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Fish and Invertebrate Community and Trophic Structure in the Gulf of Mexico Coastal Plains Watersheds across Varying Hydrologic Connectivity.

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FISH AND INVERTEBRATE COMMUNITY AND TROPHIC STRUCTURE IN THE GULF OF MEXICO COASTAL PLAINS WATERSHEDS ACROSS VARYING HYDROLOGIC CONNECTIVITY

A Dissertation

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Louisiana State University and
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in

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by
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Abstract

Subtropical Northern Gulf of Mexico coastal plains watersheds tend to have mild seasonal temperature, low topography, and fine, mostly homogenous substrate sizes; these watersheds may not follow fish and invertebrate community patterns predicted by established lotic paradigms because those paradigms tend to describe communities in either temperate or tropical regions. However, the bases of most generally accepted lotic paradigms involve evaluation of allochthonous and autochthonous contributions to lotic ecosystems via three hydrologic connectivity dimensions including vertical (hyporheic and shallow groundwater), lateral (floodplain), and longitudinal (upstream and downstream). My dissertation goals were to explore these three dimensions within Louisiana coastal plains watersheds to provide further clarity of fish and invertebrate community patterns. In Chapter Two, I explored shallow-groundwater and fish assemblage relationships to the threatened Louisiana pearlshell mussel (*Margaritifera hembeli*). Results indicated that fish assemblage data was more important at distinguishing non-mussel stream suitability than the abiotic variables measured, with the exception of the temperature difference of stream water to 25 cm hyporheic depth as well as watershed drainage density. In chapters three and four, I explored invertebrate and fish food-web structure in two Louisiana watersheds based on stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$). Chapter Three results indicated some macroinvertebrate functional feeding guilds (FFGs) showed differences among seasons and streams with varying floodplain connectivity (evaluated by data obtained from annual hydrographs). The results of Chapter Four indicated strong differences in fish food webs based on upstream-downstream connectivity differences between the Tickfaw River and Calcasieu River watersheds. In Chapters three and four, a disconnection between the

insect and crayfish/fish food webs was apparent in the $\delta^{13}C$ and $\delta^{15}N$ biplots, and may be a product of unaccounted basal sources, mainly algae. Overall, lack of clearer relationships with shallow groundwater connectivity variables across all chapters signifies a need for the development of long-term groundwater indicators to allow for detection of changes in flow regimes. Conversely, the results in chapters three and four act as a baseline in stable isotope patterns for other subtropical coastal plains lotic food webs to better understand trends future anthropogenic disturbances especially in light of future climate change.

Chapter 1. Introduction

1.1. Introduction

Streams and rivers are strongly influenced by connections to adjacent terrestrial habitats, which complicates our understanding of the structure and function of these lotic systems. Numerous hypotheses have been developed to describe the factors that shape the energy, trophic, habitat, and physicochemical characteristics of stream systems, but no all-encompassing concept has been developed. Integration of standing paradigms such as the Flood Pulse Concept (FPC, Junk et al. 1989; Tockner et al. 2000; Thorp et al. 2006), and the River Continuum Concept (Vannote et al. 1980; Doretto et al. 2020) have led to a general consensus that geology and hydrology are dominating factors affecting stream processes. However, most paradigms have been developed for temperate or tropical systems, so applicability may be limited for streams in other climate regimes. Aquatic ecosystem diversity is high in Louisiana and the northern Gulf of Mexico coast, with many subtropical, coastal plain watersheds contained entirely within the state's boundaries. Variability in geology and hydrology, as well as a significant east-west biogeographical barrier to dispersal (the Mississippi River; Douglas 1974; Barr and Chapin 1981; Robison 1986; Kaller et al. 2013) contribute to the biogeographical patterns of Louisiana's fish and invertebrate assemblages, which are characterized by high species diversity and moderate endemism. These streams, therefore, provide an excellent opportunity to elucidate the applicability of lotic structure hypotheses to the biotic composition of coastal plain streams. The goal of this dissertation is to better understand the hydrologic factors influencing the abundance and distribution of fish and invertebrates of Gulf of Mexico coastal plains streams and rivers, with special emphasis on energy flow, food webs, and hydrologic connectivity.

1.2. Lotic Paradigms and Applicability to Northern Gulf of Mexico Coastal Plains

Lotic ecology can probably be best understood in terms of hydrologic connectivity, which includes vertical (hyporheic/subsurface), longitudinal (upstream and downstream movement), and lateral (riparian zone/floodplain) compartments (Ward 1989). The importance of each compartment regarding their influence on stream biota varies depending on the season and location within a watershed. Lotic ecosystems form complex dendritic networks of smaller order streams, rivers, distributaries, and deltas that exhibit various connections of flow, physicochemistry, and biota (Fausch et al. 2002; Brown and Swan 2010). Discharge is the volume of water moving through a channel per unit time and is determined by inputs from groundwater and runoff from precipitation after removals for evapotranspiration, soil storage, and aquifer recharge (Fausch et al. 2002). Runoff and shallow subsurface flows from precipitation events create peaks in the annual hydrograph (hourly or daily discharge depicted over the course of a year), whereas groundwater sustains baseflow (Poff et al. 1997).

Discharge has a predictable relationship with watershed precipitation; as precipitation accumulates over time, especially in spring, the soil becomes saturated and reduced storage capacity leads to shallow groundwater movements. These groundwater movements combine with surface runoff to increase stream stage, and as water levels rise, discharge may be pushed past bankfull (Leopold 1994) and enter the floodplain, where water, inorganic, and organic materials are exchanged. Once precipitation subsides, discharge decreases and waters recede from the floodplain (Junk et al. 1989).

This spatial and temporal variation in discharge determines the extent of floodplain inundation, which in turn influences the amount of energy or carbon sources that are exchanged between the aquatic and adjacent terrestrial systems (Polis and Winemiller 1995). Stream carbon sources can be allochthonous and/or autochthonous sources (Doi 2009). In smaller streams, allochthonous inputs of particulate organic matter (POM), dissolved organic carbon (DOC), and CO_2 (Cummins 1974; Hershey et al. 2017) from the riparian zone are particularly important as stream energy sources. During the 20th Century, the view of terrestrial and aquatic systems as separate ecological entities transitioned to a holistic understanding of watershed function with increased knowledge of the importance of terrestrial-aquatic linkages (Welcomme 1970).

Autochthonous primary producers in lotic systems include phytoplankton, surface attached algae, and macrophytes (Cummins 1974). Phytoplankton densities tend to be limited because of turbulence and downstream transport but can occur in slow moving rivers and floodplains with low turbidity. Although macrophytes are generally considered to provide inferior forage for most fishes and macroinvertebrates (Wolters et al. 2018), they do contribute to the coarse particulate organic matter (CPOM) pool as they decompose (Allan and Castillo 2007) and can act as an important substrate for attached periphyton (Soszka 1975). Although biomass levels can be low, algae may be an important autochthonous energy source in lotic food webs because algae are a labile food source with high turnover rates related to stream discharge (high flows scour algal mats) and macroinvertebrate consumption (Vadeboncoeur and Power 2017). Watershed area, local geology, natural and anthropogenically modified landcover, soil characteristics, topography, connection to the floodplain, and riparian vegetation characteristics all influence the relative importance of allochthonous and autochthonous energy sources in streams (Poff 1997; Patil and Stieglitz 2011).

Ultimately, stream structure and function depend on the relative strengths of vertical, longitudinal, and lateral connections with upstream, adjacent terrestrial, and hyporheic environments. Vertical connectivity largely concerns interactions with the hyporheic zone (the area just beneath the stream bed), which is particularly important in lower order streams. The 'hyporheic biotope’ was first recognized by Orghidan (1959) and Schwoerbel (1967). Within the hyporheic zone, three types of surface-groundwater interactions can occur: 1) surface water over impermeable stratum with no exchange; 2) extended sediment layer over impermeable layer, which only maintains the hyporheic zone via advected surface flows; and 3) a deep sediment layer with connection to groundwater and subsurface water. An exact definition of the size and boundary of the hyporheic zone does not exist because measures of chemistry, temperature amplitudes (both daily and annual) and velocities vary seasonally. However, these physiochemical features tend to be distinct from the stream and benthic zone (Brunke and Gonser 1997). For instance, groundwater receives energy in the form of DOC and POM from its stream, and in turn the stream receives inorganic nutrient inputs from groundwater. Dissolved oxygen in the hyporheic zone tends to be independent of the stream, and in fine grained sediments, pore spaces can be filled with POC, which creates an anoxic zone (from decomposition) below a small, oxygenated layer. CO_2 is higher in the hyporheic zone because of lack of photosynthesis and atmospheric exchange, leading to lower pH in interstitial spaces (Brunke and Gonser 1997). Streambed topography also affects hydrological exchange in hyporheic zones, with low-pressure points (upwelling) occurring at the end of riffles and high-pressure points (downwellings) at the end of pools. Other features like gravel bars, macrophytes, large woody debris, or fish nests can also influence vertical connectivity with the hyporheic zone

(White 1990; Brunke and Gonser 1997; Mutz et al. 2007). In areas lacking obvious riffle and pool sequences, i.e., low relief watersheds, identification of hyporheic exchange sites can be difficult, but some studies have demonstrated the importance of vertical connectivity to the distribution and abundance of lotic biota (Boulton et al. 2010).

Longitudinal hydrologic connectivity mainly focuses on downstream transport of water and materials, although some organisms have the ability to move in either direction (i.e., fishes and adult flying stages of aquatic insects). The River Continuum Concept (RCC) was developed to describe geomorphic and energy relationships in streams along a watershed continuum (Vannote et al. 1980; Doretto et al. 2020). Upstream processing of allochthonous coarse particulate organic matter (CPOM) typically results in downstream availability of fine particulate organic matter (FPOM). In larger stream reaches and small rivers, the importance of allochthonous materials to the stream trophic web declines as the stream edge to stream area ratio decreases, elevating the importance of autochthony as canopy cover declines. In turn, trophic webs in larger rivers are reliant on upstream inputs of organic matter delivered from headwater and mid-reaches in the watershed. These inputs are particularly important determinants of the taxonomic and functional feeding guild composition of macroinvertebrate assemblages (FFGs, described below), as well as fish functional traits and assemblage structure. Although the predominately unidirectional character of longitudinal hydrologic connectivity seems obvious, empirical measurements of upstream-downstream impacts on biotic structure have proven difficult to measure (Thorp and Delong 1994; Jackson et al. 2001). However, predictions of the RCC regarding longitudinal connectivity and biotic structure have generally held across evaluations (Larsen et al. 2019; Doretto et al. 2020).

As streams converge into rivers and move into lower gradients, lateral hydrologic connectivity with the floodplain becomes more important to ecology of resident biota, particularly fishes (The Flood Pulse Concept; Junk et al. 1989). Flooding, or periodic inundation of the floodplain, increases lateral connectivity, ultimately linking lotic to lentic (floodplain) systems and allowing development of unique life histories based on seasonal exploitation of flooded habitats (Junk et al., 1989; Tockner et al. 2000; Thorp et al. 2006). Overall, The Flood Pulse Concept and RCC predict that larger order mainstem rivers will have low production to respiration ratio, indicating lower productivity (Dettmers et al. 2001). However, given connections to the floodplain, especially at predictable times of the year, increases in nutrients on the floodplain during inundation ultimately increase primary production, and in turn provide nutrients, organic material, and biota to the river as floodwaters recede (Junk et al. 1989). Floodplains provide a variety of services including nutrient exchange, increased spawning habitat, food (e.g., seeds, pollen, fruit and terrestrial insects), and refugia for younger fishes (Junk et al. 1989; Harris and Gehrke 1994). These habitats tend to be more heterogeneous with more variable velocities than the middle of the channel, providing more suitable conditions for development of macrophytes that can support diverse macroinvertebrate assemblages (Junk et al. 1989; Colon-Gaud et al. 2004; Fisher et al. 2012). Lateral floodplain connections and complex lateral trophic interactions in large rivers are quite distinct from trophic dynamics in most lower order streams and their differential connections to adjacent terrestrial landscapes (Schlosser 1991). For example, floodplain inundation might be minimal and highly periodic in a headwater stream relative to an annual flood pulse in a downstream river, with concurrent differences in nutrient inputs, energy flow, and biotic composition. Flooding still plays an important role in stream function, but the distinction between sub-bankfull flow pulses versus supra-bankfull flooding is an important distinction between low- and high-order systems (Thorp and Delong 1994).

Discharge is considered to be the ‘Master Control Variable’ limiting distributions and abundances of riverine biota (Poff 1997; Fausch et al. 2002; Thorp et al. 2006; Hershey et al. 2017; Poff 2018; Hitt et al. 2022). Moreover, hydrograph characteristics can be as important as other local habitat metrics in determining species presence and absence (Poff 1997, 2018). The Natural Flow Regime (NFR, Poff et al. 1997; Poff 2018; Palmer and Ruhi 2019) focuses on the quantity and timing of streamflow, and the influence of flow magnitude, frequency, duration, timing, and rate of change on fluvial morphology and stream/riparian biota (Poff and Ward 1989; Poff, 1997, 2018).

Spatiotemporal scales are important to consider in the application of ecological paradigms to stream function. The influence of biotic interactions on biodiversity may be extremely important at small spatial scales (stream reach) and be strongly influenced by variability in hydrologic connectivity, whereas at the ecosystem (watershed) level, abiotic factors and biogeographical influences are often cited as exerting a stronger control on community structure (Tockner and Ward 2000). For example, the Murray-Darling Basin (MDB) spans a large geographic range in temperate regions of Australia, causing a heterogeneous climate and unpredictable flooding, which reduces development of life history traits in endemic fishes that would otherwise evolve to capitalize on predictably-available floodplain resources (Humphries et al. 1999; King et al. 2003). Conversely, the Atchafalaya River basin has a complex but reasonably predictable annual spring flood pulse (usually March to June) that creates physiochemical conditions that strongly influence habitat quality for resident fishes and invertebrates (Alford and Walker 2013; Kaller et al. 2011, 2015).

The paradigms discussed above were developed from studies in temperate (RCC, NFR) or tropical (FPC) regions of the world and may not be applicable to other climate regimes (Winterbourne et al. 1981; Minshall et al. 1983; Malicky 1990; Thorp et al., 2006; Roebuck et al. 2019). Importantly, these models of stream function are mostly based on high-gradient headwater streams in defined stream channels that depend on allochthonous inputs of terrestrial organic matter from plant senescence in fall and winter (Cummins 1974). Subtropical Gulf of Mexico coastal streams often function very differently, which offers an excellent opportunity to explore the applicability of lotic paradigms in a unique biome. Coastal plain streams in the northern Gulf of Mexico tend to be sandy-bottomed, with low pH, periodic hypoxia, muted seasonal environmental fluctuations, and high turbidity, which limits autochthonous algal productivity (Kaller et al. 2013). The predominance of sand and silt substrates may result in colmation or the clogging of the top layer of sediments restricting groundwater flow and vertical hydrologic conductivity (Brunke and Gonser 1997), unlike streams in higher gradient regions (e.g., Buffington and Tonina 2009; Tonina and Buffington 2009). Like temperate lotic systems, large woody debris (LWD) is recalcitrant with little consumption by aquatic organisms aside from obligate xylophagic taxa (Hoffmann and Hering 2000). Because northern Gulf of Mexico coastal plain streams lack large substrates such as rocks and boulders, LWD is more important geomorphologically in terms of channel stability and creating heterogeneous habitats in both rivers and streams (Montgomery et al. 2003; Dolloff and Warren 2003), resulting in macroinvertebrate assemblages that are highly reliant on stable woody debris as cover and foraging habitat (Drury and Kelso 2000; Kaller and Kelso 2006a; Kaller and Kelso 2007).

In addition to climate and microhabitat differences, northern Gulf of Mexico coastal plain streams tend to have flashier hydrographs compared to temperate stream systems. This flashiness results from more extreme precipitation events and more gradual changes in topography that create less steep river and stream beds gradients (Felley 1992; Isphording and Fitzpatrick 1992). This topography allows some streams to have functional floodplains, with periodic access to leaves, herbaceous plants, seeds (more labile materials) and grasses during supra-bankfull flooding (Felley 1992; Thorp and Delong 1994). However, even with the low topography, some streams still do not have significant lateral connection to floodplain areas because of stream incision; such streams are ‘underfit’ in reference to their bankfull discharge and fluvial geomorphological relationships (Hupp 2000). Moreover, sub-bankfull spates can scrape algal mats from the stream beds, especially in fine sediments that already provide limited attachment substrates (Mulholland et al. 2000; Cardoso-Leite et al. 2015).

1.3. Biogeography of Louisiana Freshwater Ecosystems

The physical attributes listed above make northern Gulf of Mexico coastal plain streams unusual, but the biogeographical history also adds another layer of complexity in understanding the effects of varying hydrologic connectivity on stream biota. The southeastern U.S. has high aquatic diversity, especially fishes, which is a product of past and current climate and geology (Hocutt and Wiley 1986; Muneepeerakul et al. 2008). The Pleistocene glaciation significantly changed the geomorphology of the Mississippi River, with lower river basin and the rest of the southeastern U.S. providing an unglaciated refuge (Hocutt and Wiley 1986). This allowed five

Archean families to persist in this region, including the Petromyzontidae (lampreys), Acipenseridae (sturgeons), Polyodontidae (Paddlefish), Lepisosteidae (gars) and Amiidae (Bowfin). High diversity is also due to significant radiation of many teleosts, including the Cyrpinidae (minnows), Catastomidae (suckers), Ictaluridae (catfishes), Percidae (perches), Centrarchidae (sunfishes) (Hocutt and Wiley 1986).

The post-glaciation period experienced high rainfall and ice melt, which created new glacial lakes that overflowed into new channels, providing connections to previously disconnected drainage systems and corridors for dispersal of many fish species. Increases in mainstem Mississippi River and tributary river sizes restricted movements of many stream fishes across the mainstem river. For example, the Arkansas and Tennessee rivers are the most diverse eastern and western tributaries of the Mississippi River (Hocutt and Wiley 1986), although these two rivers have little overlap in species composition. Biogeographic studies indicate the lower Mississippi River Basin (MRB) and alluvial floodplain functions as a barrier to east-west dispersal of fishes and macroinvertebrates (Howden 1969; Douglas 1974; Barr and Chapin 1988; Kaller et al. 2013). Significant landscape diversity exists east and west of the Mississippi River, as evidenced by the six level III US EPA ecoregions of MRB, which include the Mississippi Alluvial Plain, Mississippi Valley Loess Plain, South Central Plain, Southern Coastal plain, Southeastern Plains, and Western Gulf Coastal Plain. This landscape diversity further selects from the regional taxonomic pool, resulting in considerable differences among streams and rivers along the Gulf of Mexico Coast. Although biogeographical units such as river basins are a better scale than ecoregions for explaining fish distributions, both river basins and ecoregions are associated with macroinvertebrate distributions, likely because of the terrestrial dispersal mechanisms in many aquatic taxa, particularly insects (Hoeinghaus et al. 2007; Kaller et al., 2013).

1.4. Functional Traits and Food Webs in Lotic Ecosystems

Flora and fauna that occupy instream and floodplain habitats are continually subjected to various predictable (seasonal) and unpredictable fluctuations in water flow and have evolved complex life histories associated with the timing of these flow events (Poff 1997, 2018; Hitt et al. 2022). Several conceptual models have been proposed to relate flow variability to biotic composition and abundance in lotic systems. Stochastic disturbances in stream discharge include short-term pulses, such as spates, steadily increasing ramps, such as drought, and presses, which involves a chronic disturbance that persists through time, like sedimentation from a landslide (Lake 2000). Fish and macroinvertebrates in the coastal plain region of northern Gulf of Mexico are subject to many types of natural and anthropogenic disturbances, including highly fluctuating hydrographs (Hupp 2000), hurricanes, (O'Connell et al. 2014; Patrick et al. 2020), channel alterations (Hupp et al. 2009), urbanization (Carstens and Amer 2019), eutrophication (Jarvie et al. 2013), and invasive species (Kaller and Kelso 2006b), all of which impact species persistence.

Although the species is the fundamental unit of ecology and is typically the basis of most studies of lotic community composition, functional traits (e.g., reproductive strategies, feeding guilds) have also been studied as alternative metrics of community structure (Keddy 1992; Cummins 1974; Johnson et al. 1996; Lytle and Poff 2004). Life history traits and reproductive strategies related to hydrologic connectivity include abilities to capitalize on floodplain inundation for foraging and spawning, or the use of discharge as a spawning cue (Mims and Olden 2013). Although reproductive strategies have been successfully related to flow regimes across the U.S. and Australia (Winemiller 2005), relationships of fish and macroinvertebrate functional feeding guilds (Grossman et al. 1982; Rodríguez-Lozano et al. 2016) to stream discharge characteristics have received much less attention. Reproductive traits are more directly linked to population persistence and abundance, and thus more research has focused on understanding those trait relationships to the flow regime (Mims and Olden 2013). In contrast, studies of trophic traits have tended to focus more on short-term changes related to disturbance events (Lytle and Poff 2004).

Trophic dynamics determine energy exchange and biotic production in aquatic systems- (Johnson 2000), and food webs typically reflect direct relationships between species (Fry 2006). Energy is lost through metabolism with each increasing trophic level (Elton 1927), reducing the number of foraging strategies as trophic level increases. Within stochastic and food-limited ecosystems like streams (Vannote et al. 1980; Legrue et al. 2011), opportunistic foraging strategies (i.e., generalists) are more common at higher trophic levels (MacArthur 1955). Resource partitioning leads to specialist strategies to reduce competition (Schoener 1974), but true specialists (i.e., those surviving on only one prey type) are infrequent in stochastic environments like streams (Townsend 1989; Lake 2000). Conversely, individuals possessing multiple traits, such as having the ability to forage on more than one trophic level, increases the diversity of functional traits at the community level (Cadotte et al. 2015). Relating stream resilience to food web composition involves investigating the relative abundances of generalists versus specialists, food web complexity, and ontogenetic shifts in those webs (Saint-Béat et al. 2015).

Many tactics have been used to study stream food webs including direct observation, gut analyses, experiments, energy budgets, and stable isotopes, which has become a widely used technique (Hershey et al. 2017). Defining trophic levels of aquatic consumers can be difficult because both vertebrates and invertebrates can be grazers and/or detritivores, but they also ingest microbes, which can place them between trophic levels (Hershey et al. 2017). Similarly, predators can also ingest detritus and diatoms with their prey, placing them between trophic levels. Fishes tend to be mostly omnivorous; however, invertivory is the primary feeding mode for most stream fishes (Goldenstein and Meador 2004). Therefore, grouping fishes by feeding traits is based on feeding habits rather specific prey (i.e., species with subterminal mouths feeding on the benthic zone versus species with terminal mouths feeding in the water column) mouths. Streams can have up to four fish trophic levels, but systems subject to frequent disturbance usually have less complex food webs (Hershey et al. 2017). Sabo et al. (2010) demonstrated that fish assemblages in smaller streams have shorter food chain lengths than their larger river counterparts, and cited watershed size as an attenuating factor regarding hydrologic disturbance.

Although most trophic designations in macroinvertebrates are indistinct (i.e., polyphagy is employed over monophagy) and great overlap among species occurs (Allan and Castillo, 2007), macroinvertebrates have been placed in functional feeding guilds (FFGs) based on feeding habit, morphological features, and particulate size ingested (Cummins 1974; Wallace and Webster 1996; Allan and Castillo 2007; Cummins et al. 2019). In most small streams, food webs are based on coarse particulate organic matter (CPOM), which consists mainly of allochthonous deciduous leaves that are later colonized by bacteria, hyphomycete fungi, and protozoans (Chauvet and Suberkropp 1998; Gulis and Suberkropp 2003). Macroinvertebrate shredders feed on this colonized CPOM (Wallace and Webster 1996; Cummins et al. 2019) as a principle food source. As CPOM becomes recalcitrant (a higher carbon to nitrogen ratio) after leeching, microbes become reliant on additional dissolved inorganic and organic nitrogen sources (Cummins 1974). Fine particulate organic matter (FPOM) comes from both the partial break down of CPOM through stream mechanics, as well as leeching of CPOM and DOC in runoff and subsurface groundwater, dead aquatic plants, and sloughed microbial cells (Cummins 1974). Macroinvertebrates that consume FPOM are collectors, which include early instars of aquatic insects. Shredders and collectors allow the energy and nutrients in allochthonous materials to be available to higher trophic levels, whereas algal scrapers provide an avenue for autochthonous materials to move to higher consumers (Wallace and Webster 1996; Cummins et al. 2019), although periphytic biofilms often contain both autochthonous and allochthonous materials.

As stated previously, allochthonous/autochthonous contributions are determined by stream size, shading, substrate, and other variables, which are on a continuum in the watershed (Vannote et al. 1980; Fausch et al. 2002; Dorretto et al. 2020). Headwater streams tend to be shaded and CPOM from leaf senesce is processed by shredders including crayfish, amphipods, freshwater shrimp, snails, and immature stages of insects (Roeding and Smock 1989; Wallace and Webster 1996; Allan and Castillo 2007; Balibrea et al. 2020). Shredders rely on CPOM, but only convert 40% to their own body tissue and CO_2, with the remainder egested as feces, which is considered FPOM (Cummins 1974; Cummins et al. 2019). Mid-reaches have less shading with more algal production and a greater predominance of scrapers, given typical turbidity levels that permit subsurface photosynthesis. Large rivers have high turbidity and less autochthonous production, so collectors are important and dependent on FPOM (Wallace and Webster 1996; Cummins 2016; Gholizadeh and Heydarzadeh 2020). Collectors utilize FPOM throughout the watershed, though FPOM sources may be difficult to determine. Sloughed biofilm/periphyton seems to be the most nutritious food source, whereas FPOM from processed CPOM may not be as nutritious (Allan and Castillo 2007).

The reliability of such FFG classifications has been argued, i.e., collectors and scrapers often ingest other smaller animals along with the targeted algae, and predators often ingest detritus with their prey (Cummins 1974; Cummins et al. 2019). Unionid mussels seem to rely heavily on bacteria in FPOM, and some acquire 80% of their food from deposited materials, although microbial and algal components still make up a portion of their diets (Allan and Castillo 2007). Ontogenetic diet shifts and omnivory, for example among crayfish, can also make it difficult to reliably assign a FFG (France 1996). In streams, ponds, and lakes, crayfish often play an important role as keystone consumers because they feed on live/dead animal as well as terrestrial plant materials (France 1996; Nyström and Strand 1996). However, despite potential downfalls, FFGs can still be useful for determining the most prevalent food source in a stream and how disturbances can alter food sources and biotic assemblage structure (Allan and Castillo 2007; Cummins et al. 2019).

Microbial and meiofauna compartments of lotic food webs play huge roles in nutrient and energy fluxes. Bacteria and fungi transform organic carbon (DOC) to CO_2 and allow higher consumers (macroinvertebrates) to consume the allochthonous carbon (Allan and Castillo 2007). With the help of macroinvertebrate shredders, microbes are responsible for most of the breakdown of CPOM to FPOM. Most production occurs in the benthic zone, where exopolymers secreted by bacteria on the leaf biofilms are eventually consumed by macroinvertebrates ingesting amorphous detritus. Meiofauna including microcrustaceans, rotifers, early instars of macroinvertebrates, protozoans, oligochaetes, nematodes, gastrotrichs, and microtuberllarians, consume bacteria as suspension feeders or from the biofilm, whereas fungi are consumed in the leaf matter by macroinvertebrates (Majdi et al. 2017). The meiofaunal community in Gulf Coast lotic systems is likely limited because of the lack of large interstitial spaces within the predominantly sandy sediment, analogous to low meiofaunal productivity reported in coastal plain streams of the Atlantic Coast (Smock et al. 1992).

Nutrient flux and recycling are also important in the context of food webs and ecosystem resilience (Deangelis 1980). O'Neill (1976) demonstrated that ecosystems with high rates of biomass turnover, such as ponds, have higher resilience than ecosystems with lower rates of turnover, like the tundra. Sabo et al. (2010) demonstrated that with increased hydrologic disturbance, especially in smaller streams, a reduction in food chain length occurred. Marks et al. (2000) found river food chain length of basal sources and macroinvertebrates was a product of interannual variability in flow, and years with more disturbances or high spates tended to have more trophic levels. Marks et al. (2000) concluded that food chain length was a product of the hydrologic regime and is not directly related to nutrient input and productivity. These observations may also pertain to the effects of disturbance on fish assemblage composition. When an ecosystem is not resilient, a disturbance may cause a shift to an alternative state. For instance, Power et al. (1985) demonstrated state changes in food web structure following a spate, with algae-limited pools being dominated by stone rollers (*Campostoma* spp.), and algal-rich pools dominated by black basses (*Micropterus* spp.). Autochthonous sources can contribute significantly to the instream secondary production, which would change the structure of stream food webs.

Because of spatiotemporal variability in physical forces (i.e., flow), subtropical lotic systems may not be expected to follow the temperate food web paradigm, where the microbial food web is connected to meiofauna compartment and then to macroinvertebrates and fishes (Schmid-Araya and Schmid 2000; Schmid-Araya et al. 2016). Subtropical coastal lotic food webs may be more disjunct than expected in terms of meiofauna and microbes contributing directly to the food web from riparian allochthonous sources, so a better understanding of energy flow and hydrologic connectivites in these systems is needed.

1.5. Goals of Dissertation and Objectives of Each Chapter

The overarching goal of this dissertation is to test the applicability of generally accepted lotic paradigms, described in high-gradient, temperate streams, or large tropical river systems, to subtropical coastal plains watersheds of the northern Gulf of Mexico. I set out to explore the ways in which energy and nutrients are delivered across a set of subtropical northern Gulf of Mexico coastal plain watersheds, and how variability in the hydrologic avenues affect a single species (Chapter Two), benthic aquatic insect assemblages and food webs (Chapter Three), and fish and macroinvertebrate food webs (Chapter Four). Hydrologic avenues include lateral (floodplain/riparian zone, subsurface flows), vertical (subsurface flows, hyporheic zone), and longitudinal (upstream/downstream movement) inputs, as well as hydrologic disturbances (spates and flooding) that can affect the magnitude of each avenue. The dispersal barrier formed by the

Mississippi River for fishes and aquatic macroinvertebrates also allows me to explore differential functionality of food webs in eastern and western Louisiana streams (Chapters Two and Three).

Chapter Two is study performed on the federally threatened Louisiana pearlshell mussel (*Margaritifera hembeli*) in the Kisatchie National Forest. This freshwater mussel is restricted to central Louisiana, with disjunct populations occurring north and south of the Red River, and a smaller population in southwestern Arkansas (Sikes et al. 2019). The purpose of this chapter is to identify streams that may have groundwater connectivity for potential translocation of this imperiled mussel. Other mussel research has identified groundwater as a significant contributor to healthier freshwater mussel populations (Arbuckle and Downing, 2002; Andrisoa et al. 2019). My first objective was to determine groundwater/subsurface flows in relation to presence/absence or abundance of *M. hembeli*. I used hydrologic sampling methods that include habitat survey data, GIS Hillslope analysis, hyporheic temperature recordings and annual hydrographs, and fish assemblage data as predictor variables. For my second objective, I evaluated the suitability of streams located near but outside of *M. hembeli*'s historical range for translocation by analyzing five streams containing mussels and ten additional non-mussel streams scattered throughout the Kisatchie National Forest.

Chapter Three is focused on macroinvertebrate assemblages among eight streams, four each east and west of the Mississippi River. My main goal was to understand how hydrologic connectivity to the riparian zone (lateral connection) creates active floodplains that contribute to the structure of the macroinvertebrate food webs. Lateral hydrologic connection can be brief (i.e., hours to days) as in flashy streams, or longer (i.e., days to weeks) in floodplain streams and may be surficial or sub-surficial. Study streams exhibit obvious differences in visible functional floodplains versus simple riparian zones, indicating variability in lateral hydrologic connectivity among streams.

Chapter Three is separated into two parts. In part one, I focused on exploring relationships with taxa and FFGs in relation to lateral hydrologic connectivity in all eight streams, as well as the effects of the Mississippi River, basin geology, and basin geomorphology (Daigle et al. 2006). However, it is not clear whether on taxonomic and physical differences ecosystem function, especially in the less taxonomically rich western streams and rivers (Barr and Chapin 1981; Kaller et al. 2013). I expected to observe seasonal differences in abundances in aquatic insect FFGs between eastern and western streams as a product of varying levels of lateral connectivity.

Part two involved exploring lateral hydrologic connectivity via seasonal flooding. A subset of streams, two each east and west of the Mississippi River, was chosen to perform stable isotope analysis (δ^{13}C and $\delta\ ^{15}$N) of plant and animal tissues. Predictor variables were flooding categories developed from stream HOBO data loggers, as well as δ^{13}C of different riparian basal sources. Flooding categories were based on the number of sub- and supra-bankfull floodings on an annual and seasonal bass. Supra-bankfull flooding exceed bank height and spreads onto the floodplain, increasing the overall surface area of the stream bed. Basal sources include pine needles and deciduous tree leaves, as an example of traditional temperate allochthonous sources, as well as grass and herbaceous plants that represent possible floodplain specific contributors. In fall, more senesced leaves are available on the floodplain to be washed into streams, introducing greater amounts of allochthonous energy sources. Conversely, sub-bankfull flooding in the summer may be more impactful to algal food resources because of hydraulic scouring.

Chapter Four expands on chapter Three to include a watershed focus to better understand longitudinal hydrologic connectivity. I set out to understand the relationship between headwaters, mid-reaches, and mainstem rivers of two river basins (Calcasieu River basin and the Tickfaw River) in terms of fish food web structure. The Tickfaw River is a scenic river with no impoundments, and the selected sites on the Calcasieu River are above any impoundments or major anthropogenic impacts with limited leveed sections, making these floodplain rivers comparable in this study. From the eight streams previously mentioned in Chapter Three, four were selected (two for each basin) and were sampled in the fall of 2017 and 2019. The rivers were sampled in the fall of 2018 and 2019, and I added a mid-reach site in fall 2019 to gain a clearer picture of headwater/river relationships.

I sampled fish (backpack and boat electrofishing, and hoop nets), macroinvertebrates (woody debris, Hess) and allochthonous/autochthonous basal samples that were identified, enumerated, and used in stable isotope analysis. Because taxonomic turnover occurs as one precedes down a watershed, I selected fishes that represented guilds of the food web (i.e., detritivores, omnivores, intermediate and top predators). I used the information gained from Chapter Three to designate lateral connectivity of the headwater streams and how it affects the macroinvertebrate food webs. Combined with the fish information collected for this chapter, I investigated how groundwater inputs (vertical connectivity) and connections to the floodplain (lateral connectivity) influenced stream food web structure and if those patterns could be detected downstream (longitudinal connectivity). My hypothesis were centered around discovering differences in headwaters between watersheds, in lower sites between watersheds, and whole comparisons of the Calcasieu River and Tickfaw River watersheds.

I finish with a discussion highlighting important results of this dissertation, as well as a discussion of how the structure and function of subtropical, Gulf of Mexico coastal plain watersheds may be affected by future anthropogenic disturbances, especially climate change. My hope is that this dissertation will inform managers and biologists regarding the applicability of existing paradigms to new environments to refine, refute, or reinforce the use of these important models in understanding coastal stream and river systems.

Chapter 2. Groundwater Connectivity and Fish Community Characteristics within Threatened Louisiana Pearlshell Mussel (*Margaritifera hembeli*) Streams

2.1. Introduction

Freshwater mussels (superfamily Unionoidea) are filter-feeding, relatively sessile bivalves occurring in lakes, streams, and rivers (Vaughn et al. 2008). Compared to other marine bivalves, freshwater mussels are unique in that they have evolved a parasitic larval stage, glochidia, that encysts on the gills of a host fish for dispersal in watersheds that tend to be naturally highly fragmented (Bauer 1987). Freshwater mussels are important ecosystem engineers that transfer nutrient and energy from the water column to benthic zone (Vaughn et al. 2008). Additional benefits to stream communities include stream bed stabilization created by dense mussel beds as well as mussel shells act as an attachment substrate for periphyton, biofilm and other invertebrates (Vaughn et al. 2008; Haag and Williams 2014; Vaughn 2018). Because freshwater mussels purify water by filter-feeding, freshwater mussels are particularly sensitive to high siltation rates, and contaminants such as heavy metals, which has led researchers to use freshwater mussels as bioindicators of good water quality (Van Hassel and Farris 2007; Al-Mamun and Khan 2011). Freshwater mussels are in decline because of past and current habitat alterations such river channelization, impoundments, and watershed fragmentation (Vaughn et al. 2008; Böhm et al. 2021; Garrison et al. 2021). Drought can also have deleterious effects, i.e., changing thermal and stream discharge regimes, to mussel populations, especially in the south-eastern United States where drought is expected to increase in the future (Gough et al. 2012). Freshwater mussels are one of the most vulnerable groups of aquatic organisms mainly because of their life history traits mentioned above (Giest 2010). About 71.7% of North American species are federally listed as endangered, threatened or of special concern, and further decline of freshwater mussels, both in biomass and species loss, could of devastating effects for the entire structure of the aquatic community (Williams et al. 1993).

Among the Unionoidea, freshwater pearl mussels (genus *Margaritifera*, family Margaritiferidae) consist of 13 species found throughout the Holarctic biome and may have been widely distributed. Plate tectonic movement and competition with other Unionids restricted the range of Margaritiferids mostly to headwater streams (Araujo et al. 2017). In those streams, Margaritiferids develop large beds consisting of anywhere from 100 individuals/m^2 (Ted Soileau, United States Department of Agriculture (USDA) Forest Service, personal communication) to 400/m^2 (Young and Williams 1984a), which may benefit reproduction (Bauer 1987).

Margaritiferids are long-lived, with records of individuals reaching 60 to 93 years old (Linnaeus 1758; Conrad 1838; Bauer 1987; Johnson and Brown 1998). Long lifespans along with low juvenile recruitment (Bolden and Brown 2002) has led to most Margaritiferids having ‘geriatric’ populations (Johnson and Brown 1998; Hastie et al. 2000; Orueta et al. 2007; Rudzīte 2005; Hastie and Toy 2008). Historic and on-going anthropogenic pressures have put populations in decline, with all 13 Margaritiferid species listed under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. Historically impacted by overharvesting for manufacture of buttons and knife blades and cultivation for oyster pearls (Gómez and Araujo 2008), most populations are currently threatened by habitat degradation, commercial harvest, invasive species (especially other Mollusks), and overall deterioration of water quality, particularly fine sediment deposition (Bauer 1987; McMahon 1991; Williams et al. 1993; Nakamura et al. 2022). Overexploitation of glochidial fish hosts and habitat fragmentation may further exasperate these deleterious effects (Johnson and Brown 1998).

Conservation efforts include examining population genetic structures, monitoring fish host populations, and cultivating captive-bred individuals for reintroduction and translocation (Boon et al. 2019). Successful reintroductions of mussels into streams requires identifying important macro- and microhabitat features. Freshwater pearl mussels may have stricter water-quality requirements, especially in relation to siltation, than other Unionids (Gómez and Araujo 2008). Fine sediment and silt can be detrimental by suffocating adults and juveniles. Fine sediment can also fill interstitial spaces in the hyporheic zone, which limits groundwater connectivity, a process that is locally important for maintaining nutrient and energy exchanges between the stream and hyporheic zone (Buddensiek et al. 1993; Hastie et al. 2000). Within streams, mussel beds tend to be patchily distributions and associated with a mixture of loose sand and some gravel (Hastie et al. 2000; Bolden and Brown 2002) and in artificial canals (Gómez and Araujo 2008). Larger substrates, e.g., cobble (64–246 mm) and boulders (> 256 mm) stabilize the stream bed, allowing sand and gravel to collect in pockets, which prevents individuals or entire mussel beds from being washed out by spates (Vannote and Minshall 1982; Johnson and Brown 2000; Hastie et al. 2000). Johnson and Brown (2000) reported shallow, compacted substrate with some gravel and good flow act to stabilize substrates for *M. hembeli* when larger sediment sizes are lacking.

Adult mussel bed stability and juvenile recruitment is dictated by the geomorphology and hydrological regimes of streams, which have profound control over sediment deposition and the hydraulics of stream beds (Hastie et al. 2000; Moorkens and Killeen 2014, Quinlan et al. 2015). Most margaritiferids prefer riffles rather than pools that tend to collect fine sediment that trap organic detritus and limit water flow creating anoxic conditions (Johnson and Brown 2000; Bolden and Brown 2002; Gómez and Araujo 2008). Conversely, high shear stress may inhibit juvenile deposition (Johnson and Brown 2000), which results in variability among streams regarding margaritiferid preferences for riffle depth and stream velocity (Roscoe and Redelings 1964; Hastie et al. 2000; Quinlan et al. 2015). Margaritiferids are capable of small movements to mitigate problems associated with changes in water depth (the Louisiana pearlshell mussel, *M. hembeli* was reported to move 7 m upstream in a one-year period, Johnson and Brown 2000), but minimum flow requirements are important to maintain stream populations. Shallow groundwater connectivity or subsurface flows can alleviate effects of drought as well as maintain critical habitat for juveniles, which remain in the hyporheic zone for up to five years (Blackburn et al. 2021; Hyvärinen et al. 2021).

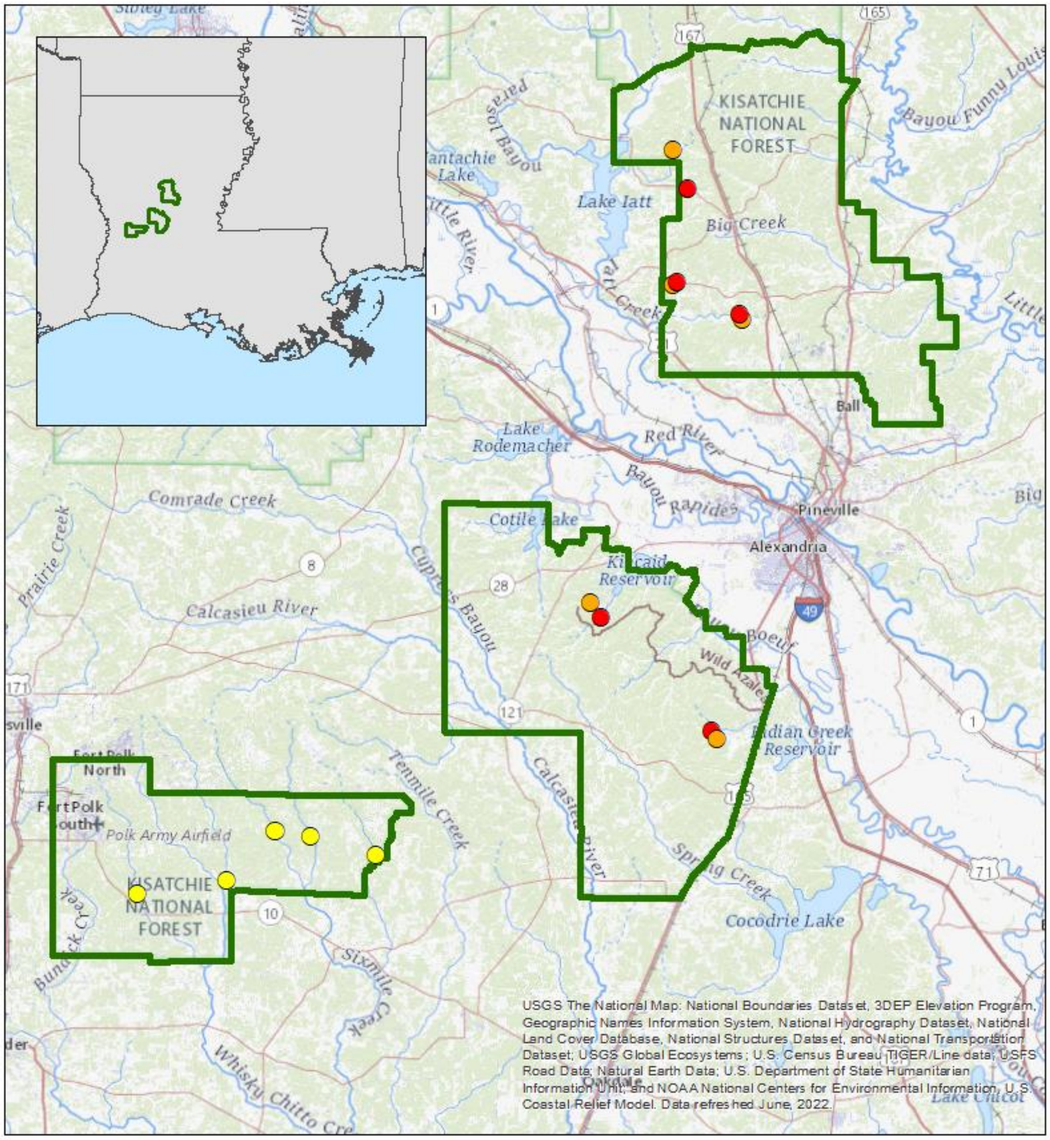
The Louisiana pearlshell mussel (*M. hembeli*) is currently listed as Threatened under the Endangered Species Act but is predicted to recover with proper management (U.S. Fish and Wildlife 2019). Bolden and Brown (2002) identified important microhabitat features associated with presence of *M. hembeli* by successfully translocating adults to new sites within the same natal stream. Presently, a captive breeding program is underway for *M. hembeli* at the United States Fish and Wildlife Service (USFWS) Natchitoches National Fish Hatchery. Successful cultivation of *M. hembeli* juveniles from wild gravid females and Redfin Pickerel *Esox americanus americanus* hosts has been documented.

Although relocation of *M. hembeli* is considered to be an important component of restoration efforts in Louisiana streams, important environmental variables related to mussel presence at the stream level have not been clarified. Consequently, my first objective was to compare occupied and unoccupied streams within the historical geographic distribution of *M. hembeli* to identify differences in fish assemblages, geomorphological features, and shallow groundwater connectivity. Secondarily, I wanted to evaluate stream suitability near, but outside the historical range of *M. hembeli* for potential translocation. These studies are particularly important in Louisiana because of significant difference in environmental characteristics relative to other margaritiferid species ranges (i.e., boulders are very rare in Louisiana streams). Importantly, however, stream and watershed-level characteristics (i.e., overall streambed stability; Hegeman et al. 2014; Blevins et al. 2017) may be similar among study streams. Fish data were collected to assess species associations with occupied habitats, and geomorphological data were analyzed to address measures of stream bed stability, including sediment regimes and riffle abundances. I also measured variables intended to indicate groundwater connectivity. This combination of variables have not previously been investigated in assessments of the life history and environmental associations of *M. hembeli*.

2.2 Methods

2.2.1 Site Information, and field sampling

The Louisiana Pearlshell Mussel is found in only 22 (2nd and 3rd order) streams, all of which drain north and south to the Red River in central Louisiana (Johnson and Brown 2000; USFWS 2019 Species Status Assessment for *Margaritifera hembeli*). I selected five streams occupied by *M. hembeli* paired with five unoccupied streams within this region (Figure 1.1, Table 1.1). I selected five additional streams outside the geographical range of *M. hembeli* as possible translocation sites, all located in the Vernon unit of the Kisatchie National Forest (hereafter, these streams are denoted as out-of-range streams). Streams in central Louisiana tend to have low slope, soft water, low pH, and are dominated by sandy substrates (Budnick et al. 2018; Reuter et al. 2019). Habitat features and fish were sampled in the summer and fall of 2018 with groundwater variables measured (described below) in the winter of 2018/2019.



USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed June, 2022.

Figure 2.1. Locations of study sites (circles) within the Kisatchie National Forest (green polygons). Red circles indicates *M. hembeli*-occupied study streams and orange indicate *M. hembeli*-unoccupied study streams within the Catahoula ranger district (upper polygon) and Calcasieu ranger district, Evangeline unit (center polygon); yellow indicate *M. hembeli*-unoccupied study streams within the Calcasieu ranger district, Vernon unit (Lowest polygon). Map was created in ArcGIS 10.7.1 (ESRI 2019).

Fish were sampled via a double-pass DC backpack (Halltech HT-2000, Ontario, Canada) electrofishing depletion method in a blocked 100 m stream reach. For streams containing mussels, I selected reaches that were in between but not overlaying any known mussel beds because I did not want to disturb any previously established beds. All fishes were identified and enumerated; those individuals that could not be identified were placed on ice while traveling from study sites and brought back to the Freshwater Ecology Lab, School of Renewable Natural Resources, LSU, to be identified later. Fishes were collected and handled following IACUC A2021-15.

Table 2.1. Study streams included 10 streams that were occupied or unoccupied by mussels within the range of *M. hembeli* and an additional five streams that were out of range. All study streams were contained within the Kisatchie National Forest, LA.

Kisatchie National Forest District, Unit	Mussel Status	Stream
Calcasieu District, Evangeline Unit	Occupied	Bayou Clear
Catahoula District	Occupied	Beaver Creek
Catahoula District	Occupied	Trib. of Cress Creek
Catahoula District	Occupied	Trib. of Grey's Creek
Calcasieu District, Evangeline Unit	Occupied	Valentine Creek
Catahoula District	Unoccupied	Black Creek
Catahoula District	Unoccupied	Cress Creek
Catahoula District	Unoccupied	Grey's Creek
Calcasieu District, Evangeline Unit	Unoccupied	Hospital Bayou
Calcasieu District, Evangeline Unit	Unoccupied	Stracener Branch
Calcasieu District, Vernon Unit	Out-of-Range	Big Brushy Creek
Calcasieu District, Vernon Unit	Out-of-Range	Bird's Creek
Calcasieu District, Vernon Unit	Out-of-Range	Drake's Creek
Calcasieu District, Vernon Unit	Out-of-Range	East Fork of Six-mile Creek
Calcasieu District, Vernon Unit	Out-of-Range	West Fork of Six-mile Creek

Habitat features were measured following the USDA Forest Service protocol for stream surveys within Kisatchie National Forest (Byrd 2005). At 15 transects 10 m apart, depth (m) and velocity (m/sec) were measured at 25, 50 and 75% of stream width along with wetted width (m) of each transect. Sediment was collected via a standardized scoop at two fast velocity and two slow velocity sites to capture the range of sediment sizes. Sediment samples were later dried and sieved through a modified Wentworth series (32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.63 mm). Stream gauges equipped with hobo pressure transducers (Onset HOBO U20L) were placed in a subset of streams to gain baseline data of stream hydrographs, which allowed for comparison of occupied, unoccupied, and out-of-range streams.

Shallow groundwater connectivity was evaluated through a series of field and GIS methods (Figure A.1). I first recorded stream and hyporheic temperatures (°C) every 3 m for 40 transects, with anomalous differences indicating possible upwellings and downwellings (Baskaran et al. 2009; Krause et al. 2012). Temperature was measured by inserting a Cole-Palmer Thermometer with probe into the stream bed and recording temperature at 25, 50 and 75 cm depth, as well as the temperature within the stream water column. Two sites that did not have temperature differences between the water column and hyporheic zone were randomly selected, along with four irregular sites were selected for further sampling. I used a piezometer and peristaltic hand pump to extract water in the hyporheic zone at a standardized 50 cm depth. I recorded specific conductance (mS/cm), dissolved oxygen (mg/L) and pH in the stream and the piezometer via a YSI handheld probe meter (ProSolo Digital Water Quality Meter) to further explore stream/hyporheic differences that may indicate shallow groundwater connectivity.

2.2.2. Variable Development

Fish community metrics used to assess differences among occupied, unoccupied, and out of range streams included overall species richness and abundances of taxa. I calculated catch per unit effort (CPUE) for electrofishing (#/hour) as a proxy for individual species counts for each stream. To calculate species richness, I developed a rarefaction curve to estimate the species richness based on small sample sizes (Program R, vers. 4.2; R Core Team 2022 vegan package; Oksanen et al. 2019). A list of specific taxa was compiled to compare across streams. Infrequent species that were represented across all streams were analyzed by genus. Any species not historically found over the entire sample region (e.g., Striped Shiner-*Luxilus chrysocephalus* and Creek Chub-*Semotilus atromaculatus*), and rare species that comprised less than 5% of all fishes collected and 5% frequency of occurrence were eliminated from analyses; these procedures resulted in seven taxonomic groups for subsequent analyses (Table 2.2).

Measured fluvial-geomorphic variables included average stream width, riffle count within a stream, streambed complexity, and sediment particle size. Streambed complexity is associated to increased groundwater connectivity and mussel presence (Geist and Auerswald 2007), with most measurements relating to riffle to pool sequences (Kasahara and Wondzell 2003); however, most central Louisiana streams lack these geomorphological features, therefore, I developed a variable that captures streambed complexity based on rugosity measurements similar in coral reef studies (Gratwicke and Speight 2005). In each study stream, from the measurements of the 15 transects, I calculated the length of the streambed for each transect by use of Pythagorean theorem (Figure A.2). The final variable to indicate streambed complexity was the ratio of stream wetted width to streambed transect length. For sediment, the proportion by weight for each size category compared to overall sample weight was calculated. I used the multivariate ordination technique Detrended Component Analysis (DCA, Program R, vers. 4.2; R Core Team 2022 vegan package; Oksanen et al. 2019) to reduce and identify important sediment sizes among all study streams to capture large regional differences in sediment composition. In ArcGIS (ESRI 2019), I also calculated watershed area (km^2), drainage density (km/km^2) and slope.

Table 2.2. Variables considered in the random forests analysis of streams containing *M. hembeli* and streams that did not contain *M. hembeli* but could be potentials sites for translocation. Sediment variables are based on the proportion of that sediment size over all sediment sizes (percentage); greater than or equal to (≥) and less than or equal to (≤) indicate the cumulative proportion of all sediments sizes that are above or below, respectively, the sediment size. All Delta (Δ) values are the difference between stream water and water collected at 25 cm depth within the hyporheic zone.

Variable Type	Variable	Measured in
Fish Assemblage	*Fundulus spp.*	Field
	Notorus spp.	Field
	Ichthyomyzon gagei	Field
	Lepomis macrochirus	Field
	Lepomis megalotis	Field
	Lepomis marginatus	Field
	Lythrurus spp.	Field
	Species Richness	Field
Geomorphology and Watershed	Sediment ≥ % 8mm	Laboratory
	Sediment % 1mm + % 0.5 mm	Laboratory
	Sediment ≤ % 0.25 mm	Laboratory
	Wetted Width (m)	Field
	Streambed Complexity	Field
	Riffle Count	Field
	Stream Slope	GIS
	Watershed Area (km^2)	GIS
	Drainage Density (km^{-1})	GIS
Groundwater Connectivity	Average UAA: Stream length	GIS
	CoV Δ pH	Field
	CoV Δ Dissolved Oxygen (mg/L)	Field
	CoV Δ Specific Conductance (mS/cm)	Field
	CoV Δ Temperature (°C)	Field

Shallow groundwater connectivity was summarized by the coefficient of variation (CoV) of stream-hyporheic temperatures of all 40 transects per stream. From the subset of transects, CoV of stream-hyporheic pH, specific conductance, and dissolved oxygen for the six piezometer samples for each stream were also used as variables to assess differences in the hyporheic zone and stream indicating possible shallow groundwater connectivity. I calculated Upslope Accumulation Area (UAA) with ArcGIS (ESRI 2019) and SagaGIS (Conrad 2015) following methods outlined in Grabs et al. (2010). UAA indicates the potential of the surrounding area to contribute subsurface flow to a streams (Grabs et al. 2010). After calculating total stream length (ft), I converted UAA from square meters to square feet for each side of the stream (left and right banks). Then, I was able to standardize UAA values to compare across stream by taking the ratio of the UAA to stream length and averaging for both sides. The final variable was the ratio of average UAA to stream length. All predictor variables that were explored in the following Random Forest analyses are listed in Table 2.1.

2.2.3. Statistical Analyses

Random forests (Program R, vers. 4.2; R Core Team 2022 Caret package; Kuhn 2008) is a machine-learning technique that produces an ‘average classification tree’ to categorize new datasets (i.e., project). The output tree produced is the average of each important variable across all trees. Random forests uses a portion of the dataset to train the model and then the remaining data becomes the test dataset to understand model performance. Models were evaluated by their accuracy, which is the proportion of correct classifications in the training dataset, as well as Cohen’s Kappa, which also indicates the proportions of correct classifications but also compares to an expected or random chance accuracy. Accuracy and Kappa values of 1 indicate correct classification by the Random Forest model. Individual variables were evaluated by their Values from the model output, with higher Values indicating the rank of importance of variable in categorizing streams correctly. I developed the Random Forests classification tree from the 10 occupied/unoccupied streams to classify the out-of-range streams as suitable for *M. hembeli*.

2.3. Results

The 15 study streams yielded 45 fish species in 20 genera and 10 families (Table A.1), which included the seven taxonomic groups used in random forest analysis (Table 2.2). Species richness estimated by rarefaction curve analysis was highest for the tributary of Cress Creek (an occupied mussel stream) with 30 estimated species, and East Fork Six Mile (out-of-range stream) was the lowest with 13 (Table 2.3). The five out-of-range streams had overall lower species richness compared to the in-region study streams. The most northern district in the Kisatchie National Forest, the Catahoula district, had higher species richness than the Calcasieu district (Evangeline unit), regardless of occupied/unoccupied assignment.

Based on DCA results (Table 2.4, Figure A.3), from DCA 1, larger sediment sizes (8mm and 16mm) were best at explaining variability among streams. The natural break between (1 mm - 0.5 mm) and (≤ 0.25 mm) sediment size percentages in detrended DCA 2 was also important because their negative association. Therefore, reduced sediment variables used in the random forest analysis were percent ≥ 8mm, percent ≤ 0.25mm and the sum of percent 1mm and 0.5mm (DC1 eigenvalue = 0.41, Axis length = 2.11, DC2 = 0.28, = 1.75).

Sampling all 15 streams for groundwater measurements occurred, but within most streams, especially mussel-occupied streams, temperature at depths of 50 and 75 cm could not be recorded because an impenetrable layer, potentially either dense gravel or clay pans restricted measurement with the temperature probe. Overall, patterns in abiotic variables between occupied, unoccupied and out-of-range seemed to not be obvious, although large difference between all streams occurred. Sediment sizes, geomorphology, and GIS data are summarized in Table A.2, and shallow groundwater data are in Table A.3. The hydrographs for the subset of streams with data loggers data loggers are presented in Figure A.4, and also appear to show large variability among all study stream types.

Table 2.3. Rarefied species richness for each study stream located within the Kisatchie National Forest is presented below.

District, Unit	Mussel Status	Stream	n	Species Richness
Calcasieu, Vernon	Out-of-Range	East Fork of Six-Mile	24	12.72
Calcasieu, Vernon	Out-of-Range	West Fork of Six-Mile	28	13.81
Calcasieu, Vernon	Out-of-Range	Drake's Creek	38	16.07
Calcasieu, Vernon	Out-of-Range	Big Brushy Creek	60	19.57
Calcasieu, Vernon	Out-of-Range	Bird's Creek	68	20.54
Calcasieu, Evangeline	Unoccupied	Hospital Bayou	91	22.8
Calcasieu, Evangeline	Occupied	Valentine's Creek	91	22.8
Calcasieu, Evangeline	Occupied	Bayou Clear	107	24.05
Calcasieu, Evangeline	Unoccupied	Stracener Creek	107	24.05
Catahoula	Unoccupied	Cress Creek	124	25.18
Catahoula	Occupied	Beaver Creek	130	25.55
Catahoula	Unoccupied	Black Creek	147	26.49
Catahoula	Unoccupied	Grey's Creek	195	28.66
Catahoula	Occupied	Trib. of Grey's Creek	198	28.78
Catahoula	Occupied	Trib. of Cress Creek	230	29.93

Table 2.4. The scores of the Detrended Correspondence Analysis with 10 different sediments size percentages corresponding to the 15 study streams.

Sediment Size	DCA 1	DCA 2
16 mm	2.1186	0.2525
8 mm	2.0519	0.3002
4 mm	1.7441	0.5044
2 mm	1.4147	0.7975
1 mm	1.152	1.2964
500 µm	-0.6236	1.0086
250µm	-0.1874	-0.6678
125 µm	0.0192	-1.3255
63 µm	0.9489	-1.5055
Pan (leftover)	0.8857	-1.3674

Table 2.5. Variables considered in the random forests analysis of *M. hembeli*-occupied and unoccupied study streams. Higher values indicates the rank of importance of variable in categorizing streams correctly. Accuracy and Kappa indicate model performance, and all variables were 1 for both Accuracy and Kappa.

Most Important Variables	Value
Ichthyomyzon gagei	100
CoV Δ Temperature (°C)	95.12
Lythrurus spp.	76.59
Drainage density (km^{-1})	75.04
Noturus spp.	74.46
L. marginatus/L. megalotis	73.56
Species Richness	68.76
Sediment ≥ % 8mm	43.88
Sediment ≤ % 0.25 mm	29.57
Wetted Width (m)	22.77
Riffle Count	6.20
Streambed Complexity	0

The best random forest model contained 12 variables (Table 2.5), all with an Accuracy and Kappa of 1, suggesting that the analysis was overfit, i.e., the model developed was too complex compared the number observations used in the training data set or the number of parameters was higher than the number of observations. Fish taxonomic and richness variables appear to be more important based on their values than measured abiotic variables in describing mussel presence, aside from CoV Δ Temperature (°C) and drainage density (m^{-1}). The CoV Δ Temperature (°C) is more variable in the unoccupied streams (0.49 ±0.04), while it is about equal in the occupied (0.38 ±0.04) and out-of-region (0.36 ±0.04) streams. Drainage density on average was similar between the occupied and unoccupied streams, while it was much lower in the out-of-range streams (Table 2.6). The most important variable to accurately classify occupied and unoccupied mussel streams was abundance of Southern Brook Lamprey *I. gagei* abundance. The out-of-range streams had a higher average *I. gagei* abundance than unoccupied streams

(Table 2.7). The out-of-range streams had lower average abundances of *L. marginatus*, *L. megalotis* and *Lythrurus* species. The average *Noturus* abundance in the out-of-region streams was 1.98 (±1.33), while the occupied and unoccupied streams' average *Noturus* abundances were much higher (17.24 ±3.25; 16.09 ±4.91, respectively). The random forest model applied to out-of-range steams indicated Big Brushy, Bird's Creek and Drake's Creek were suitable for mussel translocation, whereas East and West forks of Six Mile Creek were not suitable.

Table 2.6. Estimated average (± standard error) abundances of the remaining important abiotic variables measured in study streams that are occupied or unoccupied by *Margaritifera hembeli* and study streams found out of the range of *M. hembeli*.

Variable	Total	Occupied	Unoccupied	Out-of-Range
CoV Δ Temperature (°C)	0.41 (±0.03)	0.38 (±0.04)	0.49 (±0.04)	0.36 (±0.04)
Drainage density (km^{-1})	3.45 (±0.23)	3.94 (±0.2)	3.71 (±0.44)	2.7 (±0.32)
Wetted Width (m)	3.92 (±0.29)	3.5 (±0.38)	3.36 (±0.29)	5.15 (±0.34)
Riffle Count	0.46 (±0.13)	0.6 (±0.36)	0.55 (±0.28)	0.36 (±0.14)
Streambed Complexity	0.97 (±0)	0.97 (±0)	0.97 (±0.01)	0.96 (±0)
Sediment ≥ % 8mm	6.51 (±1.69)	2.84 (±1.51)	8.75 (±3.12)	3.65 (±2.12)
Sediment ≤ % 0.25 mm	52.83 (±3.77)	55.8 (±6.51)	50.56 (±6.08)	56.85 (±8.3)

Table 2.7. Estimated average (± standard error) abundances of the seven important taxa found in study streams that are occupied or unoccupied by *Margaritifera hembeli* and study streams found out of the range of *M. hembeli*.

Variable	Total	Occupied	Unoccupied	Out-of-Range
Ichthyomyzon gagei	4.76 (±2.06)	8.87 (±6.05)	2.06 (±0.86)	3.35 (±0.93)
Lepomis marginatus	8.09 (±2.24)	12.41 (±5.12)	9.41 (±3.48)	2.44 (±1.18)
Lepomis megalotis	6.02 (±1.93)	6.63 (±4.2)	5.88 (±3.79)	5.56 (±2.64)
Lythrurus spp.	4.92 (±1.19)	6.86 (±2.73)	4.79 (±2.22)	3.13 (±0.85)
Noturus spp.	11.77 (±2.63)	17.24 (±3.25)	16.09 (±4.91)	1.98 (±1.33)

2.4. Discussion

This study defined important yet subtle differences between headwater streams within and adjacent to the small geographical distribution of *M. hembeli*. This study focused on aspects of fish communities, fluvial geomorphology (i.e., riffle habitat and sediment diversity) and groundwater connectivity to support conservation and restoration efforts, including on-going translocation efforts. Within the mussel's range, the occupied streams differed from unoccupied streams in tighter stream-hyporheic zone temperature relationship, coarser substrate composition, in greater estimated abundance of a potential indicator fish species, *Ichthyomyzon gagei*, and qualitatively shallower hyporheic zones on top of clay hardpan. The out-of-range streams that were suitable for mussel translocation possessed: higher fish species richness, and abundance of specific fishes and abiotic factors of importance included sediment sizes, and geomorphological features (drainage density, streambed complexity, riffle count), which indicate possible measures of stream bed stability and complexity. Therefore, conservation and restoration efforts should focus on prioritizing streams with characteristics favorable to mussel bed establishment.

2.4.1. Fish Communities

From the random forest model, species richness and four taxonomic groups (*I. gageli, Noturus spp., Lythrurus spp.* and *L marginatus/L megalotis*) were important in classifying the streams. Species richness was overall higher for the in-region streams, indicating that higher diversity is important for *M. hembeli*. However, Johnson and Brown (1998) observed differences in *M. hembeli* recruitment in streams with similar species richness but varying abundances of common species, which may indicate that species richness is not as important as abundance of specific taxa. Lack of higher abundances of *Noturus Spp., L. marginatus*, *L. megalotis* and *Lythrurus*, in addition to, lower species richness in the out-or-range streams compared the occupied/unoccupied mussel streams indicates that the Vernon Unit of the Kisatchie National Forest may not be suitable for *M. hembeli*. However, the out-of-range streams had a higher average *I. gagei* abundance compared to unoccupied streams, and since *I. gagei* is the most important variable from the random forest model, ruling out the out-of-range streams in the Vernon Unit may not be advisable. Furthermore, in the Pacific Northwest of the U.S., studies show that the Western Pearlshell Mussel (*Margaritifera falcata*) and Pacific Lamprey (*Lampetra tridentata*) larva occupy the same functional feeding guild (i.e., filterers), but exhibit commensalism. The lamprey filter smaller particle sizes compared to mussels (partitioning of resources) and both benefit from the added pseudofeces increasing bacteria growth (Limm and Power 2011). Alternatively, *I. gagei* may share habitat association(s) with *M. hembeli* (i.e., both responding positively to one or more habitat components), potentially shallow groundwater connectivity, which is an important habitat component for many lamprey species (Morman et al., 1980; White 1990; Brunke and Gonser 1997). Whether commensalism or shared habitat association, similar important mechanisms may be at work here because lamprey abundances varied greatly in these streams, with three out of the five mussel-occupied streams having lamprey present.

The relationship between *M. hembeli* and the remaining three taxonomic groups from the random forest model is not immediately obvious. Notorids and Lythrurids are not considered to be hosts of *M. hembeli* because this mussel's life cycle has not been demonstrated to be carried to completion, i.e., encysting glochidia to full maturation as it has in Redfin Pickerel in the laboratory and hatchery conditions. One possible explanation is the host fish need to withstand a certain glochidial parasitic load. Redfin Pickerel have maximum body-sizes much larger than Notorids and Lythrurids, so Redfin Pickerel are potentially the better suited host. Additionally, glochidia have been detected on the gills of the Brown Madtom (*Noturus phaeus*), and Redfin Shiner (*Lythrurus umbratilis*) which are also small-bodied (Hill 1986; Johnson and Brown

1998). Glochidial presence indicates these fishes were at least adjacent to spawning mussels. A deep and distinct relationship between Notorids and Lythrurids with *M. hembeli* may exist, by serving as prey for their Redfin Pickerel host or as a physiological indicator to spawning. I eliminated Striped Shiner and Creek Chub because they were only found in streams within *M. hembeli's* range (i.e., occupied/unoccupied mussel streams). These two species may be highly associated with each other, as Striped Shiners have been observed to use the nests built by Creek Chub during spawning. Records of Striped Shiner (*L. chrysocephalus*) having glochidia on their gills also exists (Hill 1986). These two fishes may also have a deeper underlying ecological relationship to *M. hembeli* that leads to a more stable community. The evidence that these two fish species are not present in the out-of-range streams, may indicate these new streams to be unsuitable for *M. hembeli* introduction as well. Likewise, *L. marginatus/L. megalotis* were the last important taxa group identified in the random forest model. No records exist of glochidia using these species as a host, but the higher abundances of both species within mussel-occupied streams may indicate a healthy ecosystem that is adequate for a sensitive, sessile species like *M. hembeli*.

2.4.2. Sediment, Mussel Bed Stability and Groundwater Connectivity

A diversity of sediment sizes is important to mussel bed development and persistence (Geist and Auerswald 2007). It was expected that within this study mid-sized substrates (0.5 mm-4 mm) ranging from fine to coarse sand and small gravel were not important in classifying streams, which was shown by my DCA and random forest model. Most streams in central Louisiana consist of sandy substrates, but very fine and larger substrates occur among streams. Bolden and Brown (2002) found when translocating *M. hembeli* within the same Louisiana stream, even when placed in their preferred habitat (i.e., riffles with adequate gravel), some mussels were washed out by spates which indicates overall stream bed stability may be more important. Beds with high densities can change the flow dynamics within stream beds which helps to stabilize *M. hembeli* beds and prevent washouts from spates (Johnson and Brown 2000). Large substrates like boulders act to stabilize *M. margaritifera* (Hastie et al. 2000), but substrates of this size are lacking from the streams in my study. In many of the mussel-occupied streams sampled, temperature probe penetration past 25 cm was limited by an impenetrable layer, suggesting that the reduced hyporheic depth was somehow beneficial to mussels, potentially by increasing exchange. Penetration meters have been used in other *M. hembeli* studies to better understand the compaction of sediment within the stream bed with respect to juvenile recruitment (Geist and Auerswald 2007). Future studies on *M. hembeli* should include a combined method to detect clay pans and compaction within streambeds.

The hyporheic zone provides important nutrient cycling and refugia to stream inhabitants including *M. hembeli*. Fine sediment (i.e. ≤ 0.25 mm) can fill the interstitial spaces within the hyporheic zone and limit shallow groundwater connectivity. Geist and Auerswald (2007) noted streams that mix well with their hyporheic zones are better for juvenile *M. margaritifera* in Europe. However, it was difficult to detect groundwater connectivity and mixing in these streams. Two reasons might explain why the measured variables, aside from fine sediment and differences in hyporheic and surface stream temperature, were not useful variables to characterize differences between occupied and unoccupied streams, and thus were not included in my random forest model. First, small topographic relief within and among central Louisiana watersheds, and the overall small geographical distribution of *M. hembeli* limit the ability to detect appropriate habitat requirement cutoffs (i.e., the range of observations was narrow and with low variability). Consequently, the hillslope analysis was also not included in the final random forest model, but this method may be applicable in other Margaritiferid studies where extreme differences in topographical relief can occur (Johnson and Brown 2000). Furthermore, a consensus of the exact phylogeny of Margaritiferidae does not yet exist, partially because acidic water degrades Margaritiferid shells, making them difficult to trace though the fossil record (Gómez and Araujo 2008). Therefore, Margaritiferid species might be distantly related despite being in the same genus/family, and application of one species' microhabitat factors may not apply to another species.

Secondly, targeting adult presence to indicate stream level processes, such as groundwater connectivity, may not accurately represent population recruitment. Juvenile and adult *M. hembeli* may have different habitat requirements, with juveniles requiring more strict water quality parameters (Gómez and Araujo 2008). Adults are large and conspicuous, with more than half their shell exposed above the sediment, whereas juveniles reside within the sediment for up to 5 years (Geist and Auerswald 2007). Adult mussel presence, with individuals reaching 93 years old, could be a relic of past stream conditions (Hastie et al. 2000; Geist and Auerswald 2007). Furthermore, large spates move exposed adults to places where juveniles are not present; therefore, some adult presence is a response to stochastic events, versus stricter habitat requirements, further convoluting identification of juvenile versus adult habitat needs (Hastie et al. 2000). Perhaps a better approach for future studies evaluating if groundwater connectivity at the stream level as an important predictor for mussel recruitment is to focus on streams with juvenile recruitment.

2.4.3. Translocation factors

Margaritiferids in general have combated stochastic events with life history tactics. After reaching maturity around age 20, these mussels have consistently high fecundity across all ages classes until death (Bauer 1987). Low heterozygosity in Margaritiferids was once thought to be a relic of past population bottlenecking, but evidence for a metapopulation structure exists (Curole et al. 2004; Garrison et al. 2021). In this case, mussel beds are considered a population and the stream or watershed level is the metapopulation. Johnson and Brown (1998) saw the most apparent differences in growth, densities, body sizes, ages, and recruitment rates of *M. hembeli* at the stream level, meaning abiotic factors may be more at play than density dependent factors (Curole et al. 2004). Hermaphrodism, which can also lead to low genetic variability, is another tactic some Margaritiferids use which allows females to self-fertilize in beds with low male numbers (Bauer 1987).

The Louisiana pearlshell mussel (*M. hembeli*) has specific threats, including a smaller geographic distribution and a non-anadromous *Esox* host, compared to other Margaritiferids, which makes captive breeding and reintroduction a viable conservation strategy (e.g., Geist et al. 2021). Garrison et al. (2021) also saw low heterozygosity in *M. hembeli* but used a SNPs analysis to observe a deeper genetic structure. These mussels sampled from streams south of the Red River were more genetically similar to each other than mussels north of the river. A deeper genetic structure also exists at a smaller scale (sub-watersheds), indicating care must be taken when rearing mussels from broodstock for reintroduction to maintain genetic structure (Garrison et al. 2021). Low genetic diversity can be detrimental to species that are habitat specific, especially in the face of climate change, which is expected to drastically change the hydrologic cycle (Johnson and Brown 1998). Scouring spates that cause 5-10% mortality might become more frequent reducing the population enough over time to where birth rate is less than death rate which is especially important to consider for populations with older populations that take a long time to mature. Increased droughts in which mussels are unable to move to an adjacent refugia or are also expected to occur, with increased siltation being especially adverse for juveniles (Curole et al. 2004; Geist and Auerswald 2007). Captive breeding is a temporary solution to maintaining viable populations until a better understanding of habitat requirements for restoration and relocation of adults is achieved (Bolden and Brown, 2002; USFWS 2019). It may be more important to identify crucial biotic and abiotic factors that limit *M. hembeli* and apply those to habitat restoration because successful captive breeding is still being refined (Garrison et al. 2021; Geist et al. 2021). These results should aid natural resource managers in charge of this threatened mussel to identify streams for translocation but also further identify important drainage level characteristics of mussel streams to help in future habitat restoration efforts.

Chapter 3. Invertebrate Food Web Structure among Louisiana Coastal plains Streams with Varying Lateral Connectivity

3.1 Introduction

Basal energy sources for stream and river food webs are either of autochthonous, or allochthonous origin (i.e., produced instream from algae and macrophytes or from outside of stream, often in the form of deciduous tree leaves; Allan and Castillo 2007). However, the palatability and bioavailability of these two energy sources to macroinvertebrate consumers differs (Arsuffi and Suberkropp 1989; Parker et al. 2007; Labed-Veydert et al. 2021). Allochthonous deciduous leaves need to be colonized by bacteria, hyphomycete fungi and protozoans prior to being consumed by macroinvertebrates (Chauvet and Suberkropp 1998; Gulis and Suberkropp 2003), and very few taxa can digest woody debris that enters streams, whereas algae and periphyton are readily digested and preferred forage (Kühmayer et al. 2020). Macroinvertebrate have been placed in functional feeding guilds (FFG) based on their use of allochthonous/autochthonous sources, which is based on feeding habit, morphological features, and ingested particle size (Cummins1974; Wallace and Webster 1996; Allan and Castillo 2007; Cummins et al. 2019). Shredders consume colonized leaf material or coarse particulate organic matter (CPOM; Roeding and Smock 1989; Wallace and Webster 1996; Allan and Castillo 2007; Balibrea et al. 2020), whereas collectors ingest smaller-sized particles through gathering or filtering mechanisms (Wallace and Webster 1996; Cummins et al. 2019). Shredders and collectors transfer allochthonous energy to higher trophic levels, and scrapers feeding on algae provide an avenue for autochthonous materials to move into the food web, although periphytic biofilms often contain both autochthonous and allochthonous materials (Wallace and Webster 1996; Cummins et al. 2019).

In temperate streams, seasonal leaf senescence falling directly into streams introduces a food pulse to the food web that is broken down and digested by macroinvertebrates throughout the winter. As air and water temperatures increase in warm seasons, algae and periphyton become more available, especially during low stream flow (Wallace and Webster 1996; Cummins et al. 2019). However, in subtropical climates such as the Northern Gulf of Mexico (hereafter Gulf) Coastal Plains, temperate seasonal patterns of allochthonous and autochthonous availability may not occur. For example, streams in Gulf Coastal Plains watersheds have lower stream slopes, flashy hydrographs (Felley 1992, Isphording and Fitzpatrick 1992, Hupp 2000), and mild cool season temperatures, potentially leading to greater riparian access during flooding and increased availability of riparian materials throughout the year. When seasonal leaf senescence occurs, some material falls directly into or is immediately washed into streams where it can be processed. Material remains on the floodplain/riparian zone or buried in sandy stream substrates to be accessed later in the year via supra- and sub-bankfull floodings. Supra-bankfull flooding exceeds bank height and spreads onto the floodplain, increasing the overall surface area of the stream bed and access to allochthonous materials. Mild terrestrial temperatures in the riparian zone allow terrestrial microbes to colonize leaf matter that will eventually enter the stream. These allochthonous leaves are in a different decompositional state than senescenced leaf matter that moved quickly from trees to the stream (Kcohi and Kagaya 2005). Mild temperatures also allow labile herbaceous plants and grass to grow adjacent to streams year-round, which can increase the amount of terrestrial materials delivered to streams during floods. Conversely, fine/sandy substrates limit algal production at certain times of year in which spates or sub-bankfull floodings occur, scouring stream beds. (Villeneuve et al 2010; Schneck et al. 2011;

Luce et al. 2013).

Studies on other subtropical (in China, Wen et al. 2010) and tropical (in Brazil, Neres-Lima et al. 2017) streams have shown strong allochthonous detrital pathways whereas others have shown autochthonous benthic algae as the main carbon source in subtropical streams (in Taiwan, Huang et al. 2007; in New Zealand, Hadwen et al. 2010). Collector, shredder, and scraper organisms are key organisms supporting stream nutrient cycling, as well as converting plant biomass and energy into animal biomass to support higher level consumers (Covich et al. 1999). The microbial route is potentially a more important pathway in determining energy fluxes compared with macroinvertebrates (Demars et al. 2021) that tend to have strong omnivory in subtropical climates (Huang et al. 2007). However, deciphering autochthonous and allochthonous pathways within microbes may be difficult because as microbes breakdown terrestrial materials and respire CO_2, in-stream autotrophs have the ability to fix this allochthonously-derived materials to subsidize any nutrient inadequacies within streams, convoluting $\delta^{15}N$ and $\delta^{13}C$ signature interpretations of higher trophic levels (Demars et al. 2021).

The role macroinvertebrates play in Gulf Coastal Plains stream food-webs needs clarification in relation to allochthonous/autochthonous food source usage. Moreover, the Mississippi River is a natural biogeographical barrier to dispersal (Connor and Suttkus 1986; Swift et al. 1986) and as a result, east-west differences in macroinvertebrate and fish assemblage composition have been observed (Connor and Suttkus 1986; Feeley 1992; Kaller et al. 2013). Utilization of macroinvertebrate FFG assignments could reflect functionally similar assemblage structures across subtropical Gulf Coastal Plains streams and rivers despite known taxonomic differences. Importantly, macroinvertebrate FFG data would allow predictions of future changes and management options for aquatic food webs, given growing human populations in coastal zones, increasing urban and agricultural water use, rising global temperatures, and pervasive changes in precipitation, stream hydrology, and water availability (Mankin et al. 2019; Martinich and Crimmins 2019; Powell et al. 2019).

The objective of this study was to clarify macroinvertebrate food-web structure and allochthonous/autochthonous usage in Gulf Coastal Plains streams with varying floodplain connectivity. Floodplain connectivity was evaluated by flooding and geomorphological data, while I employed stable isotope analysis (SIA, $\delta^{13}C$ and $\delta^{15}N$) of macroinvertebrate and plant tissues to trace energy (carbon) pathways and assess trophic niche space of food webs (Fry 2006). Many studies have used $\delta^{13}C$ and $\delta^{15}N$ to examine sources of primary productivity (e.g., Rosenfeld and Roff, 1992; Rounick et al. 1982; Zah et al. 2001; da Silva Reis et al. 2020 but see Goebel et al. 2010) and to assess sources of variability in trophic structure (e.g., Daniel et al. 2015; Smucker et al. 2018). Therefore, SIA offers a powerful and rich methodology to examine food webs across floodplain connectivity and geomorphology. For this study, streams were located east and west of the Mississippi River and selected from regions with known macroinvertebrate taxonomic differences (Figure 1; Kaller et al. 2013) but the study streams were also believed to have varying floodplain connectivity based on visual observation and differences in landscape level characteristics (i.e., geomorphology, geology, landcover, etc.) and would be representative of stream types in the coastal plain (Felley 1992, Isphording and Fitzpatrick 1992, Hupp 2000).

Variation in floodplain connectivity among study streams may contribute to different autochthonous/allochthonous availability to the macroinvertebrate food web. Thus, my first hypothesis is that western stream food webs are driven more by allochthonous productivity, because those streams have more connections to their floodplains than eastern streams, which are typically more incised. Secondly, I hypothesize scrapers and FFGs related to autochthonous production will be more pronounced in eastern food-webs because sub-bankfull floodings that ‘rejuvenate’ streams i.e., flushing out stagnant water and dead, amorphous material with little nutritional value, resetting the base of the food web, occur more frequently than western streams. (Blenkinsopp and Lock 1994; Power et al. 2013), but on fewer occurrences, supra-bankfull floodings introduce additional allochthonous materials. Thirdly, I hypothesize that seasonal differences in individuals abundances of FFGs between regions to occur as a product of varying levels of lateral connectivity (floodplain/riparia) caused by varying flooding regimes, as evidence by stream loggers. Finally, I hypothesize that I will observe different seasonal use of overstory plants (pine needles and deciduous tree leaves) compared to understory plants (herbaceous plants, and grasses; Thorp and Bowes 2017) by macroinvertebrates based on macroinvertebrate $\delta^{13}C$ signatures and FFGs between regions and streams with varying flooding regimes.

3.2. Methods

3.2.1 Study sites and field collection

Four study streams (each) east and west of the Mississippi River to were selected from Gulf Coastal Plains watersheds within similar ecoregions (USEPA level 4) within Louisiana (Figure 3.1). Study streams were 3rd and 4th order pairs within the same watershed (USGS HUC 8) and had variable lateral (floodplain) connectivity based on visual observations of active floodplain indicators (e.g., presence of isolated pools of water adjacent to stream, low bank angles, etc.). Each stream was sampled quarterly from September 2017 to November 2019, except fall 2018.

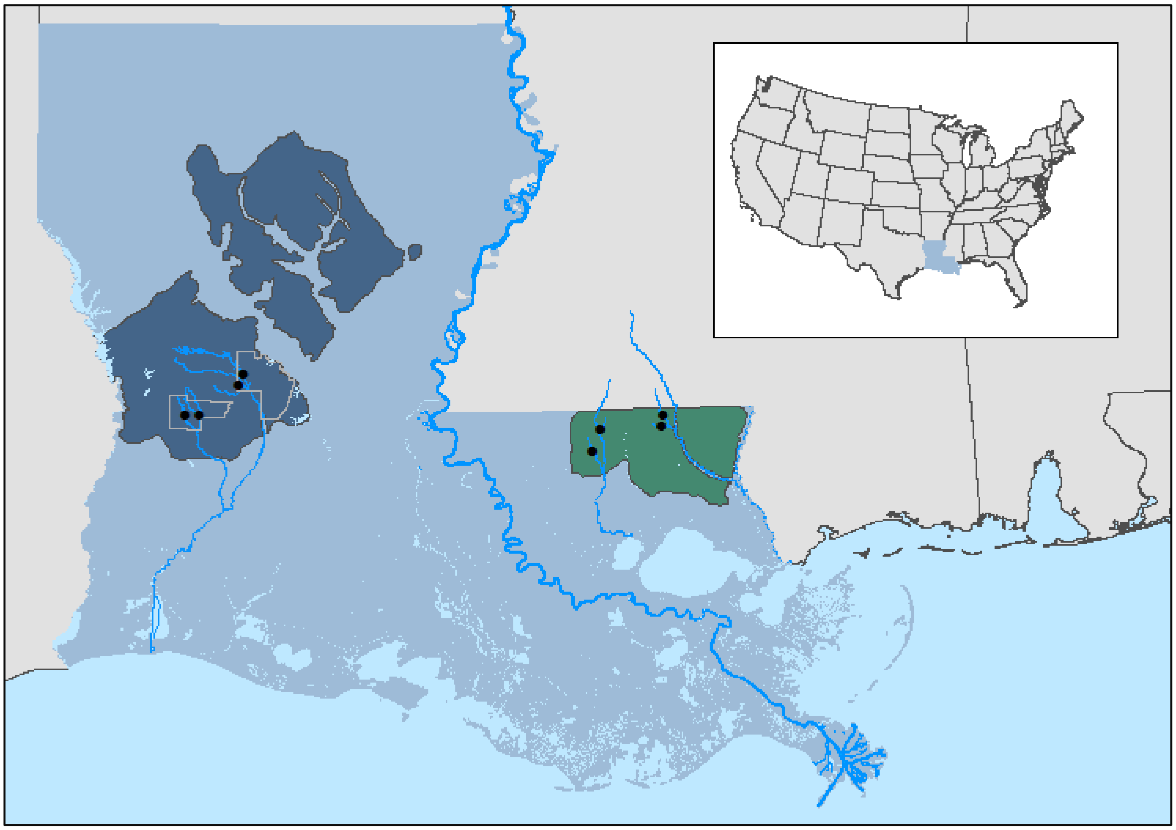


Figure 3.1. Locations of study sites (black circles) within their USEPA ecoregions (Level 4) Gulf Coastal Plains. From east to west, the study streams were contained within the Calcasieu River, Tickfaw River and Bogue Chitto River watersheds. From east to west study streams were Drake's Creek, Bird's Creek, Big Creek, Cypress Bayou, Twelvemile Creek, Crittenden Creek, Little Silver Creek and Silver Creek. The western ecoregion, depicted in dark gray blue, is the Southern Tertiary Uplands and the eastern ecoregion is the Southern Pine Plains and Hills depicted in green. Open polygons outlined in gray indicate the Kisatchie National Forest boundaries (Map was created in ArcGIS 10.7.1 (ESRI 2019).

During each sampling event water, plants and macroinvertebrates were collected at bank-to-bank transects at 25 m, 50 m, and 75 m within a 100-m reach. Two pairs of water samples were collected in 500 mL glass jars at 25 m, and 75 m transects for total dissolved organic carbon (DOC) and dissolved nitrogen (DN) analysis. Prior to water collection, jars were placed in a combustion oven at 400°C for 1 hour (5310B Standard Methods Protocol for TOC/TIC to obtain TDOC; APHA/AWWA/WEF 2018) to remove any carbon that could have contaminated the sample.

Macroinvertebrates and primary producers were collected for SIA of $^{13}C/^{15}N$ during each sampling period. Macroinvertebrates were sampled by collection of small woody debris in a 0.25mm mesh grab bag (see Kaller and Kelso 2007), and with a modified Hess sampler (see Kaller and Kelso 2006). All material in the Hess sample was filtered into a 500 mL collection cup. To compare to wood samples, Archimedes' principle of displaced volume of each piece of wood per sample was used, and the number of individuals was standardized to be out of 500 mL. Each sample type was used once at each transect (2 sample types (wood and Hess) x 3 transects x 8 streams x 8 seasons = 384 total samples). Leftover organic material from the Hess sample was rinsed and used as the detritus or particulate organic matter (POM) component for SIA. Backpack electrofishing (methods modified from Budnick et al. 2018) was added to each sampling event starting winter 2019 to acquire additional individuals to have enough tissue for SIA analysis. All organismal samples were placed on ice and then transferred to a freezer until processing. Allochthonous food sources collected from the riparian corridor (< 5 m from stream) included tree leaves (pine and deciduous) as well as herbaceous plants and grasses.
Algal mats and filamentous algae was rarely observed, and collection was difficult on sandy substrates; therefore, starting in sample year two, three sets of four unglazed ceramic tiles arranged vertically in PVC-constructed racks were placed in streams at each transect and retrieved two weeks later to obtain an autochthonous representative.

Stream catchment or watershed area, drainage density, sinuosity and percent landcover, were calculated in ArcGIS for each stream (ESRI 2019). Geomorphological features that may indicate floodplain connectivity, such as average stream slope, and entrenchment ratio were measured with a laser level and stadia rod (Topcon® AT-B Series) during summer 2019, (transects every 10 m for a 1500 m reach). Stream pressure and temperature loggers (Onset HOBO U20L) housed in PVC pipes were placed in Cypress Bayou, Drake's Creek, Crittenden Creek, and Silver Creek to monitor stream level or stage every hour from summer 2019 through 2020.

Table 3.1. The taxonomic composition of the Functional Feeding Guilds (FFGs) used in Stable Isotope Analysis across study streams.

Class	Order	Family	Functional Feeding Guild
Insecta	Coleoptera	Elmidae	collector/gatherer
	Diptera	Chironomidae	multiple guilds
	Diptera/Tipulidea	Tipulidae	shredder/detritivore
	Ephemeroptera	Heptageniidae	scrapers
		Isonychiidae	collector-filterers/
	Odonata	Aeshnidae	predator/engulfer
		Macromiidae	predator/engulfer
		Gomphidae	predator/engulfer
		Coenagrionidae	predator/engulfer
		Cordulegasteridae	predator/engulfer
		Corduliidae	predator/engulfer
	Plecoptera	Perlidae	predator/engulfer
	Trichoptera	Hydropsychidae	collector/filterer
Malacostraca	Decapoda	Cambaridae	omnivore
Gastropoda	Architaenioglossa	Viviparidae	snail scraper

3.2.2. Laboratory processing and SIA

Particulate matter was removed from water samples by filtering 100 mL of sample water through a glass fiber filter (GFF) prior to being sent to Wetland Biogeochemistry Analytical Services at Louisiana State University for DOC/DN analysis (5310B Standard Methods Protocol for TOC/TIC to obtain TDOC; APHA/AWWA/WEF 2018). Periphytometer tiles were processed in a similar manner, with removal of invertebrates and debris followed by scraping with a wire blush and rinsing with DI water into a collection jar, where the contents were filtered through a GFF. Material on the GFF was then processed for SIA. Leaves, pine needles, grass and herbaceous plants were rinsed in DI water, and roots were removed. Macroinvertebrates were identified to the lowest practical taxonomic level, enumerated, and most were assigned to a FFG according to Merritt et al. (2019). Composite samples of taxa that represented a range of FFGs (Tables 3.1 and B.1) consisted of whole insects or tail muscle from crayfish (Hicks 1997).

All SIA samples were dried at 60°C until constant mass was achieved and were ground with mortar and pestle (macroinvertebrates) or a Wiley Mill (plants) into a powder (Winemiller et al. 2011; Daniel et al. 2015). Values of SIA are denoted as $\delta^{13}C$ and $\delta^{15}N$, where δ is the differences in ratios (R) than the expected 1. R is ratio of heavy isotope (*H*) of an element (*X*) (C and N) to light isotopes of either sample or global standard (Vienna Pee Dee Belemnite and Air for C and N, respectively; Fry 2006):

$$\delta^{H}X = \left[R_{sample}/R_{standard} - 1\right] * 1000$$

This value is then multiplied by 1000 because differences tend to be very small. For bulk $\delta^{13}C$ and $\delta^{15}N$ SIA, most of fall 2017 samples were processed by the Stable Isotope Facility [PDZ Europa 20-20 Isotope ratio mass spectrometer (IRMS; Sercon Ltd., Cheshire, UK) with a PDZ Europa ANCA-GSL elemental analyzer] at the University of California at Davis, and all other samples were processed by Stable Isotope Ecology Laboratory [Delta Plus XP IRMS and Delta V Advantage IRMS with elemental analyzers (Costech ECS 4010 and Thermo Scientific Flash EA)] in the Department of Oceanography and Coastal Sciences, College of the Coast and Environment at Louisiana State University.

3.2.3. Categorization of annual hydrographs

Data from the *in-situ* Onset HOBO loggers were uploaded into the HOBOWARE program for data processing. Data were corrected for changes in atmospheric pressure, which can vary greatly among data points and distort stream stage levels. The local Hammond and Alexandria International Airports, LA, and the Fort Polk Army Airfield, LA barometric pressure records, which are publicly available through NOAA, were used for Crittenden Creek and Silver Creek, Cypress Bayou, and Drake's Creek, respectively. Stage boards were placed in the stream, the bank height on the board or bankfull stage was recorded, and stream stage was recorded at each sampling event. Those values were related back to the exact time and day of the HOBO data, the difference in the stream stage from the board and from the HOBO logger data were averaged across sampling times, and that correction value was applied to the entire HOBO dataset. I assigned streams to a flood category based on the occurrence of supra-bankfull and sub-bankfull floodings. This entailed finding the proportion of data points (stream level at every hour) that were above bankfull stage (supra-bankfull flooding) and between 75% of bankfull stage and bankfull stage (sub-bankfull floodings) to overall data points on a quarterly basis and for the entire year. Data points below 75% of bankfull stage were not considered floodings. Based on these data, I qualitatively assigned streams to flood categories of yearly supra-flooding, winter/spring flooding, yearly sub-bankfull flooding, and spring sub-bankfull flooding (Table 3.2).

Cypress Bayou tended to have the highest and longest floodings and was categorized as yearly supra-flooding for models. Drake's Creek had mainly sub-bankfull floodings especially in winter and spring with some supra-bankfull floodings and thus was categorized as winter/spring flooding. Crittenden Creek had the least amount of detectable flooding and was categorized as spring sub-bankfull flooding, whereas Silver Creek was categorized as yearly sub-bankfull blooding.

Table 3.2. The proportion of supra- and sub bankfull floodings based on hourly stream stage values for four study streams and flooding categories are presented below.

Flooding Category		Cypress Bayou	Drake’s Creeks	Silver Creek	Crittenden Creek
		yearly supra-flooding	Winter/Spring Flooding	yearly sub-bankfull Flooding	spring sub-bankfull Flooding
Bankfull Height (ft)		6.86	8.47	5.39	6.67
Sub-bankfull flooding height (ft)		5.15	6.36	4.04	5.00
Ratio of Bankfull Floodings	Annual	0.2868	0.0057	0.0095	0.0003
	Winter	0.3695	0.0171	0.0354	0.0000
	Spring	0.1760	0.0059	0.0023	0.0014
	Summer	0.1908	0.0000	0.0000	0.0000
	Fall	0.4015	0.0000	0.0000	0.0000
Ratio of Sub-Bankfull Floodings	Annual	0.1448	0.0290	0.0203	0.0024
	Winter	0.3068	0.0383	0.0486	0.0046
	Spring	0.1435	0.0488	0.0146	0.0050
	Summer	0.0366	0.0000	0.0150	0.0000
	Fall	0.0928	0.0282	0.0027	0.0000

3.2.4. Statistical Analyses

Estimated abundances of different macroinvertebrate taxonomic groups were calculated from Hess and wood samples based on a CPUE of numbers per 500 mL. I calculated Shannon-Weiner Diversity and richness after performing a rarefaction analysis (Program R, vers. 4.2; R Core Team 2022 vegan package; Oksanen et al. 2019). I then used a generalized linear mixed model [GLMM, Gamma distribution (link=log); Equation 1] and a linear mixed model (LMM; Equation 2) to investigate differences in diversity and richness among seasons and regions as fixed effects, with year as a random variable (Program R, vers.4.2.1; R Core Team 2022; lme4 package; Bates et al. 2015). To determine which model (probability distribution and link function combination) best fit the invertebrate δ13C values, I used AIC and quasi ĉ-hat for model selection.

Equation 1

$$y_i \sim Gamma(\mu_{ij}, \phi)$$

$$\mu_{ij} = \alpha_i + \beta_{1j}x_1 + \beta_{2j}x_2$$

$$\beta_j \sim Normal(\mu_\beta, \sigma_\beta^2)$$

In equation 1, y_i is either estimated richness or diversity, μ_{ij} is the expected value of richness or diversity given the random effect of year, β_{1j} is the parameter estimate for season given the random effect of year, β_{2j} is the parameter estimate for region given the random effect of year, and β_j is the random effect allowing for different estimates of season and region by year.

Equation 2

$$y_i = \alpha_i + \beta_{1j}x_1 + \beta_{2j}x_2$$

$$\beta_j \sim Normal(\mu_\beta, \sigma_\beta^2)$$

In equation 2, y_i is either estimated richness or diversity, β_{1j} is the parameter estimate for season given the random effect of year, β_{2j} is the parameter estimate for region given the random effect of year, and β_j is the random effect allowing for different estimates of season and region by year.

I also used multivariate generalized linear models [MVGLMs, Poisson distribution (link =log); Program R, vers. 4.2.1, R Core Team 2022; mvabund package; Wang et al. 2022; Equation 3] to discern differences among taxa and FFGs based on the predictor variables (e.g., region and season). Rare taxa, i.e., those having less than 10% for frequency of occurrence and 20% of total count, were removed, resulting in 37 taxonomic groups for the taxonomic

MVGLM. A MVGLM (Poisson distribution (link=log)) was also run for FFGs with the same previously mentioned predictor variables.

Equation 3

$$Y_{ij} \sim Poisson(\lambda_{ijk})$$

$$\lambda_{ijk} = \alpha_i + \beta_{1j}x_1 + \beta_{2j}x_2$$

In equation 3, Y_{ij} is either a matrix of taxa or FFG (*j*) at each site (*i*), λ_{ijk} is the estimated matrix of taxa or FFG(*j*) at each site (*i*) given fixed effects (*k*), β_{1j} is the parameter estimate for each taxa or FFG (*j*) for each season, and β_{2j} is the parameter estimate for each taxa or FFG ($_j$) for each region.

Isotopic analysis involved first removing extreme outliers (outer quartiles ±1.5* Interquartile Range within boxplots) and applying a trophic fractionation factor to whole insects (0.4‰) and crayfish tail muscle tissue (0.8‰; Atkinson et al. 2018). Because the main interest of study was not to understand individual taxa prey selection, but to understand energy flow and food web structure in relation to floodplain connectivity, I used GLMs [Gaussian distribution (link=identity); Equation 4] to investigate the relationship of invertebrate $\delta^{13}C$ and: 1) flood categories, FFGs, grass/herbaceous $\delta^{13}C$, and their interactions; and 2) flood categories, FFGs, pine/leaf $\delta^{13}C$, season, and their interactions. To determine which model (probability distribution and link function combination) best fit the invertebrate $\delta^{13}C$ values, I used AIC and quasi ĉ for model selection.

Equation 4

$$y_i \sim Normal(\mu_i, \sigma_i^2)$$

$$\mu_i = \alpha_i + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_{3,4} x_{3,4} + \beta_{4,5} x_{4,5} + \beta_{3,5} x_{3,5} + \beta_{3,4,5} x_{3,4,5}$$

In equation 4, y_i is the value of invertebrate δ^{13}C, μ_i is estimated value of invertebrate δ^{13}C given fixed effects, β_3 is the parameter estimate for flood category, β_4 is the parameter estimate for FFG, and β_5 is the parameter estimate for each δ^{13}C source.

3.3. Results

Study streams varied in watershed area, drainage density and entrenchment ratio, but not sinuosity, and stream slope, with no obvious patterns in eastern or western regions (Table 3.3). The percent forest landcover was higher in western study streams whereas agricultural percent landcover was higher in the eastern study streams (Table 3.4). Annual hydrographs were visually different among the four streams (Figure 3.2, Table 3.2).

Table 3.3. Geomorphological metadata for the selected study streams. Region indicates east and west of the Mississippi River. Watershed area and Drainage Density were calculated in ArcGIS, and the remaining variables were estimated on site.

Stream	Region	Watershed Area (km^2)	Drainage Density (km/km^2)	Sinuosity	Stream Slope (m/m)	Entrenchment ratio
Crittenden Creek	East	26.63	1.55	1.20	0.011	2.76
Twelvemile Creek	East	52.15	1.82	1.66	0.003	1.41
Bird's Creek	West	52.44	2.19	1.69	0.010	4.84
Drake's Creek	West	56.30	1.84	1.84	0.006	1.62
L. Silver Creek	East	75.42	2.64	1.29	0.006	2.15
Silver Creek	East	105.80	1.75	1.21	0.005	1.67
Big Creek	West	106.08	4.45	1.12	0.007	1.50
Cypress Bayou	West	436.43	3.17	1.33	0.002	4.97

Table 3.4. The Landscape indicators for the eight study streams. Region indicates streams east or west of the Mississippi River.

Stream	Region	Forest (%)	Agricultural (%)	DOC/DN (Ave (±SE))	Basal δ^{15}N (Ave (±SE))
Crittenden Creek	East	29.41	15.50	6.38 (±0.55)	1.08 (±0.61)
Twelvemile Creek	East	37.36	6.39	6.80 (±0.54)	-1.01 (±0.28)
Bird's Creek	West	52.87	0.15	12.55 (±0.99)	0.9 (±1.43)
Drake's Creek	West	63.76	1.90	14.06 (±0.99)	-0.14 (±0.63)
L. Silver Creek	East	21.06	31.15	3.39 (±0.27)	1.4 (±0.55)
Silver Creek	East	23.49	31.68	3.59 (±0.38)	2.16 (±0.45)
Big Creek	West	38.10	4.93	13.81 (±1.25)	-0.87 (±0.58)
Cypress Bayou	West	38.96	5.70	18 (±0.89)	2.01 (±0.39)

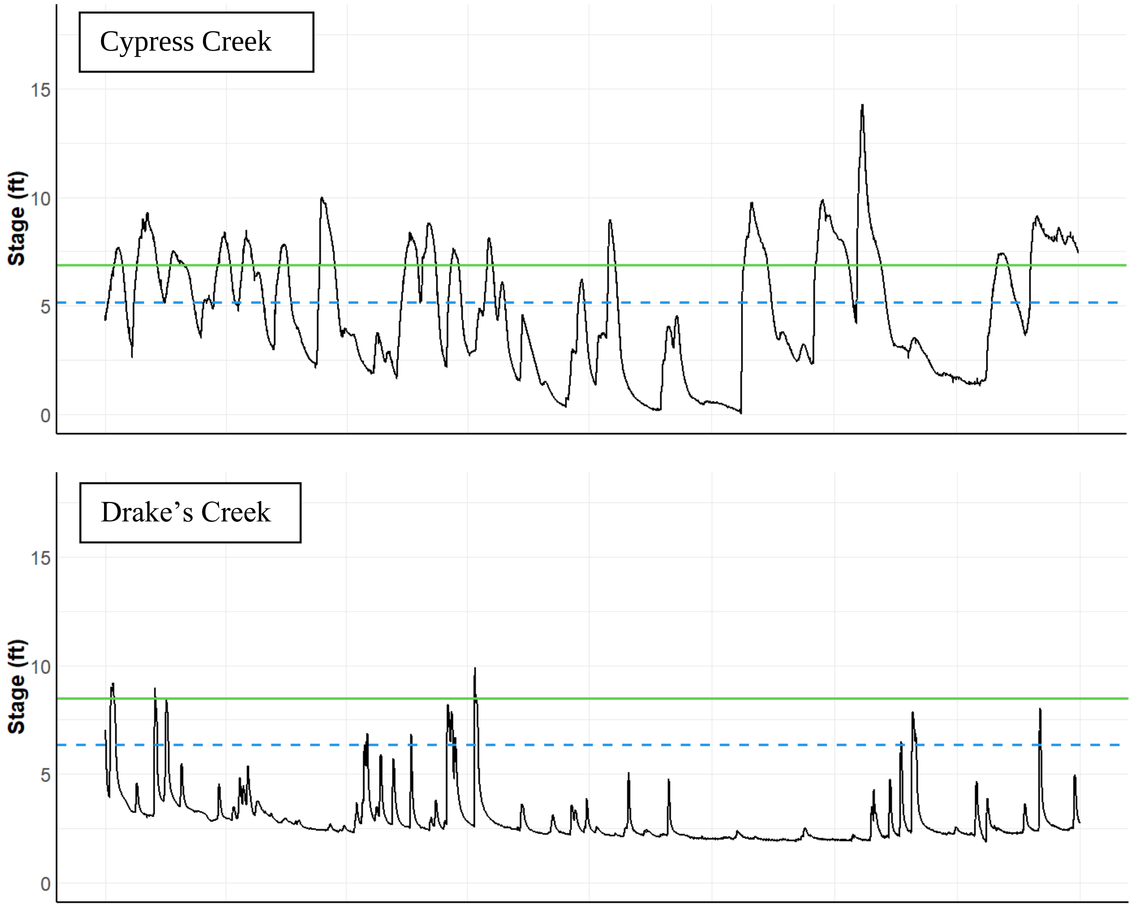


Figure 3.2. Annual Hydrographs for Cypress Bayou and Drakes Creek that were west of the Mississippi River. Blue dash line indicates the stage at which sub-bankfull flooding occurred estimated at 75% of bankfull stage. Green solid line indicates supra-bankfull floodings. Note that Drake's Creek data was obtained from 2019, not 2020. (fig. cont'd.)

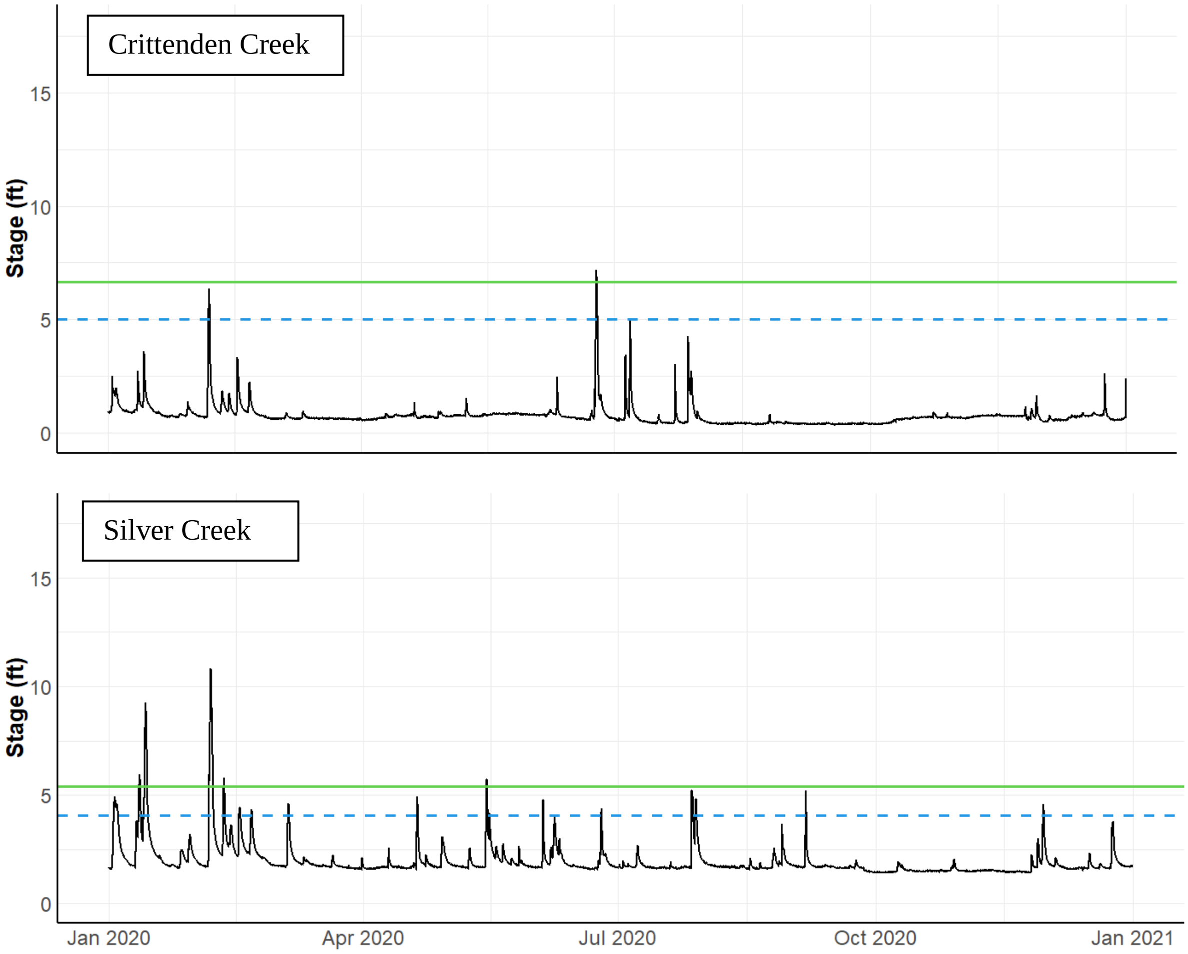


Figure 3.2. Annual Hydrographs for Crittenden and Silver Creeks that were east of the Mississippi River.

The best LMM model for macroinvertebrate taxonomic richness included regions, seasons, and the interaction (Likelihood Ratio Test χ^2 = 22.25, P < 0.01); however, macroinvertebrate taxonomic richness was significantly lower only in winter compared to other seasons [β_1 = -2.17 (SE ± 0.94), t = -2.30, P = 0.03], but not for region [β_2 = 0.44 (SE ± 1.240, T = 0.5, P=0.73]. Similarly, the best GLMM for Shannon-Weiner diversity also included regions, seasons, and the interaction (Likelihood Ratio Test χ^2 = 24.53, P < 0.01), again, with only winter being significantly lower than the remaining seasons (β_1 = -0.26 (SE±0.10), t =-2.70, p < 0.01).

Table 3.5. Multivariate analyses of taxonomic composition detected numerous differences attributable to season and region. The direction of effect is indicated by (+) and (-) for the coefficients.

Taxa	East Fall	West Fall	East Winter	West Winter	East Spring	West Spring	East Summer	West Summer
Caenidae	-	+			+		+	
Gerridae	-						+	+
Hirudinea	-	+						
Oligocheata	-	+	-		+	-	+	
Perlodidae	-		+		+	+	+	+
Simuliidae	-	+	+		+		+	
Zygoptera	-	+	+			-	+	
Anthericidae		-				+		+
Coenagrionidae					-		-	-
Corydalidae					-			
Gyrinidae		-	-	+		+		+
Isonychiidae								-
Leptoceridae		-		+				+
Limoniidae		-		+		+		+
Macromiidae		-						+
Philopotamidae					-			
Tabanidae		-						+

Table 3.6. Multivariate analyses of functional feeding groups detected numerous differences attributable to season and region. The direction of effect is indicated by (+) and (-) for the coefficients.

Functional Feeding Guild	East: Fall	West: Fall	East: Spring	West: Spring	East: Summer	West: Summer	East: Winter	West: Winter
Collector/Filterer	+	-	-	+	+	+	-	-
Collector/Gatherer	+	-	-	-	+	-	-	-
Detritivore	-	+	+	-	+	-	+	-
Omnivore	+	-	-	+	+	+	+	+
Predator/Engulfer	+	-	+	+	+	+	+	+
Predator/Piercer	+	+	+	-	+	+	+	-
Scraper	+	+	-	-	+	+	+	-
Shredder	+	-	+	+	+	+	-	+
Shredders/Detritivores	+	-	-	+	+	+	-	+

Results of the MVGLM's indicated differences in abundance among regions and seasons (Wald $P_{3,63} < 0.01$) for many taxa (i.e., coefficients > ±15 cutoff for taxa; Table 3.5). For FFGs, significant differences also occurred among regions and seasons (Wald $P_{3,63} < 0.01$, Table 3.6). In winter, detritovore/shredders and shredders were more abundant in western streams, and scrapers were more abundant in eastern streams. In spring, collector/filterers were more abundant in western streams, and shredder/detritovores were more abundant in eastern streams. In summer, collector/gatherers, scrapers, and shredders were more abundant in eastern streams, and no FFG was more abundant in western streams. In fall, detritivores were more abundant in western streams, and collector/gatherers, collector/filterers, shredders, and shredder/detritovores were more abundant in eastern streams. Omnivores and predators did not show any obvious patterns related to season or region.

Basal δ^{15}N appeared to follow no pattern among the streams and regions, whereas basal resources exhibited obvious distinctions in δ^{13}C signatures (Figures 3.3 and 3.4, Table 3.7). The top macroinvertebrate δ^{13}C models for the grass/herb δ^{13}C and pine/deciduous leaf δ^{13}C separate analyses included interactions with flooding type and FFGs (Table 3.8). Between these top models, differences in relationships of FFGs and measured environmental variable were apparent (Tables 3.8 and 3.9). For model 1, predators, gastropod scrapers, collector/gatherers, and shredder/detritivores FFG assignment, as well as a grass/herb δ^{13}C were positively associated with individual macroinvertebrate δ^{13}C from streams with winter/spring supra- and sub-bankfull floodings, whereas collector/filterer FFG assignment were only associated with individual macroinvertebrate δ^{13}C values from streams with yearly supra-bankfull floodings. For model 2, Pine/Leaves δ^{13}C and shredder/detritivore FFGs were associated with individual macroinvertebrate δ^{13}C from streams with yearly supra-bankfull floodings and winter/spring supra- and sub-bankfull floodings, while individual macroinvertebrate δ^{13}C assigned scraper FFG were negatively associated with pine-Leaves δ^{13}C, regardless of flooding regime.

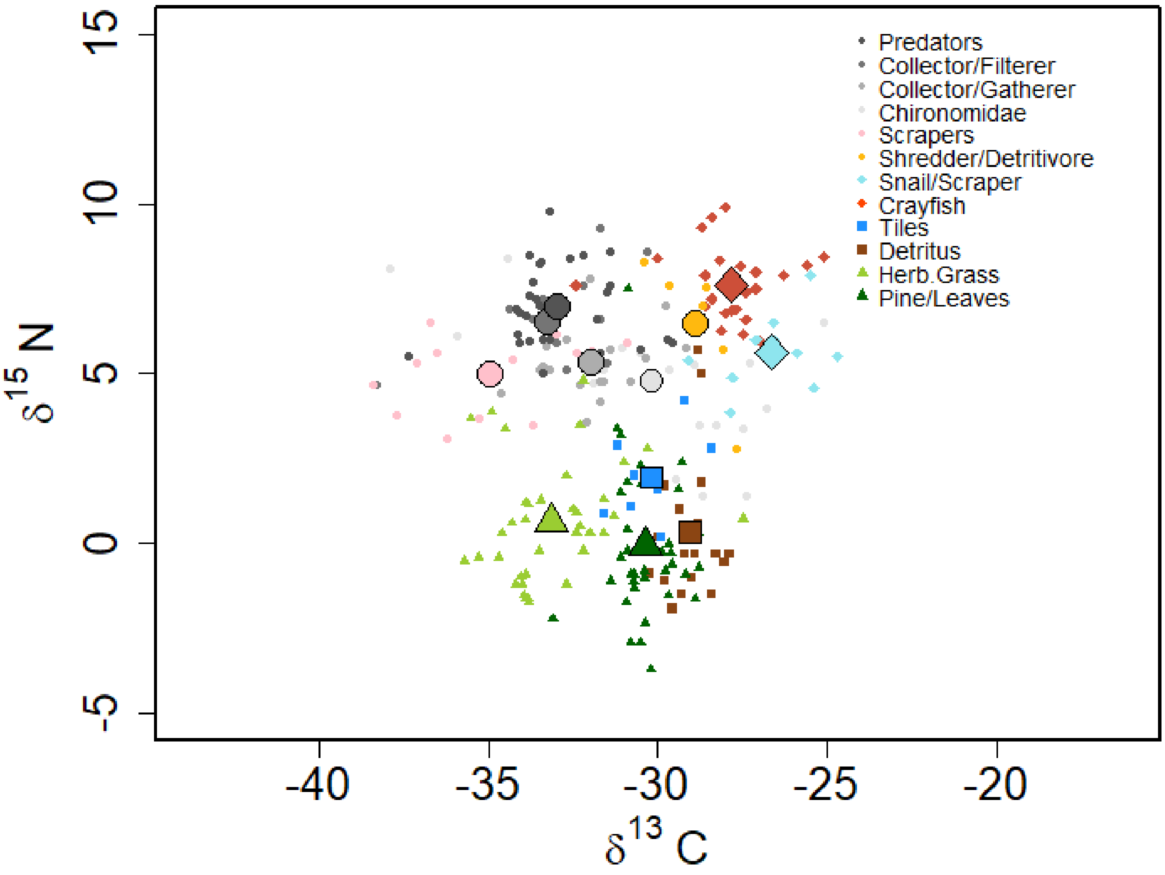


Figure 3.3. Results of the Stable Isotope Analysis of macroinvertebrates and basal samples for two study streams west of the Mississippi River. Functional feeding guilds and basal samples are denoted by the colors; diamonds indicate non-insect animals, while circles indicate insects squares indicate in-streams samples, and triangles indicate allochthonous sources.

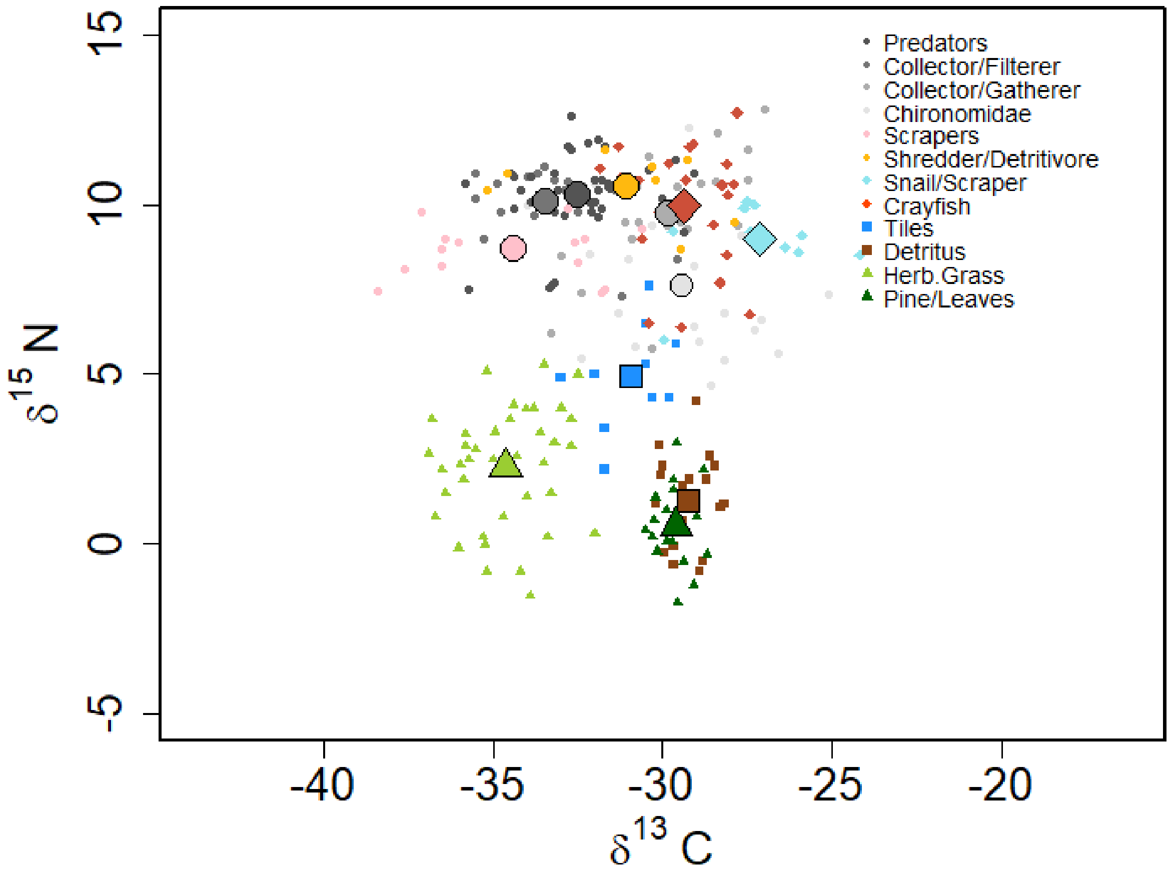


Figure 3.4. Results of the Stable Isotope Analysis of macroinvertebrates and basal samples for two study streams east of the Mississippi River. Different functional feeding guilds and basal samples are denoted by the colors; diamonds indicate non-insect animals, while circles indicate insects squares indicate in-streams samples, and triangles indicate allochthonous sources.

Table 3.7. Stream macroinvertebrates δ^{13}C signatures explained by flooding types, functional feeding guilds (FFG), season, pine needles/deciduous leaves, grass/herbaceous predictor variables. Streams with yearly supra-flooding included Big Creek, and Cypress Bayou. Bird's and Drake's Creeks had winter/spring flooding. Crittenden and Twelvemile Creeks had spring sub-bankfull flooding. Silver Creek and Little Silver Creek had yearly sub-bankfull flooding.

	Competing Models	Quasi ĉ	AIC
Model 1	δ^{13}C ~ Flooding Type*FFG*Grass/Herb	2.66	2037.5
	δ^{13}C ~ FFG*Grass/Herb	3.16	2083
	δ^{13}C ~ FFG	3.25	2089.7
	δ^{13}C ~ Grass/Herb	8.37	2574.3
Model 2	δ^{13}C ~ Flooding Type*FFG*Pine/Leaves	2.65	2034.6
	δ^{13}C ~ FFG*Pine/Leaves	3.18	2086.6
	δ^{13}C ~ FFG	3.25	2089.7
	δ^{13}C ~ Pine/Leaves	8.43	2578

Table 3.8. Significant variables from the top generalized linear model of macroinvertebrates δ^{13}C signatures explained by flooding type, grass/herbaceous sources, and functional feeding group. Flooding types are denoted as W/T = winter/spring supra- and sub-bankfull, Y/S = yearly supra-bankfull. Estimate is the model parameter estimate and standard error for the variable combination.

Variables	Estimate (±Std. Error)	t	P
Y/S * δ^{13}C Grass/Herb * Collector/Filterer	1.69 (±0.83)	2.03	<0.05
W/T * δ^{13}C Grass/Herb * Collector/Gatherer	2.08 (±0.63)	3.29	<0.005
Y/S * δ^{13}C Grass/Herb * Collector/Gatherer	1.46 (±0.70)	2.09	<0.05
W/T * δ^{13}C Grass/Herb * Predator/Engulfer	1.32 (±0.55)	2.39	<0.05
W/T * δ^{13}C Grass/Herb * Shredder/Detritivore	2.08 (±0.89)	2.33	<0.05
W/T * δ^{13}C Grass/Herb * Gastropod Scraper	1.92 (±0.80)	2.41	<0.05

Table 3.9. Significant variables from the top model top generalized linear model of macroinvertebrates δ^{13}C signatures explained by flooding type, pine/leaf sources, and functional feeding group. Flooding types are denoted as W/T = winter/spring supra- and sub-bankfull, Y/S = yearly supra-bankfull. Estimate is the model parameter estimate and standard error for the variable combination.

Variables	Estimate (±Std. Error)	t	P
δ^{13}C Pine/Leaves * Scraper	-2.69 (±1.31)	-2.04	<0.05
W/T * δ^{13}C Pine/Leaves * Shredder/Detritivore	5.86 (±2.35)	2.50	<0.05
Y/S * δ^{13}C Pine/Leaves * Shredder/Detritivore	5.56 (±2.15)	2.59	<0.05

3.4. Discussion

Assigned annual flooding type of the study streams and timing appeared to be the most important factor in structuring food resources and the functional composition of macroinvertebrate assemblages in Gulf Coastal Plains streams. Despite previously documented regional differences in taxonomic composition, differences in function among macroinvertebrate assemblages (i.e., FFGS) among stream types across this area were undocumented. Analyses revealed that flooding type and timing were more important in determining relative densities of collector/filterers, collector/gatherers, shredder/detritovores, and gastropod scrapers within streams than biogeographic patterns, as strong relationships between flooding type with FFGs were detected in both regions. Although each flooding type was described by a single stream, these streams are representative of stream types across the Gulf coastal plain (Felley 1992, Isphording and

Fitzpatrick 1992, Hupp 2000). Therefore, these results should generalize across the Gulf coastal plain and similar subtropical ecosystems.

Results of this study provide a glimpse into macroinvertebrate food web structure in Gulf Coastal Plains streams. Patterns emerged between streams among the two regions (east and west of the Mississippi River) across the different datasets explored. Western study streams tended to have higher percent forest landcover and C/N ratios, while eastern study streams had more agricultural landcover and lower C/N ratios. In contrast to my original hypothesis, no obvious patterns in measured geomorphic features emerged that might have indicated variation in floodplain connectivity attributable geographic regions. Richness and Shannon-Weiner diversity Index were also not different among regions and seasons, which was unexpected, but may indicate that interannual variability in overall macroinvertebrate composition exceeds variation explained by season or region. However, multivariate analyses still indicated differences in abundances of specific taxa among regions and seasons. These results indicate diversity and richness measures are not capturing nuanced spatiotemporal differences in macroinvertebrate assemblage characteristics, which are reflected in taxonomic and functional group analyses.

Interestingly, insect scrapers δ^{13}C were not associated with any flooding regime nor region but were negatively associated with pine needles and deciduous leaves δ^{13}C. A lack of a positive association with biofilm δ^{13}C values may indicate scrapers were exploiting a food source that was not well represented on the ceramic tile biofilms. However, results of previous studies of allochthonous/autochthonous food sources have been equivocal at best i.e., algal/periphyton δ^{13}C samples have been reported to have more depleted signatures than the allochthonous samples (Rosenfeld and Roff, 1992; Rounick et al. 1982), or vastly different among different algal groups within a stream (Zah et al. 2001), or not different from allochthonous sources at all (Goebel et al. 2010). Although it is possible that Heptageniid scrapers were utilizing an unknown algae source, it is also possible they were scraping biofilms that contained depleted carbon produced from methane reducing bacteria (Kohzu et al. 2004; Jones and Grey 2011; Sampson et al. 2019). As stated previously, much of the leaf material is buried by the shifty sand substrate and stored year-round in the study streams. Although unmeasured in this study, because there is little interstitial space within the hyporheic zone, an anoxic reduced environment may be potentially with bacteria that show methane-derived carbon signatures. Methane is naturally produced in headwaters and wetted floodplains (e.g., Pulliam 1993; Robinson et al. 2021; Zhu et al. 2022), and long periods of warm temperatures in subtropical regions may be conducive to high methane production (Pulliam 1993). Thus, these bacteria may act as another energy source for scrapers and other macroinvertebrates.

Previous reports indicate collector/gatherer and collector/filterer macroinvertebrates are inconsistent in their response to increased stream flooding and drying (Statzner and Beche 2010), both resisting (Vieira et al. 2004) and negatively responding (Burcher et al. 2007; Vandewalle et al. 2010; Sefick et al. 2018) to altered streamflow. The results of this study are consistent with Piedmont sandy streams where relationships among flow, organic matter, and abundance of collector/gatherers were apparent (Sefick et al. 2018), although carbon sources were not explicitly measured in those streams. Importantly, positive or negative relationships among collector/gatherers and carbon sources and flooding were not detected in taxonomic analyses, suggesting carbon sources and flooding were more important than region or season in explaining distributions of these organisms. Given the importance of these taxa in multiple aspects of stream trophic webs (i.e., laterally by incorporating biomass delivered by connectivity with floodplains and riparian zones, and longitudinally by-passing biomass and energy downstream), ensuring natural flooding regimes in light of expected changes to coastal streams and rivers is important.

It was unexpected that shredders and shredder/detritivore FFGs exhibited relationships with season and region, but relationships were not associated with selection of allochthonous sources in the SIA. This may indicate more allochthonous material was available to shredders in eastern streams in winter, boosting shredder productivity, but overall shredders did not preferentially consume different carbon sources among seasons. However, differences in macroinvertebrate $\delta^{13}C$ assigned to shredder/detritivore FFG and associated pine/leave $\delta^{13}C$ were still detected within yearly supra-bankfull floodings and winter/spring supra- and sub-bankfull floodings compared to other streams' flooding type. This suggest a tighter relationship to allochthonous carbon sources in western flooding regimes. It has been shown in other studies that riparian grasses, macrophytes, and herbaceous plants contributed more to the allochthonous vegetative sources of CPOM than leaf litter (Leberfinger et al., 2011). Cruz and Post (1977) demonstrated that pine versus deciduous leaf litter had differing caloric and elemental (C, H, N, and P) levels that varied seasonally as well in a Mississippi coastal plains stream. Given that Cruz and Post (1977) and Leberfinger et al. (2011) detected differentiation in signatures between the two allochthonous sources (i.e., pine/leaves versus herb/grass), it is interesting that shredders and shredder/detritivore FFGs did not consistently exhibit clearer relationships with the either riparian measured food source.

Overall, snail scrapers (Viviparidae) appeared to be utilizing an unknown, less-enriched carbon source that does not overlap with insect scrapers, nor is tied to any autochthonous/allochthonous source measured in this study. However, interestingly, snail scrapers were positively associated with grass/herb C^{13} sources in the winter/spring flooding assigned category. Viviparidae have high productivity, are considered detritivores in Louisiana streams (Richarson and Brown 1989) and are important at organic transfer in other subtropical food webs (Huang et al. 2007). Crayfish also appeared to be utilizing a carbon resource not sampled in this study, and that may also slightly overlap snails. Similar results of consumer signatures outside of the sampled basal sources ranges have been reported (Hadwen et al. 2010). In streams, ponds, and lakes, crayfish often play an important role as keystone consumers because they feed on live/dead animal as well as terrestrial plant materials (France 1996; Nyström and Strand 1996). Longer life span of these individuals may lead to unaccounted trophic fractionation, biasing interpretation of signatures (Fink et al. 2012), and there is the possibility that mixing is occurring (i.e., snail and crayfish diets are a combination of two different carbon sources, one of which is definitely unknown) that could not be explored via stable isotope mixing models in this study. Similar to the findings of Wen et al. (2010) in China, Neres-Lima et al. (2017) in Brazil, and Demers et al. (2021) in Scotland, investigating the food sources for these two groups specifically might provide additional clarity on the detrital pathway in subtropical and lowland stream systems.

3.4.1. Conclusion

This study provides a baseline assessment of trophic structure within a series of Gulf Coastal Plains streams that varied in flooding regimes. It appears usage of autochthonous/allochthonous sources is more convoluted than other temperate regions. Moreover, the microbial compartment may play a larger role in carbon flux than macroinvertebrates. Importantly, flooding regimes were strongly associated with food resources and some aspects of the functional structure of the macroinvertebrate assemblage. The structure of the macroinvertebrates food webs ($\delta^{13}C/\delta^{15}N$ biplots) of the two regions appear to be very similar. Evidence that food webs between regions appeared similar in structure, but with differences in overall taxonomic comparison, reiterates the premise that functional composition is conserved, and the most common macroinvertebrate groups drive trophic structure.

In river systems, climate change and associated environmental impacts are expected to homogenize taxonomic diversity, especially in coastal zones (Vörösmarty et al. 2010; Brauns et al. 2022), increasing the importance of understanding, protecting, and conserving stream function (Mouton et al. 2020). Therefore, in light of future human population expansion, climate change, and increasing water use, efforts to conserve and restore freshwater stream and river habitats (Acreman et al. 2020) should emphasize natural flooding regimes and a better understanding of the microbial/detrital pathway in these systems to conserve both taxonomic and functional diversity. These data support restoration efforts (e.g., Acreman et al. 2020), especially those efforts that target restoring river flows (e.g., Whipple and Viers 2019), that focus on reinforcing the linkage between hydrogeology and biological function. Hydrologic connection to the floodplain, whether it be brief (i.e., hours to days) as in flashy, smaller-ordered streams or longer (i.e., weeks to days) in larger streams/rivers, allows for exchange of energy and this may create a more-complex food web, promoting resistance and resilience to disturbance.

Chapter 4. Longitudinal Connectivity Investigated via Food Web Structure among Two Louisiana Coastal Plains Watersheds

4.1. Introduction

Watersheds by nature are connected hydrologically, and the role of hydrologic connectivity on aquatic systems depends on location within the watershed and upstream contributions of water and nutrients (e.g., the River Continuum Concept (RCC), Vannote et al. 1980). Three dimensions of hydrologic connectivity include longitudinal, lateral (floodplain), and vertical (subsurface/hyporheic; Ward 1989) and influence allochthonous/autochthonous contributions to lotic trophic webs. However, detecting effects of each hydrologic connectivity dimension on the food web is a challenge but can be better determined by stream size, shading, substrate type, and other variables that are distributed on a continuum within the watershed (Vannote et al. 1980; Fausch et al. 2002; Dorretto et al. 2020). Vertical and lateral connectivity can be investigated by exploring differences in physiochemical parameters between surface and hyporheic water within a site.

Differences in dissolved organic carbon (DOC) between the hyporheic zone and stream surface water in headwater streams can indicate overland flow and riparian inputs (lateral connectivity) as well as periodic subsurface and deeper groundwater flows, with less DOC contribution from deep groundwater (Birkel et al. 2014; Hosen et al. 2018). Dissolved organic nitrogen (DON) as well as DOC to DON ratios (C:N) of the hyporheic/stream water differential in headwaters can indicate vertical hydrologic connectivity and processing that occurs before downstream transport (longitudinal connectivity; Brookshire et al. 2005). DON is much less influenced by lateral inputs than DOC is and thus, differences in DON between the stream and hyporheic zone are assumed to reflect varying magnitudes of exchange between surface and groundwater.

Evaluating longitudinal connectivity and contributions to local and watershed level food-webs is much more convoluted and is evaluated by more direct biotic response. For instance, insect functional feeding guilds vary with stream size and temperature regime, and the different guilds possess morphologies that can capitalize on different-sized food particles of allochthonous or autochthonous origin (Cummins et al. 2019). Relative to macroinvertebrates, fishes exhibit less magnitude in taxonomic turnover within watersheds, and species presence is more a product of thermal limitations, behavior, and barriers to dispersal (Wang et al. 2003; Quist et al. 2004; Buckwalter et al. 2018; Bouska 2018). Headwater streams tend to be shaded and receive substantial inputs of coarse particulate organic matter (CPOM) from leaf senesce, which is consumed by invertebrate shredders including crayfish, amphipods, freshwater shrimp, snails, and immature stages of insects (Cummins 1974, Roeding and Smock 1989; Wallace and Webster 1996; Allan and Castillo 2007; Cummins et al. 2019; Balibrea et al. 2020). Mid-reaches of larger streams and rivers have less shading with more algal production and a greater predominance of invertebrate scrapers, given typical turbidity levels that permit subsurface photosynthesis. Larger rivers have higher turbidity and less autochthonous production, so invertebrate collectors are important and dependent on fine particulate organic matter (FPOM; Wallace and Webster 1996; Cummins 2016; Gholizadeh and Heydarzadeh 2020). Thus, prey resources available to fishes differ from upstream to downstream, and the availability of differing types of prey is an important factor structuring fish assemblages within watersheds (Curtis et al. 2018).

In addition to allochthonous and autochthonous food resources, invertebrate and vertebrate prey resources are also influenced by local and landscape conditions (Martin et al.

2021; Champagne et al. 2022; Hartman and Kaller, in press). As a consequence, conservation and management of riverine fishes must consider watershed position as well as the associated physicochemical and habitat conditions. Given that rivers are considered some of most vulnerable ecosystems to changing climate and increasing water demands (Vörösmarty et al. 2010; Brauns et al. 2022), a greater understanding of trophic structure and the role of stream-connectivity would enhance opportunities for conservation and restoration of river systems (Bouska et al. 2019; Acreman et al. 2020; Mas et al. 2022). Furthermore, watershed paradigms have tended to originate in temperate regions exhibiting four distinct seasons and year-round gravity-driven flow. Expected patterns in the structure of lotic invertebrates and fishes that occur in temperate regions (Doretto et al. 2020) may not apply in different climatic and geologic conditions (Meyer and Edwards 1990; Roebuck et al. 2020).

The north-central Gulf of Mexico Coastal Plains (Gulf) region is in a subtropical climate with little topographic relief (Isphording and Fitzpatrick 1992; Feeley 1992; Hupp 2000). Moreover, these rivers support or have supported numerous resident and migratory fishes of conservation concern (e.g., American Eel *Anguilla rostrata,* Gulf Sturgeon *Acipenser oxyrinchus desotoi*, and Alabama Shad *Alosa alabamae*) and deliver critical nutrients and sediment to coastal wetlands. Modeling predicts upland and inland wetland migration in response to climate change and sea level rise globally, with the greatest potential in the northern Gulf (Osland et al. 2022). Therefore, there is potential for considerable change to rivers in this region, and information about trophic structure and connectivity will inform management and restoration responses to change in the northern Gulf and in other coastal regions.

Based on preliminary measurements (Chapter Three) and evidence from the literature

(Isphording and Fitzpatrick 1992; Feeley 1992; Hupp 2000), headwaters of the Tickfaw River watershed have less lateral connectivity than headwaters of the Calcasieu River watershed. Both watersheds have variability in vertical connectivity, although the Tickfaw River watershed generally have more groundwater connections. Therefore, these two watersheds provided the opportunity to test hypotheses about vertical and lateral connectivity and the effects on watershed longitudinal connectivity.

My objective for this study was to better understand longitudinal connectivity (upstream-downstream) as it relates to food-web structure in small Gulf watersheds that differ in lateral (floodplain), and vertical (subsurface/hyporheic) hydrologic connectivity. My hypotheses are: food-web structure of headwater sites between the Tickfaw River and Calcasieu River watersheds are different because of differing magnitudes of lateral connectivity (Headwaters Hypothesis), while intermediate (sites wider and deeper than wadable streams and smaller than commercially navigable rivers) and lower river sites are more similar between the two watersheds because of larger landscape factors in effect (Lower Watersheds Hypothesis); and the Calcasieu River watershed has strong lateral connectivity in the headwaters which makes the headwater, intermediate and river sites are more similar in food web structure along the longitudinal continuum upstream to downstream, whereas the Tickfaw River watershed more closely follows predictions of the RCC (Whole Watersheds Hypothesis). In this study, food webs were characterized by stable isotope analysis (SIA of δ^{13}C and δ^{15}N) of fishes, invertebrates, and basal resources.

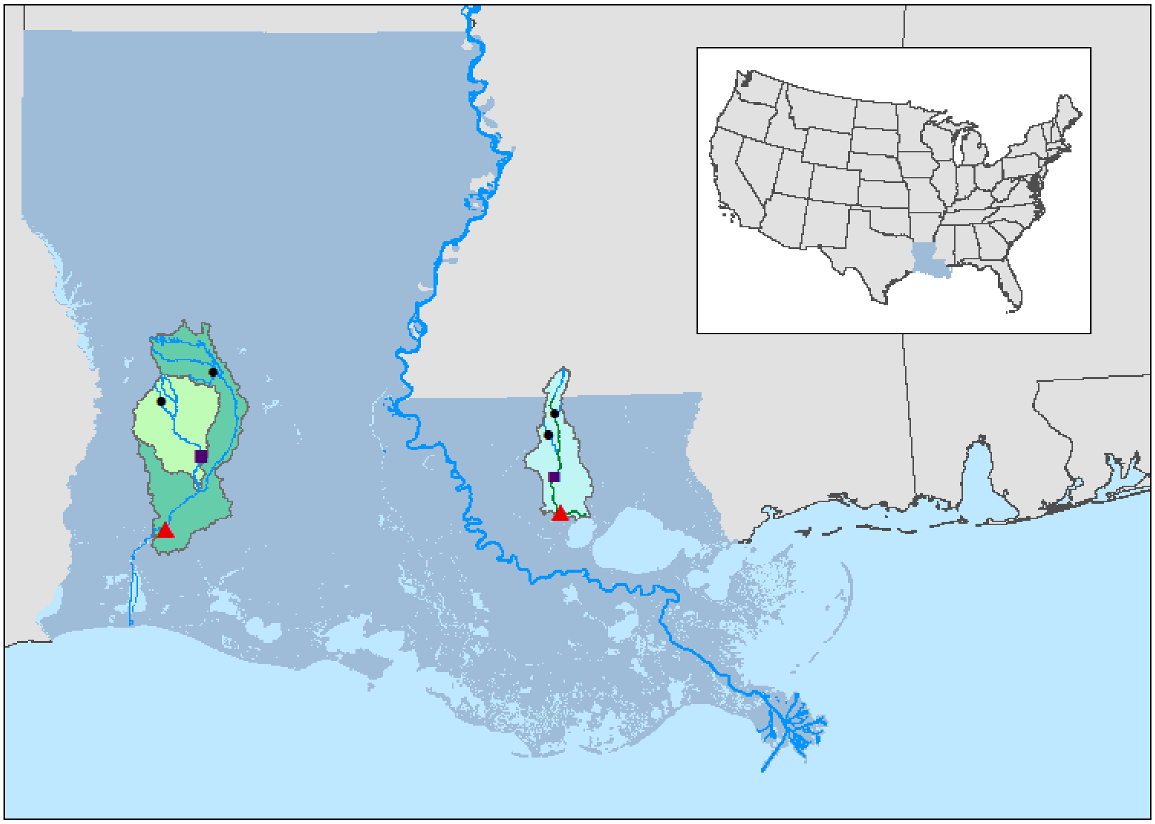


Figure 4.1. Locations of study sites across two watershed in the Gulf Coastal Plain region of Louisiana. Headwater streams (S), intermediate (I) and river sites are depicted as black circles, purple squares, and red triangles, respectively. The Calcasieu River watershed is west of the Mississippi River and contains, from north to south, Big Creek and Drake's Creek (S), Ouiska Chitto (I), and River sites. Tickfaw River watershed is east of the Mississippi River and contains, from north to south, Crittenden Creek and Twelvemile Creek (S), Tickfaw River Intermediate (I) and River sites. (Map was created in ArcGIS 10.7.1 (ESRI 2019).

4.2. Methods

4.2.1. Study sites and field collection

Two watersheds were selected for this study (Figure 4.1). Both the Tickfaw River and Calcasieu River were sampled in segments designated as Natural and Scenic by the Louisiana Department of Wildlife and Fisheries (https://www.wlf.louisiana.gov/page/scenic-rivers-descriptions-and-map) to reduce confounding anthropogenic influence and increase comparability. The Tickfaw River is not impounded and does not have levees. All samples in the Calcasieu River occurred upstream of channel modifications. Four streams were selected (two for each watershed) and were sampled in the fall of 2017 and 2019. The rivers were sampled in the fall of 2018 and 2019, and I added a mid-reach, intermediate site in fall 2019 to gain a clearer picture of headwater/river relationships. The intermediate site within the Tickfaw River watershed was located on the Tickfaw River, up-river from the river site. Ouiska Chitto River is a smaller river draining into the Calcasieu River and was the intermediate site for the Calcasieu River watershed.

Fish, macroinvertebrates, and allochthonous/autochthonous basal samples were collected and later used in SIA at all sites. Headwater (third and fourth order) fishes were sampled via backpack DC electrofishing (Halltech HT-2000, Scarborough, Ontario, Canada) adjusted to 300 volts and 3-4 amps; prior to sampling, block nets were deployed upstream and downstream to isolate a 100-m reach. At each site, two passes with the electrofisher were conducted. Intermediate sites were sampled via DC backpack electrofishing and hoop nets. Because intermediate sites tended to be wide and deep in areas, electrofishing efforts were focused on key habitat types that would encompasses habitat needs of a variety of fishes. Intermediate and river sites were sampled via boat DC electrofishing (Smith-Root 7.5 GPP electrofishing unit, Vancouver, Washington) adjusted to approximately 700 volts and 9-12 amps. Depending on site type, point-based (intermediate and river) and continuous (headwater, intermediate, and river) electrofishing methods were employed (Trumbo et al. 2016). The proportion of woody debris, vegetation, anthropogenic, open, inner bend, and outer bend habitats in the littoral zones in river sites were determined by employing the timed qualitative assessment method (Snedden et al. 1999). This method entailed driving a set distance in the river (1000 m) and at every 20 m the dominant habitat type was noted for a total of 48 habitat data point and based on the proportion of each habitat type, 15 points were randomly selected for electrofishing point sampling. At each point, the entire habitat type or 20 m, whichever was smaller, was sampled via electrofishing for 60 seconds of power-on time. Hoop nets (0.9m diameter, 13 mm bar knot-less nylon net; Memphis Net and Twine, Memphis, Tennessee) were set and retrieved after 24 hours.

All fish were retrieved, enumerated, identified, and lengths recorded, except during boat electrofishing, when only fish under 150 mm were counted. Upper dorsal tissue (1 cm^3) was extracted from a subset of individuals for SIA (Miller et al. 2015). Target numbers were 10 individuals of each species or guild of interest.

To sample macroinvertebrates, three modified Hess samples (see Kaller and Kelso 2006) and three 0.25-mm mesh grab bag samples of woody debris (see Kaller and Kelso 2007) were collected at 3 locations at 25 m, 50 m, and 75 m within each100 m electrofishing reach (streams and intermediate sites), and at 3 randomly chosen electrofishing point samples (river sites). At those same locations, basal resources, specifically terrestrial plant matter (leaves and herbaceous plants), were collected. Unglazed ceramic tiles were placed in headwater streams, within intermediate sites, and near a boat launch for river sites and were retrieved two weeks later and analyzed to represent the autochthonous food source. Water was collected from the water column (all sites) and the hyporheic zone (headwaters sites only) via a drive point piezometer (Rivett 2008) at similar locations to the Hess and wood samples for dissolved organic carbon and dissolved nitrogen (DOC/DN) analysis. All samples were placed on ice and stored in a freezer in the lab until processing.

4.2.2. Laboratory processing and SIA

Water samples were filtered through glass microfiber filters (1.5µm size particle retention) to remove particulate matter. Particulate matter was removed via filtering 100 mL of sample through a glass fiber filter (GFF) prior to being sent to the Wetland Biogeochemistry Analytical Services at Louisiana State University for DOC/DN analysis (5310B Standard Methods Protocol for TOC/TIC to obtain TDOC; APHA/AWWA/WEF 2018). Ceramic tiles were processed in a similar manner, i.e., any invertebrates and debris were removed from the tiles, which were then scraped with a wire blush and rinsed with DI water into a collection jar, and the contents were filtered through a GFF. Material on the GFF was then processed for SIA, denoted as the 'filter' sample, henceforth. Roots of grass and herbaceous plants were removed, and all plant material (deciduous leaves, pine needles, grass, and herbaceous plants) collected from each site's riparia were rinsed in DI water prior to drying.

Table 4.1. Landscape-variables and averages (standard errors) of C:N, and Basal δ^{15}N signatures for headwaters and river sites. Agriculture landcover is abbreviated Ag.

Site	Region	Watershed Area (km^2)	Forest (%)	Ag. (%)	DOC/DN (Mean (±SE)	Basal δ^{15}N (Mean (±SE)
Crittenden	East	26.63	29.41	15.50	6.38 (±0.55)	1.08 (±0.61)
Twelvemile	East	52.15	37.36	6.39	6.80 (±0.54)	-1.01 (±0.28)
Drake's	West	56.30	63.76	1.90	14.06 (±0.99)	-0.14 (±0.63)
Big	West	106.08	38.10	4.93	13.81 (±1.25)	-0.87 (±0.58)
Calcasieu	West	6194.34	35.28	14.53	22.945 (±1.53)	1.19 (±0.47)
Tickfaw	East	1880.29	26.00	11.40	9.25 (±1.92)	4.16 (±1.13)

Macroinvertebrates were identified to lowest practical taxonomic level, enumerated, and most were assigned to a functional feeding group (FFG) per Merritt et al. (2019). Composite samples of taxa that represented a range of FFGs (Table 4.1, Table C.1) consisted of whole insects or tail muscle from crayfish (Hicks 1997). For the first round of sampling in fall 2017, I processed most fish tissues and produced an isotopic specimen for each feeding guild (i.e., detritivores, omnivores, and top predators; Goldenstein and Meador 2004; Mueller et al. 2013; Lima et al. 2017). Because of cost and time limitations, a focus was placed on fish that seemed to show idiosyncrasies in their SI signatures between streams for 2018 and 2019 samples. These included the detritivores Spotted Sucker (*Minytrema melanops*) and Blacktail Redhorse (*Moxostoma poecilurum*), a nocturnal insectivore, Pirate Perch (*Aphredoderus sayanus*), Longear Sunfish (*Lepomis megalotis*) as an omnivore and Spotted Bass (*Micropterus punctulatus*) as a top predator. For intermediate (fall 2019) and river (fall 2018 and 2019) sites, similar feeding guilds were targeted, including detritivores (Catastomids), omnivores (Lepomids), and top predators (Largemouth Bass *Micropterus salmoides* and *M. punctulatus)*, as well as guilds present only in larger rivers including piscivores (Spotted Gar *Lepisosteus oculatus*, Bowfin *Amia calva*) and a plankitvores (American Gizzard Shad *Dorosoma cepedianum*).

All SIA samples were dried at 60°C until constant mass was achieved and ground with mortar and pestle (macroinvertebrates) or a Wiley Mill (plants) into a powder (Winemiller et al., 2011; Daniel et al. 2015). Values of SIA are denoted as $\delta^{13}C$ and $\delta^{15}N$, where δ is the differences in ratios (R) than the expected 1. R is ratio of heavy isotope (*H*) of an element (*X*) (C and N) to light isotopes of either sample or global standard (Vienna Pee Dee Belemnite and Air for C and N, respectively; Fry 2006):

$$\delta^{H}X = \left[R_{sample}/R_{standard} - 1\right] * 1000$$

This value is then multiplied by 1000 because differences tend to be very small. For bulk $\delta^{13}C$, $\delta^{15}N$ SIA most of fall 2017 samples were processed by the Stable Isotope Facility at the University of California at Davis; all other samples were processed by Stable Isotope Ecology Laboratory in the Department of Oceanography and Coastal Sciences, College of the Coast and

Environment at Louisiana State University.

4.2.3. Statistical Analyses

Analyses of my data regarding my hypotheses and trophic structure within Gulf coastal plains employed three methods. First, I examined overall patterns in tissue stable isotopes with a Bayesian multivariate approach. Second, I qualitatively examined trophic trajectories from basal resources to fish. Finally, I tested my first two hypotheses by generalized linear models separately for $\delta^{13}C$ and $\delta^{15}N$.

I compared food webs of fish among and within the Tickfaw River and Calcasieu River watersheds by stable isotope Bayesian ellipses (SIBER; Jackson et al. 2011; Program R vers. 4.0.0, R Development Core Team 2022). SIBER computes the credible intervals of Layman's terms (Layman et al. 2007) which are based on $\delta^{13}C/\delta^{15}N$ biplots and values that provide a fish assemblage-wide view and comparison of food webs. The range of $\delta^{15}N$ (NR) provides information regarding trophic length, whereas $\delta^{13}C$ range (CR) yields insights to basal resource diversity. The Total Area (TA) of the group ellipses indicates assemblage niche width, whereas mean distance to centroid (CD) indicates the degree of trophic diversity. Mean nearest neighbor distance (MNND and Standard Deviation NND) provides an idea of the density or packing of a species or guild of interest. To address my hypotheses, I performed a series of comparisons between two assemblages. First, I compared headwaters assemblages among watersheds then grouped intermediate and river sites by watershed and compared the resulting assemblages among watersheds. I then compared headwaters to intermediate and river sites within each watershed and finished with an overall comparison of watersheds. Four guilds for each assemblage comparison were used to create a large enough sample size and included invertivores, omnivores, Lepomids, predators (see A1 for taxa in each group). Fish assemblages were compared based on credible interval of each Layman's term estimated by MCMC Bayesian inference with uninformative priors for $\delta^{13}C$ and $\delta^{15}N$ means and a covariance matrix prior based on the Wishart distribution following Sturbois et al. (2022). For each comparison, I used the percentage of the first assemblage's estimated posterior distributions that did not overlap with assemblage two for each of the of each Layman's term to better understand differences among the different site comparisons. I also used the methods of Turner et al. (2010) and Sturbois et al. (2022) to plot and qualitatively describe the path trajectories of each food web guild and primary producers through each watershed. Stable isotope trajectory analysis (Sturbois et al. 2022) is based on a multivariate integration of SIA values across compartments and may provide a more robust and easier to delineate trajectory analysis than the methods of Turner et al. (2010).

In stable isotope studies, $\delta^{13}C$ and $\delta^{15}N$ are often modeled separately due to nondefinite estimates in error matrices (i.e., covariances are difficult to estimate because ranges of values of $\delta^{13}C$ and $\delta^{15}N$ do not overlap), which prevents the use of typical multivariate generalized linear models. In the previous analyses, these issues were resolved by permutations, however, I chose to test my hypotheses following the established process of employing conventional generalized linear models (e.g., Walsh and Tucker 2020). For the Headwaters Hypothesis, a set of candidate generalized linear models and mixed models were constructed with three link-probability distribution combinations (identity-normal, log-quasipoisson, and inverse-Gamma) and with and without year sampled as a random covariable. Fixed effects were watershed, a measure of lateral connectivity, and two measures of vertical connectivity. Lateral connectivity was estimated by the difference between surface and groundwater DOC, and vertical connectivity was estimated by differences in DN and C/N ratios. For the Lower Watersheds and Whole Watersheds hypotheses, fixed effects in the generalized linear models (without year covariable) and mixed models (with year covariable) were watershed and location (headwater or downstream) nested within watershed were employed. For each analyses, model selected for interpretation entailed the lowest AIC and quasi-c-chat closest to one.

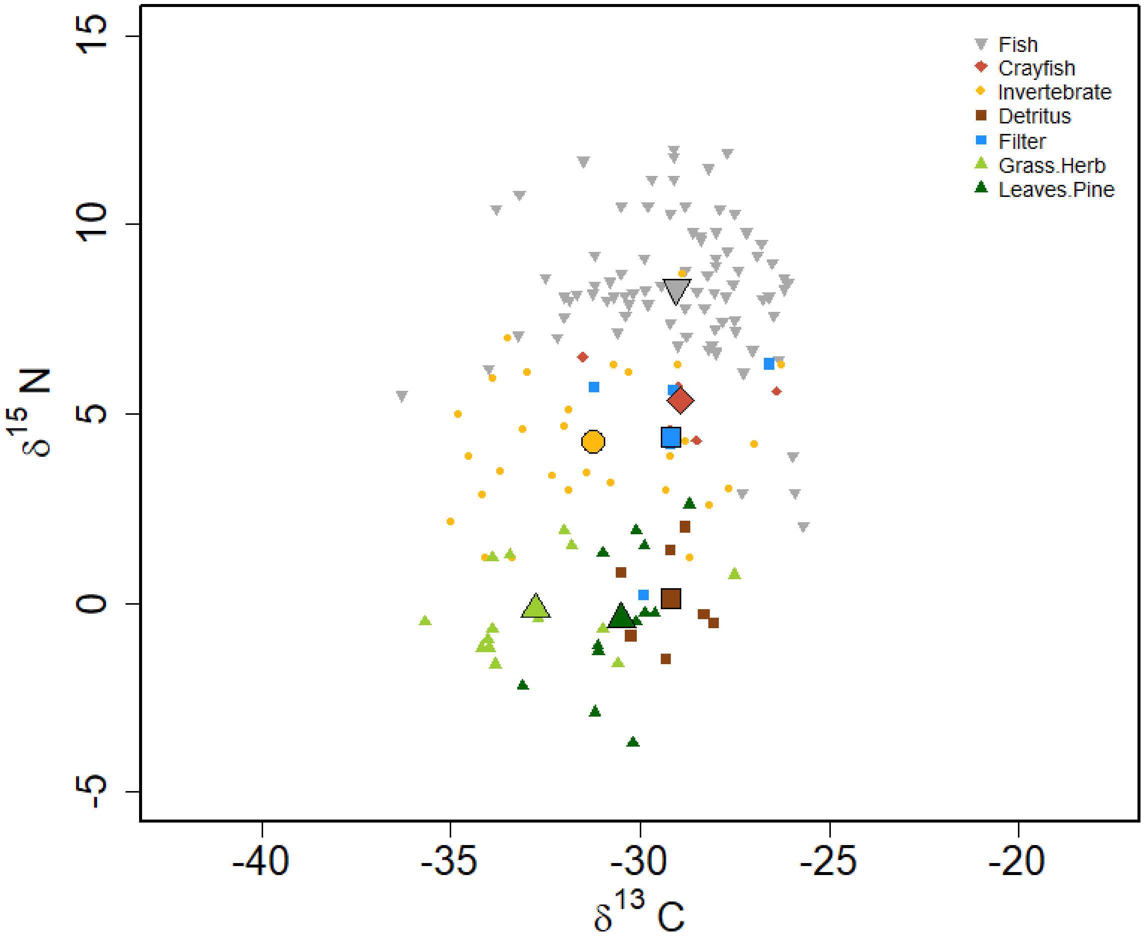


Figure 4.2. The δ^{13}C and δ^{15}N signature biplot depicts individual food-web guilds and primary producers (colors and shapes) for all sites within the more laterally connected Calcasieu River watershed. Larger shapes are the centroids of the guilds and smaller shapes are the individual composite samples.

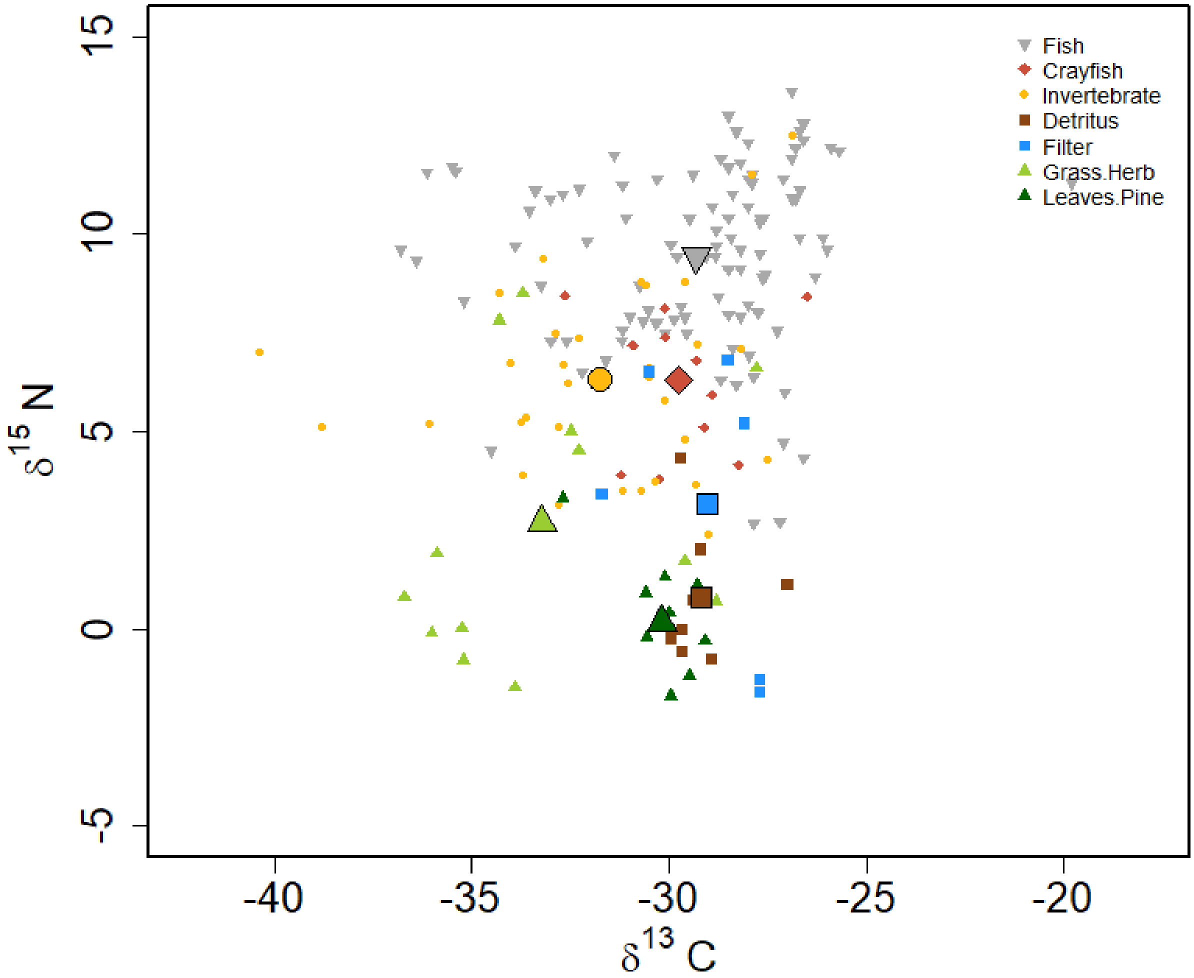


Figure 4.3. East: The δ^{13}C and δ^{15}N signature biplot depicts individual food-web guilds and primary producers (colors and shapes) for all sites within the less laterally connected Tickfaw River watershed. Larger shapes are the centroids of the guilds and smaller shapes are the individual composite samples.

4.3. Results

Overall, differences in the food web structure between the Tickfaw River and Calcasieu River watersheds were obvious (Figures 4.2 and 4.3), with allochthonous and detrital sources having lower δ^{15}N, followed by slightly more enriched values for the filtered samples. Invertebrates tended to be at the same levels as the filtered samples, and as expected, fish were the highest trophic level. There appeared to be some differentiation between herbaceous/grass and leaf samples, but not between these samples and the autochthonous filtered samples. More complicated relationships were evident among streams within watersheds (Table 4.1, Figure 4.4). Variability in $\delta^{13}C$ occurred across different food web compartments (i.e., basal sources, invertebrates, fishes), but a watershed-level pattern was not apparent. In contrast, $\delta^{15}N$ values tended to increase as stream size increased across all food web compartments, with highest values at the Tickfaw river site.

Table 4.2. The results of the SIBER analysis (Jackson et al. 2011) for fish assemblages across different comparisons and Layman's terms (Layman et al. 2007). Layman's terms included $\delta^{15}N$ Range (NR), $d^{13}C$ range (CR), Total area (TA), Mean distance to centroid (CD), Mean nearest neighbor distance (NND), and Standard deviation of (SD_{NND}). Percentages indicate the proportion of the posterior distribution of the first assemblage that do not overlap with the second assemblage. CH = Calcasieu River headwaters. CIR = lower Calcasieu River. TH = Tickfaw River headwaters. TIR = lower and intermediate Tickfaw River. TICK = Tickfaw River watershed. CAL = Calcasieu River watershed.

Assemblage Comparisons	n vs n	NR	CR	TA	CD	NND	SD_{NND}
TH/CH	65/49	62.73%	45.43%	71.68%	67.73%	56.58%	56.38%
TIR/CIR	38/37	85.85%	98.90%	98.05%	98.20%	97.68%	46.55%
TH/TIR	60/37	95.90%	1.80%	37.40%	15.58%	27.80%	60.18%
CH/CIR	46/36	98.83%	77.28%	97.25%	94.08%	97.40%	59.70%
TICK/CAL	98/82	84.98%	99.70%	95.05%	99.25%	93.90%	26.43%

Results of the SIBER analyses (Table 4.2 and Figures 4.1-4.5 and C.1) indicated most assemblages had little overlap in the credible intervals of the six Layman's terms within each comparison, especially in the overall watershed comparison (TICK/CAL, Figure C.2). Headwater (TH/CH, Figure C.3) and Tickfaw River upper/lower watershed (TH/TIR, Figure C.4) comparisons shared more overlap (lower percentages) of Layman's terms than the other comparisons. The Calcasieu River headwater to upper/lower comparison (CH/CIR, Figure C.5) showed less overlap than the Tickfaw River comparison (TH/TIR), especially in the Tickfaw River $\delta^{13}C$ range, and mean distance to centroid differences were only 1.80 and 15.58%, respectively. Additionally, the lower site comparison across watersheds (TIR/CIR, Figure C.1) had less overlap in Layman's terms than the upper site (TH/CH) comparison.

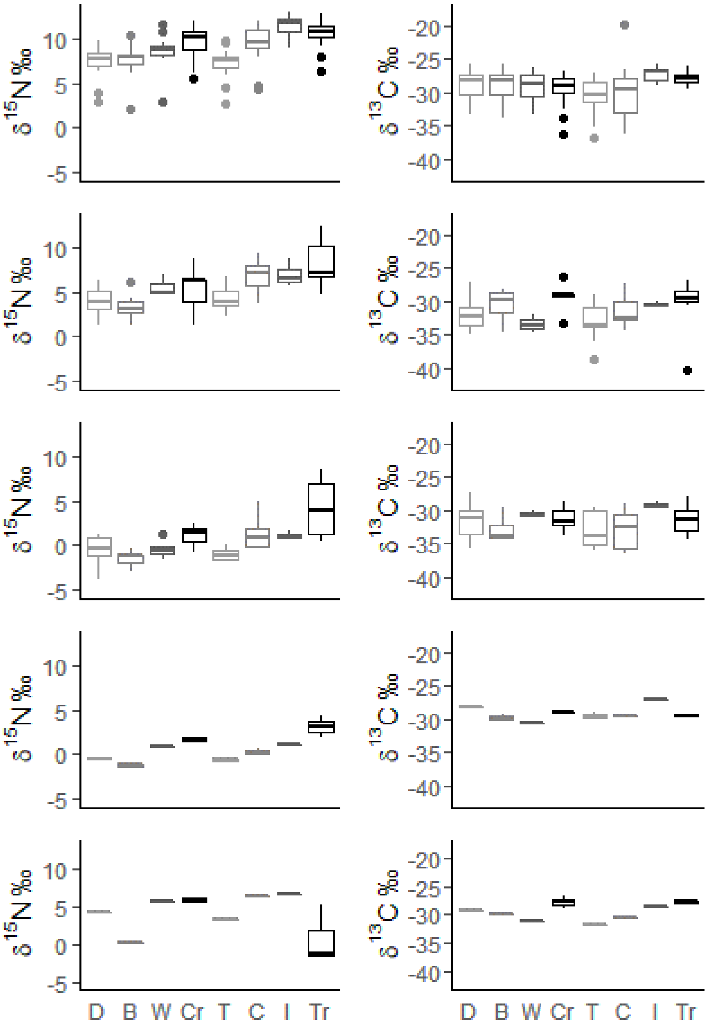


Figure 4.4. Stable isotope box plots of δ^{13}C and δ^{15}N signature for each study sites. Boxes indicate the interquartile range (IQR), the line inside the boxes is the median, and the whiskers outside the box indicate the upper and lower quartile bounds ; points outside whiskers are outliers. From the top row of graphs to bottom: fish, invertebrates, allochthonous sources (grasses, herbacous, leaves, pine needles), detritus, and tiles (autochthanous production) are presented. Lightest gray boxes indicate the stream sites, medium grey are the intermediate sites and black boxes are the river sites. "Drake's Creek" = "D", "Big Creek" = "B", "Calcasieu Int." = "W", "Calcasieu River"= "Cr", "Twelvemile Creek"= "T", "Crittenden Creek"="C", Tickfaw Int." = "I", "Tickfaw River"= "Tr")).

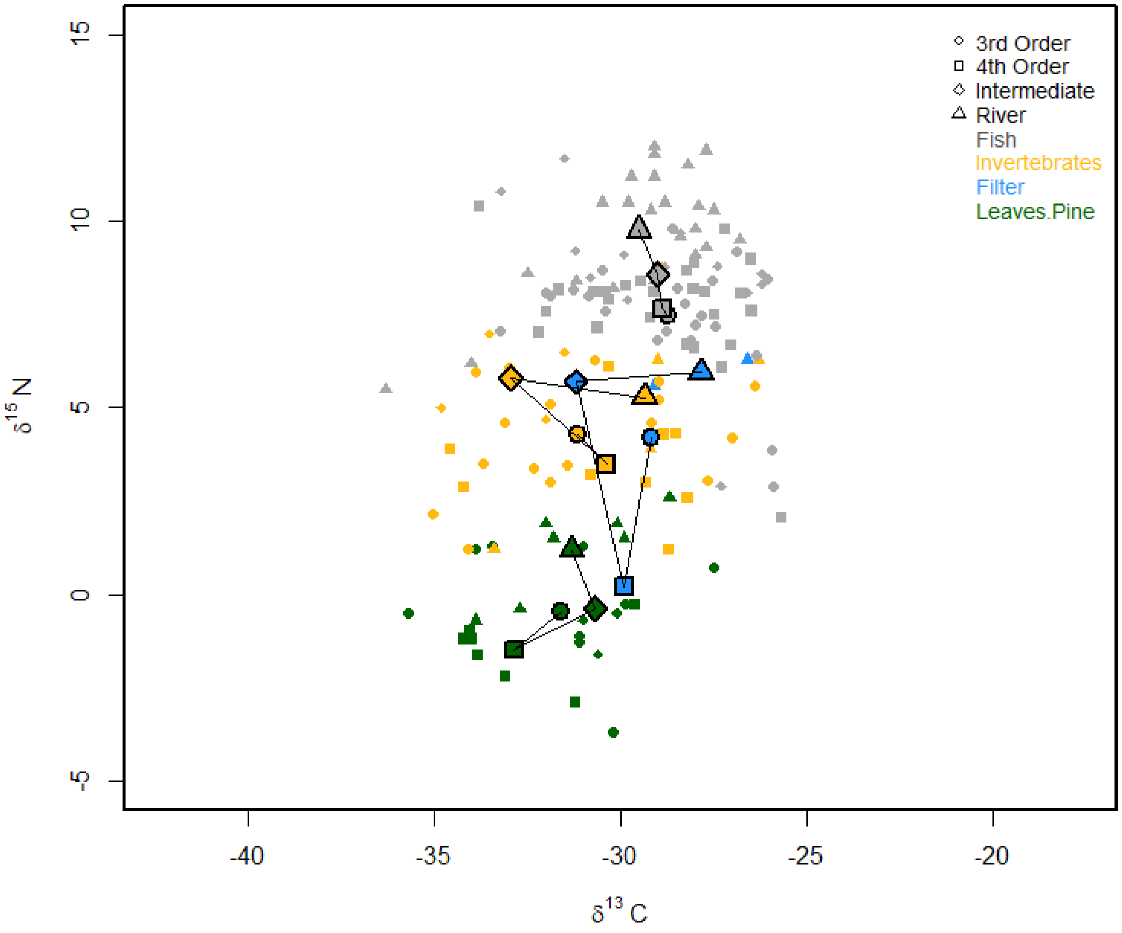


Figure 4.5. The δ^{13}C and δ^{15}N signature trajectory biplot (Turner et al. 2010) of the Calcasieu River watershed. Larger shapes are the centroids of the guild (colors)/site (shapes) groupings and smaller shapes are the individual composite samples.

Overall, the Calcasieu River and Tickfaw River watersheds seem to have little similarity in trajectory paths (Figures 4.5, 4.6, and 4.7). Both watersheds exhibited little overlap among the fish, invertebrates (including crayfish), and even lower food web compartments, filter, and leaves/pine, and although data were highly variable, there appeared to be no obvious patterns in relation to the site location within a watershed that would allude to changes in usage of allochthonous/autochthonous sources (Figures 4.5 and 4.6). SITA results (Figure 4.7) also agree in that the trophic web trajectories differed between watersheds.

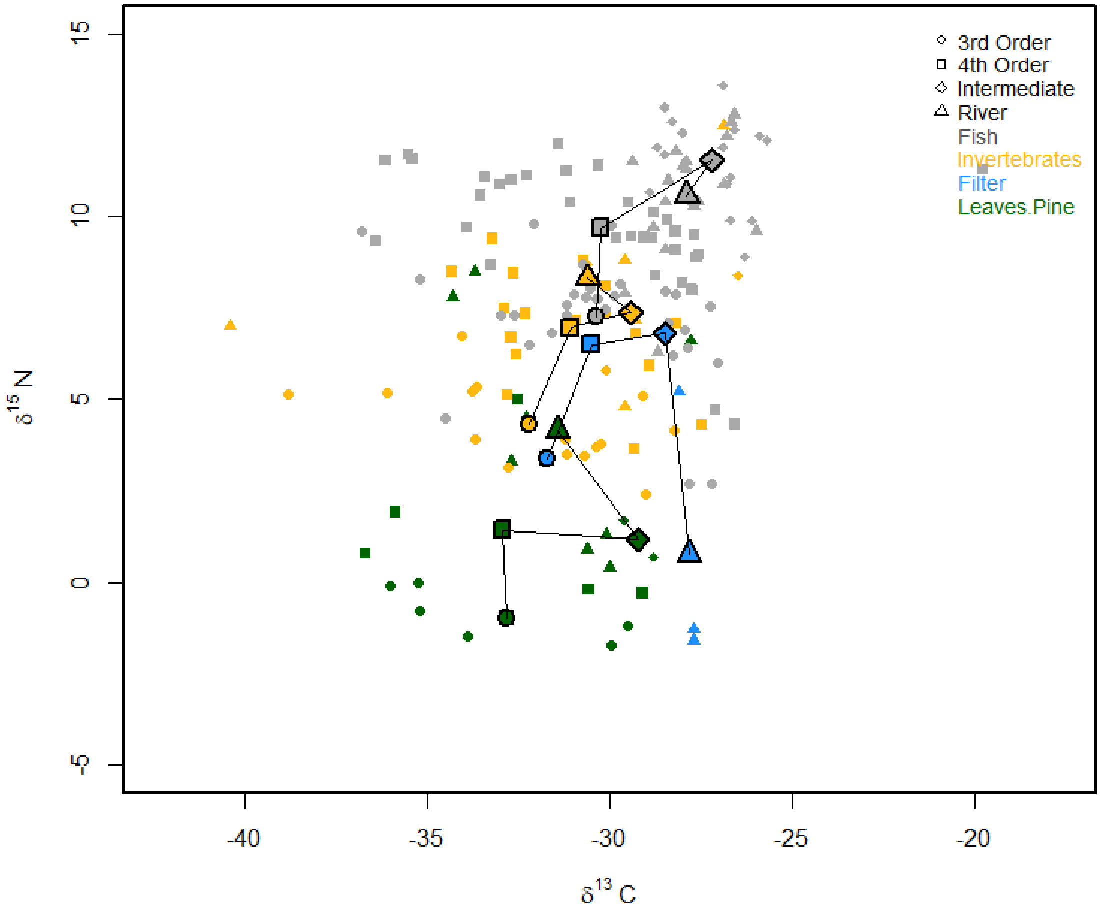


Figure 4.6. The δ^{13}C and δ^{15}N signature trajectory biplot (Turner et al. 2010) of the Tickfaw River watershed. Larger shapes are the centroids of the guild (colors)/site (shapes) groupings and smaller shapes are the individual composite samples.

The GLMs quantified some of the differences in trophic web structure illustrated in the SITA and SIBER analyses. The best fitting model to describe δ^{13}C signatures in headwater streams between watersheds included differences in surface/subsurface DOC, DN, and C:N and watershed (family= Gamma, Quasi ĉ =0.007203, AIC 1010.6), but none of these predictor were significantly different ($P \geq 0.05$; Table 3). For δ^{15}N model, the same predictor variables were also included (family = quasipoisson, Quasi ĉ =0.85); both ΔDOC and ΔDN were important in describing community δ^{15}N signatures ($P<0.05$, Table 4.3) and after back transforming, parameter estimates were 1.04 (SE ±0.02), and 0.93 (SE ±0.03), respectively. Results of the $\delta^{13}C$ model (family = quasipoisson, Quasi ĉ = 0.19) showed that headwater site signatures within the Tickfaw River watershed were significantly more enriched in ^{13}C (back-transformed parameter estimate = 1.09 (SE ±0.04) than the lower Tickfaw River sites, and all Calcasieu River sites. Results of the $\delta^{15}N$ model (family = quasipoisson, Quasi ĉ = 0.91) showed that headwater site signatures within the Tickfaw River as well as the Calcasieu River watersheds were significantly more enriched in ^{15}N [Tickfaw/headwater back-transformed parameter estimate = 0.92 (SE ±0.04); Calcasieu/Headwaters back-transformed parameter estimate = 0.90 (SE ±0.04] than the lower Tickfaw River sites, and all Calcasieu River sites.

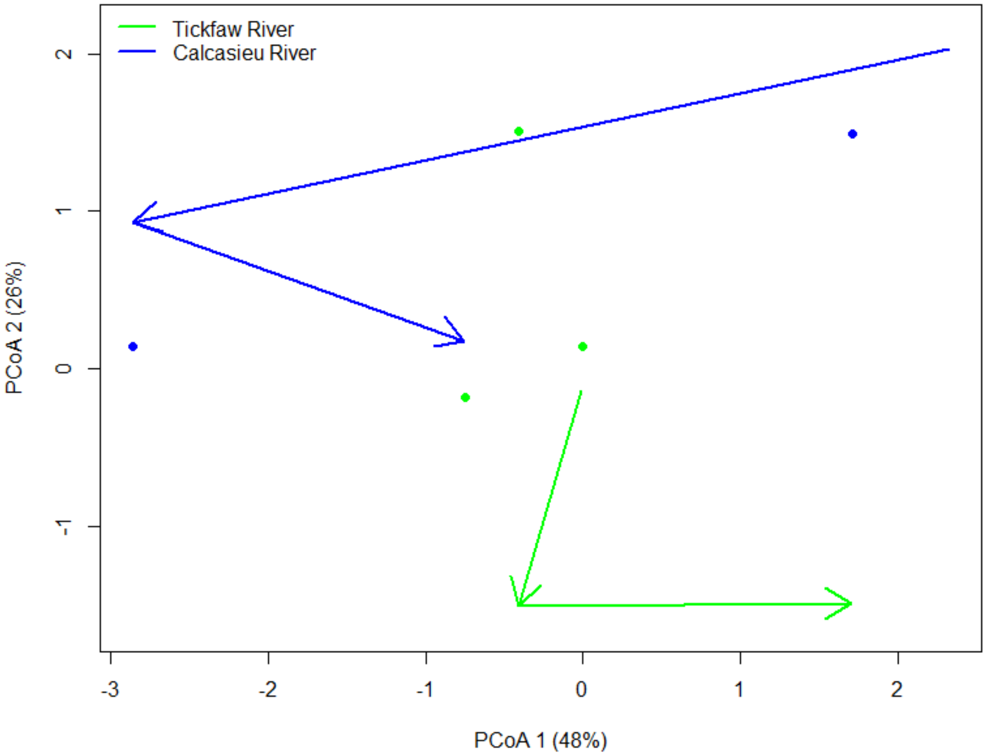


Figure 4.7. The $\delta^{13}C$ and $\delta^{15}N$ signature combined trajectory biplot (Sturbois et al. 2022) of both watersheds. Axes are the first and second principal components (PCoA) from a principal coordinate analysis embedded in the Stable Isotope Trajectory Analysis. Dots are centroids of headwater, intermediate, and lower river sites based on the principal coordinate analysis of Layman's (2007) terms.

Table 4.3. Generalized linear model results of important variables included in the $\delta^{13}C$ (family = Gamma) and $\delta^{15}N$ (family = quasipoisson) community-wide signature models between the Tickfaw River and Calcasieu River headwaters (Headwaters Hypothesis).

Variables Response	 Predictor	Estimate (±Std. Error)	t	P
$\delta^{13}C$ ~	Watershed	-3.73e-04 (±7.13e-04)	-0.52	0.60
	ΔDOC	1.96e-05 (±2.03e-04)	0.10	0.92
	ΔDN	-1.74e-05 (±4.17e-04)	-0.04	0.97
	ΔC:N	1.58e-04 (±2.15e-04)	0.73	0.46
$\delta^{15}N$ ~	Watershed	0.02 (±0.06)	0.28	0.78
	ΔDOC	0.04 (±0.02)	2.38	0.02
	ΔDN	-0.07 (±0.03)	-2.09	0.04
	ΔC:N	0.03 (±0.02)	1.92	0.06

Table 4.4. Generalized linear model results of important variables included in two models (family = quasipoisson) that described community-wide $\delta^{13}C$ and $\delta^{15}N$ signatures between the Tickfaw River and Calcasieu River watersheds with sites nested within (Lower Watersheds and Whole Watersheds Hypotheses).

Variables Response	 Predictor	Estimate (±Std. Error)	t	P
$\delta^{13}C$ ~	Tickfaw	-0.05 (±0.02)	-3.24	0.001
	Calcasieu/Headwater vs Lower	0.01 (±0.01)	0.89	0.37
	Tickfaw/Headwater vs Lower	0.09 (±0.01)	6.31	<0.001
$\delta^{15}N$ ~	Tickfaw	0.09 (±0.04)	2.21	0.03
	Calcasieu/Headwater vs Lower	-0.11 (±0.04)	-3.08	0.002
	Tickfaw/Headwater vs Lower	-0.08 (±0.04)	-2.22	0.027

4.4. Discussion

The Tickfaw River and Calcasieu River watersheds are in relatively close proximity (< 160 km) and are both within subtropical Gulf of Mexico coastal plains ecoregions, yet they differ in food-web structure based on lateral (floodplain) and vertical (subsurface/hyporheic) hydrologic connectivity. Observed SIA patterns partially supported my hypotheses regarding differences in trophic web structure between the selected watersheds attributable to connectivity. However, statistical (SIBER fish assemblages, GLMs for overall community patterns as it relates to DOC, DN, and C:N) and trajectory analyses revealed much more complexity at the site level than expected. Headwater trophic structure appeared to be influenced by both broader biogeographic and geologic patterns and by local hydrologic connectivity, suggesting partial support for the Headwaters Hypothesis. Similarly, data also partially supported the Lower Watersheds Hypothesis in that differences in headwater food-web structure between watersheds would be greater than differences in lower watershed sites. Finally, watershed-level comparisons did provide evidence that lateral connectivity influenced trophic structure with much more distinct trophic patterns evident in the more laterally connected Calcasieu River watershed than in the Tickfaw River watershed. Interestingly, these observations were inconsistent with the River Continuum Concept, as there did not appear to be patterns in assemblages switching from allochthony then autochthony with increasing drainage size or stream order and then back again to allochthony at the river sites.

My first two hypotheses were not supported by the results of the Fish SIA SIBER analyses. Layman's terms of the headwater assemblage comparison of the Tickfaw River and Calcasieu River watersheds showed great overlap in isotopic niche space compared to other site comparisons, indicating strong similarities in the food webs, which was unexpected. Fish assemblages in lower (intermediate and river) sites were more dissimilar than in headwaters between watersheds, which also was surprising. I expected lower sites to be more similar to each other because of similar lateral connections and floodplain allochthonous inputs occurred between watersheds, which should have led to similarly structured food webs in the lower sites (Vannote et al. 1980; Doretto et al. 2020). Although detectable differences in Layman's terms occurred across different assemblage comparisons, results of the trajectory analysis did not show parallel relationships in basal resources and invertebrate structure from headwater to lower sites between watersheds. Overall, these results were unexpected, but other studies of stream and watershed comparisons had varying results as well. Stream comparisons between watersheds in the tropics of India showed trophic structural differences (Mondal and Bhat 2021). Sampled rivers in tropical forests of Mexico had differences in discharge, climate, and riparian forest cover also had differences in food chain lengths (Pease et al. 2019). Within a large subtropical river in China, drastic changes in food web structure occurred especially $\delta^{15}N$ signatures, as a product of anthropogenic effects (Wang et al. 2021). Therefore, my results in subtropical coastal plains suggest more similarity between these systems with other global subtropical systems than with continental temperate systems that were the foundation of the River Continuum Concept.

Headwater site differences in $\delta^{15}N$ were locally modified by lateral and vertical connectivity, reflected by differences in energy source (DOC) and trophic level (DN) between surface and hyporheic measurements. The results suggest that increased lateral connectivity, as reflected by DOC, provides more carbon resources enhancing consumer prey trophic complexity (i.e., more types of consumers, such as scrapers, collectors, and filterers). Additionally, decreased vertical connectivity, as indicated by DN, increases trophic levels potentially through increased and more complicated prey resources (i.e., fish and predatory invertebrates have multiple levels of prey) in streams with less groundwater inputs and more surface (lateral) inputs. Balcombe et al. (2005) also demonstrated that fish diets contained a greater diversity of prey items associated with lateral connectivity. However, those relationships were potentially masked in fishes by larger landscape factors lower in the watersheds such as land use practices and large functional floodplains, or by more generalist fishes that were able to exploit a greater diversity of prey resources, resulting in greater isotope differences among invertebrates. Comparisons of trophic differences between crayfishes and fishes (Figures 2 and 3) were more pronounced in the watershed with greater overall connectivity, and followed the expected pattern of enrichment, as predatory black basses frequently prey on crayfish in lotic systems (Scalet 1977; Sammons 2012; Alford and Heimann 2016). Conversely, in the less connected Tickfaw River watershed, $\delta^{15}N$ values show considerable overlap between crayfishes and fishes, suggesting less connectivity reduces resource partitioning and increases dietary overlap. Although agricultural and other anthropogenic activities can enrich $\delta^{15}N$ in aquatic habitats (Mulholland et al. 2000; Diebel and Vander Zanden 2009; Daniel et al. 2015; Brooks et al. 2019), this is unlikely in this study. Diebel and Vander Zanden (2009) and Daniel et al. (2015) reported that anthropogenic activities elevated $\delta^{15}N$ beginning with autochthonous primary producers and continuing upward through the assemblage. The apparent paucity of autochthonous production in these study streams suggests that $\delta^{15}N$ differences are probably attributable to connectivity.

Obvious differences occurred in the Layman's terms that supported the Whole Watersheds Hypothesis regarding lateral connectivity and longitudinal similarity in trophic web structure. Surprisingly, the headwater to lower watershed comparison demonstrated more similarities (especially $\delta^{13}C$ range and mean distance to centroid) in the Tickfaw River watershed than the Calcasieu River watershed. The similarity in $\delta^{13}C$ range indicates that within the Tickfaw River watershed, the same types of allochthonous and autochthonous materials are being incorporated into food webs along the length of the river. In contrast, there appears to be a switch in carbon sources used in the headwaters compared to the lower Calcasieu River watershed. Importantly, watershed area was lower in the Tickfaw River system, and site locations were much closer to each other than in the Calcasieu River watershed. Differences in carbon range of the fish assemblage in the Calcasieu River may not be due to food-web structural differences but may simply be due to natural variability within allochthonous/autochthonous signatures over the larger spatial scales (Finlay 2004).

Trajectory plots showed substantial variability, even among primary producers, which masked trophic web patterns within a watershed, although δ^{13}C and δ^{15}N GLMs indicated watershed position was the main driver of SIA signature differences with local modifications, at least in the headwaters. Ceramic tiles, which were used to estimate signatures of autochthonous producers, were normally covered by a thick biofilm that included higher trophic levels (i.e., microbes). These organisms are likely obtaining carbon from multiple sources, possibly biasing interpretation of δ^{13}C signatures (Augspurger et al. 2008; Demars et al. 2021). Despite the potential inaccuracy of primary producer signatures, however, the overall analyses did show exhibited demonstrable differences in trophic structure among invertebrates and fishes between sites and watersheds.

Differences in trophic structure between these watersheds were subtle, but this study provided a baseline assessment of food web structure for two Gulf of Mexico coastal plain watersheds. Relative to other temperate stream systems, these coastal plain streams were characterized by lower gradients and finer substrates. As a consequence, it appeared headwater trophic webs resembled their larger intermediate/river sites, even with taxonomic differences (i.e., functional web structure is similar). Several results do not support trophic web predictions of the River Continuum Concept, even considering potential issues with primary producer signatures. For example, planktivorous Gizzard Shad were isolated in resource space from the rest of the food webs at both river sites, suggesting phytoplankton may have different isotopic signatures than the sampled allochthonous sources or other algal sources not sampled, as seen in other studies (Babler et al. 2011; Coulter et al. 2019). Likewise, some omnivorous and predatory fishes appeared to not be directly tied to the allochthonous sources measured. Moreover, as top predators and omnivores, fish tissue reflects assimilation of carbon sources by their prey, making these results surprising given the importance of allochthonous food to invertebrates in these systems (Post 2002; Fry 2006; Hayden et al. 2016).

These results suggest stream and river organisms may be relying on more than two basal sources, i.e., instream algae, and deciduous leaf matter, and are foraging on organisms that are exploiting other carbon (i.e., microbial decomposition). The microbial route is potentially a more important pathway in determining energy fluxes in streams relative to macroinvertebrates (Demars et al. 2021). Additionally, carbon associated with microbial decomposition of peat has been detected in aquatic organisms (Schell 1983), and experimental additions of dissolved organic carbon has fueled microbial pathways and influenced consumer carbon signatures (Robbins et al. 2020). A better understanding of carbon sources and their incorporation into lotic food webs is clearly an area of needed research (Guo et al. 2016).

4.4.1.Conclusion

This study provided some evidence that hydrologic connectivity can shape trophic structure in coastal plain watersheds. At the local scale, headwater reaches were more influenced by both lateral and vertical connectivity, and watershed-wide expectations were modified by local conditions. Downstream sites continued to show differences associated with connectivity with lateral connectivity appearing to play an important role throughout the watershed. Given expected changes to coastal freshwaters systems, these results will provide a baseline and a method for assessing and mitigating change.

Chapter 5. Discussion

In this dissertation, I studied relationships of lateral, vertical, and longitudinal hydrologic connectivity to an individual species (*Margaritifera hembeli*), macroinvertebrate food-webs in headwaters, and aquatic community food webs throughout watersheds. Results of my research into vertical (Chapter 2), lateral and vertical (Chapter 3), and longitudinal (Chapter 4) hydrologic connectivity influences on species and food webs are discussed in the following sections. I finish with addressing how climate change may directly affect subtropical coastal plains watersheds of Louisiana and other regions, and possible management solutions.

My Chapter Two entailed identifying critical characteristics of vertical connectivity, fish assemblages, sediment size, and other factors pertinent to maintaining Louisiana Pearlshell Mussel (*M. hembeli*) populations. Assessing vertical connectivity of shallow groundwater was difficult in this study. Alternative solutions to detect shallow groundwater input to streams is needed, such as permanent temperature loggers, recording through time might allow for a clearer picture of groundwater input (Baskaran et al. 2009; Krause et al. 2012). Long term monitoring of another indicator organism associated with of groundwater input i.e., Lamprey (Petromyzonidae; Limm and Power 2011) could be useful with future climate change. My data suggested alternative streams that may be potential translocations sites, but a deeper understanding of the groundwater relationships in Kisatchie National Forest that harbors populations of *M. hembeli* is needed.

The lateral (floodplain) connectivity detected in the study streams in Chapter Three appeared to show trends with some selected macroinvertebrate functional feeding guilds (FFGs) based on stable isotope analyses (δ13C and δ15N). Interestingly, there appeared to be no apparent patterns between the two watersheds, even with the known Mississippi River biogeographical barrier (Howden 1969; Swift et al. 1985; Connor and Suttkus 1986). Collector/filterers, collector/gatherers, shredder/detritovores, and gastropod scrapers were associated with specific flooding regimes, while insect scrapers and shredders did not show any relationship with flooding regimes and differed in their δ13C signatures from other FFGs. This may indicate more insect scrapers are reliant on an unsampled basal source, and allochthonous leaf material was available year-round to shredders.

The results of Chapter Four indicated strong differences in food web structure and longitudinal connectivity between the Tickfaw River and Calcasieu River watersheds. However, these results appeared to support my hypotheses only partially in this chapter. It was surprising that the headwater sites tended be more similar between watersheds than the similarity of sites in the lower reaches of the two watersheds based on stable isotope values (δ^{13}C and δ^{15}N). In headwaters, greater lateral and lesser vertical connectivity appeared to provide more types of δ13C sources and more complex (i.e., more enriched δ15N suggesting more trophic levels; Post 2002) prey resources, which may have overwhelmed expected geographic differences in trophic structure. However, at lower reaches, differences in δ13C sources between the watersheds were more pronounced, resulting in greater differences between them.

Overall, these results provided some important insights into the trophic ecology of subtropical coastal plain systems. Insects seem to have a looser relationship with their FFGs, which may be a product of a wider variety of basal source available as has been noted in acid streams (Dangles 2000). The microbial compartment of these food webs seems to be very important in cycling energy to higher trophic levels (i.e., crayfish and fishes), and insects may be less important in these food webs compared to temperate regions. Crayfish seem to have an important role in the food webs because they occupy similar ranges of δ13C of fishes. In other stream, pond and lake studies, crayfish can play an important role as a keystone energy transformer' (France 1996; Nyström and Strand 1996; Parkyn et al. 2001; Ollson et al. 2008). Thus, although there are unaccounted basal sources, mainly algae, there still seems to be a disconnected food web between insects and crayfish/fishes.

An added layer of complexity in understanding lotic food webs arises when climate change is predicted to have rapid effects in the near future (Woodward et al. 2010; Hobday and Lough 2011) is considered. Two major ways in which climate change will affect freshwater ecosystems includes the actual temperature rise of water itself, and alterations in precipitation that will ultimately affect flow regimes. Global scale assessments of altered flow regimes are limited because of lack of research (Doll and Zhang 2010), but there is a growing body of research pertaining to current negative anthropogenic impacts on altered flow regimes (Bunn and Arthington 2002; Palmer and Ruhi 2019; Poff and Zimmerman 2010). In the case of subtropical coastal plains, many “Big Squeeze” effects are expected to occur including sea level rise, and saltwater intrusion that will push biota north/upstream (Nicholls and Cazenave 2010; Inman, M. 2011; Ferguson and Gleeson 2012; Glick et al. 2013). In oceanic deltas, such as the Mississippi River Delta, relative sea-level rise will accelerate as compared to other coastal lands due the present sediment being compacted by new sediment laid. Furthermore, subtropical coastal plains systems may not be as reactive to disturbance as temperate regions because of high disturbance history (hurricanes, droughts, and other tropical storms), but those events are expected to increase.

Climate change will have varying effects on different compartments of subtropical food webs. A 4°C increase in water temperature may shift thermal regimes of current ecosystem north 680 kilometers (422 miles) (Poff et al. 2002). Freshwater mussels, like *M. hembeli*, are relatively sessile organisms, which means understanding temperature thresholds is key for the conservation of single species. Decreased runoff could mean decreased water levels, potentially removing the littoral zone, productive nursery grounds, and perched floodplains disconnecting important allochthonous sources impacting macroinvertebrates and fishes. Extreme and unpredictable weather patterns leading to more and higher magnitude spates will likely transform streams geomorphologically, disrupted primary production (more scouring spates) and disrupt food-web dynamics (Poff et al. 2002). Food chain lengths may be shortened, making these stream ecosystems more vulnerable to other future anthropogenic effects (Sabo et al. 2010). Fish that use floods as ques to spawn might have a phenological mismatch of critical ontogenetic resources (Lytle and Poff 2004).

5.1.1. Conclusions and Management Directions

The management of subtropical coastal plain watersheds should have a climate change focus, factoring in changes to flow regimes. Resource managers need to continue better understand the natural flow regime in these systems, in order to designate the minimum flow required to maintain ecosystem services (Poff et al., 2002; Whipple and Viers 2019). I provided two suggestions to monitor and manage subtropical coastal plain watersheds of Louisiana and other regions. I focused on macroinvertebrate and fish community level patterns among study sites and watersheds, but future research could focus on specific taxonomic groups, such as *Micropterus spp.* or crayfish, throughout a study watershed to act as a monitoring tool to better understand future climate change impacts. Likewise, focusing on Lamprey (Petromyzontidae) as an indicator of shallow groundwater connectivity and appropriate mussel streams, could be useful when planning translocations of sessile organisms, such as imperiled mussels, in light of future climate change. Further, the installation of more stream loggers over longer periods of time provide more accurate hydrographs and allow for detection of changes in flow regimes. My research can act as a baseline for some of these different fish, insects, and ecosystems so that we may better understand future anthropogenic affects via disruptions in stable isotope signatures caused by disturbances (e.g., ‘Trophometer’ as described in Fry 2006; Alp and Cucherousset 2022).

Appendix A. Supplemental Data for Chapter 2

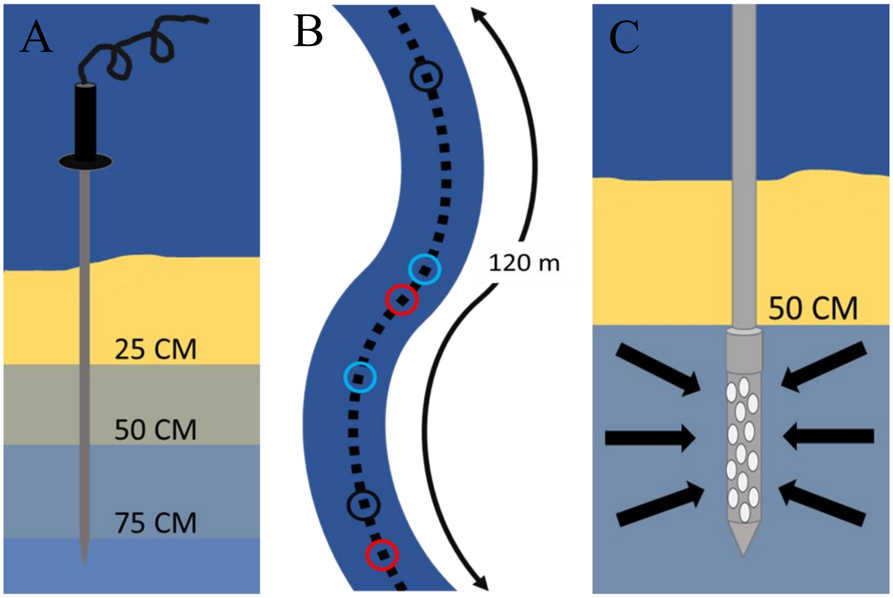


Figure A.1. A) Vertical profile of streambed in which temperatures (°C) at 25, 50, and 75 cm hyporheic depth were recorded; B) planar view of study stream in which temperatures (°C) at 25, 50, and 75 cm hyporheic depths were recorded at 40 sites (black squares) every 3 m within study streams and circled sites for warm (red) and cool (blue) anomalies or no change (black) sites were selected for C) piezometer sampling of water in which pH, dissolved oxygen (mg/L) and specific conductivity (mS/cm) were recorded.

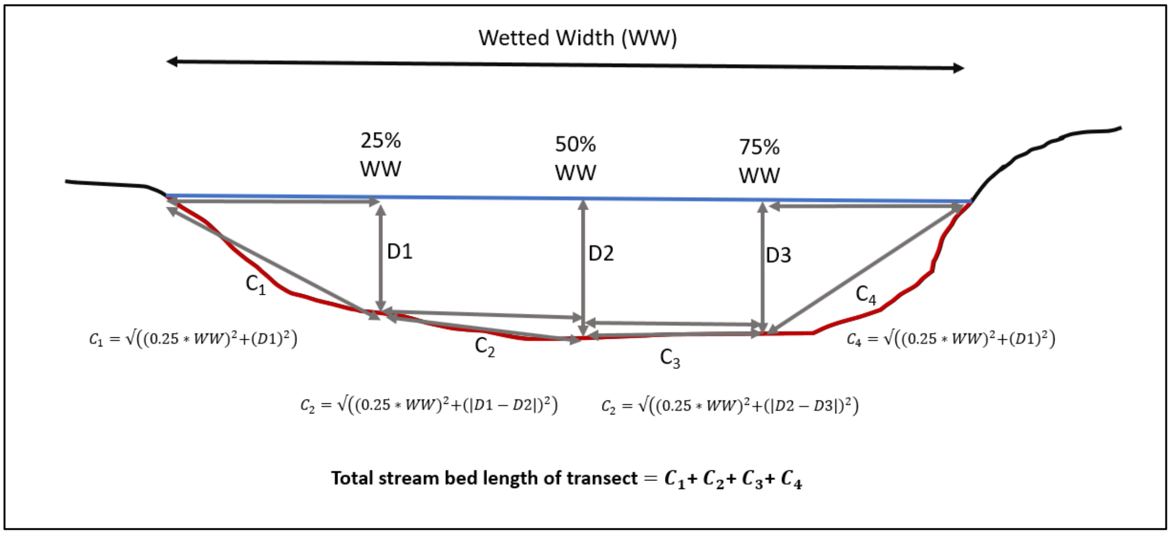


Figure A.2. The measurements and equations to calculate the ratio of Wetted Width (m) to stream bed length (m) for 15 transects per study stream.

Table A.1. The corrected abundances (CPUE) for each fish species across study streams.

Stream	Total	Beaver Creek	Black Creek	Cress Creek	Trib. of Cress Creek	Greys Creek	Trib. of Greys Creek
Ameiurus natalis	10	0	1	2	1	0	1
Aphredoderus sayanus	53	0	6	0	4	6	11
Cyprinella venusta	22	10	0	0	0	0	0
Erimyzon oblongus	26	0	2	5	4	3	4
Erimyzon sucetta	1	0	0	0	0	0	0
Elassoma zonata	2	0	0	0	0	0	0
Etheostoma artesiae	13	4	2	3	2	0	0
Etheostoma chlorosomum	6	0	0	0	0	0	0
Etheostoma histrio	3	0	0	0	0	0	0
Etheostoma proeliare	3	0	0	0	0	0	0
Etheostoma stigmaeum	1	0	0	0	0	1	0
Etheostoma whipplei	24	0	0	0	0	13	4
Essox americanus	6	0	1	0	2	0	0
Essox niger	1	0	0	0	0	0	0
Fundulus spp.	78	0	0	0	0	12	47
Fundulus olivaceus	253	38	22	49	32	25	0
Fundulus notatus	7	6	0	0	0	0	0
Gambusia affinis	44	10	1	7	0	16	0
Ichthyomyzon gagei	71	8	4	0	32	3	0
Lepomis cyanellus	4	0	0	0	0	4	0
Lepomis gulosus	32	0	1	0	0	4	6
Lepomis macrochirus	81	0	0	1	1	20	20
Lepomis marginatus	121	0	21	8	19	1	26

(table cont'd.)

Table A.1. The corrected abundances (CPUE) for each fish species across study streams.

Stream	Total	Beaver Creek	Black Creek	Cress Creek	Trib. of Cress Creek	Greys Creek	Trib. of Greys Creek
Lepomis megalotis	90	4	7	20	23	0	0
Lepomis miniatus	47	4	3	0	1	0	0
Lepomis spp	19	0	2	12	1	3	1
Luxilus chrysocephalus	257	11	24	7	71	36	39
Lythrurus fumeus	12	0	0	0	4	0	0
Lythrurus umbratilis	62	2	5	7	2	12	14
Micropterus salmoides	3	0	0	0	0	0	3
Micropterus punctulatus	16	0	0	0	0	4	1
Minytrema melanops	3	0	0	0	0	0	0
Moxostoma poecilurum	21	0	0	0	0	0	0
Notropis chalybeus	2	0	0	0	0	0	0
Notropis texanus	1	0	0	0	0	1	0
Notropis volucellus	6	0	0	0	0	0	0
Notropis spp	1	0	0	0	0	0	0
Notorus funebris	1	0	1	0	0	0	0
Noturus gyrinus	1	0	0	1	0	0	0
Noturus nocturnus	11	0	0	0	0	0	0
Noturus phaeus	163	23	19	2	27	25	14
Percina maculata	1	0	0	0	0	0	0
Percina nigrofasciata	1	0	0	0	0	0	0
Percina sciera	7	0	0	0	0	0	0
Semotilus atromaculatus	52	10	25	0	4	6	7

(table cont'd.)

Table A.1. The corrected abundances (CPUE) for each fish species across study streams.

Stream	Valentine	Hospital Bayou	Bayou Clear	Birds Creek	Big Brushy	Drakes Creek	East Fork of Six-mile	West Fork of Six-mile
Ameiurus natalis	1	0	0	1	1	0	0	0
Aphredoderus sayanus	1	0	4	5	7	4	1	2
Cyprinella venusta	0	0	0	8	0	3	0	2
Erimyzon oblongus	0	6	0	0	0	0	0	0
Erimyzon sucetta	0	0	0	0	0	0	0	1
Elassoma zonata	0	0	0	0	0	0	1	2
Etheostoma artesiae	2	0	0	0	0	0	0	0
E. chlorosomum	1	0	0	2	2	2	0	0
Etheostoma histrio	3	0	0	0	0	0	0	0
Etheostoma proeliare	0	0	0	1	0	1	0	1
Etheostoma stigmaeum	0	0	0	0	0	0	0	0
Etheostoma whipplei	4	3	0	0	0	0	0	0
Essox americanus	0	1	1	0	0	0	0	0
Essox niger	0	0	0	0	0	0	1	0
Fundulus spp.	12	0	7	0	0	0	0	0
Fundulus olivaceus	0	14	6	6	21	5	6	6
Fundulus notatus	0	0	1	0	0	0	0	0
Gambusia affinis	0	4	0	5	0	0	0	0
Ichthyomyzon gagei	0	0	4	5	1	5	3	2
Lepomis cyanellus	0	0	0	0	0	0	0	0
Lepomis gulosus	7	1	7	1	1	0	0	0
Lepomis macrochirus	16	1	0	0	0	0	0	0
Lepomis marginatus	1	13	16	1	0	5	6	1

(table cont’d.)

Table A.1. The corrected abundances (CPUE) for each fish species across study streams.

Stream	Valentine	Hospital Bayou	Bayou Clear	Birds Creek	Big Brushy	Drakes Creek	East Fork of Six-mile	West Fork of Six-mile
Lepomis megalotis	7	0	0	15	8	1	1	3
Lepomis miniatus	4	0	0	2	9	2	2	2
Lepomis spp	0	0	0	0	0	0	0	0
Luxilus chrysocephalus	6	14	33	0	0	0	0	0
Lythrurus fumeus	0	0	1	4	0	2	0	1
Lythrurus umbratilis	0	0	10	2	5	1	2	0
Micropterus salmoides	0	0	0	0	0	0	0	0
Micropterus punctulatus	2	3	1	0	1	1	0	0
Minytrema melanops	0	0	0	0	0	0	1	0
Moxostoma poecilurum	8	3	4	0	0	1	0	1
Notropis chalybeus	2	0	0	0	0	0	0	0
Notropis texanus	0	0	0	0	0	0	0	0
Notropis volucellus	0	0	0	1	1	3	0	2
Notropis spp	0	0	0	1	0	0	0	0
Notorus funebris	0	0	0	0	0	0	0	0
Noturus gyrinus	0	0	0	0	0	0	0	0
Noturus nocturnus	0	0	0	7	1	1	0	1
Noturus phaeus	11	27	12	0	0	0	0	0
Percina maculata	0	0	0	0	0	1	0	0
Percina nigrofasciata	0	0	0	0	1	0	0	0
Percina sciera	3	1	0	1	1	0	0	1
Semotilus atromaculatus	0	0	0	0	0	0	0	0

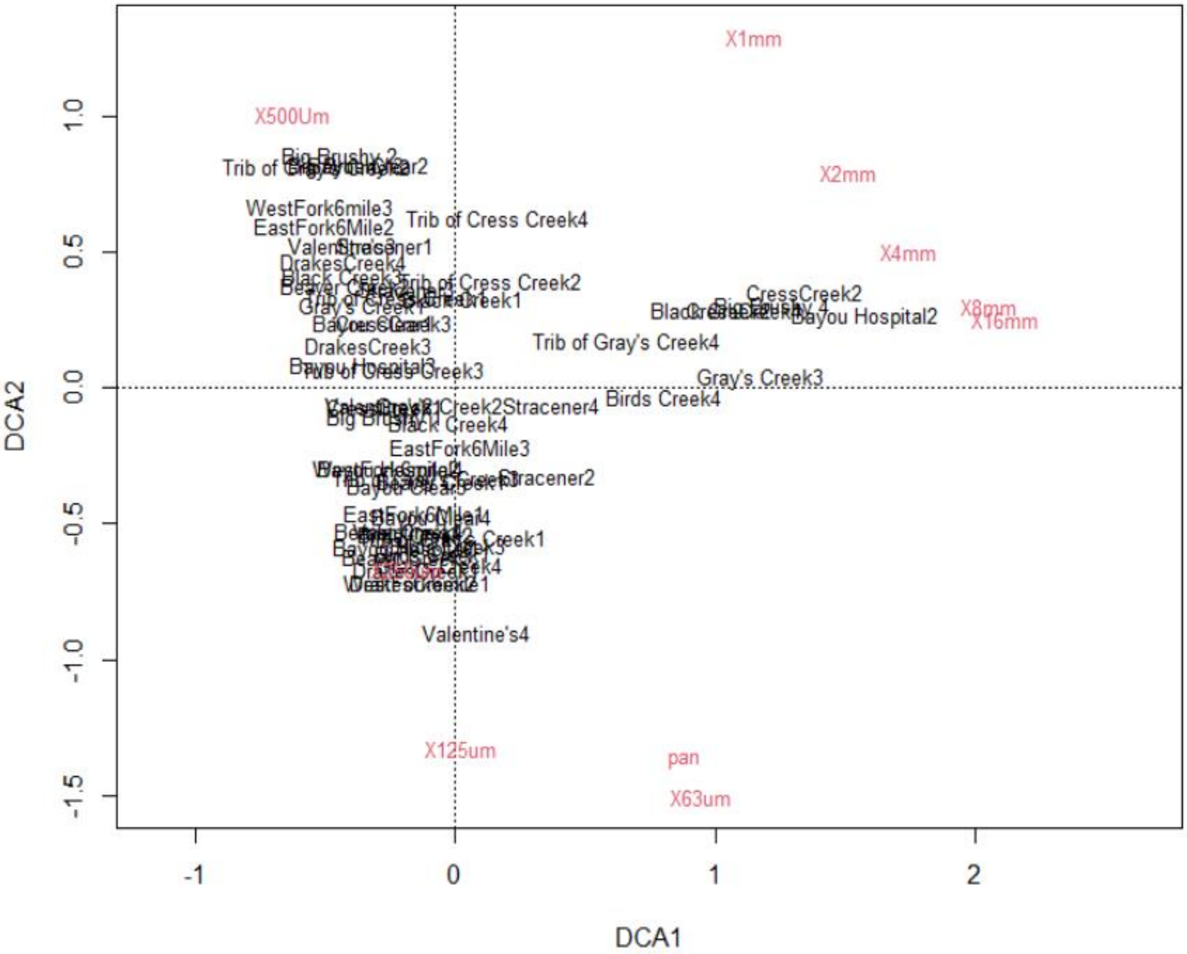


Figure A.3. The results of the DCA analysis on the different stream sites and sediment sizes.

Table A.2. Averages (± standard errors) of sediment, geomorphology, and GIS variables are presented below. Occupied streams are those that contain Margaritifera hembeli paired with five unoccupied streams within the geographical range of M. hembeli. Five additional streams were selected outside the geographical range of *M. hembeli* to determine if they were suitable for *M. hembeli*. Sediment variables are based on the proportion of that sediment size over all sediment sizes (percentage); greater than or equal to (≥) and less than or equal to (≤) indicate the cumulative proportion of all sediments sizes that are above or below, respectively, the sediment size.

Study Stream	Sediment			Streambed Complexity	Wetted Width (m)	Riffle Count
	≥ % 8 mm	% 1mm + % 0.5 mm	≤ % 0.25 mm			
Occupied	***2.84 (±1.51)***	***38.39 (±5.12)***	***55.8 (±6.51)***	***0.97 (±0)***	***3.5 (±0.38)***	***0.6 (±0.36)***
Bayou Clear	0.62 (±0.14)	43.38 (±16.19)	53.16 (±16.92)	0.97 (±0.01)	2.83 (±0.19)	0.2 (±0.14)
Beaver Creek	1.33 (±1.23)	23.85 (±13.07)	73.92 (±12.96)	0.99 (±0.01)	2.89 (±0.24)	0.6 (±0.16)
Trib. of Cress Creek	3.52 (±2.79)	54.59 (±4.55)	35.89 (±6.96)	0.97 (±0.01)	3.05 (±0.16)	0.2 (±0.11)
Trib. of Grey's Creek	8.45 (±5.99)	36.28 (±17.54)	50.74 (±16.96)	0.96 (±0.01)	3.96 (±0.25)	0 (±0)
Valentine Creek	0.29 (±0.11)	33.84 (±15.64)	65.28 (±15.58)	0.97 (±0)	4.76 (±0.13)	2 (±0.17)
Unoccupied	**13.03 (±2.67)**	**32.58 (±3.45)**	**45.84 (±4.42)**	**0.97 (±0.01)**	**3.11 (±0.16)**	**0.43 (±0.15)**
Black Creek	9.12 (±6.42)	42.25 (±10.28)	37.94 (±8.29)	0.98 (±0)	3.11 (±0.22)	0.87 (±0.24)
Cress Creek	21.19 (±12.15)	31.35 (±8.54)	34.59 (±11)	0.99 (±0.01)	2.99 (±0.13)	0.07 (±0.07)
Grey's Creek	7.32 (±6.83)	27.46 (±11.12)	55.37 (±12.49)	0.98 (±0)	3.7 (±0.29)	0.13 (±0.09)
Hospital Bayou	17.47 (±17.43)	23.44 (±9.59)	56.3 (±17.24)	0.98 (±0.01)	3.03 (±0.2)	0.47 (±0.13)
Stracener Branch	10.07 (±3.96)	38.4 (±14.14)	45 (±9.29)	0.93 (±0.01)	2.72 (±0.35)	0.6 (±0.19)
Out-of-Region	**3.65 (±2.12)**	**35.76 (±7)**	**56.85 (±8.3)**	**0.96 (±0)**	**5.15 (±0.34)**	**0.36 (±0.14)**
Big Brushy Creek	10.68 (±10.46)	56.09 (±18.5)	25.76 (±13.21)	0.96 (±0.01)	4.39 (±0.33)	0 (±0)
Bird's Creek	6.48 (±6.05)	12.83 (±0.94)	74.19 (±10.44)	0.98 (±0.01)	6.37 (±0.42)	0.73 (±0.21)
Drake's Creek	0.41 (±0.16)	31.78 (±16.85)	67.06 (±17.23)	0.95 (±0.01)	5.28 (±0.29)	0.33 (±0.13)
East Fork of Six-Mile	0.59 (±0.32)	37.9 (±16.72)	57.68 (±15.25)	0.96 (±0.01)	4.96 (±0.32)	0.09 (±0.08)
West Fork of Six-Mile	0.09 (±0.05)	40.18 (±17.57)	59.58 (±17.49)	0.96 (±0.01)	4.76 (±0.29)	0.64 (±0.21)
Total	***6.51 (±1.69)***	***35.57 (±5.11)***	***52.83 (±3.77)***	***0.97 (±0)***	***3.92 (±0.29)***	***0.46 (±0.13)***

(table cont'd.)

Table A.2. Averages (± standard errors) of sediment, geomorphology, and GIS variables are presented below. Occupied streams are those that contain Margaritifera hembeli paired with five unoccupied streams within the geographical range of M. hembeli. Five additional streams were selected outside the geographical range of *M. hembeli* to determine if they were suitable for *M. hembeli*. Sediment variables are based on the proportion of that sediment size over all sediment sizes (percentage); greater than or equal to (≥) and less than or equal to (≤) indicate the cumulative proportion of all sediments sizes that are above or below, respectively, the sediment size.

Study Stream	Stream Slope	Watershed Area (km^2)	Drainage Density (km^{-1})	Ratio of UAA to Stream length
Occupied	**3.3 (±0.42)**	**16.81 (±5.86)**	**3.94 (±0.2)**	**125.53 (±6.38)**
Bayou Clear	3.07	13.42	4.46	109.51 (±0.33)
Beaver Creek	3.00	29.67	3.90	125.2 (±0.83)
Trib. of Cress Creek	2.05	5.85	4.34	113.37 (±1.31)
Trib. of Grey's Creek	3.85	3.59	3.49	140.75 (±3.36)
Valentine Creek	4.52	31.50	3.51	138.82 (±0.95)
Unoccupied	**2.72 (±0.24)**	**11.1 (±3.87)**	**3.71 (±0.44)**	**140.31 (±19.91)**
Black Creek	1.81	26.21	2.29	213.64 (±0.86)
Cress Creek	2.99	5.20	4.55	107.75 (±0.79)
Grey's Creek	3.15	8.12	3.77	129.28 (±0.18)
Hospital Bayou	2.78	5.96	3.29	147.02 (±3.58)
Stracener Branch	2.85	10.00	4.65	103.87 (±0.91)
Out-of-Region	**3.33 (±0.5)**	**47.02 (±4.9)**	**2.7 (±0.32)**	**191.05 (±20)**
Big Brushy Creek	2.56	28.29	2.88	170 (±2.51)
Bird's Creek	5.02	56.30	2.60	188.72 (±0.05)
Drake's Creek	3.67	52.44	3.81	128.79 (±0.67)
East Fork of Six-Mile	3.28	50.77	2.12	232 (±0.13)
West Fork of Six-Mile	2.14	47.29	2.08	235.74 (±2.43)
Total	***3.12 (±0.23)***	***24.97 (±4.97)***	***3.45 (±0.23)***	***152.3 (±11.66)***

Table A.3. Shallow groundwater connectivity averages (± standard errors) are presented below. Occupied streams are those that contain *Margaritifera hembeli* paired with five unoccupied streams within the geographical range of *M. hembeli*. Five additional streams were selected outside the geographical range of *M. hembeli* as possible translocation sites. Delta (Δ) values indicate the absolute difference between stream water and water extracted from different depths (25, 50 and 75 cm) within the hyporheic zone.

Study Stream	Δ 25 cm (°C)	Δ 50 cm (°C)	Δ 75 cm (°C)	Δ DO (mg/L)	Δ SpCo (µS/cm)	Δ pH
Occupied	**2.33 (±0.38)**	**3.49 (±0.49)**	**4.21 (±0.46)**	**6.32 (±0.85)**	**0.48 (±0.16)**	**0.45 (±0.11)**
Bayou Clear	2.37 (±0.12)	3.79 (±0.1)	4.5 (±0.12)	-	0.12 (±0.05)	0.57 (±0.07)
Beaver Creek	2.08 (±0.14)	3.58 (±0.16)	4.15 (±0.18)	8.06 (±0.3)	0.47 (±0.14)	0.79 (±0.1)
Trib. of Cress Creek	2.65 (±0.28)	4.11 (±0.15)	4.83 (±0.06)	5.63 (±0)	1.08 (±0)	0.29 (±0)
Trib. of Grey's Creek	1.11 (±0.08)	1.59 (±0.1)	2.5 (±0.11)	7.64 (±0.29)	0.29 (±0.09)	0.46 (±0.16)
Valentine’s Creek	3.45 (±0.13)	4.38 (±0.09)	5.08 (±0.03)	3.94 (±1.17)	0.45 (±0.16)	0.14 (±0.16)
Unoccupied	**2.78 (±0.42)**	**3.44 (±0.49)**	**4.24 (±0.77)**	**6.15 (±0.74)**	**0.24 (±0.07)**	**0.59 (±0.22)**
Black Creek	1.37 (±0.29)	1.79 (±0.13)	1.9 (±0.13)	7.56 (±0.13)	0.41 (±0.04)	0.23 (±0.09)
Cress Creek	4 (±0.44)	4.5 (±0.22)	6.5 (±0.2)	7.83 (±0.26)	0.38 (±0.18)	1.31 (±0.1)
Grey's Creek	2.73 (±0.28)	3.11 (±0.15)	3.9 (±0.17)	6.45 (±0.76)	0.17 (±0.05)	0.03 (±0.02)
Hospital Bayou	2.7 (±0.22)	3.45 (±0.24)	3.73 (±0.24)	3.99 (±0.67)	0.06 (±0.01)	0.75 (±0.07)
Stracener Branch	3.13 (±0.25)	4.36 (±0.26)	5.14 (±0.3)	4.94 (±0.62)	0.16 (±0.02)	0.65 (±0.06)
Out-of-Region	**2.21 (±0.32)**	**3.01 (±0.4)**	**3.69 (±0.47)**	**6.84 (±0.22)**	**0.32 (±0.07)**	**1.39 (±0.47)**
Big Brushy Creek	2.23 (±0.13)	3.06 (±0.15)	3.39 (±0.21)	7.21 (±0.42)	0.36 (±0.07)	0.13 (±0.36)
Bird's Creek	2.08 (±0.13)	3.8 (±0.13)	5.15 (±0.13)	6.37 (±1.75)	0.45 (±0.14)	0.54 (±0.2)
Drake's Creek	3.08 (±0.11)	3.78 (±0.1)	4.15 (±0.08)	6.8 (±0.37)	0.46 (±0.18)	1.58 (±0.12)
East Fork Six-Mile	2.54 (±0.34)	2.79 (±0.15)	3.42 (±0.13)	7.47 (±0.24)	0.22 (±0.05)	2.65 (±0.22)
West Fork Six-Mile	1.15 (±0.09)	1.63 (±0.1)	2.33 (±0.16)	6.35 (±0.15)	0.12 (±0.05)	2.05 (±0.14)
Total	***2.46 (±0.26)***	***3.39 (±0.2)***	***4.13 (±0.22)***	***6.48 (±0.85)***	***0.3 (±0.12)***	***0.85 (±0.34)***

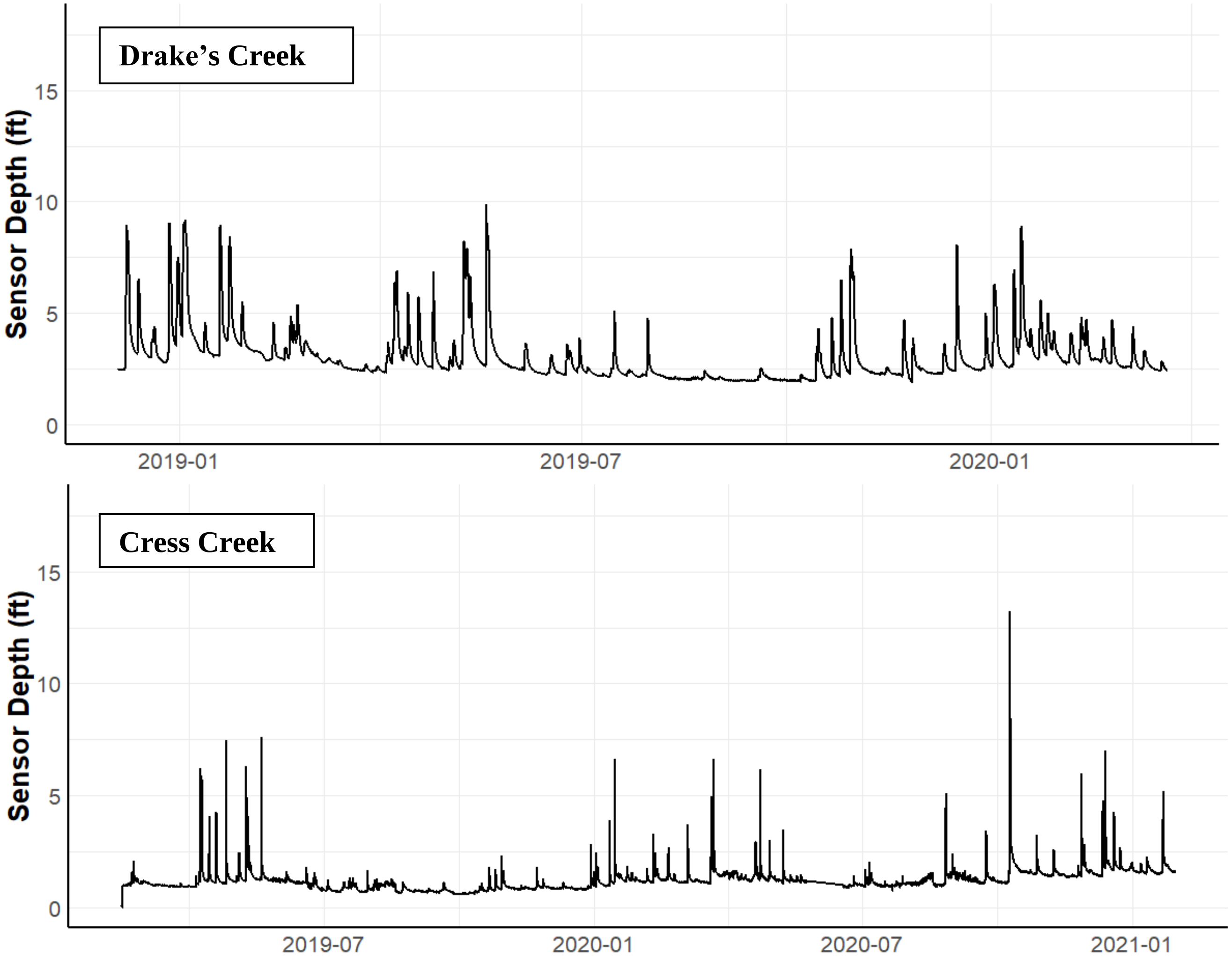


Figure A.4. Hydrographs for a subset of streams including Cress (unoccupied) and Drake's Creeks (out-of-range) from stream data loggers (Onset HOBO U20L) to monitor stream level or stage every hour starting from fall 2018/winter 2019 through 2020. (fig. cont'd.)

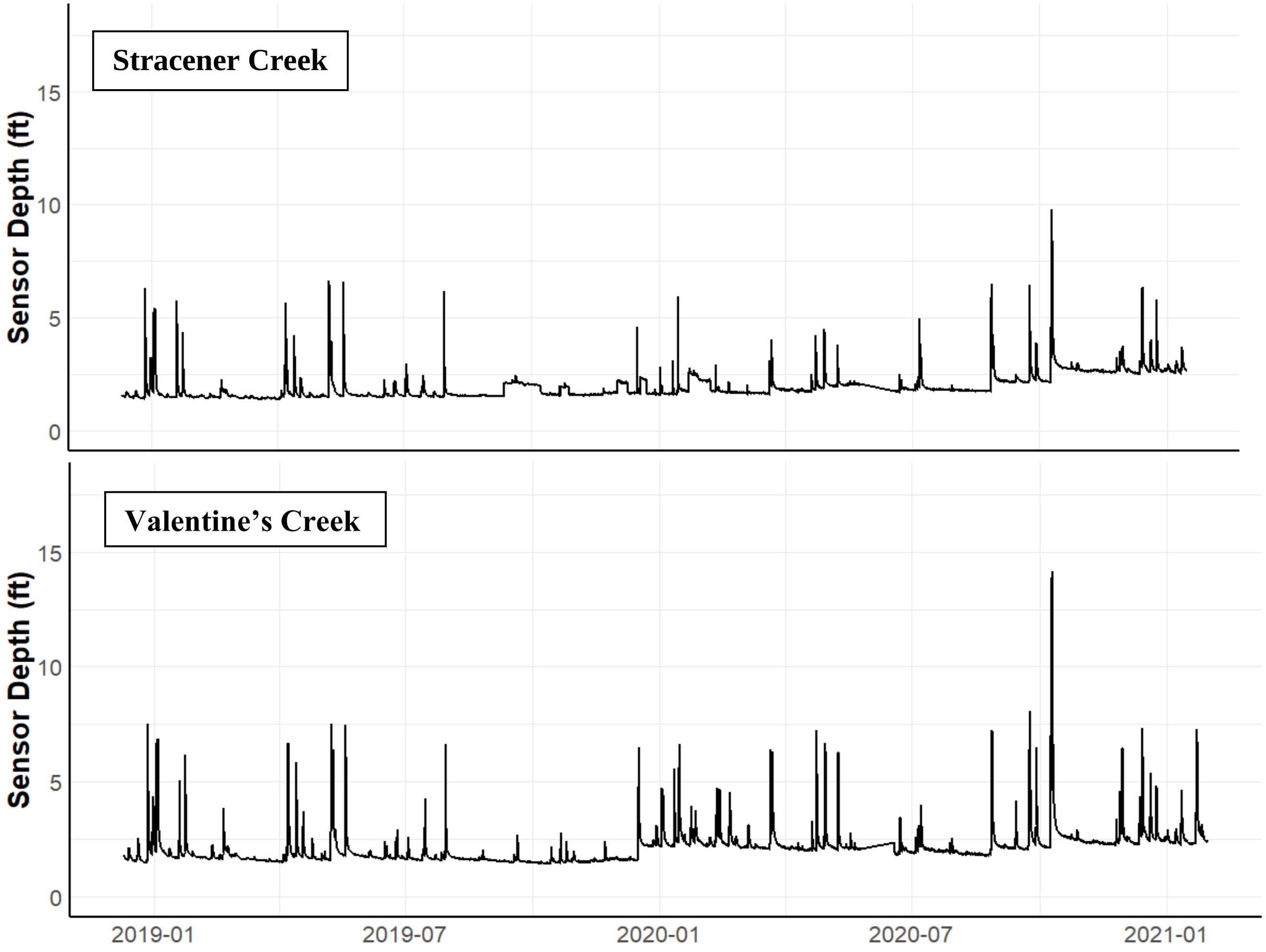


Figure A.4. Hydrographs for a subset of streams including Valentine's (occupied) and Stracener Creeks (unoccupied).

Appendix B. Supplemental Data for Chapter 3

Table B.1. The taxonomic composition of the Functional Feeding Guilds (FFGs) used in SIA across study streams.

Order	Family	Genus/Species	Functional Feeding Guild
Insecta			
Coleoptera	Elmidae	*Ancronyx*	collector/gatherer
		Dubiraphia	collector/gatherer
		Macronychoides	collector/gatherer
		Macronychus	collector/gatherer
		Microcylloepus	collector/gatherer
		Optioservus	collector/gatherer
		Stenelmis	collector/gatherer
		Unknown	collector/gatherer
Diptera	Chironomidae	unknown	Many
Diptera/Tipulidea	Tipulidae	*Hexatoma*	shredder/detritivore
		Nippotipula	shredder/detritivore
		Tipula	shredder/detritivore
		Unknown	shredder/detritivore
Ephemeroptera	Heptageniidae	*Maccaffertium*	scrapers
		Stenacron	scrapers
		Stenonema	scrapers
		Unknown	scrapers
	Isonychiidae	*Isonychia*	collector/filterers
Odonata	Aeshnidae	*Aeshna*	predator/engulfer
		Basiaeschna	predator/engulfer
		Boyeria	predator/engulfer
		Nasiaeschna	predator/engulfer
		Triacanthagyna	predator/engulfer
	Coenagrionidae	*Argia*	predator/engulfer
		Dromogomphus	predator/engulfer
	Cordulegasteridae	*Cordulegaster*	predator/engulfer
		Zoraena	predator/engulfer
	Corduliidae	*Helocordulia*	predator/engulfer
		Somatochlora	predator/engulfer
		Chauliodes	predator/engulfer
		Corydalus	predator/engulfer
		Unknown	predator/engulfer
	Gomphidae	*Arigomphus*	predator/engulfer
		Dromogomphus	predator/engulfer
		Erpetogomphus	predator/engulfer
		Gomphurus	predator/engulfer

(table cont'd.)

Table B.1. The taxonomic composition of the Functional Feeding Guilds (FFGs) used in SIA across study streams.

Order	Family	Genus/Species	Functional Feeding Guild
Odonata	Gomphidae	*Hagenius*	predator/engulfer
		Hylogomphus	predator/engulfer
		Phanogomphus	predator/engulfer
		Progomphus	predator/engulfer
		Stylurus	predator/engulfer
		Unknown	predator/engulfer
	Macromiidae	*Didymops*	predator/engulfer
		Macromia	predator/engulfer
Plecoptera	Perlidae	*Acroneuria*	predator/engulfer
		Agnetina	predator/engulfer
		Eccoptera	predator/engulfer
		Neoperla	predator/engulfer
		Perlesta	predator/engulfer
		Perlinella	predator/engulfer
		Unknown	predator/engulfer
Trichoptera	Hydropsychidae	*Cheumatopsyche*	collector/filterers
		Hydropsyche	collector/filterers
		Nytiophylax	collector/filterers
		Unknown	collector/filterers
Noninsecta			
Decapoda	Cambaridae	*Cambarus diogenes*	omnivore
		Cambarus ludovicianus	omnivore
		Foxonella clypeata	omnivore
		Orconectes creolanus	omnivore
		Orconectes h.hathawayiXh.blacki	omnivore
		Orconectes hobbsi	omnivore
		Orconectes Lancifer	omnivore
		Orconectes PalmeriXcreolanus	omnivore
		Orconectes Unknown	omnivore
		Procambarus acutus	omnivore
		Procambarus girardiella	omnivore
		Procambarus kensleygi	omnivore
		Procambarus penni	omnivore
		Procambarus pentastylus	omnivore
		Procambarus v.vioscaiXpagnei	omnivore
Gastropoda	Viviparidae	Unknown	Snail Scraper

Appendix C. Supplemental Data for Chapter 4

Table C.1. Feeding guilds assigned to fish taxa used in SIBER (Jackson et al. 2011) analysis to compare Layman's terms (Layman et al. 2007).

Invertivore	Omnivore	Lepomis spp.	Predator
A. sayanus	*A. natalis*	*C. macropterus*	*A. ariommus*
C. venusta	*D. cepedianum*	*L. cyanellus*	*A. calva*
Etheostoma/Percina sppl	*H. nigricans*	*L. gulosus*	*E. americanus*
F. olivaceous	*Ictiobus spp.*	*L. macrochirus*	*L. oculatus*
L. chrysocephalus	*M. cephalus*	*L. megalotis*	*M. punctulatus*
Nocomis leptocephalus	*M. melanops*	*L. microlophus*	*M. salmoides*
Noturus spp.	*M. poecilurum*	*L. miniatus*	
Pomoxis spp.			

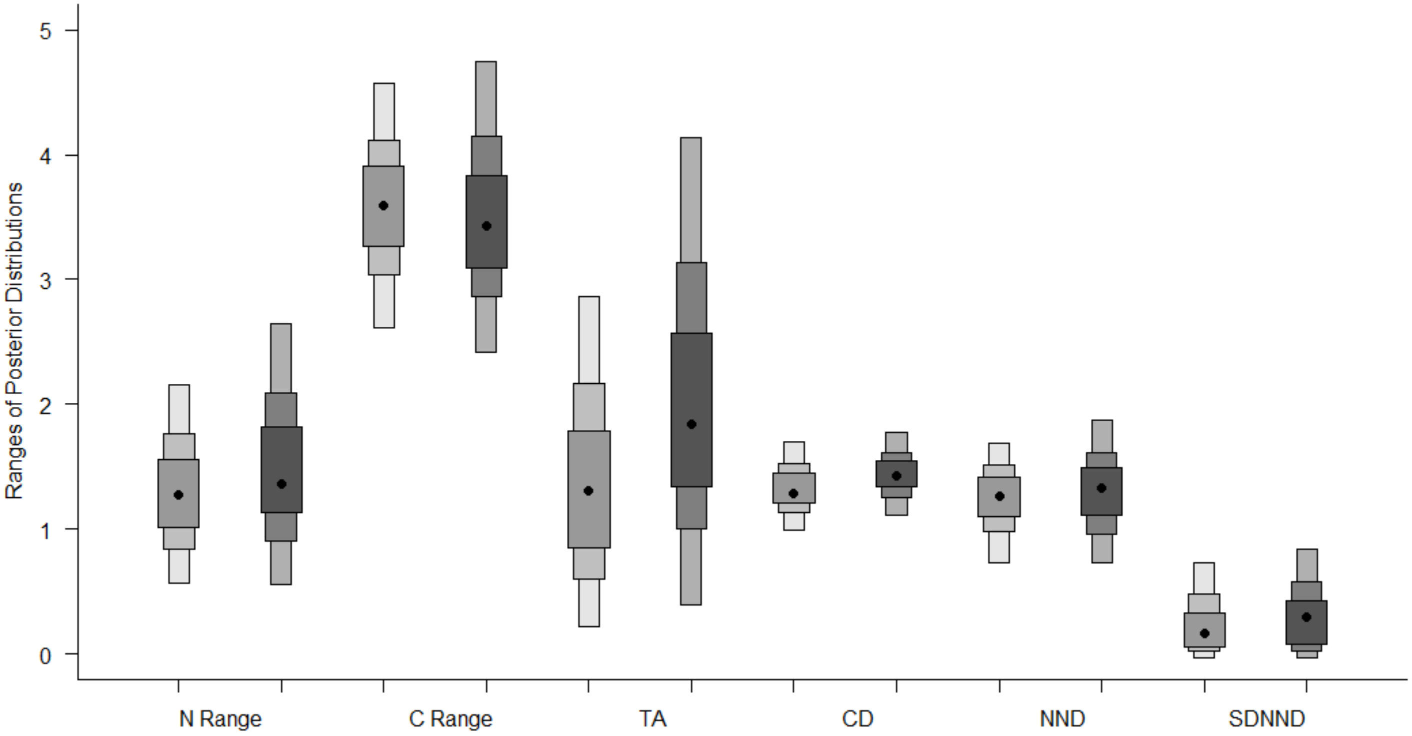


Figure C.1. Density plots of the posterior distributions of six Layman's (2007) Terms from the SIBER model comparing headwater streams of the Tickfaw River (light grey) and Calcasieu River (dark gray) watersheds. Abbreviations are: δ^{15}N range = NR, δ^{13}C range = CR, total area of the group ellipses = TA, mean distance to centroid= CD and mean nearest neighbor distance = MNND (and standard deviation= SD_{NND}).

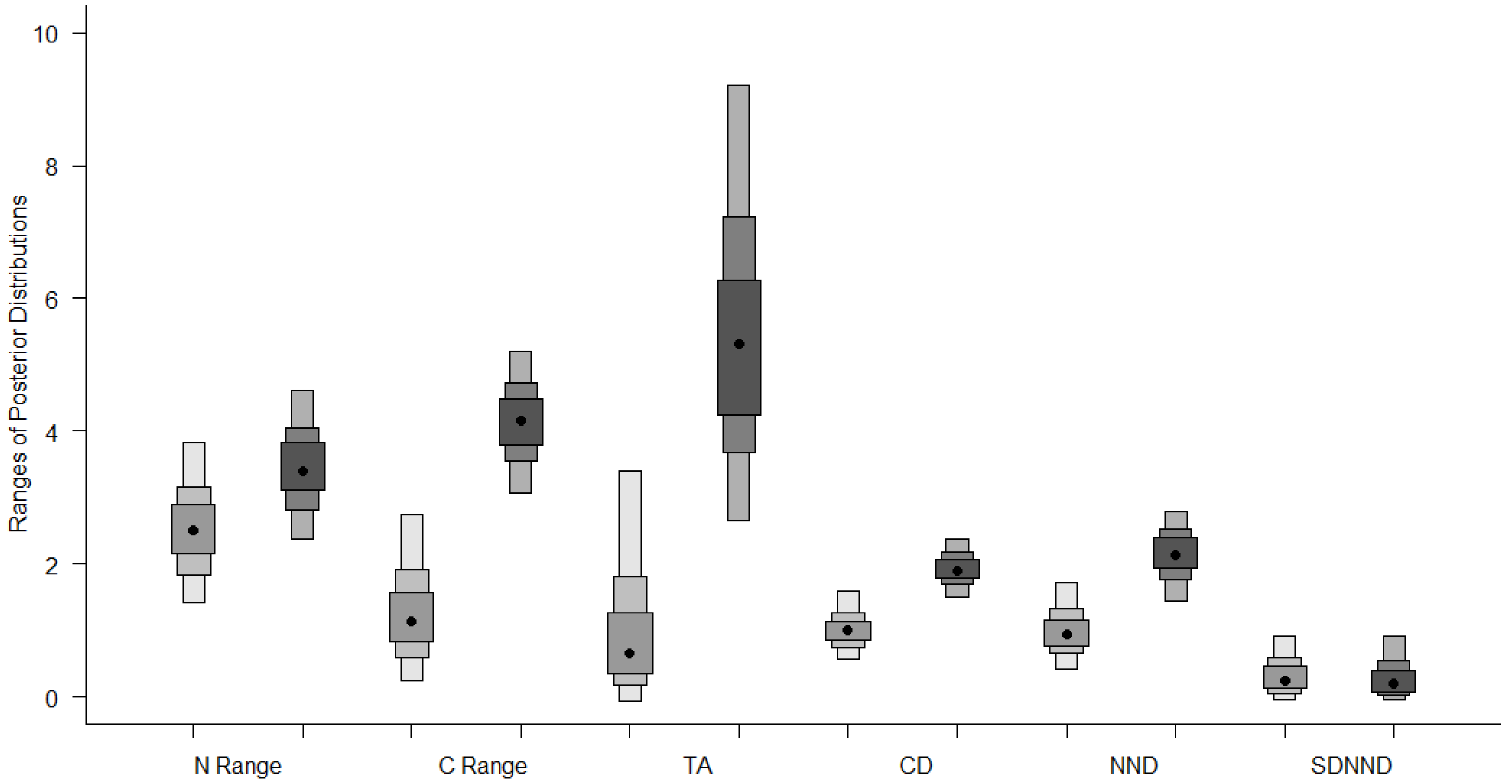


Figure C.2. Density plots of the posterior distributions of six Layman's (2007) Terms from the SIBER model comparing lower sites (intermediate and river) of the Tickfaw River (light grey) and Calcasieu River (dark gray) watersheds. Abbreviations are: δ^{15}N range = NR, δ^{13}C range = CR, total area of the group ellipses = TA, mean distance to centroid= CD and mean nearest neighbor distance = MNND (and standard deviation= SD_{NND}).

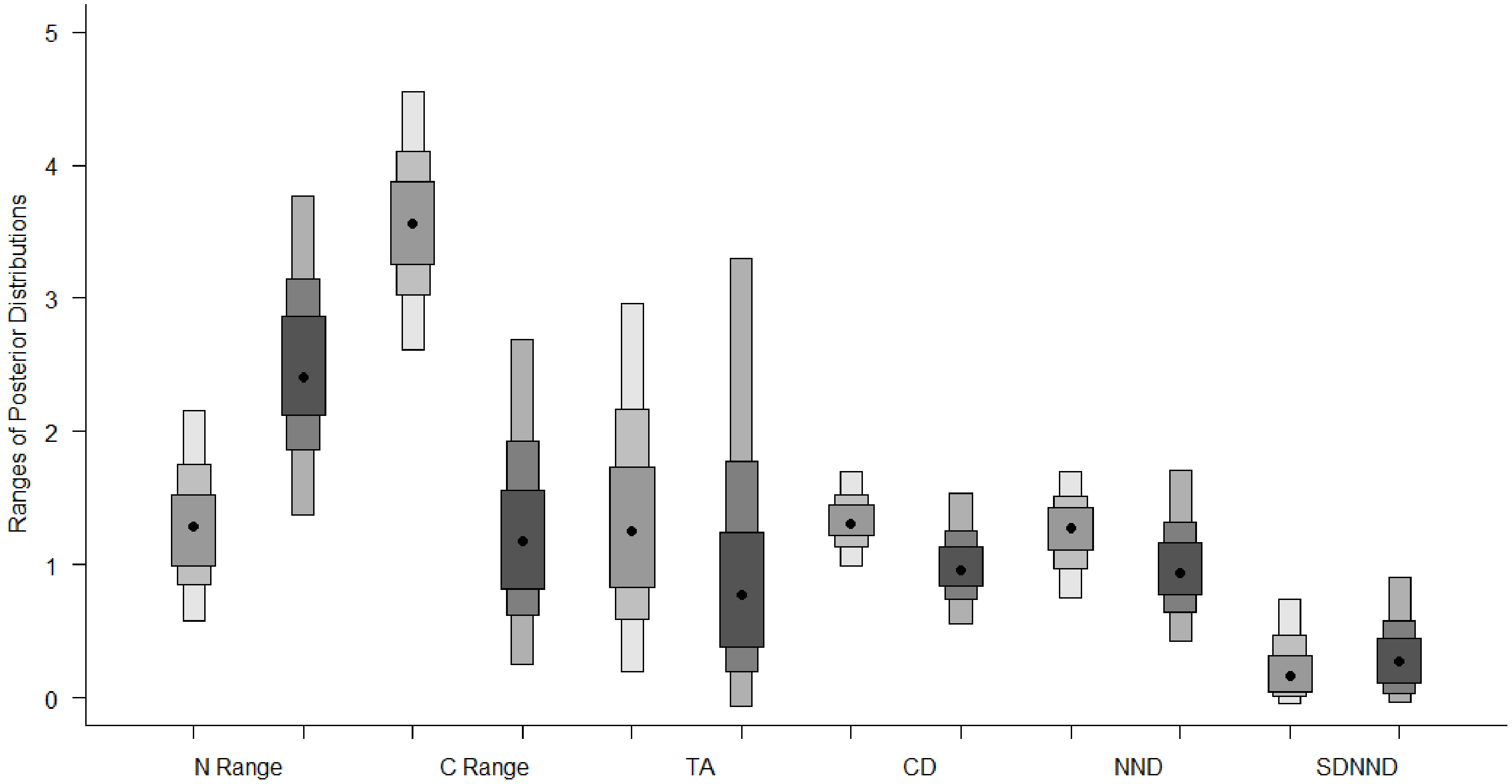


Figure C.3. Density plots of the posterior distributions of six Layman's (2007) Terms from the SIBER model comparing upper or headwater (light grey) sites to the lower or intermediate/river (dark grey) sites of the Tickfaw River watershed. Abbreviations are: δ^{15}N range = NR, δ^{13}C range = CR, total area of the group ellipses = TA, mean distance to centroid= CD and mean nearest neighbor distance = MNND (and standard deviation= SD_{NND}).

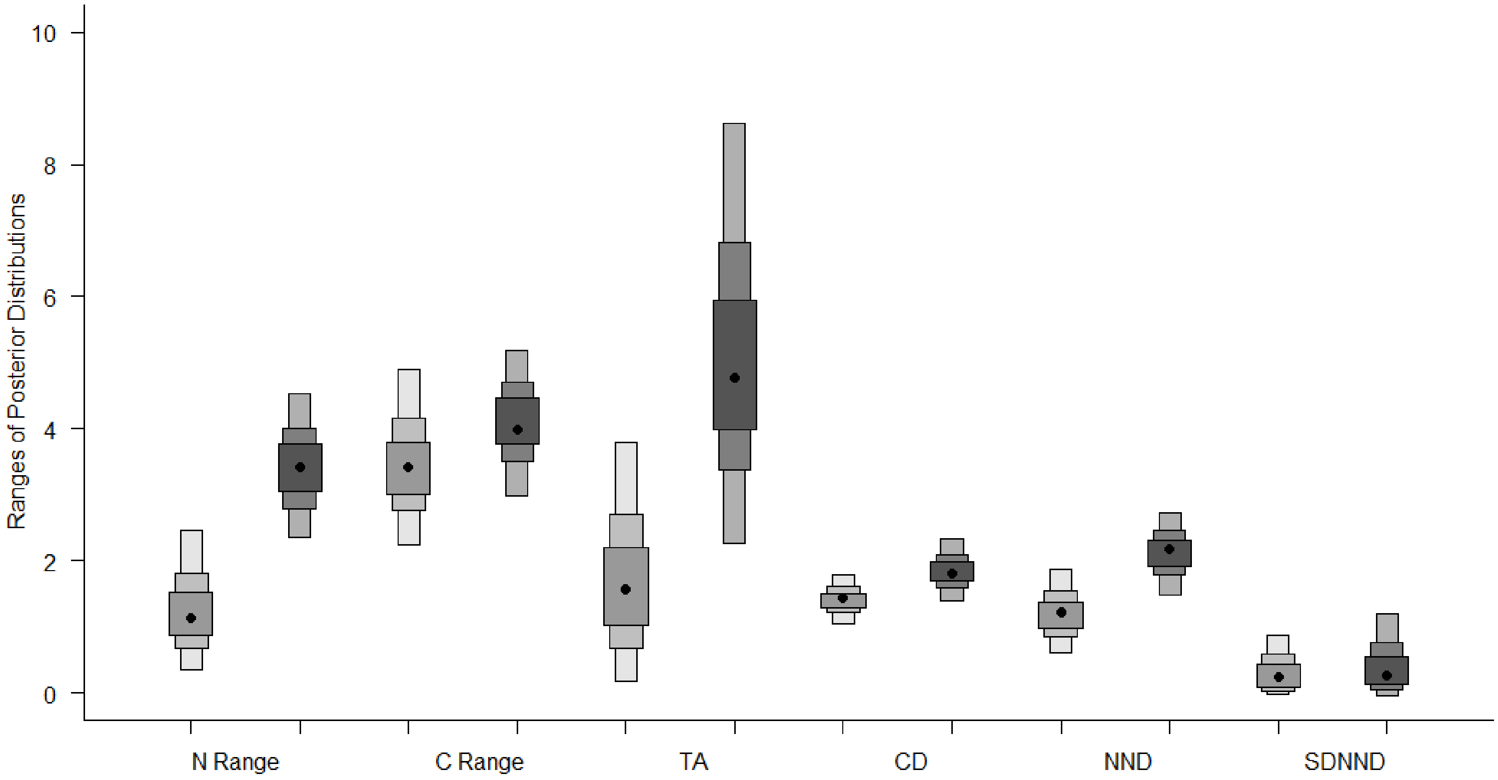


Figure C.4. Density plots of the posterior distributions of six Layman's (2007) Terms from the SIBER model comparing upper or headwater (light grey) sites to the lower or intermediate/river (dark grey) sites of the Calcasieu River watershed. Abbreviations are: δ^{15}N range = NR, δ^{13}C range = CR, total area of the group ellipses = TA, mean distance to centroid= CD and mean nearest neighbor distance = MNND (and standard deviation= SD_{NND}).

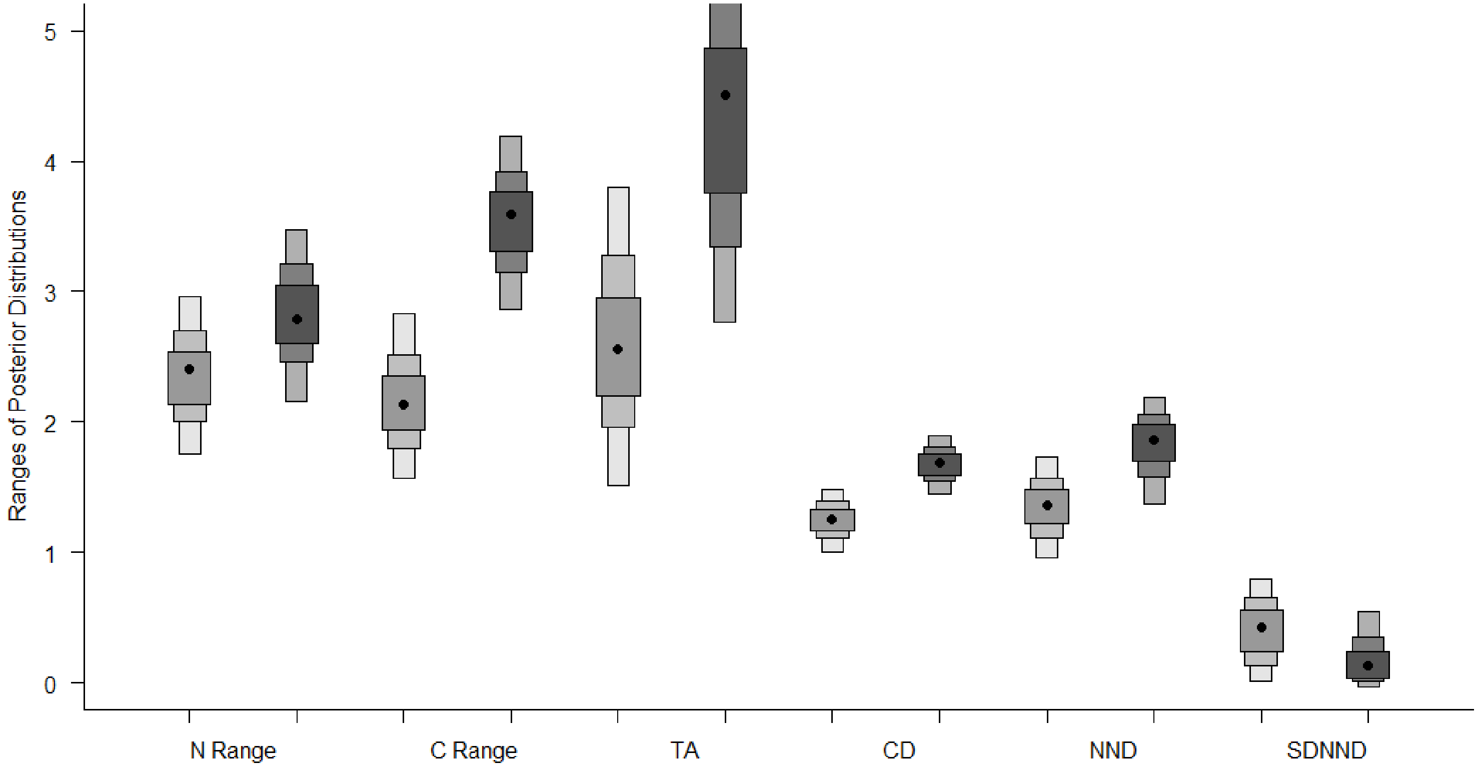


Figure C.5. Density plots of the posterior distributions of six Layman's (2007) Terms from the SIBER model comparing the Tickfaw River (light grey) and Calcasieu (dark gray) River watershed. Abbreviations are: δ^{15}N range = NR, δ^{13}C range = CR, total area of the group ellipses = TA, mean distance to centroid= CD and mean nearest neighbor distance = MNND (and standard deviation= SD_{NN}

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Vita

Erin Elizabeth Thayer grew up in western Colorado and graduated from the University of Colorado at Boulder in 2009, with a Bachelor of Arts Degree in Ecology and Evolutionary Biology and a minor in Geology. Over the course of the next three years, Ms. Thayer obtained seven technician positions ranging in water-water and trout/salmon fisheries in California and Oregon, to Alaskan commercial fisheries, and yellow perch fisheries in Illinois. In 2013, she started a Master's of Science degree with Dr. Kyle J. Hartman at West Virginia University, studying Brook Trout foraging behavior in headwater streams of the Monongahela National Forest. Upon graduating she moved to Baton Rouge, La to begin a PhD with Dr. Michael D. Kaller at Louisiana State University. Ms. Thayer focused on scaling her research to include a community view of food webs, especially invertebrates and fishes, and employed the use of stable isotopes to better understand energy and nutrients delivered from the floodplain in subtropical coastal plains lotic ecosystems. Upon graduating in December 2022, Erin is excited to start a faculty position at the Aquaculture and Fisheries Center at the University of Arkansas at Pine Bluff.