown as white circles.
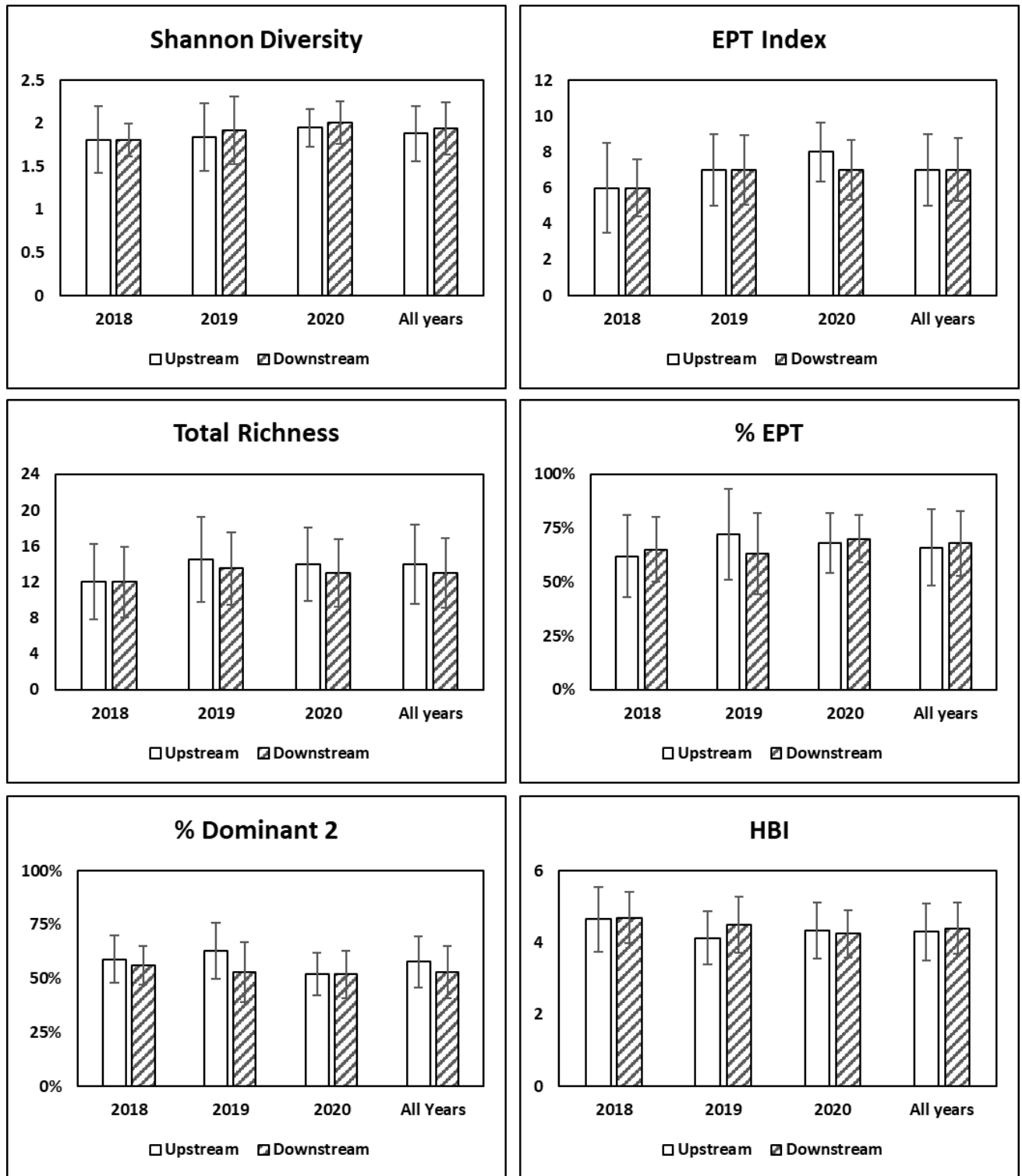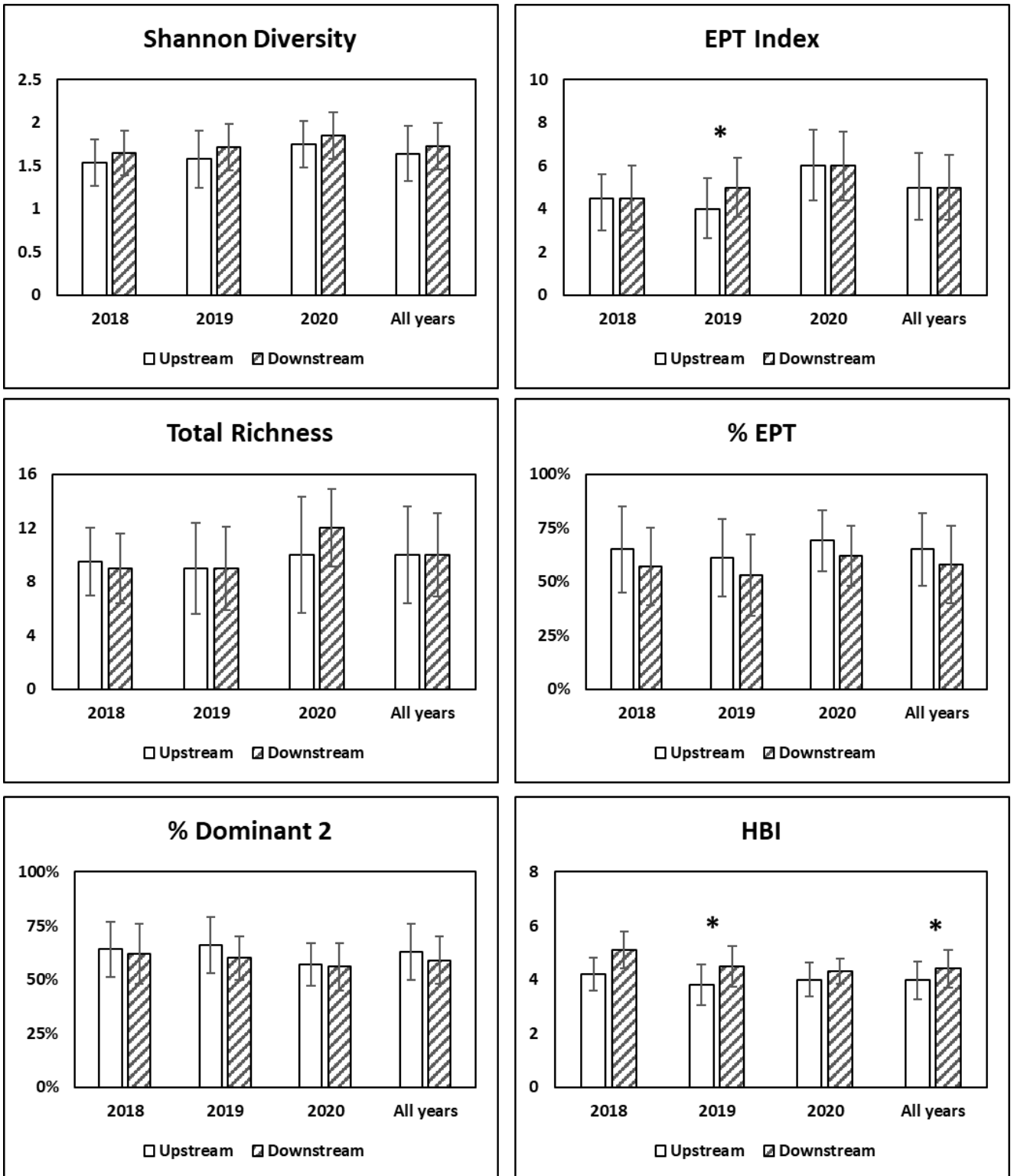
Figure 3.2. Median (+/-MAD) Shannon diversity index, EPT index, total species richness, percent
EPT, percent of the two most dominant taxa, and Hilsenhoff biotic index (HBI) for 2018 -2020
for the Ozark Highlands region. (*) represents a significant difference (p < 0.05) between
upstream and downstream sites.

Figure 3.3. Median (+/-MAD) Shannon diversity index, EPT index, total species richness, percent EPT, percent of the two most dominant taxa, and Hilsenhoff biotic index (HBI) for 2018 -2020 for the Tallgrass prairie region. (*) represents a significant difference (p < 0.05) between upstream and downstream sites.
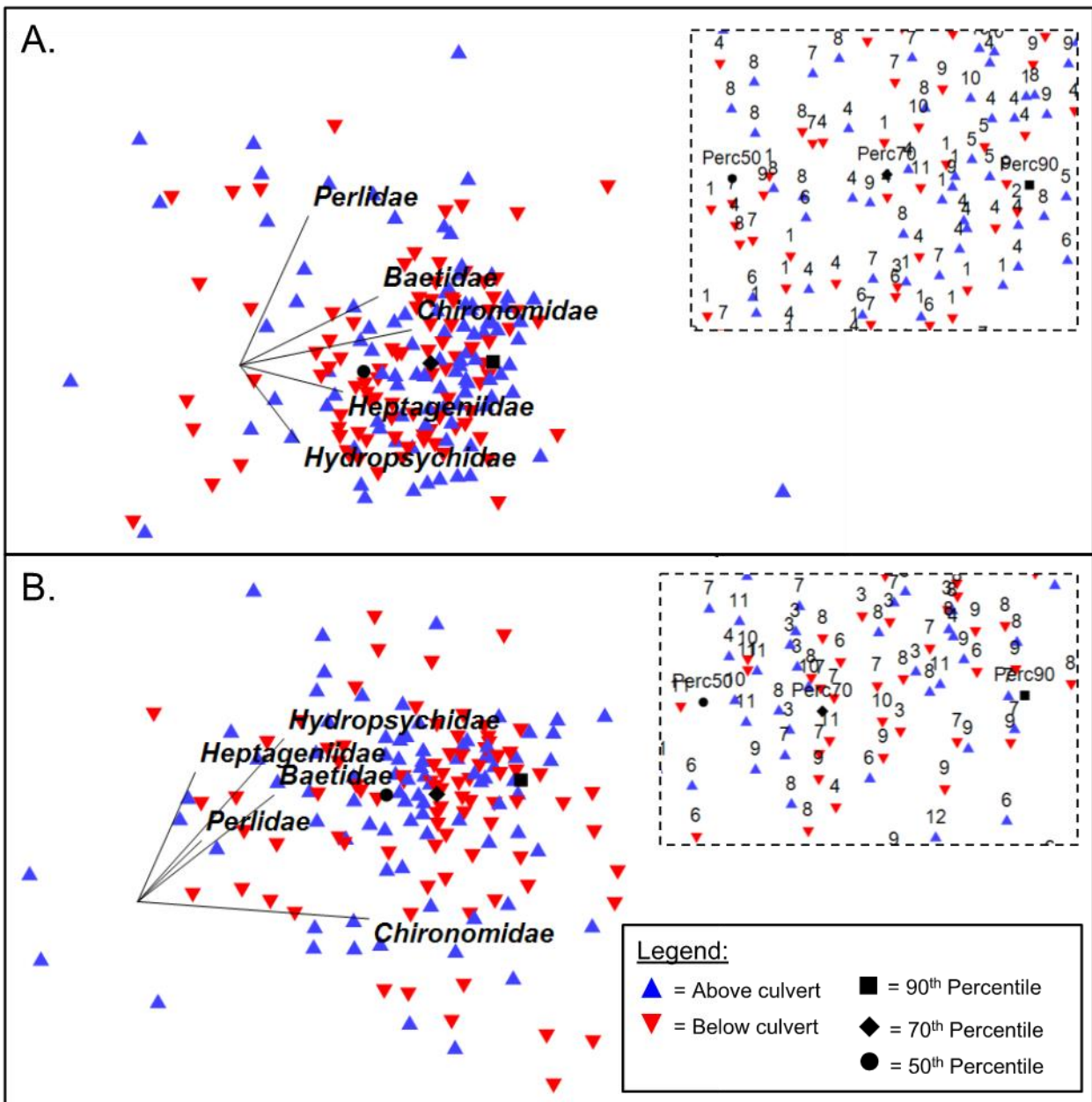
Figure 3.4. nMDS ordinations of the benthic macroinvertebrate communities sampled upstream and downstream road culverts in Ozark Highlands (A) and Tallgrass Prairie (B), the two regions surveyed. For clarity, the top five most abundant families were included: Baetidae, Chironomidae, Heptageniidae, Hydropsychidae, and Perlidae. Black symbols indicate the community abundances at a 50th, 70th, and 90th percentile gradient; callouts in the upper right represent the similarity of above and below culvert samples along this gradient. Numbers associated with the colored symbols represent the site number for each region.

CHAPTER IV

INEXPENSIVE SUBSTRATE SAMPLER FOR MACROINVERTEBRATES

INTRODUCTION

Sampling aquatic invertebrates is an important component of monitoring ecosystem health

(Barbour 1997) and is often used by citizen science groups (Edwards et al. 2017). There are many

widely accepted macroinvertebrate collection methods, which vary depending on sample site

characteristics (Mitchell, & Stapp 2000). Artificial substrate sampling devices are a type of

passive samplers that are constructed to mimic the features of a natural aquatic habitat and attract

aquatic organisms that attach to hard surfaces (Hilsenhoff 1969). Artificial substrate samplers are

an established sampling method that can be used when sampling with other types of equipment,

such as d-frame nets or kick seines, is not feasible because of habitat characteristics (Beak et al.

1973). Hester-Dendy and basket sampler are two types of devices that are widely used in standard

water quality monitoring programs and research. Previous studies (Fullner 1971; Mason et al.

1973; Kirk, & Perry, 1994) have established that these types of samplers, function equivalently

collecting similar counts, richness, and taxa groups of aquatic invertebrates; however, these

devices are expensive especially when numerous samplers are needed. In addition, leaving

expensive samplers at sites for extended periods can be disadvantageous when samplers are lost

during high stream flow events or disturbed by the public.

We aimed to create artificial substrate samplers that were efficient at collecting aquatic invertebrates, inexpensive, durable, and easy to assemble and disassemble. Therefore, we created and tested a low-cost substrate sampler using upcycled empty plastic soda bottles. We compared capture rates of aquatic invertebrates using artificial substrate sampling devices created from empty 0.59 Liter (20-ounce) plastic bottles to Hester-Dendy samplers in lentic and lotic environments.

METHODS

Soda bottle sampler construction

Our artificial substrate sampler was created from two empty 0.59 L (20 ounce) soda bottles (Figure 1). First, we cut the top 6 cm, where the curve began, from both bottles using sharp scissors. Then we burned eight holes approximately 25 mm in diameter in the lower half of each bottle using a Plaid decorative wood burning tool (Model 30725e) (Plaid Enterprises, Inc., Atlanta, Georgia, USA) with cone point tip. Next, we used a standard size hole punch to make two holes 2.5 cm apart and 2.5 cm from top of both cut bottles. Bottles were then filled with course river stones to a depth of approximately 8 cm and the open end of one bottle was slid into the open end of the other bottle while making sure to line up the two punched holes.

During sampler deployment, we threaded a zip tie through the two punched holes in the middle of the bottle sampler. Samplers were then attached to homemade concrete anchors (approximately 0.6 m x 0.4 m x 3 cm; 8 kg) using the threaded zip ties. Samplers could also be attached to the stream bed using concrete blocks or T-posts.

Study sites

To determine the effectiveness of our plastic soda bottle (PSB) samplers in a diversity of environments, we placed bottle samplers alongside Hester-Dendy samplers at seven stream sites in northern and eastern Oklahoma and at four pond sites and three lake sites in central Oklahoma.

The stream sites in northern Oklahoma were in Salt Creek and its tributaries within the Tallgrass Prairie region in Osage county. The exposed limestone formations of this rocky rolling prairie make cultivation in this area difficult, resulting in this region containing one of the largest remaining tracts of native tallgrass prairie in the world (The Nature Conservancy 2022).

The stream sites located in eastern Oklahoma were in Greenleaf Creek and its tributaries in the Ozark Highlands region in Cherokee county. This area is dominated by oak-hickory forest and woodlands (EPA 2012) and is protected by the Cherokee Wildlife Management Area. This area is underlain by cherty limestone and contains karst features with many cold, perennial, spring-fed streams.

The lentic sites located in central Oklahoma were in the Central Great Plains region in Payne County near Stillwater, Oklahoma. The pond study sites were artificial fishless ponds that were filled just prior to deployment of samplers and located at the Oklahoma State University Aquatic Ecology Research Station near Lake Carl Blackwell. The lake study sites were located at Lake McMurtry, Lake Carl Blackwell, and Boomer Lake. This area is underlain by red, Permian age sedimentary rocks and consists of rough plains that are covered by prairie grasses and eastern redcedar, oak, and elm trees (Woods et al. 2005)

Samplers

This project compared two types of artificial substrate samplers: 14-plate round Hester-Dendy samplers and PSB samplers. The Hester Dendy samplers (Hester & Dendy 1962: Wildlife Supply Company 2009) consisted of 14, 7.5 cm round plates of 0.3 cm thick tempered hardboard. Each plate has a center drilled hole separated by 2.5 cm round nylon spacers. The 14 plates were separated by 24 spacers on 0.63 cm-diameter eyebolts. The top 9 plates were separated by a single spacer. while plate 10 is separated by 2 spacers; plates 11 and 12 by 3 spacers; and plates 13 and 14 by 4 spacers. The colonization area of the Hester-Dendy samplers was approximately

13 m^2, including all surfaces of the plates.The PSB samplers contained a mixture river gravel ranging in size from 30 to 60 mm with an average stone size of 43.69 mm (SEM +/- 0.73). Stones were obtained locally from stream sample sites. The colonization area of the PSB samplers was estimated by measuring the surface area of all the stones contained in one sampler using the Rock Weight method (Cooper & Testa III 2001) and adding the interior surface of the bottles. The estimates were repeated 4 additional times to generate an average of approximately 11.61 m^2, including both the area of the stones and the area of the bottle.

Both sampler types were attached to hand-made concrete anchors with plastic zip ties, along with a waterproof identification label, and placed on the stream or lake substrate. Three replicates of each sampler type were deployed side by side at each site. At the lentic sites, foam buoys were attached to the concrete anchors with fishing line to aid in retrieval of samplers. Samplers were retrieved after a four-to-six-week colonization period. Samplers were removed from the water, and immediately put into labeled plastic gallon storage bags, and then taken back to the lab for macroinvertebrate removal and identification.

Laboratory procedure

After retrieval, PSB samplers were disassembled, and the bottles and stones were rinsed thoroughly over a white plastic bin to dislodge aquatic invertebrates. Hester-Dendy samplers were rinsed with water over white plastic bins and scraped between the plates to dislodge aquatic invertebrates. Water from the plastic bins was poured through 850-micron sieves followed by 149-micron sieves to retrieve aquatic invertebrate samples, which were preserved in 70% ethyl alcohol. Using a dissecting scope, aquatic invertebrates were counted and identified using keys by Merritt et al. (2019) and Smith (2001).

Data analysis

We calculated the following metrics using data from each sampler type with replicates per site combined: macroinvertebrate abundance, taxa richness (number of taxa collected), and Shannon-Weiner diversity index (H') (Wilhm 1972). We compared metric values between sampling methods using two sample t-tests for each metric by sampler type ($p < 0.05$) for both lentic and lotic sites.

RESULTS

Across all tests, samplers collected similar invertebrate taxa. Table 1 shows the taxa that were collected from all sites and indicates that a variety of organisms were collected by both types of samplers. At the stream sites, mean ($\pm$ 1 S.E.) macroinvertebrate abundance for the PSB samplers was 37.2 ± 12.6 and for the Hester-Dendy samplers was 34.1 ± 6.7 with macroinvertebrate abundance ranging between 8 and 161 individuals per sampler. Mean taxa richness for the PSB samplers was 5.8 ± 0.75 and for the Hester-Dendy samplers was 5.8 ± 0.81 with taxa richness ranging from 2 to 12 taxa per sampler. No significant difference was found for either macroinvertebrate abundance (t (20) = 0.216, p = 0.831) or taxa richness (t (20) = 0, p = 1) between the PSB samplers and Hester-Dendy samplers used at stream sites. Shannon Diversity Index was 2.05 for the PSB samplers and 2.55 for the Hester-Dendy samplers. The higher Shannon Diversity Index from Hester-Dendy samplers from stream samples was a result of six taxa that were not detected in the PSB samplers.

We collected 408 individuals and twelve major taxonomic groups from stream site PSB samplers. The most abundant orders collected with the PSB samplers were Ephemeroptera (54%), Diptera, (14%), and Trichoptera (12%) (Table 2A). Additionally, there were representatives of Arhynchobdellida, Bivalvia, Coleoptera, Collembola, Decapoda, Gastropoda, Haplotaxida, Odonata, and Plecoptera present in the PSB samplers. We collected 356 individuals and fifteen major taxonomic groups from the stream sites using Hester-Dendy samplers. The most abundant orders collected for the Hester-Dendy samplers were Ephemeroptera (45%),

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Diptera, (15%), and Trichoptera (15%) (Table 2A). Additionally, there were representatives of Amphipoda, Arhynchobdellida, Bivalvia, Coleoptera, Decapoda, Haplotaxida, Hemiptera, Isopoda, Neuroptera, Odonata, Plecoptera, and Tricladida collected from the Hester-Dendy samplers.

At the lake sites, mean ($\pm$ 1 S.E.) macroinvertebrate abundance for the PSB samplers was 56.7 $\pm$ 32.8 and for the Hester-Dendy samplers was 67.7 $\pm$ 42.0 with macroinvertebrate abundance ranging between 10 and 150 individuals per sampler. Mean taxa richness for the PSB samplers was 4.7 $\pm$ 0.67 and for the Hester-Dendy samplers was 4.7 $\pm$ 1.20 and taxa richness ranged from 3 to 7 taxa per sampler. Shannon Diversity Index was 1.08 for the PSB samplers and 1.05 for the Hester-Dendy samplers. No significant difference was found for either macroinvertebrate abundance (t (4) = -0.206, p = 0.847) or taxa richness (t (4) = 0, p = 1) between the PSB samplers and Hester-Dendy samplers at the lake sites.

We collected 200 individuals and seven major taxonomic groups from the PSB samplers placed in lakes. The most abundant orders collected for the PSB samplers were Diptera (49%) and Amphipoda (43%) (Table 2B). Additionally, there were representatives of Cladocera, Ephemeroptera, Gastropoda, Trichoptera, and Tricladida present in the PSB samplers. We collected 161 individuals and seven major taxonomic groups from the lake sites Hester-Dendy samplers. The most abundant orders collected for the Hester-Dendy samplers were Diptera (54%) and Amphipoda (37%) (Table 2B). Additionally, there were representatives of Gastropoda, Isopoda, Odonata, Trichoptera, and Tricladida present in the Hester-Dendy samplers.

We collected 918 individuals and eight major taxonomic groups from the pond PSB samplers over the sampling period. The most abundant order collected was Diptera (83%) (Table 2C). Additionally, there were representatives of Arhynchobdellida, Coleoptera, Ephemeroptera, Gastropoda, Hemiptera, Odonata, and Tricladida collected. No Hester-Dendy samplers were deployed at the pond sites.

DISCUSSION

For a project that requires a large quantity of artificial substrate traps, PSB samplers can be mass-produced at a low cost and require only a wood burner, scissors, and hole punch along with empty soda bottles that can be obtained from recycling bins. We spent approximately $ 14.00 US on supplies. We purchased the wood burner for $12.00 US, the hole punch for $1.00 US, and the scissors for $1.00 US. The average cost for one round plate Hester-Dendy sampler from various online supply companies (Wildco.com, Forestry-suppliers.com, Neobits.com) was approximately $38.00 US and is similar in cost to basket samplers (Wildco.com).

PSB samplers can be assembled quickly and easily with materials that are readily available. One person can construct a sampler in less than two minutes. Samplers can be fully assembled (Fig. 1G and Fig. 1H) in the lab and then transported to the field. If weight or space is a constraint, samplers can be assembled in the field by bringing unfilled sampler parts (Fig. 1D) to a site and filling empty sampler halves with stones (Fig. 1 E – H).

We also established that PSB samplers were very durable, and the samplers constructed at the beginning of sampling in 2020 lasted throughout the entire sampling period. The same sets of samplers were deployed, left at stream sites for a period of four to six weeks and re-deployed on six separate occasions. These samplers were stored in a drawer in the laboratory for approximately 6 months and then were deployed at the lake and pond sites for approximately 4 weeks on four separate occasions. In comparison, some of the hardboard plates of the Hester-Dendy samplers used for this project began to warp by the end of the study decreasing the space available for habituation between the plates. Mason et al. (1973), observed the same issue of Hester-Dendy plate warping in their comparison study of macroinvertebrate samplers.

Because the PSB samplers can be constructed easily, researchers and monitoring groups can replace samplers with new samplers at the time of retrieval. This saves time and increases

efficiency. For this project, we only had a limited number of Hester-Dendy samplers due to financial constraints. At the stream and lake sites we had to make one trip to retrieve samplers, take samplers back to the lab, remove aquatic invertebrates, and make another trip back to sites to re-deploy samplers. Removing aquatic invertebrates in the field was not an option because of the substantial amount of sediment, rocks, and organic debris lodged in between the plates of the Hester-Dendy samplers. At the pond sites, we only deployed PSB samplers and were able to replace the samplers with alternating sets of samplers at the time of retrieval. Possessing enough samplers to replace them as they are retrieved saves considerable time and resources. Additionally, if samplers are lost because of extreme weather conditions or are removed or destroyed by the public they can be easily replaced at very little cost.

Although we did not quantify the results, we found that it was simpler and less time consuming to dislodge aquatic invertebrates from PSB samplers than Hester-Dendy samplers in the laboratory. PSB samplers simply slide apart allowing the rocks to be rinsed with water over white plastic bins. In contrast, the small space between most of the plates on the Hester-Dendy samplers required the use of a dull instrument to scrape out rocks and sediment from in between the plates to dislodge the aquatic invertebrates. Yeardley et al. (2020) also documented greater effort processing Hester-Dendy samplers due to the amount of sediment in the traps in their comparison of macroinvertebrates sampling methods.

CONCLUSIONS

For this study, we created and tested an artificial substrate sampler that was efficient at collecting aquatic macroinvertebrates, was inexpensive, durable, and easy to assemble and disassemble. We found that, with a few exceptions, our homemade and easy to assemble soda bottle samplers captured the same major taxonomic groups in addition to similar abundance, richness, and macroinvertebrate diversity in lentic settings as traditional Hester-Dendy samplers; however, diversity was lower with bottle samplers than Hester-Dendy samplers in the lotic samples. The

soda bottle samplers were successful in both lentic and lotic environments.   The soda bottle

samplers we created are ideal for investigative research studies, low-budget projects supporting

teaching and research, and expanded citizen science monitoring opportunities.

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Table 4. 1. Numbers of macroinvertebrates collected by sampler type at stream, lake, and pond sites (PSB = Plastic Soda Bottle, HD = Hester Dendy)

Order	Family	Genus	Stream Sites		Lake Sites		Pond Sites
			PSB	HD	PSB	HD	PSB
Tricladida				3	2	1	7
Haplotaxida			1	5			
Arhynchobdellida			1	1			7
Gastropoda			40	19	20	7	100
Bivalvia			3	2			
Cladocera						5	
Isopoda				3	1		
Amphipoda				3	60	88	
Decapoda	Cambaridae		4	2			
Collembola			1				
Ephemeroptera	Baetidae		1				6
	Caenidae		41	33		2	
	Heptageniidae	*Maccaffertium*		2			
		Sternonema	175	106		1	
	Leptophlebiidae		4	19			
Odonata	Aeshnidae						3
	Coenagrionidae		4	12	6		1
Plecoptera	Perlidae	*Perlesta*	9	6			
		Neoperla	1				
Hemiptera	Hydrometridae		1	1			
	Mesovellidae			1			
	Notonectidae						1
	Vellidae			2			
Neuroptera	Corydalidae	*Corydalus*		3			
Trichoptera	Helicopsychidae			1			
	Hydropsychidae	*Cheumatopsyche*	14	24			
	Hydroptilidae		9	9			
	Leptoceridae		2				
	Lepidostomatidae				1	1	
	Limnephilidae				1		
	Philopotamidae	*Chimarra*	3	5			
	Polycentropodidae		22	14			
Coleoptera	Dytiscidae		2				12
	Elmidae		2	7			
	Hydrophilidae		1	3			20
	Psephenidae	*Psephenus*		1			
	Saldidae			3			
	Scirtidae		3				
	Staphylinidae	*Stenus*	2	25			
Diptera	Ceratopogonidae		2				
	Chironomidae		10	52	90	98	759
	Stratiomyidae			1			

Table 4.2. Relative abundance of major taxonomic groups by sampler (PSB = Plastic Soda Bottle, HD = Hester Dendy) type for stream sites (A), from April to September 2020, for lake sites (B), March and April 2021, and pond sites (C), October to December 2021.

A)

Taxa	HD	PSB
Ephemeroptera	45%	54%
Diptera	15%	14%
Trichoptera	15%	12%
Coleoptera	11%	3%
Odonata	6%	2%
Plecoptera	2%	2%
Hemiptera	1%	0%
Neuroptera	1%	0%
Collembola	0%	0.2%
Amphipoda	1%	0%
Isopoda	1%	0%
Decapoda	0.3%	1%
Bivalvia	1%	0.4%
Gastropoda	0%	10%
Arhynchobdellida	0.3%	0.2%
Haplotaxida	1%	0.2%
Tricladida	1%	0%

B)

Taxa	HD	PSB
Diptera	49%	54%
Odonata	0%	4%
Ephemeroptera	2%	0%
Trichoptera	1%	1%
Amphipoda	43%	37%
Isopoda	1%	0%
Cladocera	2%	0%
Tricladida	1%	1%
Gastropoda	3%	2%

C)

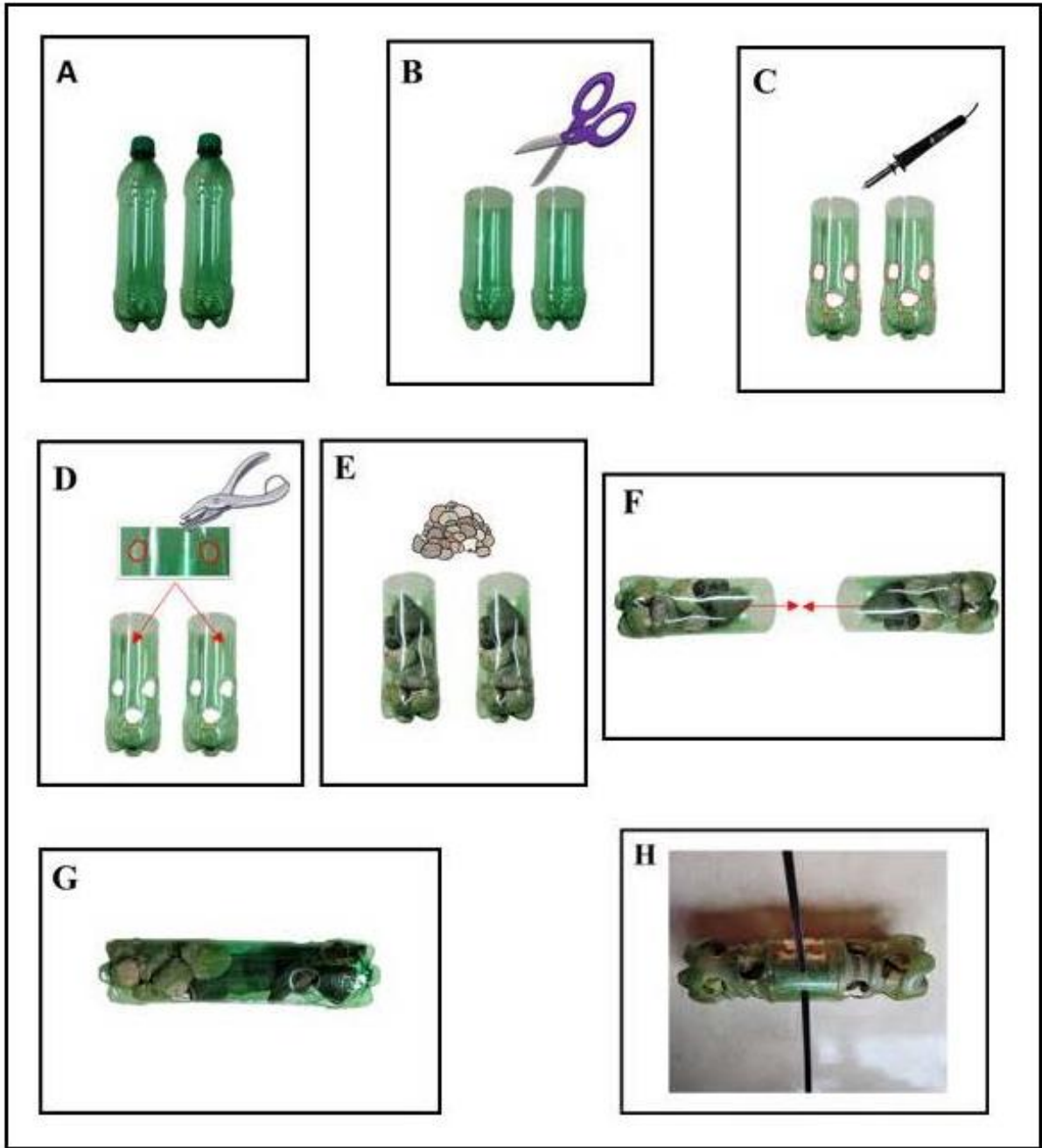| Taxa	PSB
Diptera	83%
Coleoptera	4%
Ephemeroptera	1%
Odonata	0.4%
Hemiptera	0.1%
Gastropoda	11%
Arhynchobdellida	1%
Tricladida	1%

Figure 4.1. Instructions to create plastic soda bottle artificial substrate samplers. A) To make one sampler, start with 2 empty soda bottles. B) Cut the top from both bottles. C) Use a wood burner to make eight 25 mm sized holes in the lower half of each bottle. D) Use hole punch to make two holes 2.5 cm apart and 2.5 cm down from top of both bottles. E) Fill both bottles 8 cm full of river stones. F) Slide the open end of one bottle into the open end of the other bottle making sure to line up the two holes punched to create one sampler (G). H) Thread a zip tie through the two holes punched in bottle sampler and attach to concrete anchor.

VITA

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