# Flint Hills wetlands and aquatic vertebrate responses to climate change.

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Data collected and prepared by the following students and staff at Wichita State University (alphabetical order):

**Stephanie Bristow, Shania Burkhead, Phi Hoang, Dexter Mardis, Justin Oettle, Annie Pham, Sarah Pulliam, Christine Streid, Emily Stybr, Krista Ward, Jake Wright**

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# Executive Summary

The global COVID-19 pandemic has curtailed or delayed aspects of our planned projects ("Procedures" in original proposal). However, we have still successfully completed and surpassed expectations for several aspects of our plan that were not prohibited by the pandemic. Namely, funding from the KDWPT's Chickadee Checkoff facilitated the following projects on Wichita State University's (WSU) 4,700-acre Youngmeyer Ranch (YMR):

1) **Wetland Inventory and Hydrological Characteristics**
    - Rapid Wetland Inventory & Preliminary Hydroperiod Indices
        - Completely mapped >39km of intermittent streams & pools
        - Paper in preparation for *Transactions of the Kansas Academy of Science* with 3 graduate students and 7 undergraduates at Wichita State University (WSU)
        - Critical data for 3 additional Master's Theses (see appendices)
    - Field-corrected Wetland Hydroperiods/Wetland Fill and Dry Rates
        - Despite COVID-19 shipping delays, all 15 water level loggers were placed in late June and are recording temperature & barometric pressure every 30 minutes
        - Installation of a KS Mesonet (Kansas State University) weather station with rain gage & barometric sensors was postponed by COVID-1. This prevented us from being able to assess wetland fill and dry rates.
        - Thermal data collected from the pools is being used in a Kansas NSF EPSCoR First Award Project Awarded to Dr. Luhring and for two Masters students starting in Fall 2021.
2) **Aquatic Vertebrate Inventories**
    - Aquatic Surveys
        - Systematically sampled 151 intermittent stream pools & 13 Cattle Ponds
            - >8,500 individual fish, amphibians, & invertebrates sampled
                - Summary & GPS locals on SC-076-2020 Final Collection Report
            - Landscape level presence/absence data
            - Landscape level biomass of each group (e.g., grams of amphibians/pool)
            - Landscape level community composition (e.g., co-occurrence of species)
        - This data contributed to three MS theses (will credit Chickadee Checkoff)
            - Jake Wright – Thesis Attached: ***Amphibian larvae distribution amongst intermittent stream pools in the Flint Hills covaries with vegetation and fish colonization***.
            - Krista Ward – Thesis Prospectus Attached: ***Fish distribution, diversity, and recolonization effects in intermittent stream pools***
            - Christine Streid – Thesis Prospectus Attached: ***Identifying factors that influence aquatic invertebrate use of tallgrass prairie stream pools in Kansas***
        - This data was used or will be used for the following presentations:
            - Wichita State University **Departmental Seminars** (~50 audience members)
                - Christine Streid – Fall 2020
                - Jake Wright – Fall 2020

        - Christine Streid – Spring, 2021
        - Jake Wright -  Fall 2021
        - Krista Ward – Fall 2021
        - Krista Ward – Spring 2021
    - Wichita State University **Graduate Defense Seminars** (~50 audience members)
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        - Jake Wright, Kansas Herpetological Society – November 2020 (~74 audience members)
    - **International Meetings**
        - Jake Wright, Society of Freshwater Science – May 2021 (society membership ~1,500)
        - Krista Ward, Society of Freshwater Science – May 2021 (society membership ~1,500)
- Aural Surveys (29 person-visits)
    - COVID-19 spikes in Kansas in Spring of 2020 greatly curtailed aural surveys
    - Several trips were made in 2020 and 2021 to YMR and large wetlands were sampled for fish to identify potential breeding pools.
    - No *Lithobates areolatus* were documented on the property, but potential breeding sites exist, we documented breeding choruses ~9 miles East, burrowing crayfish are present, and other large ranids (*Lithobates blairi*) breed throughout the property. Monitoring efforts will continue.

**3) Synthesis**

- Analyses conducted as part of theses (see attached) have documented several abiotic and biotic correlates to both larval amphibian presence and abundance (biomass) across intermittent stream pools. While the details of the relationships are better understood within the context of the appendices, we summarize a few findings here:
    - Nearly every wetland pool on the property was part of an intermittent stream.
    - We sampled during a potential maximal distribution of fishes on the landscape corresponding to a very wet year (12.24" above the 10-year average)
    - Fish were widely distributed and Green Sunfish in particular were adept at colonizing pools almost all the way to the uppermost reaches of drainages.
    - Amphibian larvae were largely confined to pools without fish.
    - However, fishless pools were frequently situated downstream of pools with fish.
    - Larval amphibian abundance was highest in macrophyte dominated pools.

Project-specific details are found below:

# Rapid Wetland Inventory & Preliminary Hydroperiod Indices

**Paper in Progress:** Remote mapping of the Youngmeyer Ranch's intermittent stream systems across wet and dry years using freely available historical satellite imagery. (In preparation for *Transactions of the Kansas Academy of Science*)

Thomas M. Luhring, Christine S. Streid[1], Jake T. Wright[1], Stephanie A. Bristow[2], Shania Burkhead[2], Phi Long Hoang[2], Justin Oettle[2], Annie Pham[2], Sarah Pulliam[2], Emily Stybr[2], and Krista J. Ward[1].
[1] Graduate Student, [2] Undergraduate Student

A group of three graduate and seven undergraduate students worked with Dr. Luhring in Spring 2020 to create an initial inventory of streams, intermittent stream pools, and isolated wetlands on the 1,902ha (4,700 acre) Youngmeyer Ranch. To increase utility of these resources to various stakeholders, we used a freely available satellite imagery program (Google Earth Pro©) to create maps that can be used without access to expensive proprietary software.

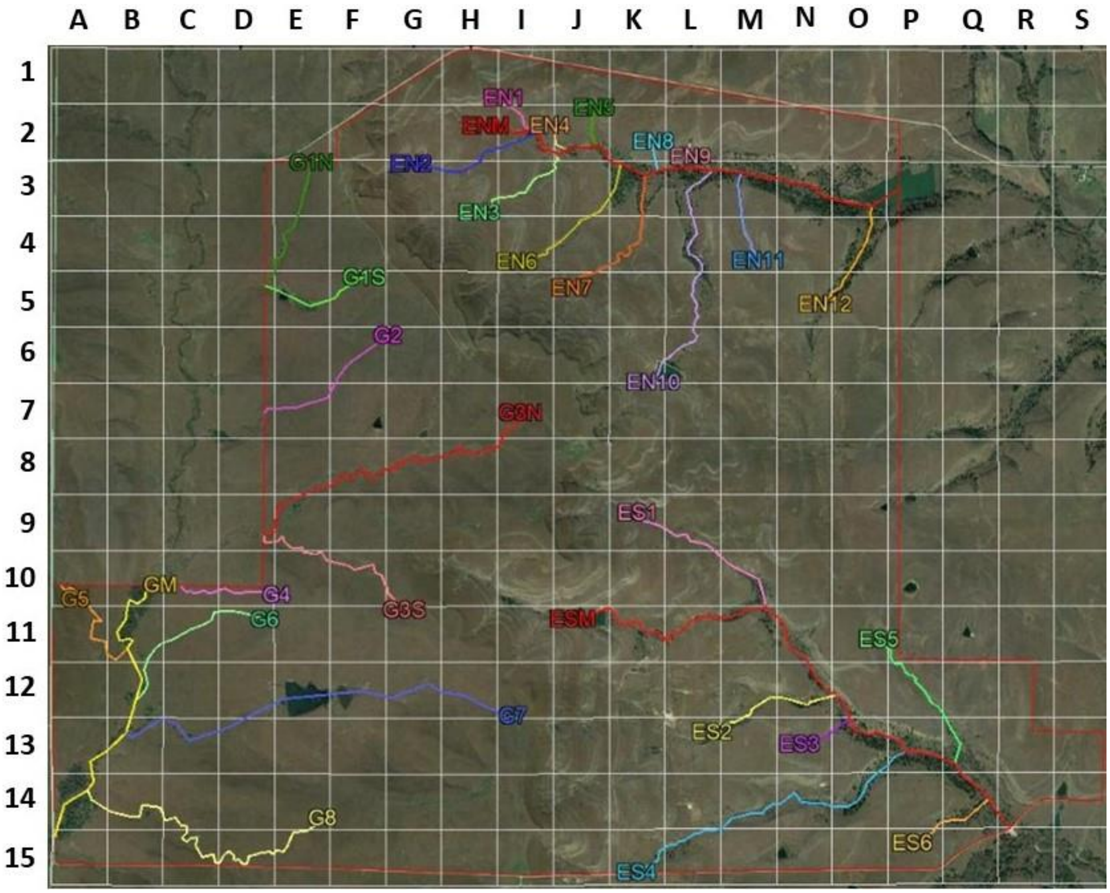
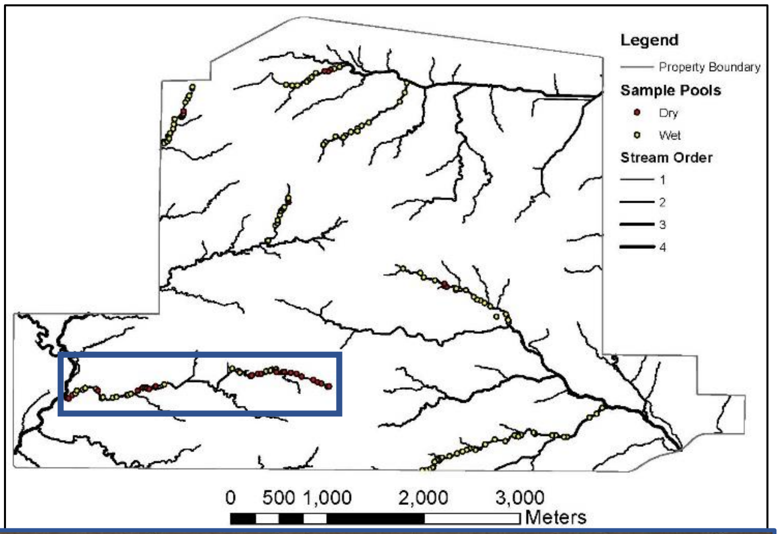


Fig. 1. Alpha-numeric grid system and stream names developed as a part of this project.

The ~39km of streams on YMR were highly dynamic across the four years where satellite data had enough resolution to assign wet/dry statuses to streams and wetlands. Details of survey efforts are summarized in each of the appendices. Briefly, in 2012 intermittent stream pools were well-connected to permanent water sources with ~76% of their downstream reaches holding water. In 2013, pools became increasingly isolated with ~14% of downstream reaches holding water. This represented a greater than 5-fold decrease in pool connectedness from one year to the next.



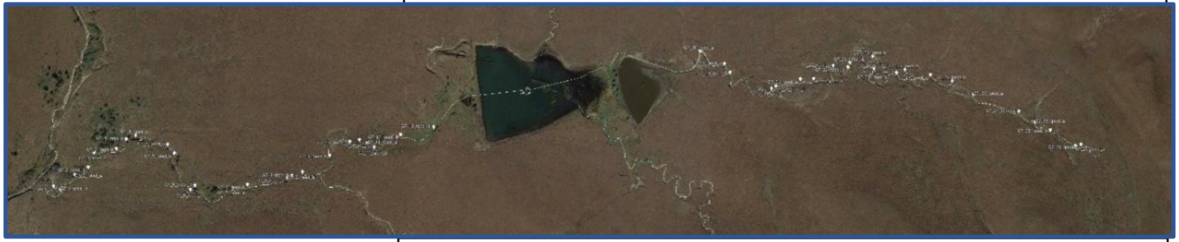


Fig. 2. Distribution of selected stream systems and pools across YMR. Blue box corresponds to satellite image with labelled pools. Two typical cattle ponds are also visible in the middle of the stream reach.

A subset of 151 randomly selected pools from seven stream reaches (Fig. 2) were designated for aquatic vertebrate inventories and characterized for their hydroperiod by satellite imagery (see theses for details). Pools showed a wide variety of hydroperiods (time holding water) with intermediate levels of permanency being more common (Fig. 3). Across the four years of satellite imagery with high enough resolution to see stream pools, the average pool held water for 2.2 out of 4 years (Figure 3). This is consistent with typical intermittent stream habitat across the Midwest and particularly prevalent in Kansas and the Flint Hills.

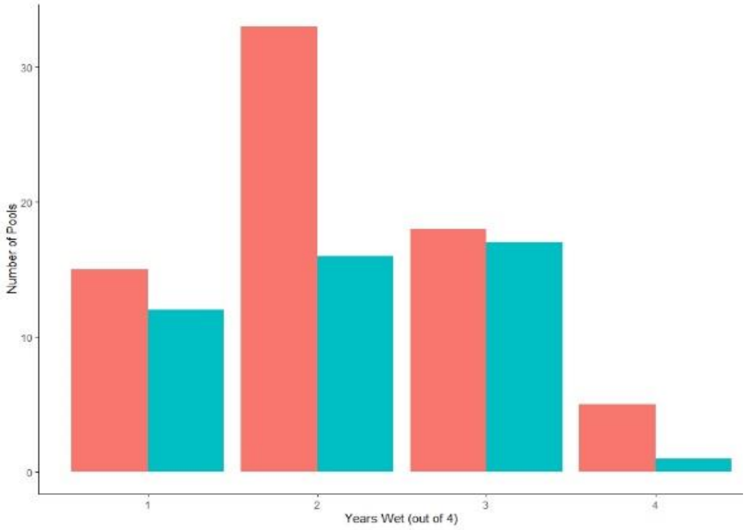
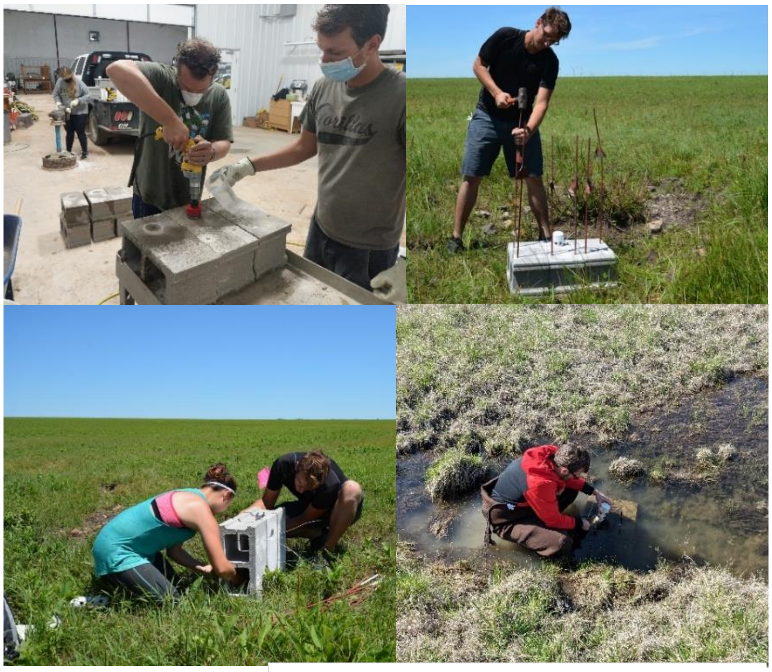


Fig. 3. Distribution of selected stream pool permanence (years wet out of 4) in eastern (red) and western (blue) drainages.

**Summary:** Collection and preparation of this data took the contributions of 7 undergraduates enrolled in 14 hours of research credit in Spring

2020, 2 paid summer undergraduate research technicians in Summer 2020, and 3 graduate students for which these data are critical components of their Master's degrees. A formal summary and analysis of this data within the context of the Flint Hills ecoregion is being prepared by Shania Burkhead, an undergraduate in Dr. Luhring's lab at Wichita State University for *Transactions of the Kansas Academy of Science*. Additionally, this approach demonstrated the utility of rapidly inventorying Great Plains stream systems with easily accessible and free software.

## Field-Corrected Wetland Hydroperiods/Wetland Fill & Dry Rates



Following COVID-19 shipping delays, six researchers constructed 15 total water level logger stations from cinder blocks. These stations were placed in 7 stream pools on the West side and 7 stream pools on the east side (corresponding to randomly selected subset of the rapid inventory pools: see above) and 1 isolated wetland on the Ninnescah Reserve. Stations were up and running and recording water temperature and barometric pressure in 30-minute increments (pressure is used to calibrate estimates of depth).

Unfortunately, the Kansas Mesonet (Kansas State University) weather station that was going to be installed in Summer 2020 was postponed and we were unable to have local rainfall or barometric pressure readings that would permit us to create our models of pool filling and drying. However, that part of the project will resume after the Mesonet weather station is installed in the near future.

Thermal data collected from the pools is being used in a Kansas NSF EPSCoR First Award Project Awarded to Dr. Luhring and for two Masters students starting in Fall 2021 to characterize the thermal profiles of small intermittent stream pools. These data show the incredible thermal ranges experienced by aquatic vertebrates in small intermittent stream pools (10-15°C in a day!) (Fig. 4).

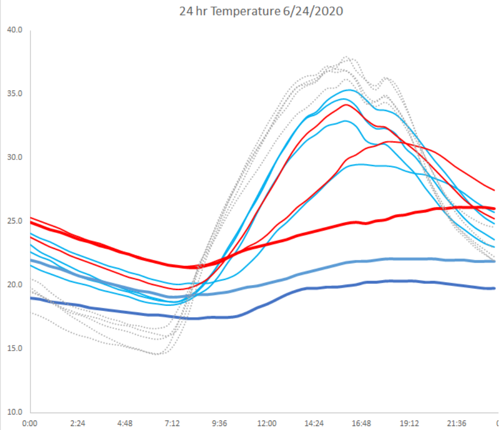


Fig. 4. Example temperature variation across 14 pools in mid-summer. Pools with water on the east side (blue) and west side (red) show considerable within-day variation that is slightly less than air temperature (dashed lines are dry pools).

# Aquatic Vertebrate Inventories: Aquatic Surveys

## Aquatic Surveys

In the summer of 2020, a group of 3 graduate students and 3 undergraduates worked with Dr. Luhring to systematically sample 151 randomly chosen intermittent stream pools (selected during the rapid inventory) & all 13 cattle ponds on the property. For each pool we recorded it's length, volume, general vegetative characteristics, and conducted visual surveys for fish counts. We conducted volume-constrained sampling (e.g., known sample volumes) to estimate the density and whole-pool biomass of aquatic invertebrates, fishes, and amphibians. In total, >8,500 animals were sampled and released (detailed in SC-076-2020 Final Collection Report).

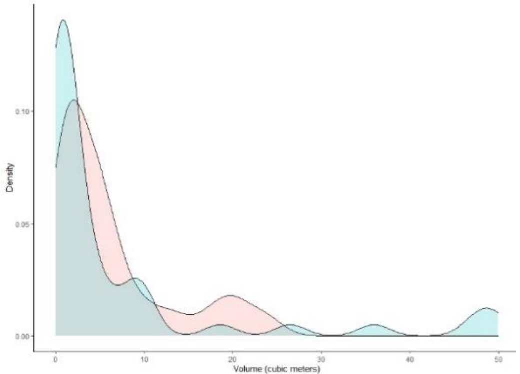


Fig. 5. Distribution of pool volumes measured across 7 stream reaches on YMR. East side (blue) and west side (red) pools were most often smaller (1 $m^3$ is approximately 264 gallons).

Jake Wright's preliminary thesis details the findings of the landscape level presence/absence data and densities of amphibians whereas Krista Ward's thesis prospectus details landscape level presence/absence data for fishes.

Overall, fish were widely distributed with more than half of all intermittent stream pools (57%) having fish (See Krista's prospectus for details). The percent of pools with fish present would have been even higher if not for large geographical barriers (e.g., waterfalls, escarpments) that prevented upstream dispersal. This suggests that following wet years (2020 sampling efforts followed an exceptionally wet period) fish can be expected to be widely distributed across the landscape. In this case, Green Sunfish were frequently found at the uppermost pool of water in streams lacking any physical upstream barriers.

The consequence of this wide distribution of fishes in wet years is seen in their effects on amphibian presence (Fig. 6). Not surprisingly, amphibian larvae were generally absent from pools with fish regardless of their connection to other bodies of water (connected/isolated in Fig. 6 refers to whether there is water flowing into or out of the pool) or total pool volume. Furthermore, 12 of the 13 sampled cattle ponds held fish and likely serve as local drought refugia.

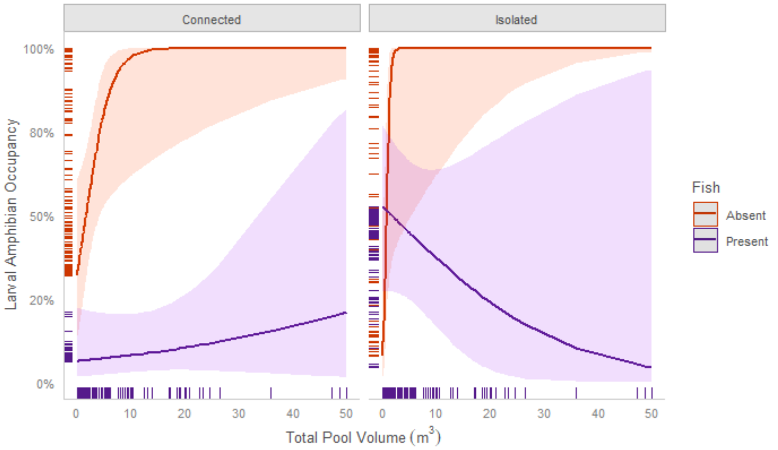


Fig. 6. The top model explaining larval amphibian abundance included an interaction amongst pool volume, the presence of fish, and whether there was water connecting it to other pools. Overall, amphibians were generally found in most isolated pools without fish, were found less often in small fishless pools when they were connected, and were mostly absent from pools with fish.

The aquatic surveys are being used in three Master's theses that will credit KDWPT Chickadee Checkoff:

- Jake Wright – Thesis Attached: ***Amphibian larvae distribution amongst intermittent stream pools in the Flint Hills covaries with vegetation and fish colonization***.
- Krista Ward – Thesis Prospectus Attached: ***Fish distribution, diversity, and recolonization effects in intermittent stream pools***
- Christine Streid – Thesis Prospectus Attached: ***Identifying factors that influence aquatic invertebrate use of tallgrass prairie stream pools in Kansas***

Additionally, these projects have been or will be presented at least 12 different seminars and meetings:

- Wichita State University **Departmental Seminars** (~50 audience members)
  - Christine Streid – Fall 2020
  - Jake Wright – Fall 2020
  - Christine Streid – Spring, 2021
  - Jake Wright - Fall 2021
  - Krista Ward – Fall 2021
  - Krista Ward – Spring 2021
- Wichita State University **Graduate Defense Seminars** (~50 audience members)
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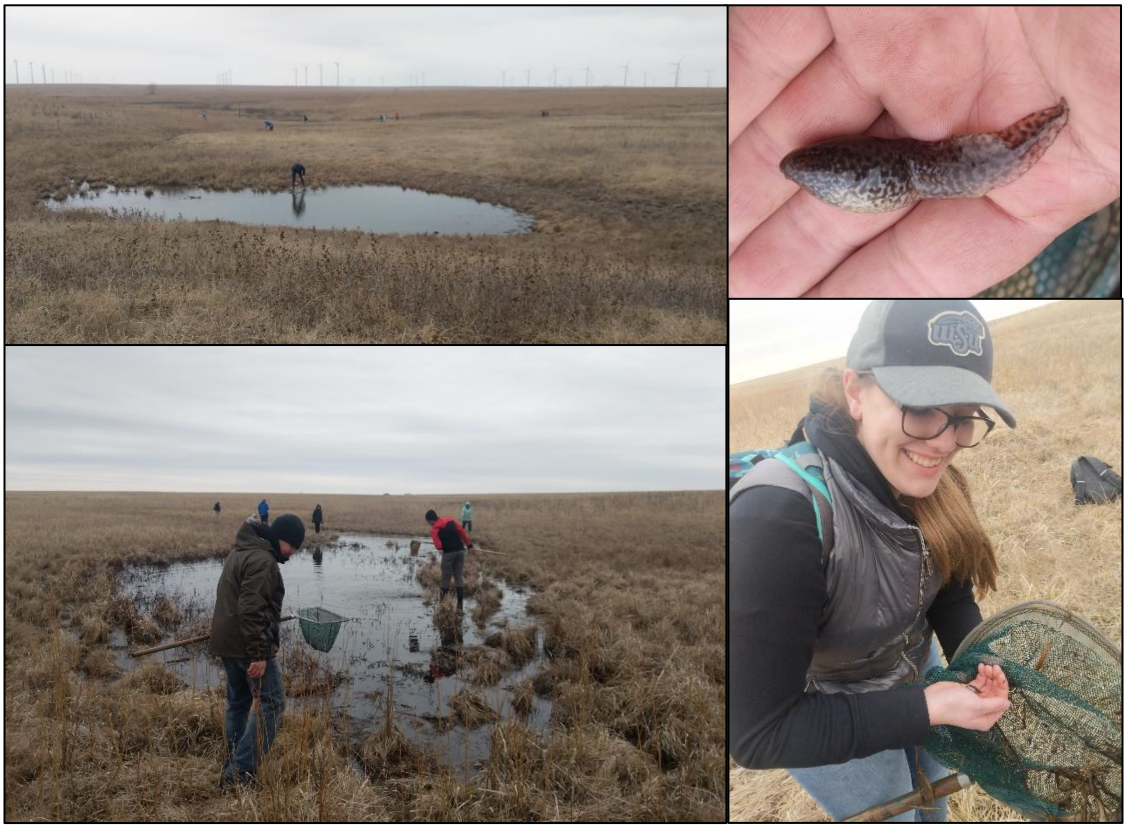
In addition to their use in obtaining the data used in the aforementioned studies, the infrastructure of fyke nets, seines and headlamps have been used for 3 mini-field trips (reduced by COVID-19 restrictions) for herpetology and will continue to be used for additional classes such as Field Vertebrate Ecology (*N*= 12-16 students each summer), Vertebrate Zoology (*N* = 40 students every other spring) and Herpetology (*N* = 30 students every other spring). Photos from these events are found on the next page.


NORTH SHORE
Wichita State
SHOCKERS

## Aquatic Vertebrate Inventories: Aural Surveys

Prior to fieldwork being temporarily suspended during the global COVID-19 pandemic, we were able to conduct several visits to YMR and the surrounding areas during the initial peak of Crawfish Frog breeding activity in South Eastern Kansas. A much reduced effort has been maintained and will continue into the foreseeable future. Expansion of these efforts will increase as precautions related to group size are no longer warranted for COVID-19 measures.

**Initial trip February 23, 2020** to check potential breeding pools on the property for the presence of fishes and Ranid tadpoles (inset pictures). Ten total people: 7 students, Dexter Mardis – WSU Field Station Manager, Dr. Peterman - visiting faculty from Ohio State University, Dr. Luhring.



**March 18, 2020**: Following rains and warmer weather, Dr. Luhring and Dexter Mardis visited several wetlands on the NW portion of the property. *Pseudacris maculata* and *Lithobates blairi* were calling throughout the property and egg masses of each were found. At least one crayfish burrow with a flattened chimney was found near water, but no Crawfish Frogs were seen or heard.

**March 25, 2020**: Following substantial rains and warmer weather, Dexter Mardis and Tyler Newman worked west from known populations and heard chorusing activity all the way until ~9 miles from the Eastern Border on Crisco Rd, Howar d, KS 0.964 miles east of 150/12 Rd. Attempted to go to southern portion of property in a Toyota Prius. Attempt unsuccessful. Listened for calls near location where burrow call was heard by D. Mardis in prior year near NW portion of property. No calls.

**March 27, 30, 2020**: Following suitable weather, Jake Wright went to the South Side of the property where *Pseudacris maculata* and *Lithobates blairi* were calling from several ponds.

**March 14, 2021:** Following the first warm rains of the spring, 13 people (mostly from the WSU Spring 2021 Herpetology Course) visited several pools on the Northwest side of the property at night to search for amphibians. *Lithobates blairi, Pseudacris maculata* and *Ambystoma texanum* were found around several pools along with ranid egg masses. No *Lithobates areolatus* were found.

**Summary:** COVID-19 restrictions on spring activities in 2020 severely limited our ability to actively sample to the extent planned. However, we were still able to log 29 person-visits (total visits by individuals) from 6 separate visits. While there were no confirmed sightings or aural recordings of *Lithobates areolatus* on YMR proper, they remain a potential species for the site that warrants continued monitoring efforts.

## Synthesis

More detailed statistical treatments of the data and interpretations are found in the accompanying theses. However, several abiotic and biotic patterns and interactions are worth mentioning. First, while we initially inventoried both isolated wetlands and intermittent stream pools, it quickly became apparent that truly isolated wetlands without intermittent connections to streams were relatively rare across the landscape. This meant that nearly all available breeding habitat for amphibians at YMR were intermittently connected as parts of continuous stream drainages. Given our sampling efforts occurred immediately following a wet year (12.24" above the 10-year average), fish distributions were likely at their peak across the landscape. Thus, the effects of fish presence and pool size were largely more important than the estimated hydroperiod length of pools (which would have very little impact given the lack of recent drying events). We will be continuing to sample these pools as part of my summer Field Vertebrate Ecology class. This continued effort will permit us to look at longer trends in amphibian and fish distributional data that encompasses regional wet and dry years.

Fish were widely distributed (over half of all pools sampled) and amphibians almost exclusively bred in fishless pools (of the 31 pools with larval amphibians, only 7 also had at least one fish). However, when there were no physical barriers to upstream movement Green Sunfish moved to the uppermost regions of each stream. This meant that in intermittent streams, amphibians appear to breed in a dynamic landscape where the suitability of a specific pool for breeding potentially varies from year to year depending on the recency of that pool's history of drying, filling, and connection to the remainder of the watershed. In other words, amphibians in the Flint Hills are ovipositing in a landscape of pools that could quickly become unsuitable given extreme precipitation events within and across years (e.g., floods permitting fish colonization, droughts drying out pools). The consequences of these dynamics is the current focus of Krista Ward's ongoing Master's research where she is simulating drying, refilling, and colonization by fish and/or amphibians in wetland mesocosms.

## Appendix I: Jake Wright Master's Thesis (Early Draft Provided to KDWPT)

# AMPHIBIAN LARVAE DISTRIBUTION AMONGST INTERMITTENT STREAM POOLS IN THE FLINT HILLS COVARIES WITH VEGETATION AND FISH COLONIZATION

A Thesis by

Jake Wright

Bachelor of Science, Pittsburg State University, 2019

Submitted to the Department of Biological Sciences
and the faculty of the Graduate School of
Wichita State University
in partial fulfillment of
the requirements for the degree of
Master of Science

2021

# AMPHIBIAN LARVAE DISTRIBUTION AMONGST INTERMITTENT STREAM POOLS IN THE FLINT HILLS COVARIES WITH VEGETATION AND FISH COLONIZATION

The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science, with a major in Biological Sciences.

______________________________________
Thomas Luhring, Committee Chair

______________________________________
Leland Russell, Committee Member

______________________________________
Andrew Swindle, Committee Member

# DEDICATION

To my wife, family, friends, and their everlasting support

# ACKOWLEDGEMENTS

I would like to thank my advisor, Dr. Thomas Luhring, for guiding me, teaching me, and improving me as a biologist. I would also like to thank my committee members, Dr. Leland Russell and Dr. Andrew Swindle for their guidance and contributions towards my thesis. I am grateful for my fellow graduate students in the lab, Krista Ward and Christine Streid, for their assistants on data collection and reviewing my work. I would like to thank the following undergraduate students for helping with remote and/or field work: Emily Stybr, Stephanie Bristow, Tiffani Holman, Justin Oettle, Shania Burkhead, Annie Pham, Phi Long Hoang, and Sarah Pulliam. I will extend my thanks to Dexter Mardis for facilitating and assisting with field work. I would also like to thank the KDWPT Chickadee Checkoff for funding my research. My gratitude goes toward the Wichita State University Biology Department faculty, staff, and students for their support.

# ABSTRACT

Climate change is expected to increase the frequency and severity of droughts and precipitation events. The connectivity and hydroperiod among stream pools are expected to change due to these climatic events, therefore influencing what organisms occupy them. Intermittent stream pools in the Great Plains present an idealized replicated system to study the impacts of variable climatic conditions on the distribution of aquatic species, including amphibians. In the Great Plains, intermittent stream pools are the most abundant spawning habitat for amphibians. We sampled 117 stream pools to investigate the effects of fish presence, stream vegetation, hydroperiod, connectivity, and pool volume on larval amphibian distribution, biomass, and density in the Flint Hills of Kansas. Larval amphibians preferred fishless, isolated pools and the effect of volume depended on the connectivity and fish occupancy of that pool ($p < 0.05$). In small pools where amphibian larvae occurred (n=31), density(g/m$^3$) was higher in pools dominated by macrophyte vegetation and decreased as pool volume increased ($p < 0.01$). We sampled during a wet year (12.24 inches above the 10-year average) which implies that fish distribution is at a maximum across the streams on this landscape and are having a large effect on the spatial distribution of amphibian larvae. Although more precipitation has the potential to increase the likelihood of larval amphibian presence by filling up more pools, this also increases connectivity among stream pools allowing for increased predatory fish colonization. Understanding how fish and amphibian larvae currently utilize intermittent streams in the Flint Hills will allow us to detect distribution shifts due to climate change.

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# CHAPTER 1

# INTRODUCTION

## 1.1 Climate Change

Shifts in precipitation and thermal regimes are particularly important to the aquatic landscape of the Great Plains, which exists in a balance of interspersed droughts and floods (Dodds et al. 2004). Effects of climate change are becoming more prevalent, including increasing temperatures, increasing precipitation intensity, and more severe droughts (Overpeck and Udall 2010; Woldeamlak et al. 2007; Walther et al. 2002). Shifts such as these will likely influence fish and amphibian distribution (Comte & Olden, 2017) and how they interact with each other (Blois et al., 2013). Understanding how fish and amphibian larvae currently utilize intermittent streams in the Flint Hills will allow us to detect distribution shifts due to climate change.

The Great Plains is an imperiled landscape (Samson et al. 2004) where few studies have focused on the intermittent stream ecosystems within it and the distribution of fishes and amphibians among them. Wetlands that are temporary but colonized by fish and wetlands that are permanent but fishless represent understudied categories of freshwater ecosystems that may influence herpetofaunal diversity (Holbrook & Dorn, 2016). This is concerning because the Great Plains intermittent stream system (GPISS) is the most abundant habitat for amphibians and fishes. Additionally, small streams constitute more than 50% of the total channel length in most watersheds (Hansen, 2001). Intermittent streams in particular can be highly susceptible to climatic disturbances (Poff & Ward, 1989).  Depending on habitat location and connectivity to water sources, such as off–stream wetlands and isolated ephemeral stream pools, flooding could introduce fishes into an aquatic habitat that previously lacked them.

It is hypothesized that hydroperiod, or the length of time an area holds water (Brooks and Hayashi 2002; Babbitt and Tanner 2000), and connectivity, or the water flow between stream pools, shapes the composition of aquatic communities. Increased connectivity may enhance the presence of fish which are known to negatively impact the occupancy of amphibian larvae in other systems (Hecnar & M'Closkey, 1997; Pilliod et al., 2010). Many commonly dispersing fishes are opportunistic predators that feed on larval amphibians (Heyer et al. 1975) and amphibians actively avoid ovipositing in wetlands with fish present (Hopey & Petranka, 1994; Kats & Sih, 1992; Petranka & Holbrook, 2006; Resetarits & Wilbur, 1989). This increased fish predation would lead to a top-down change in the trophic structure of the aquatic ecosystem (Wilkins et al., 2019).

## 1.2 Great Plains Fish Ecology

Population dynamics of fishes are affected by seasonal and interannual variations in climatic, hydrological, and geographical processes (Nakagawa, 2019). Fishes are a mobile component of stream ecosystems and their abundance is regulated by both hydrology and spatial position of aquatic resources in the landscape (Fausch et al., 2002). Many fishes of the Great Plains have adapted to the unpredictable, harsh conditions and can migrate to permanent bodies of water, reproduce fast, and can endure poor water quality in isolated pools (Labbe and Fausch 2000; Matthews 1987).

Most adult fishes in prairie ecosystems avoid areas with excessive currents produced from flooding or pools that dry frequently (Dodds et al. 2004). An example would be headwater species that move downstream to avoid harsh conditions from drought (Alford & Richards, 1999; Deacon, 1961). Effects of disturbance on prairie stream fishes can vary depending on connectivity to refugia habitat (Larimore et al. 2011) and the fishes ability to reproduce after the disturbance (Fausch &

Bestgen, 1997). Fish assemblage is influenced by species identity and life stage because swimming strength is proportional to body size and smaller individuals may not be able to resist displacement and will be washed downstream (Dodds et al., 2004; Harvey, 1987).

Timing of droughts and floods effect fish communities. Floods that occur early in the year during spawning season will flush fish eggs and larvae from the stream whereas floods in late summer will have a lower impact since most fish have spawned and their young have grown large enough to be more effective swimmers (Dodds et al., 2004). Flooding usually happens during the spring in the Great Plains, but many stream fishes can initiate spawning immediately after a disturbance (Dodds et al., 2004). Floods also increase connectivity to upper stream reaches that have valuable resources and allow fishes to move past upstream barriers to reach ephemeral pools (Dodds et al., 2004). Precipitation tends to occur less frequently as summer advances, which may lead to fishes being stranded in ephemeral pools, becoming easy prey (Dodds et al., 2004).

Droughts may have little effect on fish assemblage and recovery happens rapidly (Matthews and Marsh–Matthews 2003). Fish diversity within the Flint Hills is associated with longitude, being higher in permanent, downstream pools (Tripe & Guy, 1999). The fish that colonize the upper reaches have to move back downstream when drying occurs (Deacon 1961; Ross et al. 1985) or they would desiccate. Consecutive years of drought, however, may cause severe impacts on fish assemblage. The potential impacts of consecutive droughts on fish have not been documented in the Great Plains (Deacon 1961; Matthews and Marsh–Matthews 2003).

## 1.3 Effects of Abiotic and Biotic Factors on Amphibians

Interactions among organisms within their ecosystem depend on abiotic factors such as temperature, droughts, and floods. (Luhring et al. 2019; Thurman & Garcia, 2019), which are all expected to increase in severity due to climate change. Organisms may adapt to climate change through rapid evolution and/or by changes in behavior, physiology, and life history (Charmantier et al. 2008; Charmantier and Gienapp 2014; Anderson et al. 2012; Schaum et al. 2017; Angilletta et al. 2010; Seebacher et al. 2015; Réale et al. 2003; Padfield et al. 2016; Knies et al. 2009; Knies et al. 2006; Tseng and O'Connor 2015). Species specific responses to climate change suggest that favorable conditions should be different among species for resisting population decline and competitive exclusion (Thurman & Garcia, 2019). Moreover, biotic factors such as predator cues can strongly influence amphibian evolution, development, behavior, physiology, and life history (Flowers and Graves 1997; Mushet et al. 2012; McCollum and Van Buskirk 1996; Szuroczki and Richardson 2011).

By understanding natural stressors on larval amphibian distribution, we can accurately monitor shifts caused by climate change. In the Great Plains, amphibians prefer to breed in seasonal and semipermanent wetlands but will also use permanent wetlands if fish are absent (Petranka 1989; Sexton and Phillips. 1986; Bradford 1989; 1991; Bradford et al. 1993; Fellers and Drost 1993; Bronmark and Edenhamn 1994; Lannoo 1998). However, some amphibians can coexist well with fish, as observed with *Lithobates catesbeianus* (American bullfrog). During drought events, amphibians are excluded from seasonal and permanent wetlands since they are dried up, therefore relying on fishless, permanent wetlands for breeding (Pechmann et al. 1989; Wissinger and Whiteman 1992; Dodd 1994; Semlitsch et al. 1996; Lannoo 1998). Amphibians must adjust their breeding strategy based on shifts in the landscape to increase survival of their physiologically limited larvae. Species with longer larval stages usually are heavily dependent on water sources not drying, but some species have been documented to be more tolerable to these stresses (Crump, 2005).

Larval amphibian distribution in the Flint Hills is not as thoroughly analyzed compared to other systems. Based on studies in other systems, larval amphibians are successful in habitats with large surface areas and shallow depths (Wheeler et al., 2015), but they have also been observed to be more abundant in intermediate sized ponds since they have a long enough hydroperiod for larval development and have a lower probability to contain fish (Semlitsch et al., 2015). However, fish also persist in areas with long hydroperiods, and most amphibians cannot coexist with fish. If more rainfall increases the rate in which intermittent streams connect to water sources occupied by fishes, the chance of colonization by fishes increases. By considering the connectivity of aquatic ecosystems, we can determine which areas are more likely to have fish and thus predict the effects on the amphibian community.

## 1.4 Importance of Amphibians

Amphibians serve important roles in ecosystems through nutrient cycling, burrowing, and controlling insect populations (Hocking & Babbitt, 2014). Additionally, these ectotherms serve as indicators of environmental degradation (Halliday 2000) and are one of the most imperiled groups of vertebrates, with populations declining faster than birds or mammals (Stuart et al., 2004). These declines have been accelerating due to the impacts of climate change on their physiology and life history strategies (Ceballos et al., 2015; Wake & Vredenburg, 2008). Secondary production estimates have been conducted on a variety of organism in nature such as macroinvertebrates (Whiting et al. 2011) and fishes (Meyer & Poepperl, 2004), but can be applied to almost any taxa.

Few studies have examined larval amphibian production in nature, especially in the Great Plains. While presence/absence data may provide information on species richness, measuring secondary production is more informative for quantifying and characterizing assemblages in lotic ecosystems (Whiting et al. 2011). Further, secondary production accounts for multiple factors, such as abundance, biomass, growth, reproduction, and survivorship (Benke & Huryn, 1996).

## 1.5 Amphibian Landscape Use in the Great Plains

Climate variability is likely to alter hydrology of wetlands and streams, causing connectivity among populations to decrease during drought or increase during periods of deluge (Pilliod et al., 2015). Amphibian distribution in the Great Plains is strongly influenced by climatic events and distribution of adequate habitats. Depending on the species, amphibians utilize a variety of aquatic habitats, including wetlands, streams, ponds, lakes, ditches, and rivers. Wetlands are critical ecosystems that provide services, such as suitable habitat and breeding sites that promote amphibian populations (de Groot et al. 2012; Junk et al. 2013). How wetlands obtain their water will influence their hydroperiod, physio–chemical characteristics, vegetation composition and structure. This in turn influences the amphibians and fishes that use these wetlands (Burt and Haycock 1996; Semlitsch and Bodie 1998; Hayashi et al. 1998; Moore and Richardson 2003).

Larval amphibian response to connectivity and hydroperiod is expected to be strong in the Great Plains due to changes in suitable habitat resulting from climatic variation. Amphibians utilize isolated, ephemeral wetlands or streams that obtain their water source by rainfall compared to wetlands that are flooded into by other bodies of water that introduce fish (Greenberg et al. 2017). Water deposition in wetlands depends on the topography of the landscape, the proximity of water sources (connectivity), and the severity of climatic events.

Fishes and amphibians in the Flint Hills region of the Great Plains inhabit the intermittent (seasonal) streams, which are more abundant than wetlands and perennial streams (Dodds et al., 2004). Streams and their riparian zones promote dispersion and migration of amphibians between habitats (Dupuis et al. 1995; Rosenberg et al. 1997; Johnson 2004; Mazerolle 2005; Perkins and Hunter 2006; Semlitsch 2008). While small streams can support amphibians (Rosenberg et al. 1997; Moore and Richardson 2003), their connectivity and fish occupancy determine the suitability of habitat for larval amphibians.

## 1.6 Hypotheses

Here, we investigate the effects of hydroperiod, water connectivity, total pool volume, dominant stream vegetation, and fish occupancy (presence/absence) on larval amphibian occupancy, biomass, and density in the GPISS. Across the landscape we sampled, we test the following hypotheses: the likelihood of larval amphibian presence will decrease in the presence of fish, the likelihood larval amphibian presence will increase as hydroperiod increases, and the likelihood of larval amphibian presence will increase in isolated pools. For stream pools where amphibian larvae are present, we test the following hypotheses: larval amphibian abundance will decrease in the presence of fish, larval amphibian abundance will increase as hydroperiod increases, and larval amphibian abundance will increase in isolated pools.

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1 Study Site

Most aquatic habitats of the Great Plains has been negatively impacted by agriculture or urbanization, resulting in pollution, hydrologic disturbance, and physical modification of these ecosystems (Dodds et al. 2004). The Flint Hills of Kansas are unique because the landscape is too rugged for agriculture and most of the region is utilized for cattle grazing instead (Hickey & Webb, 1987). We used Youngmeyer Ranch (YMR), a Wichita State University field station that is 4,700 acres located in the Flint Hills of Elk County, Kansas (Figure 1). Elevation is highly variable with the west side of YMR being relatively flat at approximately 460 m in elevation, whereas the middle and east sides feature steep to gradual slopes that give way to sloped hillsides with elevations between 370–400 m (Houseman et al., 2016). The study site area is about 17.98 km$^2$ with about 39.40 km of first, second, and third order streams that flow along the elevational gradient of the site from either west to east or north to south. It has been shown that the number of fish species increases from headwaters to higher-order streams (Rahel & Hubert, 1991; Schlosser, 1987; Sheldon, 1968).

Several cattle ponds and wetlands are found throughout YMR. 12 out of the 13 cattle ponds currently have predatory game fish occupying them (Ward K., unpublished data). The station is predominantly grassland with scattered black oaks (*Quercus velutina*) along creeks and collection of trees found in creek bottoms (Houseman et al., 2016). YMR has been historically known for cattle grazing and prescribed fires that routinely occur every 1-3 year (Houseman et al., 2016).

There are potentially 65 species of fish, 12 species of anurans, and 2 species of caudates within Elk County based on current range maps (Collins et al. 2010; Kansas Fishes Committee, 2014).

## 2.2 Mapping and Hydroperiod Indices

Initial hydroperiod indices were calculated using Google Earth Pro©(Gorelick et al., 2017). Streams were mapped by creating paths in approximately 100 m increments until the stream lengths were fully mapped. Of the total 31,400 m of streams mapped, we picked a subset of 19 streams that were representative of the site to select pools for hydroperiod score collection. Stream pools are characterized by larger depths and low velocity stream flow, therefore limiting our accuracy of selection using satellite imagery. For each 100 m segment of stream, we chose two pools at random to calculate initial hydroperiod scores, for a total of 318 stream pools. Scores were calculated by recording the presence of water at each of the four most recent historical time periods (10/17/2010, 2/28/2012, 2/16/2013, 11/5/2014) that were available through Google Earth Pro© (Figure 2). We made sure to select two years where the image was taken during the early, wet season and two years representing the later, dryer season. We added an additional hydroperiod score if pools had water during the day of sampling, so pool scores could be 1-5, in which 1 is ephemeral and 5 is permanent.

## 2.3 Sampling Design

We used a variety of sampling techniques as they have been shown to be generally more effective at collecting a wider array of species (Luhring, 2007) due to the sequence of events leading up to a successful capture being susceptible to an individual’s behavior or physiology biases (Luhring et al. 2016). Aquatic surveys were completed using active sampling techniques:

dip netting and seining. To be successful, the mesh size needed to be small enough to prevent larval amphibians from escaping (Luhring et al. 2016).

Surveys took place from 3/19/2020 to 6/30/2020. We sampled a total of 117 stream pools out of 151 (34 were dry). Before pool sampling, we recorded the type of bank vegetation (grasses, shrubs, trees, bare ground), in-stream vegetation (macrophytes, grasses, bare), algae type (filamentous, mat, none), and the inflow/outflow status. We also recorded the percentage (0%, 25%, 50%, 75%, 100%) of canopy cover, vegetation litter, algae, and biofilm present. To measure stream pool connectivity, if it had inflow or outflow, the pool was considered connected, however if it lacked flow, it was considered isolated. We used seines, hand capture, and enclosures with dip nets for active capture of fishes and larval amphibians. Additionally, visual observations would help determine presence and absence of amphibians.

We used constrained volume sampling with an enclosure to subsample each pool. We used a cylindrical rubber trash can (51 X 51 X 65 cm) with the bottom cut off as our enclosure trap and it was placed throughout the open water habitat. These were randomly placed (random number generator) on either the left (1), middle (2), or (3) right side on the downstream end. If the pool was less than 14 m long, enclosure placements progressed upstream in 1 m increments. If a pool was between 14-24 m long, enclosures progressed upstream in 2 m increments. If a pool was between 24 34 m long, enclosures progressed upstream in 3 m increments.  We chose to increase increment distance as pool length increased so that large pools were not oversampled relative to smaller pools. Using a 10 m long pool with an initial placement at the middle for example, the next placements would follow a subsequent systematic design going from middle (0 m) to right (1 m), right to left (2 m), left to middle (3 m), and so on. Depth readings were taken in the center of every enclosure placement, which is how we calculated sampled volume (length and width held constant). For each placement, a round dip net with 0.3 cm mesh was swept through the water column within the enclosure until 3 consecutive dip net sweeps returned no larval amphibians, fishes, or invertebrates (this project was nested in a larger project). Captured organisms were kept in separate buckets filled with water from the habitat. Amphibian larvae were identified using *A Guide to Larval Amphibian Identification in the Field and Laboratory* (Hoverman et al., 2015). Following identification, larval amphibian mass was measured via volumetric displacement in graduated cylinders, whereby volume in milliliters was converted to grams in a 1:1 ratio based on the assumption that measured larvae had the same density as water. Organisms were released back into their habitat after the data collection.

After enclosure sampling, we completed two seine passes through the pool. The size of the pool and the amount of aquatic vegetation determined which seine size we would use. Three different sized seines were used: a 3’ width x 3’ height, small seine with fine mesh (<0.1 cm), a 10’ width x 3’ height, medium size seine with 0.5 cm mesh, and a 15' width x 6' height bag seine with a 6'x6'x6' bag in the center with 1.3 cm mesh. The data recorded and the containment and release of organisms was the same as enclosure sampling.

## 2.4 Estimating Total Pool Volume, Total Biomass, and Density

We estimated total pool volume (*TV*) by collecting cross sectional width and depth measurements every 2 m along the length of the stream pools, see Appendix C to view the protocol for measuring pool volume. We did this to extrapolate our sampled larval biomass (*SB*) within our sampled volume (*SV*) retrieved from enclosure sampling to estimate the total larval biomass (*TB*) within the entire stream pool (Equation 1). The total larval amphibian biomass is the measurement we used for analysis. Density was the concentration of sampled larval biomass ($SB$) per sampled volume ($SV$).

Equation. 1

$$\frac{SB}{SV} * \frac{TB}{TV}$$

## 2.5 Statistical Analysis

### 2.5.1 Presence/Absence

Presence/absence analysis was limited to pools that held water (n=117) across all trap types used (enclosure, seine, dip net, visual, or hand capture). We encountered five species of larval amphibians: *Anaxyrus americanus* (American toad), *Pseudacris maculata* (boreal chorus frog), *Lithobates blairi* (plains leopard frog), *Lithobates catesbeianus* (American bullfrog), *Ambystoma texanum* (small-mouthed salamander). We further analyzed all larval amphibians combined, *L. blairi* tadpoles, and *A. americanus* tadpoles since they had sufficient sample sizes (detected at 15+ stream pools).

To investigate which factors affected larval amphibian occupancy (probability of presence), we constructed candidate models including 1-3 factor generalized linear models (GLMs) with every possible combination, including interactions, between fish presence, total pool volume, and connectivity as explanatory variables. GLMs were fitted for a binomial regression using the logit function. Models were ranked using Akaike Information Criterion values corrected for small sample size (AICc). AICc weights and comparisons were calculated using ‘AICctab’ in the‘bbmle’ package (Bolker, 2013). The strongest models (AICc values < 2.0) (Burnham &

Anderson, 2002) were further analyzed using an analysis of variance (‘Anova’ in the ‘car’ package; Fox and Weisberg 2019). Type II sum of squares (SS) were used on models with no interaction effects and Type III SS for models with interactions. All statistical analyses were conducted using RStudio 3.6.1 (RStudio Team 2020).

### 2.5.2 Biomass and Density

Biomass and density analyses included enclosure captured larvae and only pools where larval amphibians occurred (n=31) due to having known sampled volumes and biomass using enclosure sampling and therefore being able to determine the known concentration of organisms per unit of volume. Rather than focusing on the distribution of larval biomass and density across the landscape (which occupancy essentially explains), we wanted to observe what influences their production and concentration where they occur. We further analyzed all larval amphibians combined, *L. blairi* tadpoles (n=21), and *A. americanus* tadpoles (n=18).

We excluded models that contained a two-way interaction between fish occupancy and stream vegetation and a two-way interaction between connectivity and vegetation due to us sampling a relatively small number of pools and not having a particular fish (present or absent) or connectivity (connected or isolated) observation across all three stream vegetation categorical observations (macrophytes, grasses, and bare). Similarly for *L. blairi* biomass, we had to avoid interactions between stream vegetation and hydroperiod. For A. americanus density we avoided models with interactions between fish occupancy and connectivity, fish occupancy and hydroperiod, and stream vegetation and hydroperiod. We used AICc model comparison and ANOVA similarly as the presence/absence analysis. Models that had dominant stream vegetation as the best predictor variable with no interaction, were further analyzed using a Tukey’s HSD post hoc test to determine if macrophytes, grasses, or bare differed from each other (using ‘glht’ in the ‘multcomp’ package; Hothorn et al. 2008).

For total larval biomass, we constructed candidate models, including 1-4 factor GLMs with every possible combination, including interactions, with fish occupancy, connectivity, hydroperiod, and dominant stream vegetation as explanatory variables and log transformed larval amphibian biomass as the response variable. Amphibian biomass was log transformed due to raw data having a right skewed distribution. We excluded total pool volume from the biomass models since total pool volume was used to estimate total amphibian biomass. The only difference for the larval amphibian density analysis is that we included log total pool volume as an explanatory variable. Larval density and pool volume were log transformed because the data had a right skewed distribution. We used fish occupancy instead of fish abundances because there were no differences in detecting significant effects on larval amphibian abundances, especially since few pools had fish (n=7) and the use of either main effect did not alter our results.

# CHAPTER 3

# RESULTS

## 3.1 Factors Influencing Larval Amphibian Occupancy

Out of the 151 pre-selected pools, 117 held water and were sampled. We detected five different species of larval amphibians across YMR in which we obtained abundance and biomass data for (Table 1). There was little overlap between larval amphibian and fish occupancy across the property, with larval amphibians present in 40 pools, fishes in 68, and only 13 pools had both. 30 pools were macrophyte dominated, 34 grass, and 53 bare.

Amphibian larvae occupancy was best explained by a model with fish occupancy, connectivity, and total pool volume as main effects, with every possible two-way interaction between them, and a three-way interaction between them ($\chi^2_1$ = 5.60, p = 0.02; Table 2). As predicted, amphibian presence was higher when fish were absent and increased with pool volume (Figure 3). In isolated pools where fish were present, the likelihood of amphibian larvae being present decreased as volume increased. This contrasts with connected pools where amphibian occupancy increased as volume increased even with fish present. Hydroperiod was not a significant predictor of amphibian presence, potentially because our sampling occurred following a wet year when fish were widespread across pools that normally dry between years.

Predictors for presence of larval amphibians were species specific. Fish had a negative effect on *L. blairi*, but where fish were present, the likelihood of larvae being present increased as pool volume increased ($\chi^2_1$ = 10.41, p = 0.001; Figure 4). *Lithobates blairi* preferred isolated habitats where fish were present but where fish were absent, they did not have a preference on connectivity ($\chi^2_1 = 4.11$, p = 0.04; Figure 5). The top model that best explained *L. blairi* occupancy had fish occupancy, connectivity, and pool volume as main effects, with every possible two-way interaction between them, and a three-way interaction between them (Table 3), however, the three-way interaction at best was only marginally significant ($\chi^2_1 = 3.12$, p = 0.08). The second-best model has two, two-way interactions from the same three variables and has a low AICc score of 0.8. A two-way interaction between fish and pool volume was significant in both models, but the two-way interaction between fish and connectivity was only significant in the top model, so including the three-way interaction provides more explanatory power but we only further analyzed the two significant two-way interactions.

Regardless of the connectivity status, fish presence negatively affected *A. americanus* tadpoles and vice versa ($\chi^2_4 = 4.07$, p = 0.04; Figure 6). Although there were other strong models (fish, and fish + connectivity), having the interaction between fish and connectivity better explained the occupancy of *A. americanus* (Table 4). Fish was significant in all three models but connectivity was only significant if the interaction with fish was included.

### 3.2 Factors Influencing Larval Amphibian Biomass

For the 20 connected and 11 isolated stream pools where larval amphibians occurred, the average estimated total amphibian biomass was 256g ± 541 ($\bar{X}$ ± SD). For the east side (n = 16), the average biomass was 41g ± 40 per pool whereas the west side (n = 15) had an average of 485g ± 718. All 16 pools were connected on the east side and the west side had 11 isolated and 4 connected stream pools. Out of the 31 stream pools where larval amphibians occurred, only 7 of the pools had fish present. 10 pools were macrophyte dominated, 18 grass, and 3 bare.

Macrophyte dominated pools had higher larval amphibian biomass ($\chi^2_2 = 38.86$, $p < 0.001$; Figure 7) compared to grass ($t_2 = 5.87$, $p < 0.001$) and bare pools ($t_2 = 4.20$, $p < 0.001$). In addition to the top model, the other model with support only had significant vegetation effects (Table 5). *Lithobates blairi* larval biomass was higher in macrophyte dominated pools ($\chi^2_2 = 23.33$, $p < 0.001$) compared to grass pools ($t_2 = 5.3$, $p < 0.001$; Figure 8). The second strongest model for *L. blairi* included an additional significant effect from hydroperiod but after further analysis, there were no significant differences among categories (Table 6). The third strongest model only had significant vegetation effects. Similarly, *A. americanus* larval biomass was higher in macrophyte dominated pools ($\chi^2_2 = 14.34$, $p < 0.001$; Figure 9; Table 7) compared to grass ($t_2 = 3.54$, $p = 0.007$) and bare pools ($t_2 = 3.12$, $p = 0.02$). Our small sample size of barren pools could not be used for meaningful conclusions. Conversely, since we only found 3 barren pools out of the 31 with larvae present, this could be due to their preference for pools with vegetation. Regardless, our sample~~s~~ size for both grasses and macrophytes were large enough to report that larvae preferred pools with macrophytes over grasses.

### 3.3 Factors Influencing Larval Amphibian Density

The average amphibian density across YMR was 28g/m$^3$ ± 102. On the east side of the property the average density was 8g/m$^3$ ± 20, while the west side had an average of 60g/m$^3$ ± 156. For the 31 stream pools where larval amphibians occurred, the average amphibian density was 63g/m$^3$ ± 139. For the east side, the average density was 37g/m$^3$ ± 106 whereas the west side had an average of 186 g/m$^3$ ± 718.

Larval amphibian density was higher in isolated pools ($\chi^2_1 = 11.46$, $p < 0.001$; Figure 10) and pools that were dominated by macrophytes ($\chi^2_2 = 22.98$, $p < 0.001$) compared to grass ($t_2 =$

4.74, $p < 0.001$) and bare pools ($t_2 = 3.24$, $p = 0.009$). Larval amphibian density was highest in small pools where macrophytes dominated ($\chi^2_3 = 11.31$, $p = 0.01$), but as pool volume increased, amphibian density decreased (Figure 11). All pool sizes above 10 $m^3$ were macrophyte dominated. Grass dominated and bare pools tended to be smaller and amphibian density was lower than macrophyte dominated pools of similar size. The two top models shared significant connectivity and vegetation main effects (Table 8). Here, we presented results from the top model which included an interaction between log pool volume and vegetation.

*Lithobates blairi* density was higher in macrophyte dominated pools ($\chi^2_2 = 6.79$, $p = 0.03$) compared to grass pools ($t_2 = 2.49$, $p = 0.05$; Figure 12). Larval *L. blairi* density was higher in isolated pools compared to connected pools ($\chi^2_1 = 16.15$, $p < 0.001$; Figure 13). The two top models are similar in that connectivity seems to be the main driver for *L. blairi* density (Table 9). Macrophyte dominated pools had higher *A. americanus* densities ($\chi^2_2 = 39.14$, $p < 0.001$; Figure 14; Table 10) compared to grass ($t_2 = 6.15$, $p < 0.001$) and bare pools ($t_2 = 4.89$, $p < 0.001$). Larval *A. americanus* density was higher when fish were absent ($\chi^2_1 = 7.76$, $p = 0.005$; Figure 15). There was only one pool where both fish and *A. americanus* tadpoles occurred which leads to an inconclusive result on the effects fish have on *A. americanus* density, but this further supports our hypothesis that amphibians tend to avoid habitats with fish present.

# CHAPTER 4

# DISCUSSION

Larval amphibian occupancy was most influenced by fish occupancy, connectivity, and pool volume. It was no surprise that fish were a strong predictor for explaining amphibian occupancy (Heyer et al. 1975; Bradford 1989; Hecnar and M'Closkey 1997). Larval amphibian occupancy was higher in isolated pools to an extent, but other factors such as fish occupancy, pool volume, affected amphibian occupancy differently in isolated and connected pools. Sensitivity to volume and connectivity were species specific, but both factors played a role in whether amphibians and/or fish were present in stream pools. Where larval amphibians occurred, the vegetation community showed strong effects on both biomass and density.

Although few studies have looked at larval stream amphibian distribution in the Flint Hills, there are studies in other systems that have shown similar results. *Rana sierrae* (Sierra Nevada yellow-legged frog) tadpole abundance in lakes showed negative responses to fish presence, although the model had high uncertainty (Wilkins et al., 2019). *Hyla versicolor* (gray treefrog) tadpoles were nine times less abundant when *Lepomis macrochirus* (bluegill sunfish) were present in experimental ponds (Smith et al., 1999). Conversely, *L. catesbeianus* tadpole abundance increased in the presence of bluegill (Smith et al., 1999). Many studies provide evidence that fishes have a negative impact on most amphibian species across all life stages (Heyer et al. 1975; Bradford 1989; Hecnar and M'Closkey 1997). Our study extends this pattern to larval amphibian species in the Flint Hills.

Habitat size has been found to be both positively and negatively correlated with amphibian richness and abundance (Asad et al., 2020; Eterovick, 2003; Strauß et al., 2010; Wilkins et al., 2019). Pool size is important, but the effect is context specific. Pool volume alone may not predict larval amphibian presence, biomass, and density, due to other factors having strong influences (e.g. fish presence, vegetation). However, pool volume is a crucial abiotic component in this system since it influences how the biota interact and should be considered in further studies to understand its relationship with predator-prey interactions.

Few studies have specifically investigated the relationship between larval or adult amphibians and stream connectivity. If connectivity from a water source that has fish inhabitants increases, observing a positive response from amphibians is unlikely. Supporting this hypothesis, amphibian occupancy and density was generally higher in isolated pools. In isolated pools with fish present, amphibian occupancy decreased as pool volume increased, most likely because larger isolated pools allow for more fish to be present. Where connected pools had fish present, pool volume had the opposite effect; amphibian occupancy slightly increased as pool volume increased. This suggests that connected pools allow fish to distribute elsewhere rather than being trapped in one pool predating on available amphibian larvae. Intermittent stream connectivity should be further evaluated since larval amphibian presence depends on how connected a stream is, especially since the flow of the stream will determine 1) distribution of amphibian eggs and larvae, and 2) colonization capability of fishes (Dodds et al., 2004; Mims et al., 2015).

Having sufficient amounts of aquatic vegetation benefit amphibians an many ways, such as cover from predators, oviposition sites, shade, oxygen production, and periphyton substrate for grazing (Babbitt & Tarr, 2002; Sredl & Collins, 1992; Stebbins & Cohen, 1995) and abundance of vegetation is positively correlated with amphibian abundance (Egan & Paton, 2004; Hamer & Parris, 2013; Hartel et al., 2007; Holzer, 2014; Shulse et al., 2010). Macrophytes may benefit larvae by providing cover from predators as a fish's ability to capture prey decreases in structurally complex habitats formed by macrophytes (Crowder & Cooper, 1982; Diehl & Kornijów, 1998; Savino & Stein, 1982). Additionally, *L. blairi* and *P. maculata* tadpoles have been observed to use aquatic vegetation as cover (Crawford et al. 2005, E.C.M., personal observation). Further, it has been shown that metaphyton (i.e. filamentous algae) are associated with habitats containing macrophytes (Iacarella et al., 2018), which also serve as an important food source for most tadpoles (Ruibal and Läufer 2012; Beiswenger 1975; Schiesari et al. 2009; Whitaker 1971). Thus, larval amphibian biomass and density in the Flint Hills likely increases in macrophyte dominated pools since they provide cover, food, and oviposition sites.

Hydroperiod length is frequently a dominant factor in other aquatic habitats (Snodgrass et al. 2000; 2001; Babbitt 2003; Rubbo and Kiesecker 2005; Mathwin et al. 2020), but it appeared to be less important among intermittent stream pools in explaining occupancy, biomass, and density trends at YMR. This is possibly due to us sampling during a wet year (12.24 inches above the 10-year average) which implies that fish distribution is at a maximum across the streams on this landscape and are having a large effect on the spatial distribution of amphibian larvae. In this system, ephemeral wetlands are colonized by fish during wet years. Therefore, high connectivity of intermittent streams may override the impacts of drying and refilling on aquatic predators seen in more isolated systems.

Larval amphibians should respond across a temporal scale. This was a natural snapshot experiment where we sampled from May 19 to June 30, 2020, when there is high climatic variation in Kansas, with a wetter climate in May and dryer climate towards the end of June. We would expect larval amphibian presence to decrease as the summer season progresses either due to dryer conditions or from larvae metamorphosing into adults. The relationship between pool volume, hydroperiod, connectivity, and the distance of pools from the mainstream could be beneficial for predicting where amphibians occur along a longitudinal gradient.

The Flint Hills is primarily used for cattle grazing therefore impoundments have been constructed to provide water for them. It would be interesting to explore how the impoundments on cattle pastures effect amphibians, by either disrupting the natural flow regime (pool volume, hydroperiod, connectivity) or by promoting predatory fishes through stocking or serving as refugia during droughts. Research indicates that tadpole density downstream of impoundments is much lower than upstream due to impoundments acting as barriers for downstream movement and that 50% of the larval population are found within the first 20% of the area upstream of the pond (Dare et al., 2020), which is likely due to impoundments causing discontinuity in the stream (Hall et al. 2011). For ponds at our field site, we may see a similar or more extreme results since they have been previously stocked with game fish that may wash out to pools downstream of ponds.

Overall, larval amphibians preferred isolated, macrophyte dominated pools where fish were absent. Although more precipitation has the potential to increase the likelihood of larval amphibian presence, it also makes pools more susceptible to fish colonization due to increased connectivity. These results are expected to vary seasonally and annually due to the highly variable climatic nature of the Great Plains which makes it an ideal system to study the effects of climate change. With both prairies (Samson et al. 2004) and amphibians (Stuart et al., 2004) imperiled and understudied, interpreting the complex landscape use of this community should be a priority to manage the Great Plains intermittent stream system effectively.

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# APPENDICES

## A. Figures

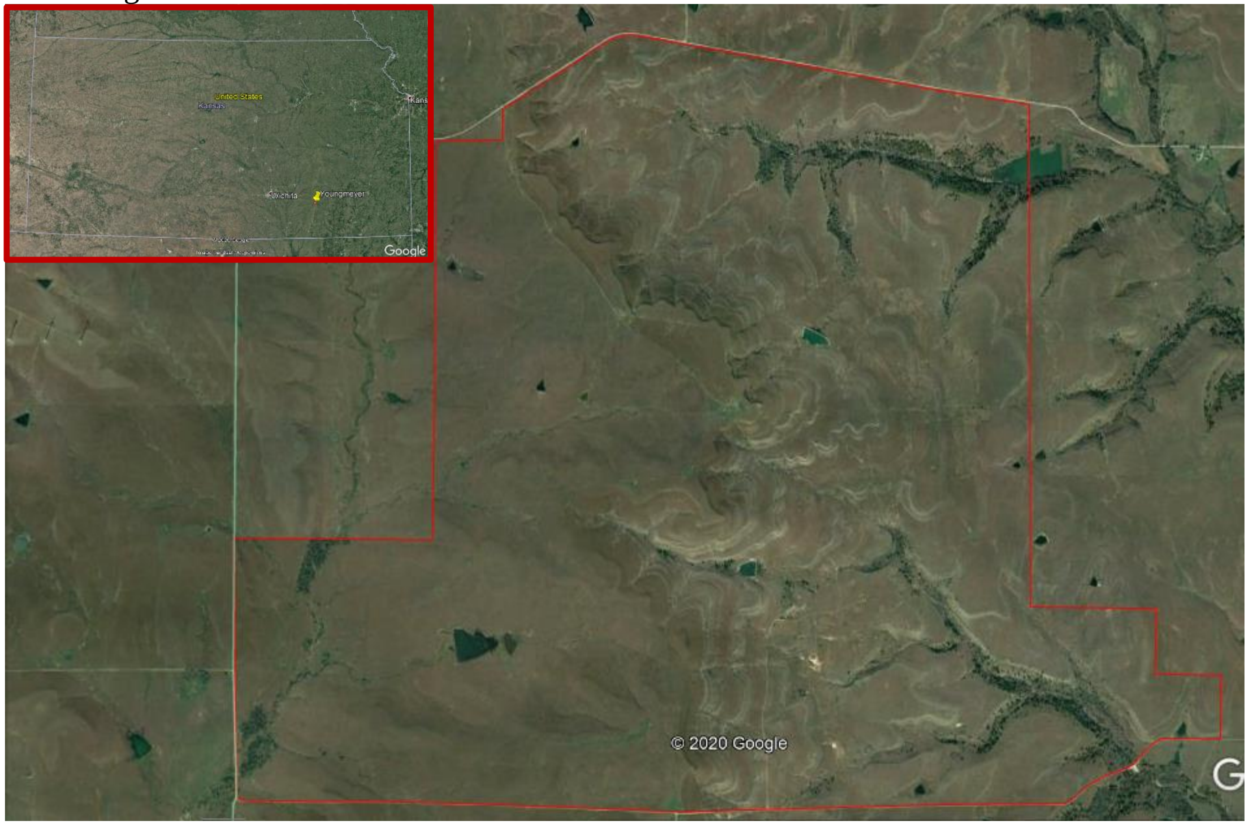


Figure 1. Location of Youngmeyer Ranch (yellow pin) in Southeast Kansas, United States (inset) with property line highlighted in red (image from Google Earth Pro©).

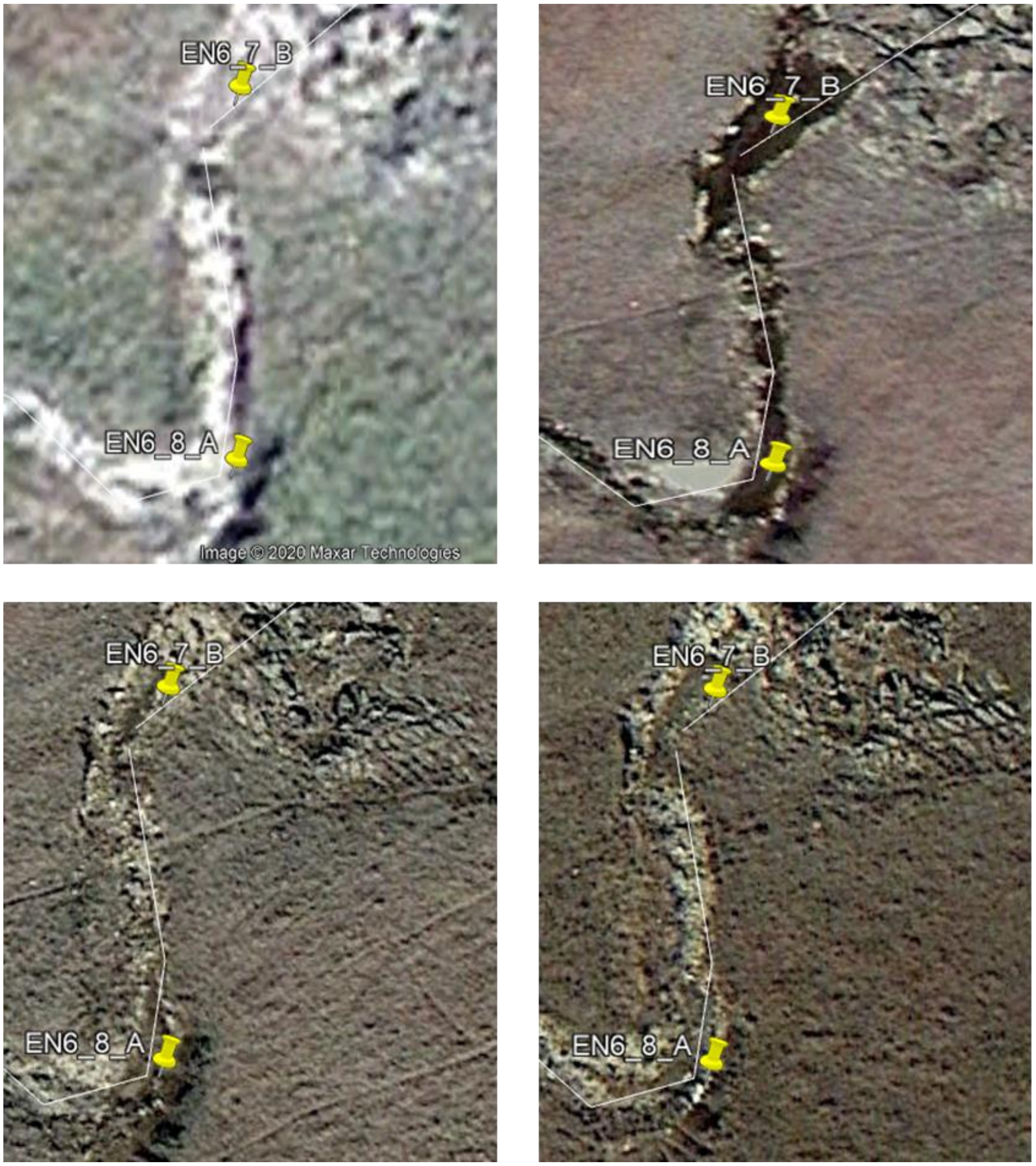


Figure 2. Example of two stream pools (yellow placemarks) where hydroperiod scores were collected using satellite imagery across four years: Oct. 17, 2010 (top left), Feb. 28, 2012 (top right), Feb. 16, 2013 (bottom left), and Nov. 5, 2014 (bottom left) (images from Google Earth Pro©).

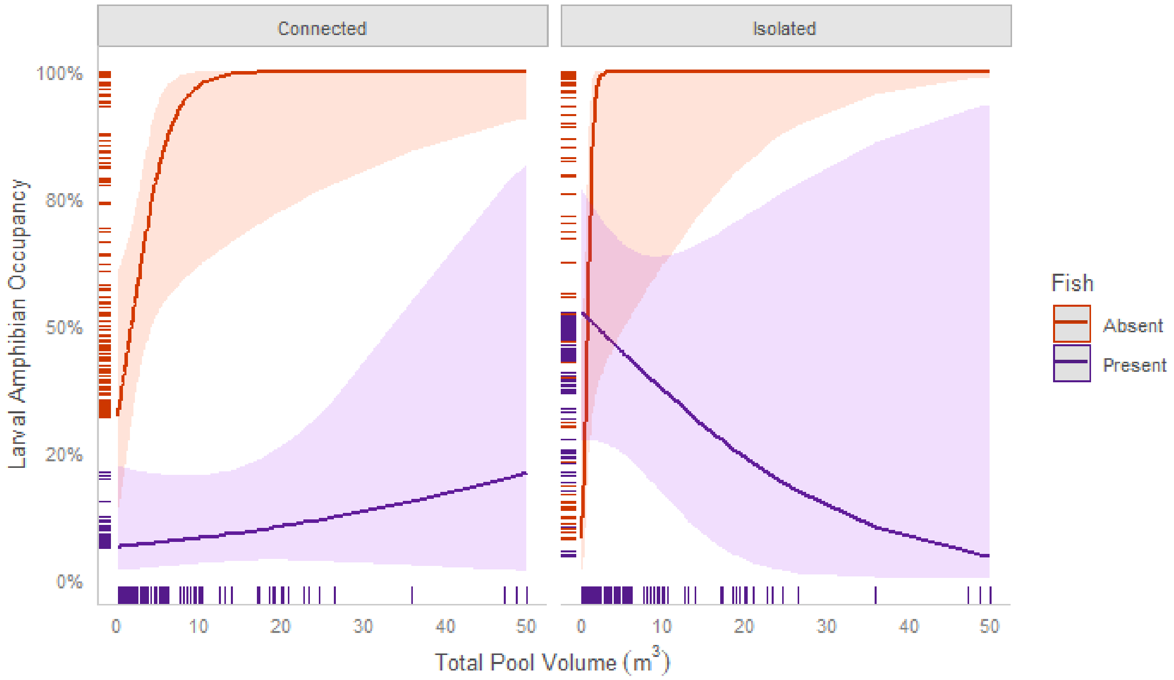


Figure 3. Probabilities of larval amphibians being present as total pool volume increases, when stream pools are connected (left graph) or isolated (right graph), and when fish are absent (orange) or present (purple).

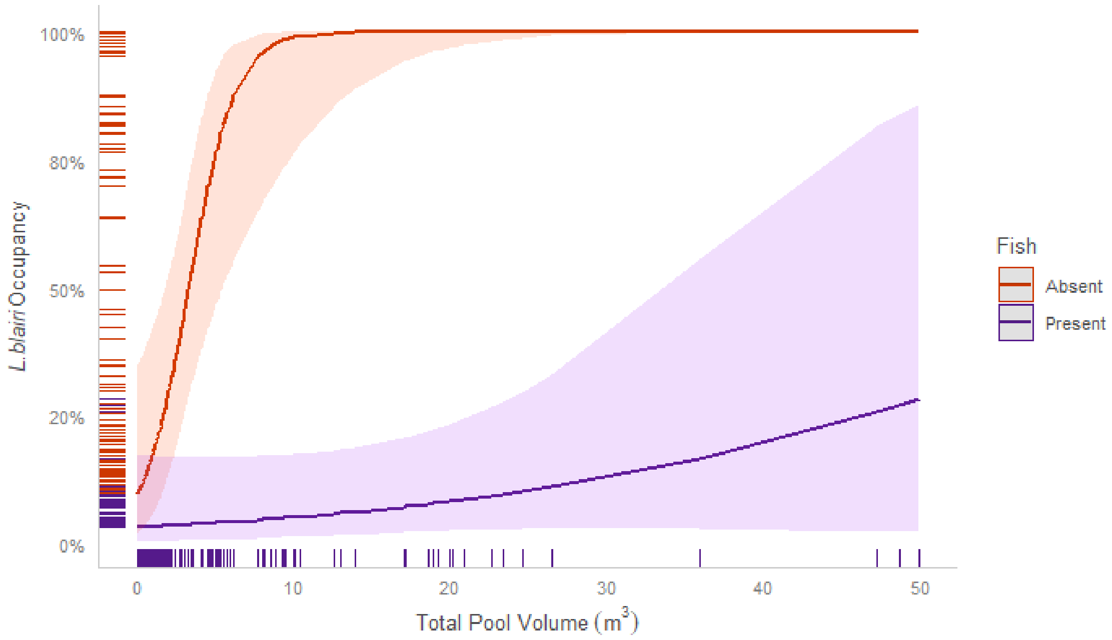


Figure 4. Probabilities of larval *L. blairi* being present when fish are absent (orange) or present (purple) as total pool volume increases.

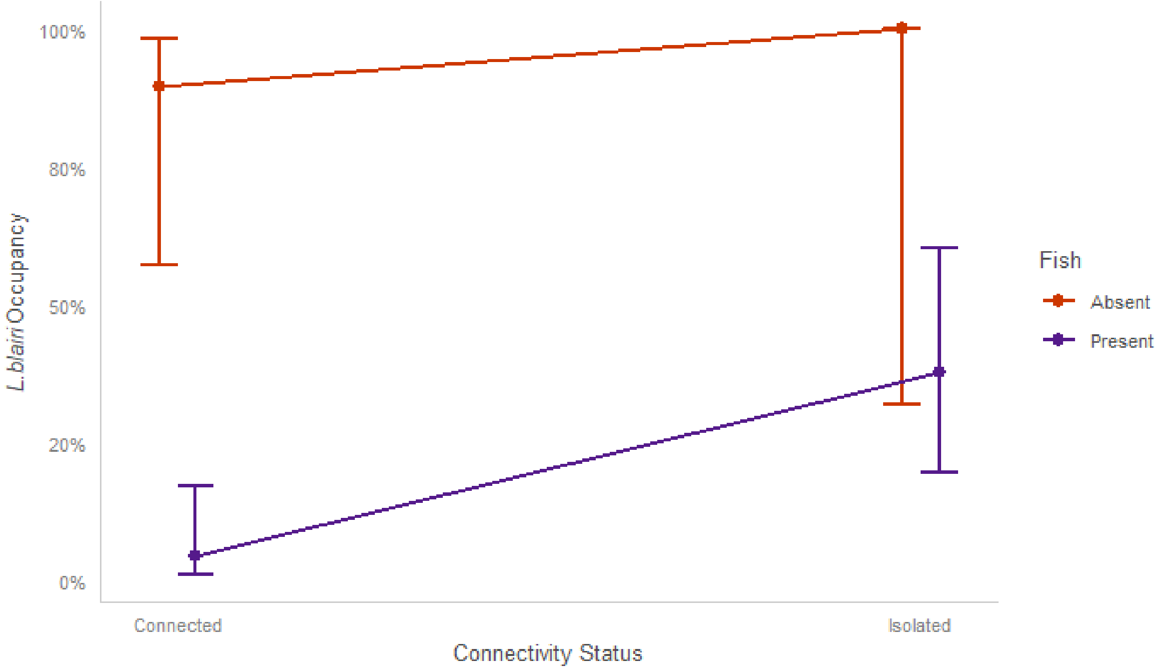


Figure 5. Probabilities of larval *L. blairi* being present when fish are absent (orange) or present (purple) and when a pool is connected (left) or isolated (right).

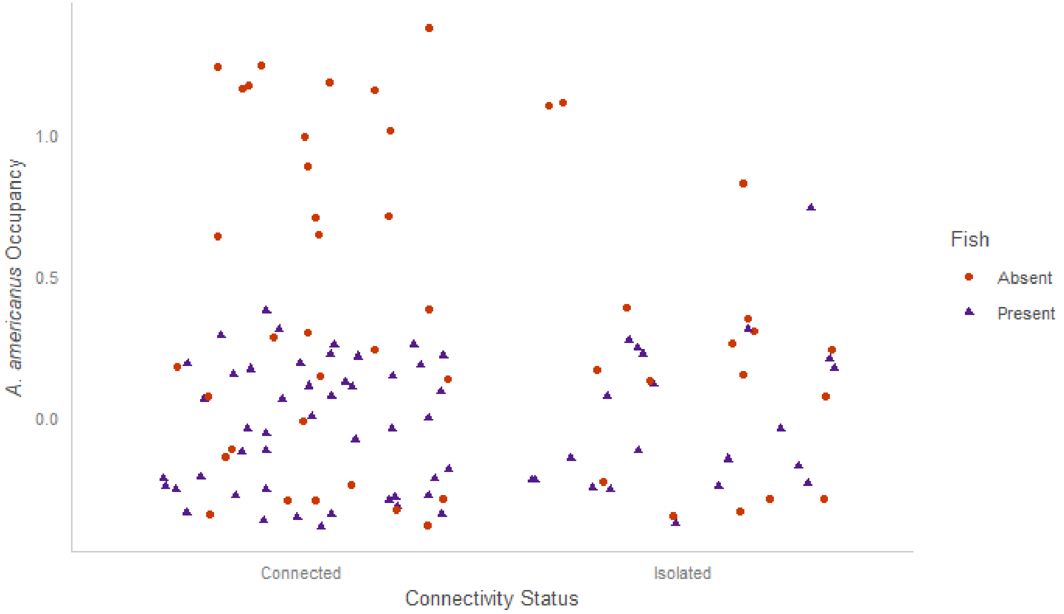


Figure 6. Probability scatterplots of larval *A. americanus* being present when fish are absent (orange circles) or present (purple triangles) and when pools are connected (right) or isolated (left).

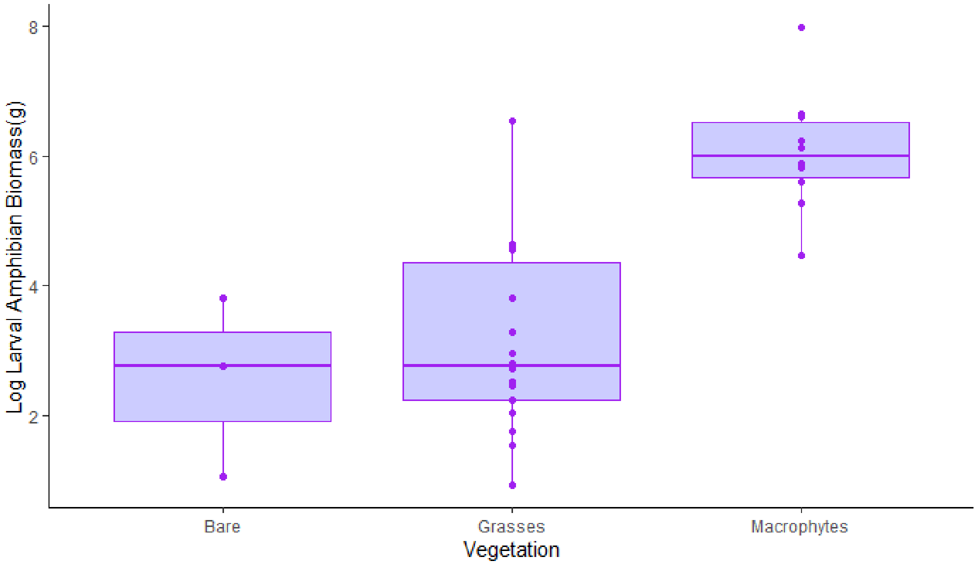


Figure 7. Boxplots of log larval amphibian biomass across bare, grass, and macrophyte dominated stream pools. Only three observation for bare pools, therefore the relationship with bare is inconclusive. The middle bar in the middle of the boxplots represents the median. The middle 50% density measurements fall within the inter-quartile range, which is the box. The values that stretch over a wider range of density than the inter-quartile range are represented by the lower and upper quartiles (outer 50%).

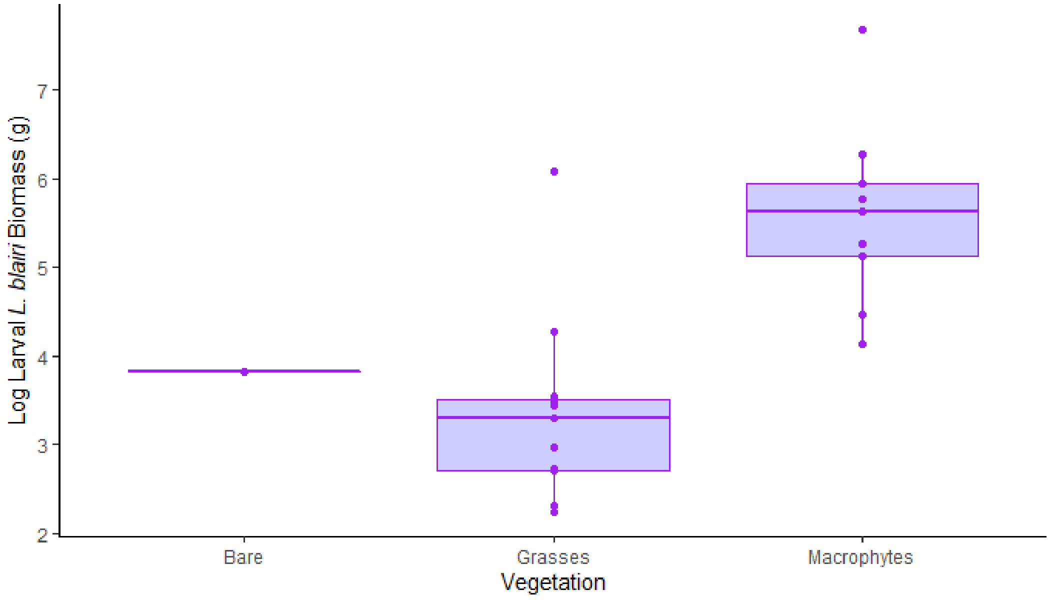


Figure 8. Boxplots of log larval *L. blairi* biomass across bare, grass, and macrophyte dominated stream pools. Only one observation for bare pools, therefore the relationship with bare is inconclusive. See Figure 7 for interpretation of boxplots.

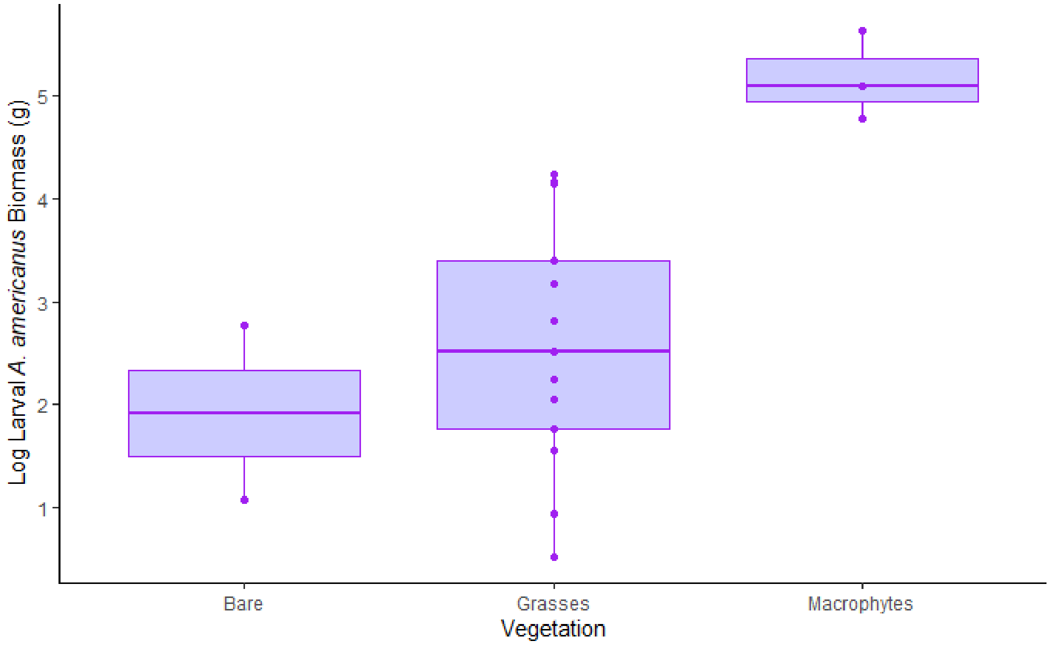


Figure 9. Boxplots of log larval *A. americanus* biomass across bare, grass, and macrophyte dominated stream pools. Only two observations for bare pools, therefore the relationship with bare is inconclusive. See Figure 7 for interpretation of boxplots.

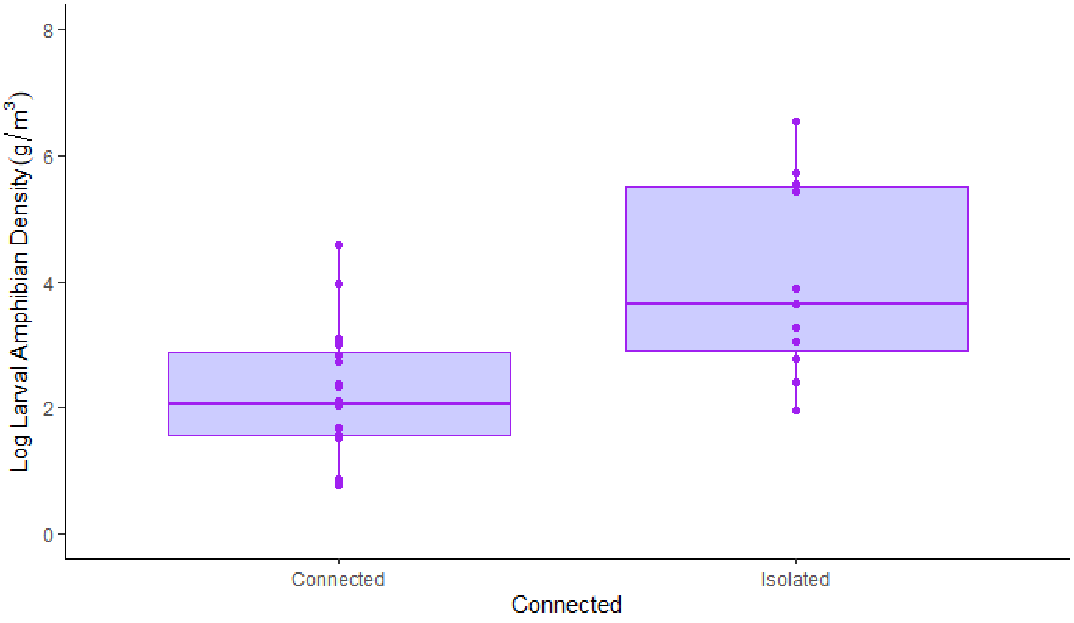


Figure 10. Boxplots comparing log larval amphibian density between connected and isolated stream pools. See Figure 7 for interpretation of boxplots.

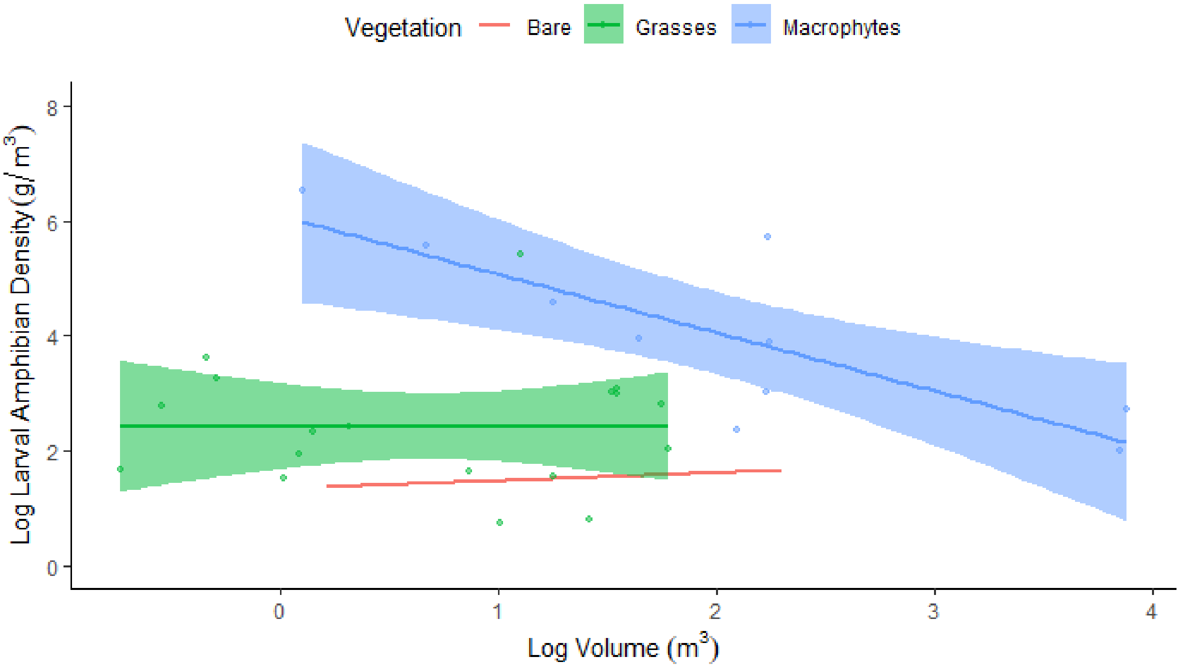


Figure 11. Interaction plot between log total pool volume and dominant stream vegetation, and their effects on log larval amphibian density. Bare pools are depicted as red, grasses are green, and macrophytes are blue. Bare pool error bars are not included due to only three observation for bare pools, therefore the relationship with bare is inconclusive.

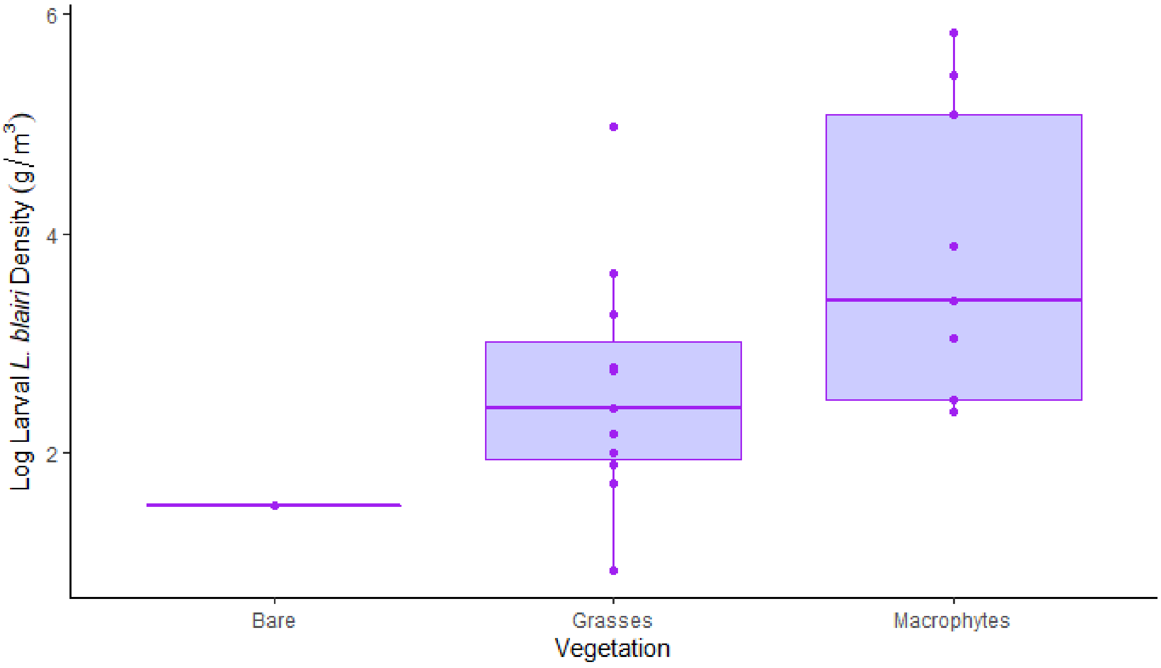


Figure 12. Boxplots comparing log larval *L. blairi* density between bare, grass, and macrophyte dominated pools. Only one observation for bare pools, therefore the relationship with bare is inconclusive. See Figure 7 for interpretation of boxplots.

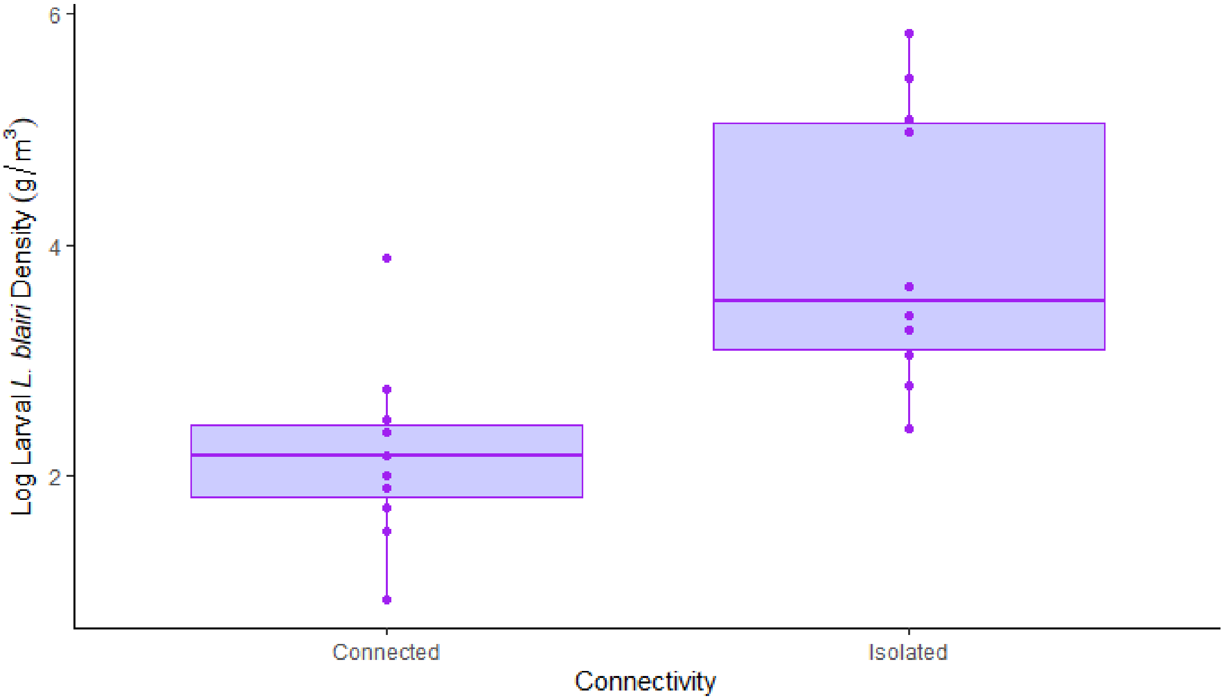


Figure 13. Boxplots comparing log larval *L. blairi* density between connected and isolated stream pools. See Figure 7 for interpretation of boxplots.

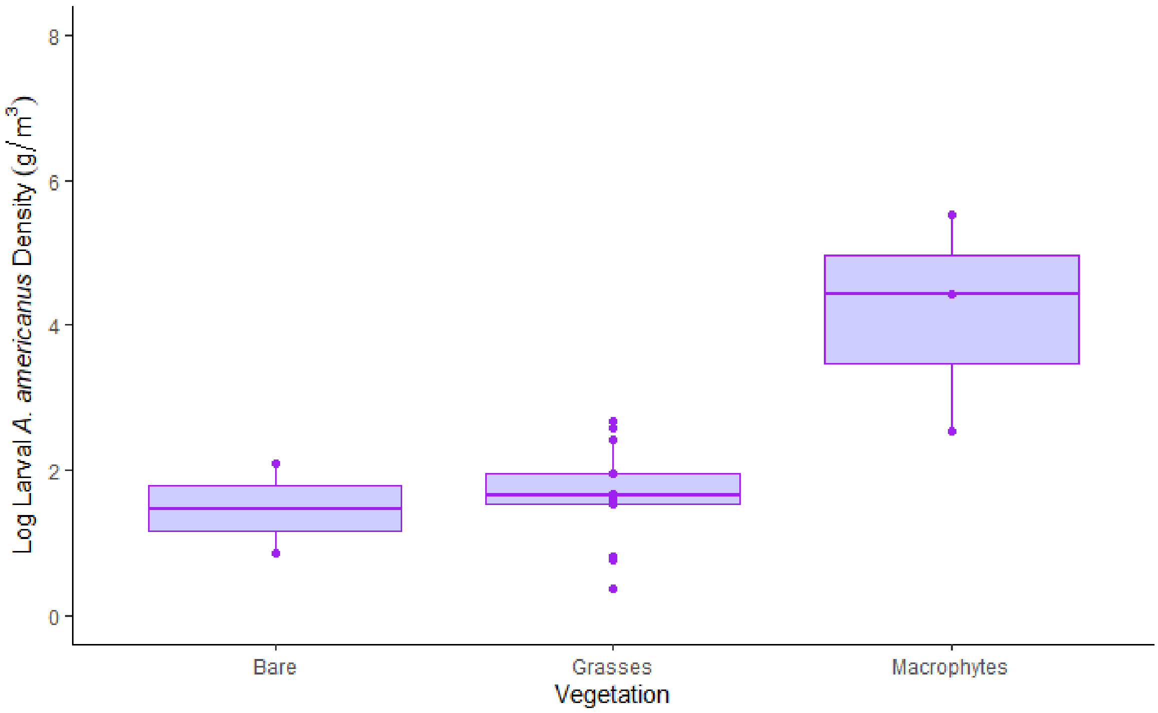


Figure 14. Boxplots comparing log larval *A. americanus* density between bare, grass, and macrophyte dominated pools. Only two observations for bare pools, therefore the relationship with bare is inconclusive. See Figure 7 for interpretation of boxplots.

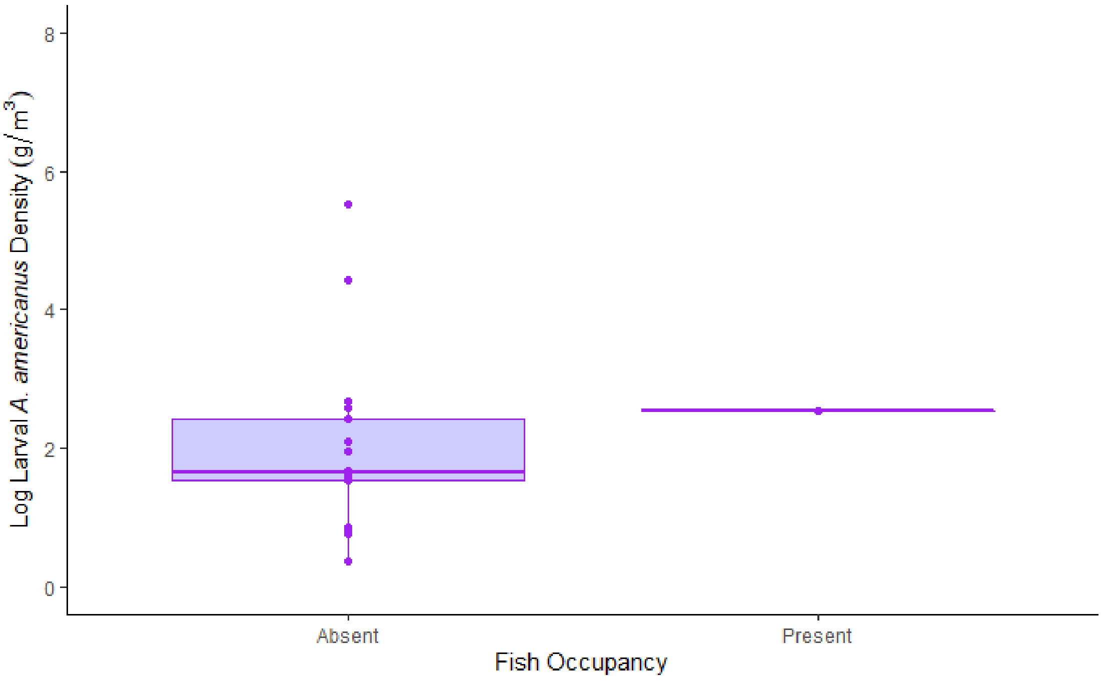


Figure 15. Boxplots comparing log larval *A. americanus* density between pools with fish present and absent. See Figure 7 for interpretation of boxplots.

## B. Tables

Table 1. Number of total larval amphibians sampled, and the total sampled biomass and density from enclosure samples across all pools.

| **Common Name** | **Scientific Name** | **Number of Individuals** | **Biomass (g)** |
|---|---|---|---|
| Small-mouthed Salamander | *Ambystoma texanum* | 64 | 44 |
| American Toad | *Anaxyrus americanus* | 1,471 | 113 |
| Plains Leopard Frog | *Lithobates blairi* | 233 | 456 |
| American Bullfrog | *Lithobates catesbeianus* | 4 | 35 |
| Boreal Chorus Frog | *Pseudacris maculata* | 48 | 14 |
| **Frogs & Toads** | **Anura** | **1,756** | **618** |
| **Amphibians** | **Amphibia** | **1,820** | **662** |

Table 2. Results of AICc model comparisons for larval amphibian occupancy. Explanatory variables are fish occupancy (Fish), connectivity status (Connectivity), and total pool volume (Pool Volume). Models with “^” tests for each main effect and each two-way (^2) or three-way (^3) interaction in the model and “:” specifies an interaction between two variables. For each model, a dAICc score is provided which was used for model comparison, models were considered strong if dAICc scores were below 2.0 and were bolded. df represents degrees of freedom and $w_i$ represents weight of the model.

| **Model** | **AICc** | **df** | **$w_i$** |
|---|---|---|---|
| **(Fish + Connectivity + Pool Volume)^3** | **0.0** | **8** | **0.82** |
| **(Fish + Connectivity + Pool Volume)^2** | 3.3 | 7 | 0.16 |
| **Fish * Pool Volume** | 8.1 | 4 | 0.01 |
| **Fish * Connectivity** | 9.4 | 4 | 0.01 |
| **Fish + Connectivity** | 15.7 | 4 | <0.001 |
| **Fish** | 16.2 | 2 | <0.001 |
| **Fish + Connectivity + Pool Volume** | 17.8 | 4 | <0.001 |
| **Fish + Pool Volume** | 18.3 | 3 | <0.001 |
| **Connectivity** | 29.9 | 2 | <0.001 |
| **Pool Volume + Connectivity** | 30.3 | 3 | <0.001 |

| | | | |
|---|---|---|---|
| **Pool Volume** | 30.3 | 2 | <0.001 |
| **Intercept** | 30.6 | 1 | <0.001 |
| **Pool Volume * Connectivity** | 32.4 | 4 | <0.001 |

Table 3. Results of AICc model comparisons for larval *L. blairi* occupancy. View Table 2 for description of explanatory variables and components of the table.

| **Model** | **AICc** | **df** | $w_i$ |
|---|---|---|---|
| **(Fish + Connectivity + Pool Volume)^3** | **0** | **8** | **0.5958** |
| **(Fish + Connectivity + Pool Volume)^2** | **0.8** | **7** | **0.3967** |
| **Fish * Pool Volume** | 8.8 | 4 | 0.0072 |
| **Fish * Connectivity** | 15.5 | 4 | <0.001 |
| **Fish + Connectivity** | 19.5 | 3 | <0.001 |
| **Fish + Connectivity + Pool Volume** | 20.9 | 4 | <0.001 |
| **Fish** | 21.3 | 2 | <0.001 |
| **Fish + Pool Volume** | 23.1 | 3 | <0.001 |
| **Connectivity** | 27.4 | 2 | <0.001 |
| **Pool Volume + Connectivity** | 29.3 | 3 | <0.001 |
| **Intercept** | 29.4 | 1 | <0.001 |
| **Pool Volume** | 31 | 2 | <0.001 |
| **Pool Volume * Connectivity** | 31.3 | 4 | <0.001 |

Table 4. Results of AICc model comparisons for larval *A. americanus* occupancy. View Table 2 for description of explanatory variables and components of the table.

| **Model** | **AICc** | **df** | $w_i$ |
|---|---|---|---|
| **Fish * Connectivity** | **0.0** | **4** | **0.44** |
| **Fish** | **1.7** | **2** | **0.19** |
| **Fish + Connectivity** | **1.9** | **3** | **0.16** |
| **Fish + Pool Volume** | 3.5 | 3 | 0.07 |
| **Fish + Connectivity + Pool Volume** | 4.0 | 4 | 0.06 |
| **Fish * Pool Volume** | 5.1 | 4 | 0.03 |
| **(Fish + Connectivity + Pool Volume)^2** | 6.4 | 7 | 0.01 |
| **(Fish + Connectivity + Pool Volume)^3** | 8.8 | 8 | 0.01 |

| | | | |
|---|---|---|---|
| **Pool Volume** | 24.8 | 2 | <0.001 |
| **Pool Volume + Connectivity** | 25.0 | 3 | <0.001 |
| **Intercept** | 26.4 | 1 | <0.001 |
| **Pool Volume * Connectivity** | 26.4 | 4 | <0.001 |
| **Connectivity** | 27.4 | 2 | <0.001 |

Table 5. Results of AICc model comparisons for log larval amphibian biomass. Explanatory variables are fish occupancy (Fish), connectivity status (Connectivity), hydroperiod score (Hydroperiod), and dominant stream vegetation (Vegetation). Models with “^” tests for each main effect and each two-way (^2) or three-way (^3) interaction in the model and “:” specifies an interaction between two variables. For each model, a dAICc score is provided which was used for model comparison, models were considered strong if dAICc scores were below 2.0 and were bolded. df represents degrees of freedom and $w_i$ represents weight of the model.

| Model | dAICc | df | $w_i$ |
|---|---|---|---|
| **Vegetation** | **0** | **4** | **0.5288** |
| **Connectivity + Vegetation** | **1.9** | **5** | **0.2048** |
| **Fish + Vegetation** | 2.7 | 5 | 0.1372 |
| **Hydroperiod + Vegetation** | 4.7 | 6 | 0.0507 |
| **Fish + Connectivity + Vegetation** | 4.9 | 6 | 0.0461 |
| **Fish + Connectivity + Hydroperiod** | 7.3 | 7 | 0.0137 |
| **Fish + Connectivity + Vegetation + Fish:Connectivity** | 7.7 | 7 | 0.0112 |
| **Hydroperiod * Vegetation** | 9.7 | 9 | 0.0041 |
| **Global** | 11 | 8 | 0.0022 |
| **Fish + Connectivity + Vegetation + Connectivity:Hydroperiod** | 14.1 | 10 | <0.001 |
| **Fish + Connectivity + Vegetation + Fish:Hydroperiod** | 15 | 9 | <0.001 |
| **Fish * Connectivity** | 17 | 5 | <0.001 |
| **(Fish + Connectivity + Hydroperiod)^2** | 17.2 | 10 | <0.001 |
| **Fish + Connectivity + Vegetation + Vegetation:Hydroperiod** | 17.3 | 11 | <0.001 |
| **Fish** | 17.9 | 3 | <0.001 |
| **Fish + Connectivity** | 18 | 4 | <0.001 |
| **Connectivity * Hydroperiod** | 19.4 | 7 | <0.001 |
| **Connectivity** | 21.7 | 3 | <0.001 |
| **Intercept** | 21.9 | 2 | <0.001 |
| **Fish + Hydroperiod** | 23 | 5 | <0.001 |

| | | | |
|---|---|---|---|
| **Fish + Hydroperiod + Connectivity** | 23.8 | 6 | <0.001 |
| **Fish * Hydroperiod** | 24.3 | 6 | <0.001 |
| **Hydroperiod** | 24.5 | 4 | <0.001 |
| **Connectivity + Hydroperiod** | 25.1 | 5 | <0.001 |

Table 6. Results of AICc model comparisons for log larval *L. blairi* biomass. View Table 5. for description of explanatory variables and components of the table.

| **Model** | **dAICc** | **df** | $w_i$ |
|---|---|---|---|
| **Vegetation** | **0** | **4** | **0.3792** |
| **Hydroperiod + Vegetation** | **0.6** | **6** | **0.288** |
| **Connectivity + Vegetation** | **1.8** | **5** | **0.1556** |
| **Fish + Vegetation** | 2.7 | 5 | 0.1001 |
| **Connectivity + Vegetation + Hydroperiod** | 4.7 | 7 | 0.036 |
| **Connectivity + Vegetation + Fish** | 5 | 6 | 0.0308 |
| **Fish + Connectivity + Vegetation + Fish:Connectivity** | 9.5 | 7 | 0.0032 |
| **Global** | 10.1 | 8 | 0.0025 |
| **Intercept** | 10.9 | 2 | 0.0017 |
| **Connectivity** | 12 | 3 | <0.001 |
| **Fish** | 12.1 | 3 | <0.001 |
| **Fish + Connectivity** | 13.5 | 4 | <0.001 |
| **Fish * Connectivity** | 15.2 | 5 | <0.001 |
| **Hydroperiod** | 16 | 4 | <0.001 |
| **Fish + Connectivity + Vegetation + Fish:Hydroperiod** | 16.4 | 9 | <0.001 |
| **Fish + Hydroperiod** | 16.8 | 5 | <0.001 |
| **Fish * Hydroperiod** | 17.5 | 6 | <0.001 |
| **Connectivity + Hydroperiod** | 18.3 | 5 | <0.001 |
| **Fish + Connectivity + Hydroperiod** | 19.9 | 6 | <0.001 |
| **Fish + Connectivity + Vegetation + Connectivity:Hydroperiod** | 23.2 | 10 | <0.001 |
| **Connectivity * Hydroperiod** | 24.5 | 7 | <0.001 |
| **(Fish + Connectivity + Hydroperiod)^2** | 32.9 | 10 | <0.001 |

Table 7. Results of AICc model comparisons for log larval *A. americanus* biomass. View Table 5. for description of explanatory variables and components of the table.

| **Model** | **dAICc** | **df** | **$w_i$** |
|---|---|---|---|
| **Vegetation** | **0** | **4** | **0.5389** |
| **Connectivity** | 2.7 | 3 | 0.1385 |
| **Connectivity + Vegetation** | 3.6 | 5 | 0.0874 |
| **Fish + Vegetation** | 3.7 | 5 | 0.0841 |
| **Intercept** | 5.8 | 2 | 0.0297 |
| **Fish * Connectivity** | 5.9 | 4 | 0.0276 |
| **Fish + Connectivity** | 5.9 | 4 | 0.0276 |
| **Fish** | 6.9 | 3 | 0.0169 |
| **Hydroperiod + Vegetation** | 7 | 6 | 0.0166 |
| **Fish + Connectivity + Vegetation** | 8.1 | 6 | 0.0096 |
| **Connectivity * Hydroperiod** | 8.4 | 6 | 0.0082 |
| **Connectivity + Hydroperiod** | 9.9 | 5 | 0.0038 |
| **(Fish + Connectivity + Hydroperiod)^2** | 10.1 | 7 | 0.0035 |
| **Hydroperiod** | 10.7 | 4 | 0.0025 |
| **Hydroperiod * Vegetation** | 11.1 | 7 | 0.0021 |
| **Hydroperiod + Connectivity + Vegetation** | 11.4 | 7 | 0.0018 |
| **Fish + Hydroperiod** | 13.7 | 5 | <0.001 |
| **Fish + Hydroperiod + Connectivity** | 14.5 | 6 | <0.001 |
| **Global** | 15.5 | 8 | <0.001 |

Table 8**.** Results of AICc model comparisons for log larval amphibian density. Explanatory variables are fish occupancy (Fish), connectivity status (Connectivity), hydroperiod score (Hydroperiod), dominant stream vegetation (Vegetation), and log total pool volume (Log Volume). Models with "^" tests for each main effect and each two-way (^2) or three-way (^3) interaction in the model and ":" specifies an interaction between two variables. For each model, a dAICc score is provided which was used for model comparison, models were considered strong if dAICc scores were below 2.0 and were bolded. df represents degrees of freedom and $w_i$ represents weight of the model.

| **Model** | **dAICc** | **df** | **$w_i$** |
|---|---|---|---|
| **Connectivity + Vegetation + Log Volume:Vegetation** | **0** | **8** | **0.5123** |
| **Connectivity + Vegetation** | **1.8** | **5** | **0.2055** |
| **Connectivity * Hydroperiod** | 3.2 | 7 | 0.1058 |
| **Fish + Connectivity + Vegetation + Fish:Connectivity** | 5.3 | 7 | 0.0362 |

| | | | |
|---|---|---|---|
| **(Connectivity + Log Volume + Hydroperiod)^2** | 5.5 | 11 | 0.0324 |
| **(Connectivity + Log Volume + Fish)^2** | 5.9 | 8 | 0.0269 |
| **(Connectivity + Hydroperiod + Fish)^2** | 6.7 | 10 | 0.018 |
| **Fish * Connectivity** | 8 | 5 | 0.0094 |
| **Connectivity** | 8 | 3 | 0.0092 |
| **Log Volume * Vegetation** | 8.4 | 7 | 0.0076 |
| **Connectivity + Log Volume** | 8.6 | 4 | 0.0068 |
| **Log Volume + Vegetation** | 9 | 5 | 0.0058 |
| **Fish + Connectivity** | 9.6 | 4 | 0.0043 |
| **(Connectivity + Log Volume + Fish)^3** | 9.9 | 9 | 0.0036 |
| **Global** | 10.7 | 9 | 0.0024 |
| **Vegetation** | 10.8 | 4 | 0.0023 |
| **Vegetation + Log Volume + Fish** | 11.1 | 6 | 0.002 |
| **Connectivity + Log Volume + Fish** | 11.3 | 5 | 0.0018 |
| **Connectivity * Log Volume** | 11.3 | 5 | 0.0018 |
| **Fish + Vegetation** | 11.9 | 5 | 0.0013 |
| **Vegetation + Log Volume + Hydroperiod** | 12.7 | 7 | <0.001 |
| **Hydroperiod * Vegetation** | 12.9 | 9 | <0.001 |
| **Hydroperiod + Vegetation** | 13.1 | 6 | <0.001 |
| **Hydroperiod + Connectivity** | 13.4 | 5 | <0.001 |
| **Log Volume + Hydroperiod + Connectivity** | 13.4 | 6 | <0.001 |
| **Connectivity + Hydroperiod + Vegetation + Connectivity:Hydroperiod + Hydroperiod:Vegetation** | 14.9 | 12 | <0.001 |
| **(Connectivity + Log Volume + Hydroperiod)^3** | 15.2 | 13 | <0.001 |
| **Fish + Log Volume + Vegetation + Fish:Log Volume + Log Volume:Vegetation** | 15.5 | 9 | <0.001 |
| **Fish + Hydroperiod + Vegetation + Fish:Hydroperiod + Hydroperiod:Vegetation** | 18.2 | 11 | <0.001 |
| **Intercept** | 18.9 | 2 | <0.001 |
| **Fish** | 20 | 3 | <0.001 |
| **Log Volume** | 21.3 | 3 | <0.001 |
| **Fish + Log Volume** | 22.5 | 4 | <0.001 |
| **Hydroperiod** | 23.9 | 4 | <0.001 |
| **Fish * Log Volume** | 24.3 | 5 | <0.001 |
| **Fish + Hydroperiod** | 25.4 | 5 | <0.001 |
| **Log Volume + Hydroperiod** | 26.8 | 5 | <0.001 |
| **Fish + Log Volume + Hydroperiod** | 28.3 | 6 | <0.001 |
| **Fish * Hydroperiod** | 28.5 | 6 | <0.001 |

| | | | |
|---|---|---|---|
| **Log Volume * Hydroperiod** | 29.4 | 7 | <0.001 |
| **(Log Volume + Hydroperiod + Vegetation)^2** | 35.3 | 14 | <0.001 |
| **(Fish + Log Volume + Hydroperiod)^2** | 38.1 | 10 | <0.001 |

Table 9**.** Results of AICc model comparisons for log larval *L. blairi* density. View Table 8. for description of explanatory variables and components of the table.

| **Model** | **dAICc** | **df** | $w_i$ |
|---|---|---|---|
| **Connectivity + Vegetation** | **0** | **5** | **0.2942** |
| **Connectivity** | **0.5** | **3** | **0.2331** |
| **Connectivity + Log Volume + Vegetation** | 2.2 | 6 | 0.0985 |
| **Fish * Connectivity** | 2.3 | 5 | 0.0919 |
| **Connectivity + Log Volume** | 3.4 | 4 | 0.0532 |
| **Fish + Connectivity** | 3.5 | 4 | 0.0503 |
| **Fish + Connectivity + Vegetation + Fish: Connectivity** | 3.8 | 7 | 0.0438 |
| **Hydroperiod + Log Volume + Vegetation** | 4.7 | 7 | 0.0284 |
| **Hydroperiod + Connectivity** | 5.1 | 5 | 0.0227 |
| **Log Volume + Vegetation** | 6.2 | 5 | 0.0134 |
| **Log Volume * Connectivity** | 6.4 | 5 | 0.0118 |
| **Hydroperiod * Connectivity** | 6.6 | 7 | 0.011 |
| **Fish + Connectivity + Log Volume** | 6.9 | 5 | 0.0092 |
| **Global** | 7.4 | 9 | 0.0073 |
| **Hydroperiod + Vegetation** | 7.4 | 6 | 0.0071 |
| **Connectivity + Log Volume + Vegetation + Connectivity:Log Volume + Log Volume:Vegetation** | 8.3 | 8 | 0.0046 |
| **Connectivity + Log Volume + Hydroperiod** | 8.3 | 6 | 0.0045 |
| **Fish + Log Volume + Vegetation** | 8.8 | 6 | 0.0037 |
| **(Fish + Connectivity + Log Volume)^2** | 9.6 | 8 | 0.0025 |
| **Log Volume * Vegetation** | 9.6 | 6 | 0.0024 |
| **Vegetation** | 10.5 | 4 | 0.0015 |
| **Intercept** | 10.9 | 2 | 0.0013 |
| **Hydroperiod * Vegetation** | 11.3 | 8 | 0.0011 |
| **Log Volume** | 12.2 | 3 | <0.001 |
| **Fish + Vegetation** | 12.2 | 5 | <0.001 |
| **Fish** | 13.6 | 3 | <0.001 |

| | | | |
|---|---|---|---|
| **Hydroperiod** | 14.1 | 4 | <0.001 |
| **(Fish + Connectivity + Log Volume)^3** | 14.4 | 9 | <0.001 |
| **Fish + Log Volume** | 14.9 | 4 | <0.001 |
| **Fish + Hydroperiod** | 16.4 | 5 | <0.001 |
| **Log Volume + Hydroperiod** | 16.8 | 5 | <0.001 |
| **(Fish + Connectivity + Hydroperiod)^2** | 17 | 10 | <0.001 |
| **Fish * Log Volume** | 18.3 | 5 | <0.001 |
| **Fish + Log Volume + Vegetation + Fish:Log Volume + Log Volume:Vegetation** | 18.4 | 8 | <0.001 |
| **Fish + Log Volume + Hydroperiod** | 19 | 6 | <0.001 |
| **Hydroperiod * Log Volume** | 19.8 | 7 | <0.001 |
| **Fish * Hydroperiod** | 20.3 | 6 | <0.001 |
| **Connectivity + Hydroperiod + Vegetation + Connectivity:Hydroperiod + Hydroperiod:Vegetation** | 21 | 11 | <0.001 |
| **Fish + Hydroperiod + Vegetation + Fish:Hydroperiod + Hydroperiod:Vegetation** | 21.7 | 10 | <0.001 |
| **(Log Volume + Connectivity + Hydroperiod)^2** | 26.2 | 11 | <0.001 |
| **(Fish + Log Volume + Hydroperiod)^2** | 34.2 | 10 | <0.001 |
| **(Fish + Log Volume + Hydroperiod)^3** | 36.2 | 12 | <0.001 |
| **(Vegetation + Log Volume + Hydroperiod)^2** | 38.3 | 12 | <0.001 |

Table 10**.** Results of AICc model comparisons for log larval *A. americanus* density. View Table 8. for description of explanatory variables and components of the table.

| **Model** | **dAICc** | **df** | $w_i$ |
|---|---|---|---|
| **Fish + Vegetation** | **0** | **5** | **0.5388** |
| **Connectivity * Hydroperiod** | 2.8 | 6 | 0.1331 |
| **Fish + Log Volume + Vegetation** | 3.2 | 6 | 0.1083 |
| **Vegetation** | 4 | 4 | 0.0724 |
| **Log Volume * Vegetation** | 4.7 | 7 | 0.0524 |
| **Connectivity** | 5.7 | 3 | 0.0314 |
| **Fish + Connectivity** | 7.1 | 4 | 0.0158 |
| **Connectivity + Vegetation** | 7.8 | 5 | 0.0111 |
| **Log Volume + Vegetation** | 7.8 | 5 | 0.0109 |
| **Log Volume + Connectivity** | 8.9 | 4 | 0.0062 |
| **Fish + Log Volume + Connectivity** | 9.2 | 5 | 0.0054 |
| **Connectivity + Log Volume + Vegetation + Connectivity:Log Volume + Log Volume:Vegetation** | 10.2 | 8 | 0.0033 |

| | | | |
|---|---|---|---|
| **Log Volume * Connectivity** | 10.2 | 5 | 0.0033 |
| **Fish + Log Volume + Vegetation + Fish:Log Volume + Log Volume:Vegetation** | 11.3 | 8 | 0.0019 |
| **Connectivity + Hydroperiod** | 11.7 | 5 | 0.0016 |
| **Connectivity + Log Volume + Vegetation** | 12.3 | 6 | 0.0011 |
| **Hydroperiod + Vegetation** | 12.5 | 6 | 0.001 |
| **(Fish + Connectivity + Log Volume)^3** | 13.6 | 6 | <0.001 |
| **Intercept** | 14 | 2 | <0.001 |
| **Connectivity + Log Volume + Hydroperiod** | 15.4 | 6 | <0.001 |
| **Fish** | 16.7 | 3 | <0.001 |
| **Log Volume** | 16.9 | 3 | <0.001 |
| **Vegetation + Log Volume + Hydroperiod** | 18 | 7 | <0.001 |
| **Hydroperiod** | 19.2 | 4 | <0.001 |
| **Fish * Log Volume** | 20.1 | 4 | <0.001 |
| **Fish + Log Volume** | 20.1 | 4 | <0.001 |
| **Global** | 22.2 | 9 | <0.001 |
| **Fish + Hydroperiod** | 23 | 5 | <0.001 |
| **Log Volume + Hydroperiod** | 23.1 | 5 | <0.001 |
| **Fish + Log Volume + Hydroperiod** | 27.7 | 6 | <0.001 |
| **(Connectivity + Log Volume + Hydroperiod)^2** | 30.8 | 10 | <0.001 |
| **Log Volume * Hydroperiod** | 32.6 | 7 | <0.001 |
| **(Fish + Log Volume + Hydroperiod)^3** | 39.3 | 8 | <0.001 |
| **(Connectivity + Log Volume + Hydroperiod)^3** | 41 | 11 | <0.001 |

## C. Estimating Pool Volume Protocol

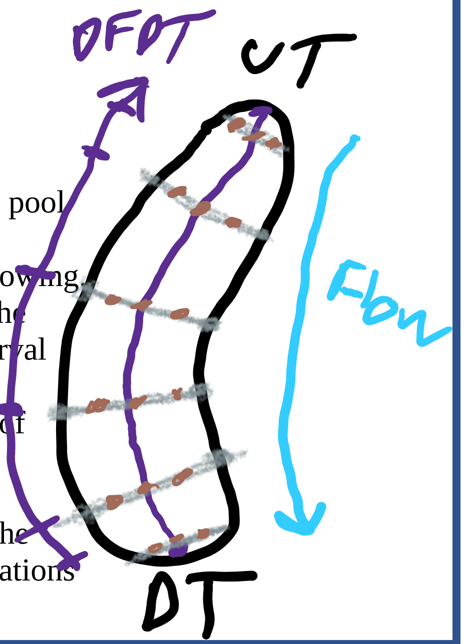
### Materials

Wetland Volume Estimation Sheet, 2-4 Measuring Tapes (metric), rebar, hammer, leather gloves
Clipboard, Pen/pencil, Metal Meterstick

### Terminology

Flow = Direction of flow for overall drainage
DT = Downstream Terminus of Pool
UT = Upstream Terminus of Pool
DFDT = Distance from downstream Terminus

Example to the right shows the boundaries of an oblong stream pool in black.
The blue line indicates the direction of flow when the pool is flowing.
The purple line outside indicates the increasing distance from the downstream terminus with hashmarks indicating a 2 meter interval where measurements are taken.
The purple line in the middle of the pool indicates the location of the measuring tape centered in the middle or deepest part of the pool (default to deepest part).
The silver lines running perpendicular to the purple line show the locations where the second measuring tap is laid to identify locations to take depth measurements (brown dots).



### Procedure (collected after all biotic samples)

***Laying out the main line***

1. Attach measuring tape to a piece of rebar or other object at the DT.
2. Unwind the tape along the center of the pool (or follow the deepest part of the pool if obvious).
    a. Anchor tape at bends with binder clips to rebar
    b. Anchor tape at UT with binder clip to rebar (or tie to rebar)
3. ***<u>Record the Pool Total Length</u>*** on the Wetland Volume Estimation Sheet

***Measuring Widths and Depths***

1. Take the first width measurement at 0.25m from the DT with a second measuring tape and ***<u>record TW & DFDT</u>*** on the datasheet. DFDT and TW can be recorded once and a line drawn down for the next two boxes on the datasheet (it will always be the same). **<u>Record "1" for sample</u>** on datasheet.
2. The first depth reading will always be at the main line whether that is in the middle or closer to a bank. Take this with a metal meterstick. ***<u>Record the depth and DFLS</u>*** on the datasheet.

3. The second depth reading will always be halfway between the mainline and the left bank (facing upstream). ***Record depth and DFLS***.
4. The second depth reading will always be halfway between the mainline and the right bank (facing upstream). ***Record depth and DFLS***
5. After the first width measurement, all subsequent measurements will be taken at a regular 2m intervals starting with 2m, 4m and so on (rather than at 2.25m). These will be **samples 2, 3, etc**.
6. The last width measurement will be taken 0.25m from the UT.

# Appendix II: Krista Ward Master's Prospectus (*Draft Provided to KDWPT*)

# Fish distribution, diversity, and recolonization effects in intermittent stream pools

Krista J. Ward

## Introduction

Intermittent streams are characterized as streams that only flow continuously for a portion of the year and exist as a series of dry segments and stream pools during low flow seasons (Fritz & Dodds 2005). Intermittent streams occur more commonly throughout the Midwest compared to perennial streams. Sixty-eight percent of streams within the state of Kansas and 70-86% of streams within Elk County and Sedgewick County are intermittent, ephemeral, or headwater streams, compared to the 58% average for the United States (Geographic Information Systems Analysis of the Surface Drinking Water Provided by Intermittent, Ephemeral and Headwater Streams in the U.S; EPA.gov). These streams typically occur in open canopy areas where their streamflow characteristics are dependent on local precipitation (which is highly variable throughout the Midwest) and connectivity to groundwater tables (Fritz & Dodds 2005). This results in series of localized flash floods and periodic droughts with high frequency and low predictability (Dodds et al. 2004; Fritz & Dodds 2005). Intermittent streams play an important ecological role by providing habitat elements such as food, cover, and nesting/breeding habitat for various wildlife species (Goodrich et al. 2018). For example, isolated pools serve as refugia for fishes during periods of drought, provide cover from certain types of predators such as wading birds, and temporary pools after a rainfall serve as breeding grounds for many amphibian species (Schlosser 1990; Snodgrass et al. 2000; Dodds et al. 2004). Additionally, runoff of intermittent streams disperses sediment and nutrients along their stream beds, and they assist recharge of alluvial and groundwater aquifers (Goodrich et al. 2018).

Intermittent streams ecosystems are extremely dynamic due to high variability of weather patterns between and within years, leading to a wide range of hydrological conditions (e.g., connectivity, habitat suitability, hydroperiod; Dodds et al. 2004; Falke et al. 2012). Hydroperiod is defined as the period of time during which a wetland is covered by water and is known to affect many biological processes including survival, reproduction, and dispersal of aquatic organisms (Pechmann et al. 1989). These dynamic characteristics affect aquatic organism presence and persistence as drying down of stream pools creates extinction pressures and subsequent refilling allows for recolonization. The variability of environmental stream conditions (e.g., flow regime, channel morphology, and physical-chemical properties) have major effects on life history characteristics of stream organisms and variation in community structure indirectly by effecting food availability, nutrient cycling, and habitat suitability and directly by lethal temperatures and dissolved oxygen concentrations (Schlosser 1990).

High variability in environmental characteristics of intermittent stream pools limits their support of large-bodied predators, meaning that smaller stream fish species comprise the highest trophic level in these systems. Therefore, stream fish are important to study because loss of a few species can lead to large ecosystem changes due to their high trophic level status, and some fish species are indicators of environmental degradation (Fausch et al. 1990). Since intermittent streams do not support large-bodied predators, Schlosser 1987 suggested that fish distribution in these systems is influenced more by physical factors instead of biological factors like predation. The distribution of fish is highly dependent on their dispersal abilities and opportunities which are moderated by species specific life history traits (e.g., body shape and size, swimming ability) and environmental characteristics that block dispersal movements (e.g., stream intermittency, physical barriers; Winston et al. 1991; Bonner & Wilde 2000; Falke et al. 2012). Changes in habitat (e.g., drying or flooding) significantly impact species distributions (Falke et al. 2012) by affecting the dispersal potential by fish. The high extinction pressures from droughts may be balanced by certain life history traits that allow for rapid colonization after disturbances and in fact, species found in upstream reaches of streams have a shorter life span, smaller body size, and earlier age at sexual maturity, all of which increase dispersal abilities (Schlosser 1987; Schlosser 1990; Falke et al. 2012). Although, intermittent streams are frequented with flood and drought disturbances, previous research shows recolonization of stream pools by fishes occurs rapidly (< 1 year; Matthews 1994; Bayley & Osborne 1993). Falke et al. 2012 found that both wet and dry sites were just as likely to contain larvae in the following year if the site contained water in the following year, supporting that recolonization of pools by fish larvae occurs rapidly. Dispersing fish benefit from seasonally abundant food resources (e.g., tadpoles) provided by intermittent streams and lack of potential predators or competitors (Baber et al. 2002; Dodds et al. 2004). However, recolonization rates depend on connectivity of the streams (Dodds et al. 2004), presence of barriers (Detenbeck et al. 1992) and distance from source populations (Sheldon & Meffe 1995; Lonzarich et al. 1998; Thornbrugh & Gido 2010). These influences affect the distribution of stream fishes among intermittent stream systems.

In general, intermittent stream communities consist of fishes, seasonal amphibians, macroinvertebrates, primary producers, and microbes. Primary productivity is high in these systems since low canopy cover allows for high levels of light penetration and microbial communities form the key component of nutrient cycling in these systems (Dodds et al. 2004). The trophic base of stream food webs shift from terrestrial organic matter to autochthonous organic matter as flow intermittency increases (Siebers et al. 2019). Compared to perennial streams, intermittent stream macroinvertebrate assemblages have lower densities, biomasses, and productions in intermittent streams (Mas-Martí et al. 2010). Also, fish density, body condition, and diet diversity were significantly lower in intermittent streams compared to permanent streams (Mas-Martí et al. 2010). However, community structure and species composition in these systems change significantly temporally over the year where species composition is characteristic of certain flow phases (Closs & Lake 1994) and species richness was highest during the spring season when precipitation and stream flow increased (Closs & Lake 1994).

Intermittent streams are under major threats including climate change, groundwater usage, water pollution, and the construction of impoundments. Negative impacts of climate change are increasing globally, affecting many ecosystem services and stability, especially in regions with high susceptibility to environmental variation such as intermittent streams (Comte & Olden 2017). While intermittent stream organisms have evolved traits for resistance and resilience of natural flooding and drying (loss of surface water) regimes, abilities to withstand drought (an unusually long dry period) are species specific and significantly limited, especially for fish (Dodds et al. 2004). Floods can displace fish from stream pools and droughts can remove pools entirely or may change water temperatures and dissolved oxygen levels beyond tolerable limits to fish (Fausch & Bramblett 1991). Now, due to climate change, more frequent and severe periods of drought are predicted, likely to result in increased unpredictability of stream flow patterns and decreased connectivity of stream systems (Jaeger et al. 2014). The annual zero flow days are predicted to increase by 27% by 2050 and the distance of flowing portions of streams are predicted to decrease by 20% (Jaeger et al. 2014). This leads to increased physiochemical stress during droughts, limits seasonally available refuges, and reduces spawning and dispersal opportunities for fishes. Additionally, changes in climate are poised to increase the spatial extent of streams that periodically cease to flow (Mas-Martí et al. 2010; Jaeger et al. 2014), meaning that more permanent streams are likely to be affected by the same flooding/drying regimes faced by intermittent streams in the future.

Other than climate change, humans impact the hydrology and connectivity of intermittent streams via impoundment of water for reservoirs or cattle ponds, streamflow diversion, groundwater usage, and urban development (Junk et al. 2013; Perkin et al. 2017; Goodrich et al. 2018). Particularly in the Midwest where agriculture is common, additional concerns result from sediment runoff from agriculture, water pollution, and erosion from cattle grazing. These threats disrupt the connectivity of streams, alter flooding/drying regimes, increase sediment loads, and alter food webs and community structures (Dodds et al. 2004). For example, heavy use of the Ogallala-High Plains aquifer has moved the water table down far enough causing streams that used to flow to remain dry most of year (Dodds et al. 2004; Perkin et al. 2017). The construction of dams and reservoirs alters physical characteristics of streams by channel degradation, substrate entrainment, and streambank erosion (Wolman 1984; Kondolf 1997). These alterations result in localized effects of habitat simplification and a reduction in native fish species diversity near reservoirs (Bonner & Wilde 2000; Falke & Gido 2006*b*; Thornbrugh & Gido 2010). The impacts of human activity and climate change threaten the integrity and stability of these stream systems. This instability of intermittent stream systems reduces their ability to perform ecosystem services effectively and has the potential to negatively impact wildlife species that rely on the refugia provided by these ecosystems. Therefore, great plains streams are important to study since they are highly endangered, understudied compared to forested perennial streams, provide major ecosystem services, and provide quality habitat to many endangered fish and freshwater mussel species (Dodds et al. 2004).

## Project 1 - Natural Snapshot of Fish Distribution in Intermittent Streams

### Introduction

The distribution of fishes among intermittent streams is influenced by a multitude of biotic, abiotic, anthropogenic, landscape factors, and the interactions between them. Intrinsic factors such body size and shape, mobility, seasonal dynamics of reproduction, affinity for home pools, predation, and competition influence stream fish occupancy and abundance (Gerking 1959; Berra & Gunning 1972; Gilliam & Fraser 1987; Schlosser 1987). For example, Ostrand & Wilde 2002 found that some stream fish species were limited in their distribution due to species-specific tolerances to salinity which differs between upstream and downstream reaches of a Texas stream. However, seasonal and spatial distribution of stream fishes is also affected by stream network spatial position such as stream order and distance from a permanent stream body (Taylor 1997; Ostrand & Wilde 2002; Smith & Kraft 2005). Ostrand & Wilde 2002 found that species diversity increased with downstream position and Smith & Kraft 2005 similarly found that species richness decreases with increasing number of stream confluences downstream from each stream segment, suggesting that upstream segments positioned after multiple confluences are likely to be less species rich. Furthermore, the local abiotic and biotic conditions (e.g., canopy cover, substrate, in-stream vegetation, pool volume, maximum depth, and water velocity) of individual intermittent stream pools also influence the fish assemblages that may occur (Taylor 1997; Baber et al. 2002; Ostrand & Wilde 2002; Smith & Kraft 2005). With regard to temporary wetlands, local characteristics of water quality, dissolved oxygen, volume, and depth affected species richness (Baber et al. 2002).

Although, physical pool characteristics may have a strong influence on the presence or absence of stream fishes, the strength of their effect may be determined by larger scale factors or an interaction of factors that predict the overall abundance and distribution of these species. Previous studies show that the relative importance of local characteristics such as canopy cover or pool volume varies based on landscape factors such as connectivity of pools, spatial position, colonization rates, and extinction pressures (Taylor 1997; Smith & Kraft 2005; Falke et al. 2012). Taylor 1997 suggested that stream fish assemblages in isolated pools were extinction driven and therefore, characteristics that lead to isolation such as distance from main stream and pool size will affect species assemblages. In contrast, Baber et al. 2002 argued that colonization dynamics were more important than extinction dynamics in determining fish assemblages of wetlands. Franssen et al. 2006 shows that spatial position in river systems plays a role in colonization and extinction where extinction risks increase in the upstream direction. Significant factors contributing to the colonization and extinction probabilities are climate, groundwater input, spatial position, and connectivity (Snodgrass et al. 1996; Baber et al, 2002; Falke et al. 2012). For example, colonization rates, along with species richness and diversity, were positively correlated with wetland inundation, connectivity (both in frequency and duration), and depth-hydroperiod (Baber et al. 2002). Altogether, these intrinsic traits of fishes and extrinsic factors of local characteristics and stream landscapes influence the natural distribution and diversity of stream fish assemblages.

## Research Objectives

This study provides ecologically relevant information on the impacts of physical pool and landscape characteristics of intermittent streams (ecosystems highly susceptible to climatic variation) across a human-altered landscape on fish distribution and diversity. We are interested in the interaction between land use alterations, impacts of climate change, and spatial distribution on fish community assemblages. We focus on quantifying fish presence, fish species richness, and species abundances in relation to the permanency of water, connectivity to nearest permanent body of water, volume of each pool, and local pool characteristics using individual stream pools since these are “discrete habitat units” for fishes (Matthews et al. 1994). By quantifying fish species presence/absence as a response variable, we can investigate which factors increase the likelihood or possibly limit the presence of fish.

Although previous studies have investigated local effects and spatial position and landscape effects on fish assemblages (Snodgrass et al. 1996; Taylor 1997; Baber et al. 2002;

Ostrand & Wilde 2002; Smith & Kraft 2005; Thornbrugh & Gido 2010), our present study is novel in several ways. One way this study is novel is that we are investigating both main effects and interactive effects of local pool characteristics (i.e., hydroperiod, pool volume, dominant in-stream vegetation) and landscape factors (distance to nearest pool of colonizers, connectivity, wetted length). Also, we are investigating the effects of connectivity and distance to nearest pool of colonizers with two different measurements, and to our knowledge, no study has investigated the combination of these effects on fish assemblages in intermittent streams. First, we are measuring the stream distance from the pool to the main stream channel. Second, we are measuring the percentage of stream length that holds surface water between the pool and the main stream channel. This second measurement of “wetted length” is also referred to as “stream permanency” throughout and provides additional ecological information since fish dispersal is limited by hydrology. The wetted length will provide a relative measure of the challenges for fish dispersal to each pool sampled. In other words, we are studying both the distance to nearest permanent body of water (pool of potential colonizers) *and* also the connectivity (i.e., wetted length) of that distance. Furthermore, our sampling period occurred after a significantly wet year (12.24 inches above the 1981-2010 average; http://climate.k-state.edu/precip/county/) which allows us to investigate fish distribution at an assumed maximum distribution since the higher precipitation likely led to increased connectivity, and therefore, increased recolonization of stream pools by fish species.

In general, we predict that landscape and local stream characteristics will affect fish distribution and species abundances. We hypothesize that fish presence, richness, and species abundances will be affected by pool water volume, permanency of water, and distance to nearest permanent body of water (source of potential colonizers). Specifically, we predict an increase in fish presence and abundance with increased pool volume, hydroperiod length, and connectivity. We also predict that distance to main stream will have a strong negative effect on stream species (ex., stonerollers, minnows, darters).

## Research Design and Methods

### *Study Site*

This study took place on Youngmeyer Ranch (YMR), a 4,700-acre Wichita State University biological field station located in the Flint Hills of Elk County, Kansas (Fig. 1). Unlike Central and Western Kansas, the Flint Hills region is utilized primarily for cattle grazing rather than agriculture due to its thin soils and rocky, steeply sloping hills (Hickey and Webb 1987). The property overlies layers of chert rock and sits at higher elevation than surrounding areas due to uplifting and decreased erosion of the resistant chert. The west side of the site consists of a gentle sloping plateau around 460 m in elevation while the east side features steep to moderate slopes that give way to rolling hillsides with elevations ranging from 370-400 m (Houseman et al. 2016). Historically, the site was predominately grassland with scattered black oaks (*Quercus velutina*) and has supported continuous cattle grazing since the land was settled. For the past 20 years, prescribed burns occur every 1 to 3 years and the site is double stocked for grazing 90-120 days during the late spring and summer (Houseman et al. 2016). Currently, the field station exists as a native tallgrass prairie of more than 500 plant species with 30 streams, 13 cattle ponds, and a few scattered wetlands located onsite. Streams flow along the elevational gradient, either west to east or north to south. Potentially 58 species of freshwater fish could be distributed on YMR according to range maps from *Peterson Field Guide to Freshwater Fishes of North America and Mexico* and *Kansas Fishes* (Page and Burr 2011; Kansas Fishes Committee et al. 2014).

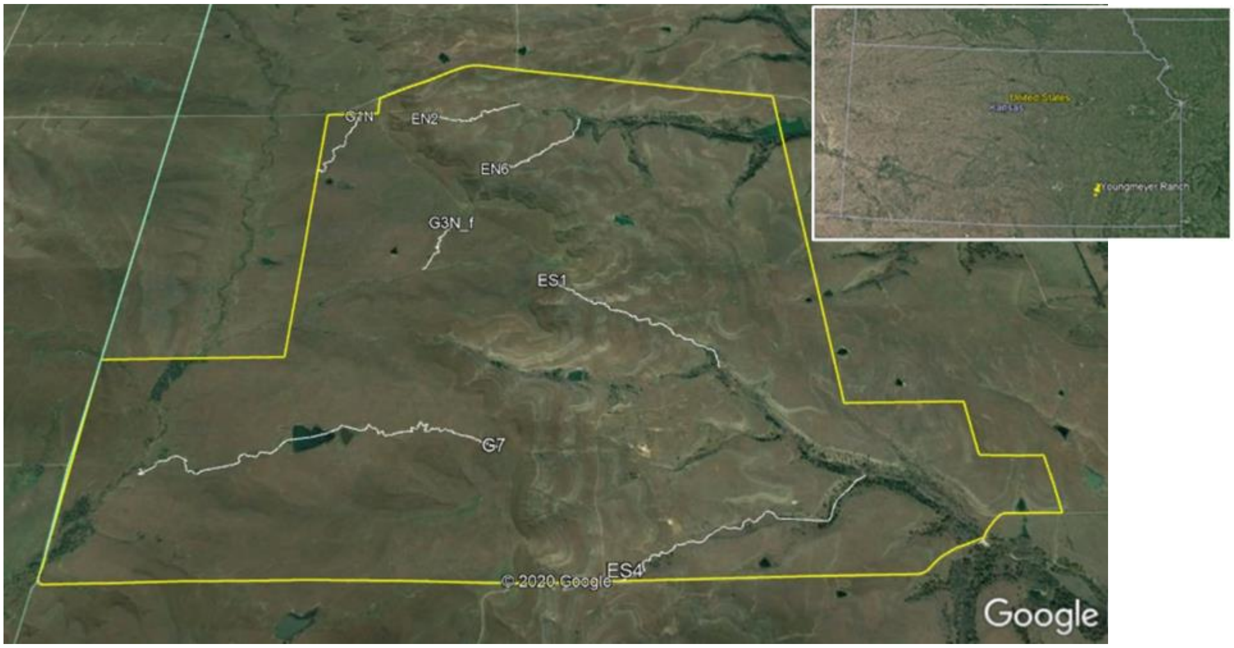


**Figure 1.** Location of Youngmeyer Ranch in Southeastern Kansas, United States (inset) with property outline highlighted in yellow. The seven streams sampled during the 2020 summer field season on Youngmeyer Ranch in Elk County, Kansas outlined in white (images from Google Earth Pro©).

*Hydroperiod Indices, Mapping, and Connectivity*

Streams within the study site were mapped using the computer program Google Earth Pro©. Streams were mapped by creating 100m increments following the line of the stream according to the satellite imagery on Google Earth Pro© until the total length of the stream was mapped. From each 100m section of stream, two stream pools were randomly selected for calculating hydroperiod scores and GPS coordinates were recorded in DMS format. A total of 31,400m of stream were mapped across 30 streams and 318 random pools were selected. Hydroperiod scores were calculated by recording the presence of water at each randomly selected pool from four time periods (10/17/2010, 2/28/2012, 2/16/2013, 11/5/2014) available through Google Earth Pro©. A hydroperiod index ($H_i$) was assigned to each pool where $P_i$ is the number of times water was present and $O_i$ is the total number of observations (Eq. 1). Connectivity of the pools to the permanent bodies of water was quantified by measuring two different measurements (stream distance to main stream and wetted length) on Google Earth Pro©. First, we measured a stream distance to main stream by mapping the stream distance from the sample site to the main stream channel. Secondly, we calculated the wetted length distance by determining the length of stream distance that contained water from the sample stream pool to the main stream channel.

Equation 1: $H_i = P_i / O_i$

*Aquatic Surveys of Species Diversity and Distribution*

We conducted aquatic surveys of 151 stream pools from 7 streams on Youngmeyer Ranch in Elk County, Kansas from May 17th, 2020 to July 7th, 2020. We chose these 7 streams (G1N, EN6, G3N_f, ES1, G7, ES4, and EN2) to sample because they covered a north to south and east to west gradient of the study site and reduced seasonal bias (Fig. 1). For each sample pool, we utilized two active sampling techniques to estimate biodiversity: enclosure sampling and seining. Enclosure sampling consisted of placing an enclosure along a transect the stream pools and immediately dip netting aquatic organisms from the inside of the enclosure (Fig. 2). We used a heavy duty, round plastic trashcan with the bottom cut out, measuring 51 mm in diameter at the top and 45 mm in diameter at the bottom. Depending on the length of the stream pool being sampled, the trashcan enclosure was placed every one or two meters along the transect to achieve around 10 enclosure samples for each pool. At each enclosure placement the depth was recorded in millimeters and aquatic organisms were dip netted, separated based on taxa (invertebrates, amphibians, and fish), and placed into 5-gallon buckets. Dip netting ceased when three consecutive sweeps of the net occurred with no aquatic organisms, and the trashcan enclosure was moved to the next increment. Seining consists of dragging a mesh netting with floats on the top and weights on the bottom of the mesh along the length of the pool (Fig. 3). We utilized one of three seins depending on the width of the stream pool: 1) Small seine- 3'long x 3'tall with fine mesh, 2) Large seine -10'long x 3'tall with 1/8" mesh, or 3) Bag seine - 15'long x 6'tall with a 6'x6'x6' bag attached to the middle with ½" mesh. All active sampling occurred in a downstream to upstream direction to minimize impacts and disturbances to the stream pools. Once the two sampling techniques were completed, we recorded the species identification, standard length (m), sex, and age class for each fish captured and the fish were released back into the pool they were captured from. Visual surveys of number and identification of fish were conducted on pools with sufficient visibility. We occasionally deployed Fyke nets, a passive bag-shaped fish trap consisting of mesh netting suspended over a series of hoops laid horizontally in the water column (Figure 4). Fyke nets were deployed and then checked later in the day or the next morning and the same data described above were recorded. For each pool, water volume estimations were measured by placing a transect through the middle of the pool from downstream to upstream. Every 1 or 2 meters (depending on total pool length) along the transect, pool width was recorded. Subsequently, depth of the deepest point, depth of the midpoint from the deepest point to the right bank, and depth of the midpoint from the deepest point to the left bank along the width were recorded. Data on physical site characteristics (e.g., weather conditions, percentage of canopy cover, percentage of emergent vegetation, etc.) were recorded for each pool.

**Figure 2:** Active enclosure sampling conducted at a stream pool on May 19th, 2020 at Youngmeyer Ranch.

**Figure 3:** Active seining: large seine (10’long x 3’ tall, 1/8” mesh) dragged through a stream pool by two researchers to capture fish and amphibians on May 19th, 2020 at Youngmeyer Ranch.

*Cattle ponds*

We conducted aquatic surveys of 13 cattle ponds located at the Youngmeyer Ranch study site from August 21st, 2020 to September 3rd, 2020. For each pond, we deployed one fyke net (27" x 39" frame with 1/8" mesh with 25' long x 27" deep, 1/8" mesh lead line; Fig. 4) and 25 minnow traps evenly spaced around the perimeter of the pond for 24 hours. Before checking the traps, we completed two seine passes from the edge of the pond down the lead line and into the mouth of the fyke net (one pass on each side of the lead line). The captured fish were separated into buckets based on how they were captured (minnow trap, seine, or fyke net) and data such as species identification, standard length (cm), and volumetric displacement (mL) were recorded. Fish were released back into their pond after processing.

**Figure 4:** A deployed fyke net (27" x 39" frame with 1/8" mesh) with the 25' long x 27" deep and 1/8" mesh netting lead line extended out.

*Permits*

This research was conducted under Dr. Thomas Luhring's WSU IACUC #277a permit for working with vertebrates at WSU Field Stations and a KS wildlife collection.

## Statistical Analysis

All analyses were performed in RStudio™. Stream pools from stream EN6 (n=14 pools) were excluded from data analyses due to a natural physical barrier downstream preventing fish movement to upstream pools. Stream pools from stream ES4 (n=32 pools) were excluded from analyses (except for *C. anomalum* analyses since 85% of their data points originate from stream ES4) since we were only able to sample the downstream portion due to field site border limitations. Data on stream distance to the main stream channel and total pool volume were log transformed to standardize the data distribution and to meet the assumptions of generalized linear models.

### *Presence/absence*

To investigate which stream pool characteristics affect fish presence, we constructed *a priori* candidate generalized linear mixed effect models (GLMERs) using all possible single, two, three, four, and five factor combination models with wetted length (the percentage of downstream stream length holding water during a wet year), total pool volume (log transformed), hydroperiod (an index score of number of years a pool holds water), dominant in-stream vegetation, and stream distance to main stream (log transformed) as explanatory variables. GLMERs were fitted for a binomial regression using the logit function. All possible two-way interaction models with these same explanatory variables were constructed and analyzed via an analysis of variance (ANOVA) and non-significant interactions were removed from further analysis. Significant two-way interactions were included in the model comparison and used to create additional models that include the interactive effect. To account for spatial autocorrelation, all models included "Stream" as a random effect. We used Akaike Information Criterion values ('AICctab' in 'bbmle' package in R) to rank models, and models with significant support (AICc values < 2) were further analyzed with an ANOVA (Burnham & Anderson 2002). ANOVAs were conducted via the 'car' package in R using type II for models without interactions and type III for models with interaction effects. We conducted a Tukey's HSD post hoc test for categorical factors within the top models to test differences between categories (Hothorn et al. 2008). These same analyses were utilized to investigate the presence/absence of the four most abundant fish species (*L. cyanellus, C. anomalum, E. spectabile,* and *Pimephales* genus) except for the exclusion of hydroperiod as an explanatory variable due to insufficient data.

### *Species richness and abundance*

To investigate which stream pool characteristics affect fish species richness, we constructed *a priori* candidate generalized linear models (GLMs) using all possible single, two, three, four, and five factor combination models with wetted length, total pool volume, hydroperiod, dominant in-stream vegetation, and stream distance to main stream as explanatory variables. All possible two-way interactions were constructed and analyzed as previously described. Significant two-way interactions were included in the model comparison and used to create additional models that include the interactive effect. Fish species richness analyses were limited to pools where fish were present. These GLMs utilized a Poisson distribution and did not include a random effect. We used the same AICc model comparison technique as described above, and top models (AICc values < 2) were further analyzed via ANOVAs and Tukey HSD post hoc tests. This same analysis was utilized to investigate the abundance of *L. cyanellus* (the most abundant species sampled) for pools where they were present.

## Results

Of the 151 pools surveyed, 117 stream pools contained water and 67 pools contained fish. We conducted visual surveys on 39 stream pools. We captured and recorded data on 981 fish from 12 species (Table 1). Incorporation of visual survey data increased the total number of fish to

2420 fish (Table 1). *L. cyanellus*, *C. anomalum*, *E. spectabile*, and *Pimephales* genus made up 88% of fish captures. For pools with fish, the number of fish averaged 36.1 fish/pool.

**Table 1.** Fish species observed during aquatic surveys of intermittent stream pools at the Youngmeyer Ranch field site from May 17th, 2020 to July 7th, 2020. These numbers include visual survey data.

| **Species** | **# Pools** | **# Fish** | **Proportion** |
|---|---|---|---|
| *Ameiurus melas* | 10 | 40 | 40/2420 = 0.017 |
| *Campostoma anomalum* | 32 | 947 | **0.391** |
| *Cyprinella lutrensis* | 1 | 5 | 0.002 |
| *Etheostoma spectabile* | 31 | 113 | **0.047** |
| *Fundulus notatus* | 5 | 14 | 0.006 |
| *Labidesthes sicculus* | 1 | 1 | 0.0004 |
| *Lepomis cyanellus* | 39 | 390 | **0.161** |
| *Lepomis humilis* | 3 | 11 | 0.005 |
| *Lepomis macrochirus* | 7 | 213 | 0.088 |
| *Micropterus salmoides* | 5 | 7 | 0.003 |
| *Notemigonus crycoleucas* | 1 | 1 | 0.0004 |
| *Pimephales* | 43 | 678 | **0.280** |
| **Total: 12** | **67** | **2420** | **1** |

### *Fish Occupancy*

Fish occupancy (presence) was best explained by stream wetted length ($\chi^2_1 = 3.53$, $p = 0.06$) and the interaction between volume and hydroperiod ($\chi^2_1 = 5.98$, $p = 0.014$; Table 2). Fish presence increased with stream wetted length (stream permanency), the percentage of downstream channel holding water (Fig. 5). In general, fish occupancy increases as pool volume increases. However, the probability of fish presence across the volume gradient differs based on pool permanency, where fish presence in ephemeral pools slightly increases with volume but fish presence in intermediate and permanent pools strongly increase with volume (Fig. 5). These results suggest that fish are most likely to be present in permanent pools with large water volumes and a high percentage of the downstream reach holding water. The second-best model contained the same interaction of volume and hydroperiod with stream distance to main stream as a main effect instead of stream wetted length. The third-best model contained the same interaction with both stream wetted length and stream distance to main stream as main effects. However, the only significant factor in each of the three top models was the interaction between volume and hydroperiod.

**Table 2**. Results of AICc model comparisons for fish occupancy. AICc results for the four top models and the intercept are shown. Explanatory variables are stream wetted length (WYPercentWet), total pool volume (Volume; log transformed), hydroperiod score (Hydroperiod), and stream distance to main stream (lSDMS; log transformed).

| **Model** | **AICc** | **df** | $w_i$ |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **WYPercentWet + Volume*Hydroperiod** | **0.0** | **6** | **0.32** |
| Volume*Hydroperiod + lSDMS | 0.9 | 6 | 0.20 |
| WYPercentWet + Volume*Hydroperiod + lSDMS | 1.5 | 7 | 0.15 |
| Volume*Hydroperiod | 2.2 | 5 | 0.10 |
| Intercept | 19.5 | 2 | <0.001 |

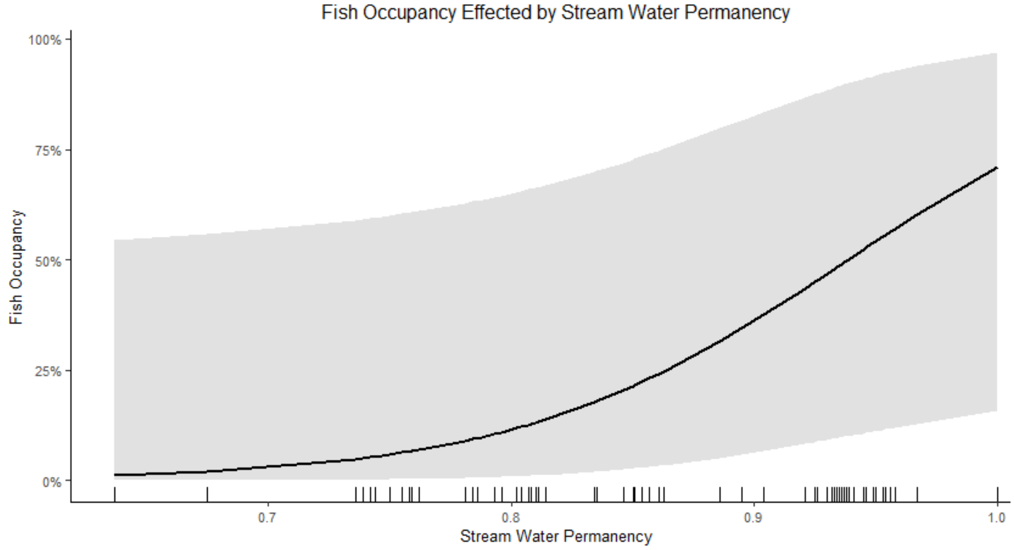


**Figure 5.** Probabilities of fish presence across a wetted length (stream permanency) gradient.

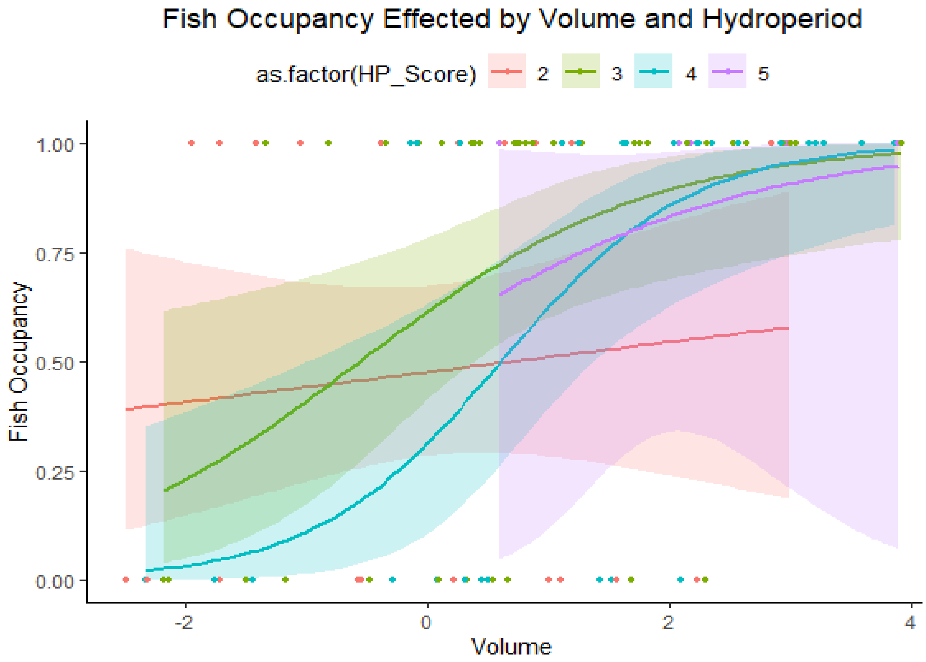


**Figure 6.** Probabilities of fish presence in hydroperiod categories (2 = most ephemeral, 5 = most permanent) as pool volume increases, showing only the sections of each hydroperiod category where data points exist.

### *Fish Species Richness*

Fish species richness per pool ranged from 1-8 species and averaged 2.24 species/pool. Species richness was best explained by stream wetted length ($\chi^2_1$= 6.73, *p* = 0.0095) and pool volume ($\chi^2_1$= 12.23, *p* = 0.0004; Table 3). Species richness increased with pool volume but decreased with wetted length, where pools with high stream permanency tended to have low species richness (Fig. 7). The second-best model included wetted length, volume, and stream distance to main stream, however, the distance to main stream was not significant ($\chi^2_1$= 0.052, *p* = 0.47).

**Table 3**. Results of AICc model comparisons for fish species diversity. AICc results for the three top models and the intercept are shown. Refer to Table 2 for description of explanatory variables.

| Model | AICc | df | $w_i$ |
|---|---|---|---|
| **WYPercentWet + Volume** | **0.0** | **3** | **0.46** |
| WYPercentWet + Volume + lSDMS | 2.0 | 4 | 0.16 |
| WYPercentWet + Volume + Hydroperiod | 2.4 | 4 | 0.14 |
| Intercept | 9.4 | 1 | 0.004 |

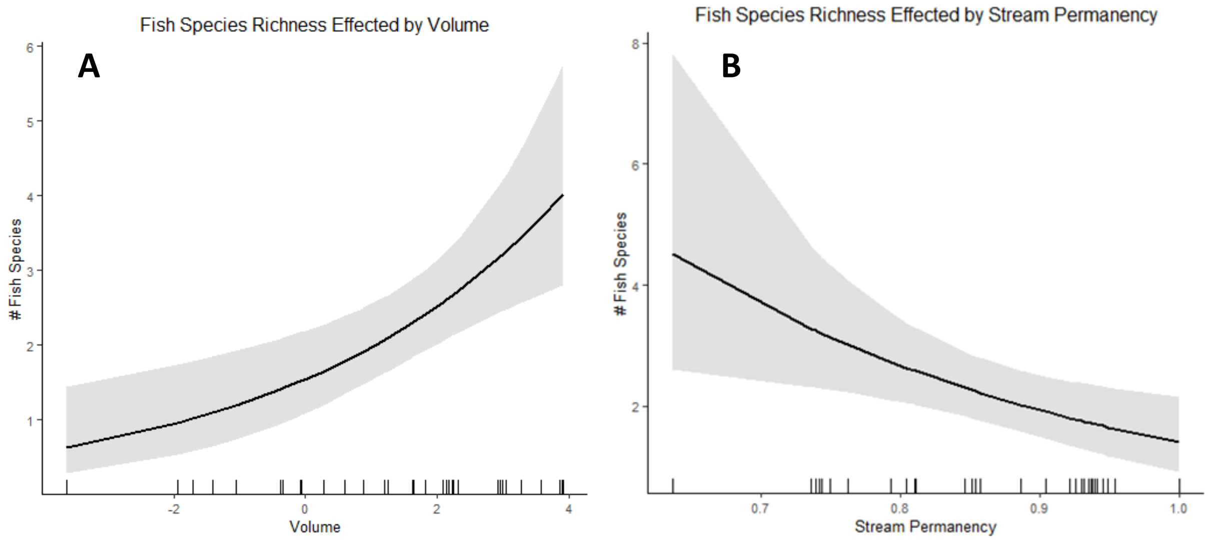


**Figure 7.** Number of fish species across a volume gradient (**A**) and a wetted length (stream permanency) gradient (**B**).

### *Lepomis cyanellus*

The presence of *L. cyanellus* (green sunfish) was best explained by an interaction between pool volume and distance from main stream ($\chi^2_1 = 8.12$, $p = 0.0044$) along with the main effect of dominant in-stream vegetation. However, dominant in-stream vegetation was not significant ($\chi^2_2 = 4.39$, $p = 0.11$; Table 4) and is not represented graphically here. When close to the main stream, green sunfish presence is highest in large volume pools, whereas their presence farther upstream is highest in small volume pools (Fig. 8). In other words, green sunfish presence is high in large, downstream pools, and high in small, upstream pools. For pools in which green sunfish were present, their abundance (number of green sunfish per pool) was best explained by a model containing three significant main effects of pool volume ($\chi^2_1 = 9.98$, $p = 0.0016$), dominant in-stream vegetation ($\chi^2_2 = 37.14$, $p = 0.001$), and distance to main stream ($\chi^2_1 = 50.25$, $p < 0.0001$; Table 5). The Tukey's post hoc test showed that "Grasses" were significantly different than both "Macrophytes" ($p < 0.001$) and "No vegetation" ($p < 0.001$). *L. cyanellus* abundance increased with pool volume, decreased with distance from main stream, and was highest in grassy pools compared to macrophyte-dominated pools and pools with no in stream vegetation (Fig. 9). However, green sunfish were only present in 4 grass-dominated pools and one of those contained the highest *L. cyanellus* abundance record from our dataset, which is why the "Grasses" vegetation category in Figure 9 is the highest but has the largest error bars. Since there is not a consistent abundance of *L. cyanellus* across grass-dominated pools (Fig. 10), we reserve judgement that green sunfish abundance will be higher in grass-dominated pools compared to macrophyte or no vegetation pools. The second-best model for *L. cyanellus* abundance contained the interaction between pool volume and wetted length (Table 5) where abundance increases with both volume and wetted length, but abundance increases faster along the volume gradient when wetted length is higher. This is displayed in Figure 10 where green sunfish abundance is highest (larger circles) in large volume pools that also have high stream permanency.

**Table 4.** Results of AICc model comparison for *L. cyanellus* occupancy. AICc results for the top three models and the intercept are displayed. Refer to Table 2 for description of explanatory variables.

| Model | AICc | df | $w_i$ |
|---|---|---|---|
| **Volume*lSDMS + Vegetation** | **0.0** | **7** | **0.50** |
| Volume*lSDMS | 0.6 | 5 | 0.37 |
| WYPercentWet + Volume*lSDMS | 2.7 | 6 | 0.13 |
| Intercept | 31.3 | 2 | <0.001 |

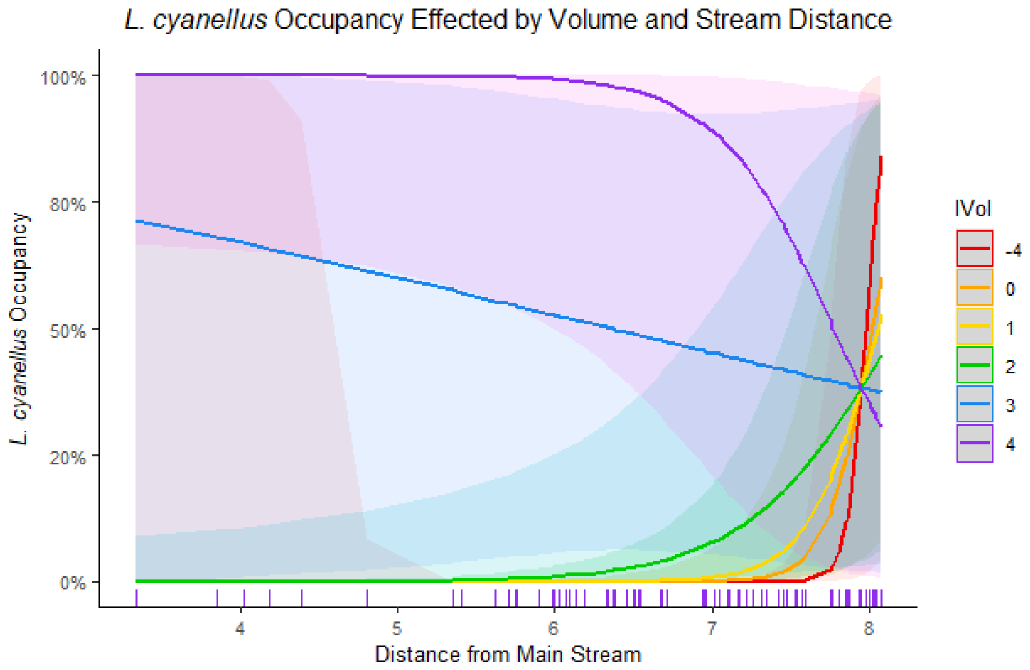


**Figure 8.** Probabilities of *L. cyanellus* (green sunfish) presence across different pool volumes (-4 = smallest volume, 4 = largest volume) as distance from main stream increases.

**Table 5.** Results of AICc model comparison for *L. cyanellus* abundance. AICc results for the top three models and the intercept are displayed. Refer to Table 2 for description of explanatory variables.

| Model | AICc | df | $w_i$ |
|---|---|---|---|
| **Volume + Vegetation + lSDMS** | **0.0** | **5** | **0.46** |
| WYPercentWet*Volume + Vegetation + lSDMS | 1.2 | 7 | 0.25 |
| Volume + Hydroperiod + Vegetation + lSDMS | 3.5 | 6 | 0.08 |
| Intercept | 140.1 | 1 | <0.001 |

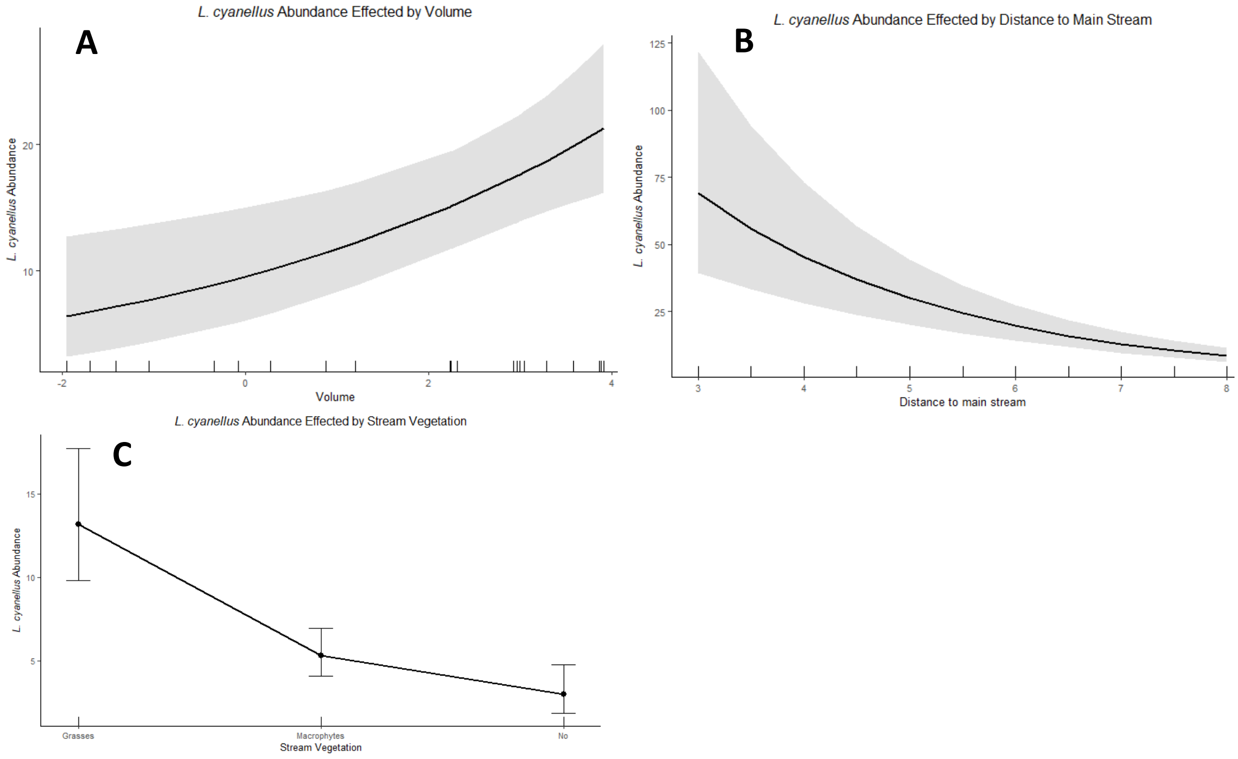


**Figure 9**. *L. cyanellus* abundance in regard to pool volume (**A**), distance from main stream (**B**), and dominant in-stream vegetation categories (**C**).

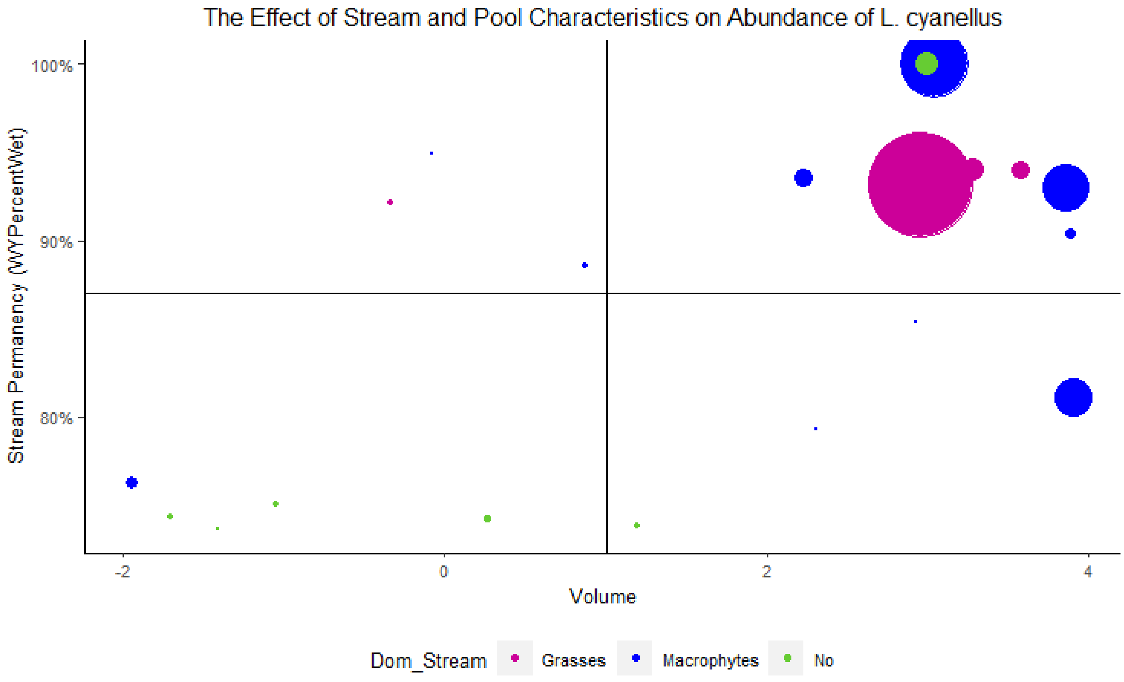


**Figure 10**. The effects of pool volume (x-axis), stream wetted length (y-axis) on abundance of *L. cyanellus* (depicted by circle size; larger circles = higher abundance) with dominant in-stream vegetation categories depicted by colors. Each quadrant represents a combination of either small or large volume pools with either low or high stream permanency (percentage of downstream channel holding water).

### *Campostoma anomalum*

Data was subset by location (East v. West) and only the East subset was used for analyses of *C. anomalum* (central stonerollers) since this species was observed only on the East side of the study site. The presence of *C. anomalum* was best explained by pool volume ($\chi^2_1$= 6.61, $p$ = 0.01) and dominant in-stream vegetation (Table 6), although vegetation itself was not significant as a main effect ($\chi^2_2$= 2.74, $p$ = 0.255). There were several other models within the "top model set" (AICc values < 2.0), however, each of these models included pool volume as a main effect which was the only significant main effect in each of these models. Therefore, C. anomalum presence with respect to pool volume is the only factor graphically represented here. *C. anomalum* occupancy increased with increasing pool volume (Fig. 11).

**Table 6**. Results of AICc model comparison for *C. anomalum* occupancy. AICc results for the top six models and the intercept are displayed. Refer to Table 2 for description of explanatory variables.

| **Model** | **AICc** | **df** | $w_i$ |
|---|---|---|---|
| **Volume + Vegetation** | **0.0** | **5** | **0.23** |
| Volume | 0.4 | 3 | 0.18 |
| Volume + lSDMS | 0.6 | 4 | 0.17 |
| Volume + Vegetation + lSDMS | 1.2 | 6 | 0.13 |
| Volume + Vegetation + WYPercentWet | 1.7 | 6 | 0.10 |
| Volume + WYPercentWet | 2.1 | 4 | 0.08 |
| Intercept | 8.8 | 2 | 0.003 |

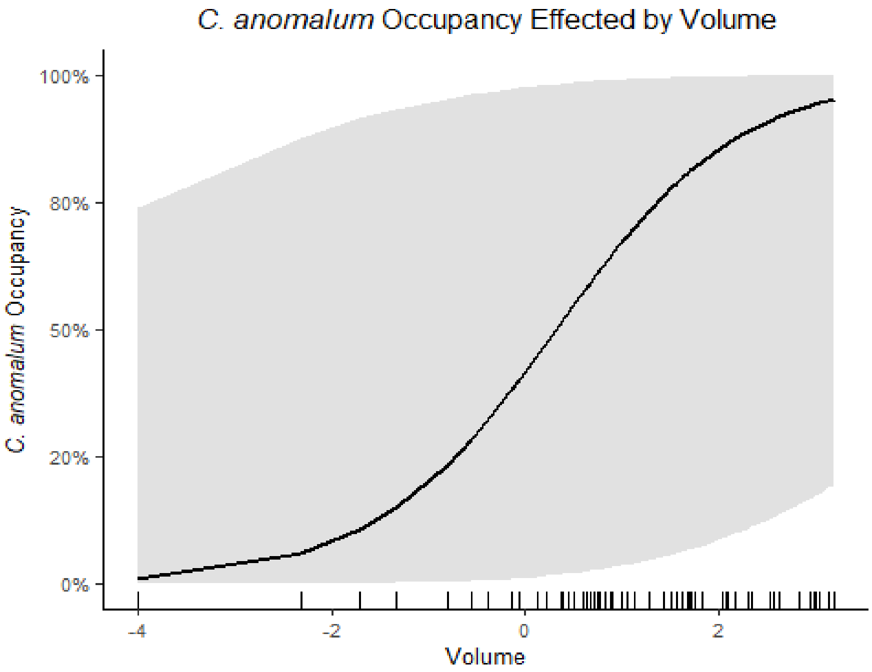


**Figure 11**. The presence of *C. anomalum* against a volume gradient.

*Etheostoma spectabile*

The presence of *E. spectabile* (orangethroat darter) was best explained by dominant in-stream vegetation ($\chi^2_2$=4.51, $p$ = 0.105) and distance from main stream ($\chi^2_1$= 2.65, $p$ = 0.104; Table 7), although neither were significant. The other two top models both included stream vegetation and distance from main stream (Table 7), but again, none of the main effects in the top three models were significant. In general, *E. spectabile* presence decreased with distance from main stream suggesting that orangethroat darters are most likely to be present in pools close to the main stream. However, this effect is not represented graphically since the main effect was not significant and graphical error bars are extensive.

**Table 7**. Results of AICc model comparison for *E. spectabile* occupancy. AICc results for the top three models and the intercept are displayed. Refer to Table 2 for description of explanatory variables.

| Model | AICc | df | $w_i$ |
|---|---|---|---|
| **Vegetation + lSDMS** | **0.0** | **5** | **0.47** |
| WYPercentWet + Vegetation + lSDMS | 1.4 | 6 | 0.22 |
| Volume + Vegetation + lSDMS | 2.4 | 6 | 0.14 |
| Intercept | 7.9 | 2 | 0.009 |

*Pimephales* Genus

All minnow species within Genus *Pimephales* (bluntnose minnows) were labeled as *Pimephales* and analyzed as a group due to identification challenges. The presence of Pimephales fish was best explained by the combination of stream wetted length ($\chi^2_1$= 2.77, *p* = 0.096), pool volume ($\chi^2_1$= 4.83, *p* = 0.028), dominant in-stream vegetation ($\chi^2_2$= 6.72, *p* = 0.035), and distance from main stream ($\chi^2_1$= 8.45, *p* = 0.0037; Table 8). *Pimephales* presence decreased with wetted length (stream permanency), increased with pool volume, was higher in grass-dominated pools compared to macrophyte or no vegetation pools, and decreased with distance from main stream (Fig. 12). In general, *Pimephales* presence is likely to be highest in large volume, grassy pools close to the main steam. However, the Tukey's post hoc test only showed a significant difference between grass and macrophyte dominated pools (*p* = 0.04) and due to the large error bars in Figure 12C, we reserve judgement that *Pimephales* presence will be prominently higher in grass-dominated pools. The second-best model shared the same AICc value as the top model (AICc = 0.0; Table 8) and shared the same factors except stream wetted length. Stream wetted length was not significant in the top model and therefore, likely does not contribute much to the overall model. This shows that even though the top model contained stream wetted length, *Pimephales* presence is mostly determined by pool volume, in-stream vegetation, and distance from the main stream.

**Table 8.** Results of AICc model comparison for *Pimephales* Genus occupancy. AICc results for the top three models and the intercept are displayed. Refer to Table 2 for description of explanatory variables.

| Model | AICc | df | $w_i$ |
|---|---|---|---|
| **WYPercentWet + Volume + Vegetation + lSDMS** | **0.0** | **7** | **0.388** |
| Volume + Vegetation + lSDMS | 0.0 | 6 | 0.387 |
| Volume + lSDMS | 2.8 | 4 | 0.10 |
| Intercept | 22.2 | 2 | <0.001 |

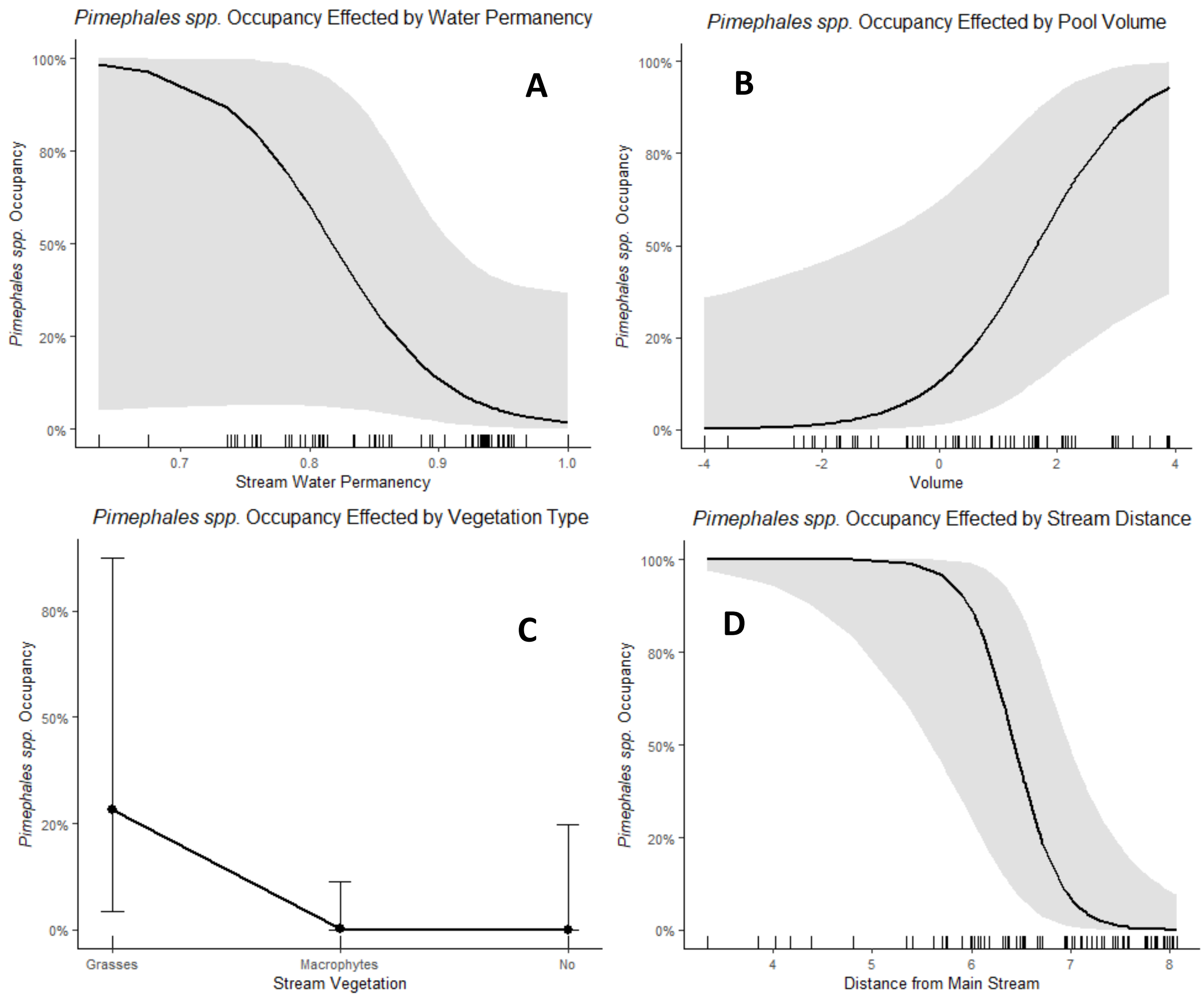


**Figure 12.** The presence of *Pimephales* genus with respect to stream wetted length (stream permanency; **A**), pool volume (**B**), dominant in-stream vegetation categories (**C**), and distance from main stream (**D**).

## Species Volume Curves

Since the presence of all four fish species increased as pool volume increased, we further investigated volume thresholds for each species and how they compared to each other. We created GLMs using only pool volume as a main effect and using only pools from stream ES4 since pools from this stream were nearly identical in all pool characteristics except for volume. The presence of *L. cyanellus*, *C. anomalum*, *E. spectabile*, and *Pimephales* all increased with pool volume, however, each had different volume thresholds for when that species was likely to be present (Fig. 13; we chose 75% as this standard). *L. cyanellus* presence of 75% occurs at the log transformed pool volume of 2.16 (8.67 $m^3$ or 2,300 gallons), *C. anomalum* at volume 0.85 (2.34 $m^3$ or 618 gal), *E. spectabile* at volume 1.93 (6.89 $m^3$ or 1,820 gal), and *Pimephales* at volume -1.17 (0.31 $m^3$ or 82 gal; Fig. 10). *Pimephales* have the lowest volume threshold and their presence is relatively high (75% and above) in low volume pools, suggesting that they can colonize and survive in smaller volume pools compared to the other species included in this study. *C. anomalum* has the second lowest threshold followed by *E. spectabile* and then *L. cyanellus* (Fig. 13), suggesting that green sunfish require the larger pool volumes for colonization and survival compared to the other stream fish species.

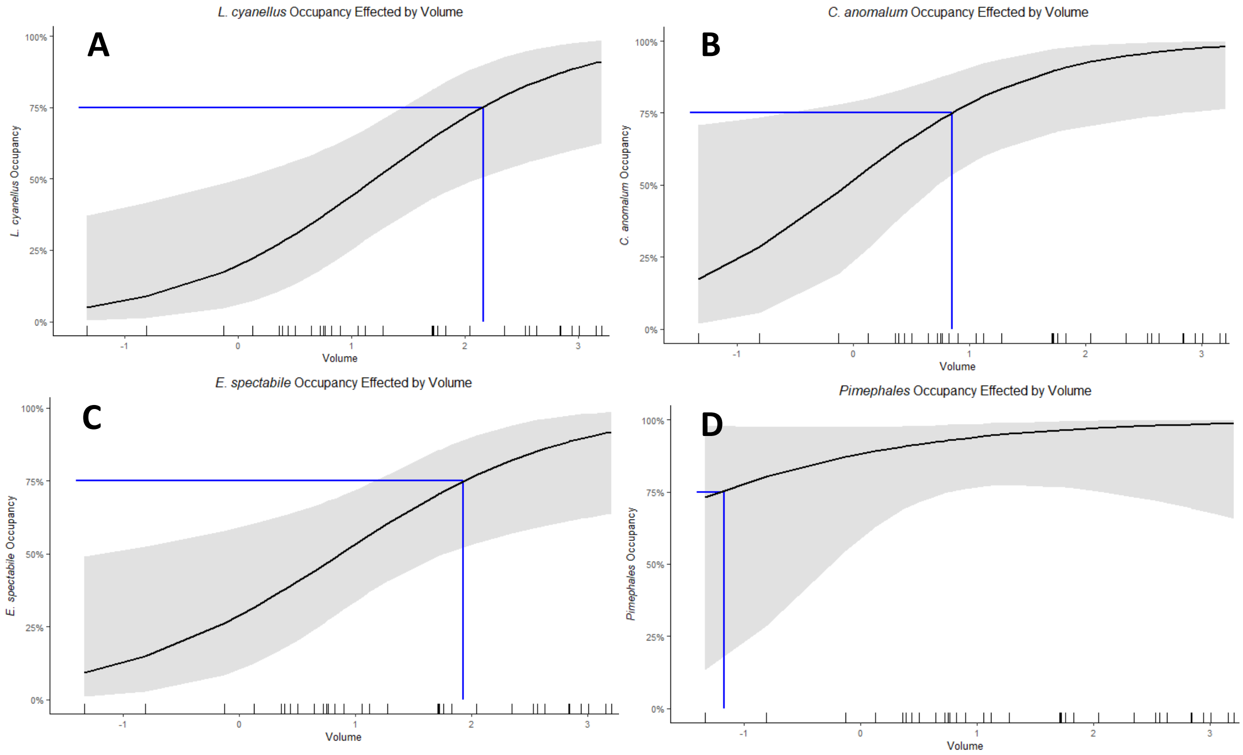


**Figure 13.** The effects of pool volume on *L. cyanellus* (**A**), *C. anomalum* (**B**), *E. spectabile* (**C**), and *Pimephales* (**D**) occupancy (presence), showing specifically the pool volume at which each species occupancies are equal to 75% (dark blue lines).

## Project 2 – Climate Change and Trophic Structure Mesocosm Experiment

### Introduction

Intermittent streams have simplified food webs and shorter food chain lengths compared to perennial streams (Mas-Martí et al. 2010; Siebers et al. 2019) with their trophic structures consisting of basal resources, primary producers, primary consumers, and secondary consumers. Algae is the main primary producer in these systems (McIntosh et al. 2017) and together, algae and detritus provide basal resources and function as the primary food source for non-predatory species (Closs & Lake 1994; Siebers et al. 2019). For streams that receive little litter input from the adjacent terrestrial ecosystem, primary food sources are based on autochthonous production of algae (Closs & Lake 1994). Due to simple trophic complexity in these ecosystems, competition rather than predation is a critical biological interaction that determines community structure (Schlosser 1987). However, predation pressure does have significant effects by increasing the intensity of competition between stream organisms by forcing them into spatially restricted refugia (Schlosser 1987). Direct interactions between two species may lead to indirect effects on a third species or group of organisms, resulting in trophic linkage and changes to the base of the food web. For example, Huang & Sih (1991) found that both larval amphibians and green sunfish individually had a direct, negative impact on isopods via predation. However, predation pressure by fish on the larval amphibians resulted in a reduction of feeding activity of the larval amphibians (Huang & Sih 1991). Therefore, when all three species were present together, fish had an indirect, positive effect on isopods by reducing larval amphibian predation rates on isopods (Huang & Sih 1991). This trophic linkage resulted in changes to intermediate and basal trophic levels and is likely to be common in intermittent streams since suitable habitat is spatially restricted which increases the magnitude of biological interactions.

Since stream fishes comprise the highest trophic level in intermittent stream systems, they can influence entire community assemblages via top-down trophic cascades, shifts in their diet, and trophic linkage. For example, bluegill impacted the community assemblage by creating a direct, negative impact on the abundance of both aquatic insects and two common larval amphibian species via predation (Smith et al. 1999). Baxter et al. (2004) found that presence of fish significantly decreased the biomass of emergent insects. Fish also create a direct, negative impact on other species in the community by reducing their activity rates and increasing their time spent in refuge (Huang & Sih 1991). Also, the presence or introduction of top feeding fish (e.g., bass, trout, sunfish) shifts the diet of other stream fish from terrestrial prey to benthic stream insects (Baxter et al. 2004). Fish can also affect the community structure indirectly by consuming alternate predators or competitors, leading to an increase in abundance of organisms in an intermediate trophic level (Smith et al. 1999). For example, bullfrog tadpoles are noxious to fish but are vulnerable to predaceous aquatic insects so the presence of fish results in a positive, indirect effect on bullfrog tadpole abundance by the negative interactions between fish and predaceous insects (Smith et al. 1999).

However, larval amphibians themselves can influence community assemblages in intermittent stream systems along with water quality and nutrient cycling. Since larval amphibians may be the first vertebrate species to recolonize a stream pool, they can act as keystone species by influencing primary productivity, decomposition, and invertebrate activity (Osborne & McLachlan 1985). They affect these processes by their feeding and excretion which influences the amount of suspended particles, shifts phytoplankton community structure, and shifts the state of nitrogen in the water (Seale 1980). Tadpoles reduce both the concentration of suspended particles and primary production of algae and also shift the state of nitrogen from particulate to dissolved nitrogen (Seale 1980). Additionally, tadpoles can influence the quality of algae communities by changing the proportions of certain algal species present. For example, tadpoles significantly reduced blue-green algae, meaning that larval amphibians have the potential to reduce the rate of natural eutrophication by reducing primary productivity, reducing nitrogen input, and exporting some nitrogen via metamorphosis (Seale 1980). Larval amphibians change their phenotypes in response to environmental stressors but can also change their nutrient assimilation and excretion (Kirschman et al. 2016). For example, phosphorus excretion and whole-body phosphorus of larval amphibians decreased with increased environmental stress cues (Kirschman et al. 2016). This can lead to overall changes in phosphorus levels in stream pools which impact nutrient cycling and decomposition rates (Kirschman et al. 2016). Overall, larval amphibians can significantly affect the community structure and water quality of streams, especially in systems with periodic drying that create the opportunity for amphibians to become keystone species.

As the predicted effects of climate change become a reality, it will be important to understand how trophic interactions/structures effect biodiversity in ecosystems extremely vulnerable to drought and flood disturbances. The most pronounced change in drying intermittent stream pools is the loss of aquatic species and associated changes in community structure (McIntosh et al. 2017). Trophic diversity is strongly affected by drying through the exclusion of large-bodied predatory species that occupy the highest potential trophic levels in these systems (Sabo et al. 2010; Takimoto & Post 2013; McHugh et al. 2015). Therefore, harsh environmental conditions such as drying limit the vertical dimensions of trophic structure (i.e., food chain length) by limiting high trophic level consumers (Sabo et al. 2010, McHugh et al. 2015; Siebers et al. 2019). Reduced water flow may affect fish at the individual level (i.e., body condition) or at the assemblage level (Mas-Martí et al. 2010). For example, Mas-Martí et al. (2010) found that droughts caused changes in macroinvertebrate assemblages which caused fish diets to shift towards less nutritious prey, resulting in changes in fish trophic ecology and a reduction in individual fish body condition. Nutrient processing becomes spatially patchy as surface waters decrease and stream pools become disconnected from their adjacent terrestrial ecosystem (Bernal et al. 2013; McIntosh et al. 2017). Specifically, there is a shift in diet of primary consumers from allochthonous organic matter to autochthonous sources of algae and periphyton as intermittency increases (Siebers et al. 2019). However, the proportional contribution of different food sources is highly spatially and temporally variable (Siebers et al. 2019), therefore, these general trophic changes are often cyclic, temporary, and vary seasonally with local weather conditions (Closs & Lake 1994; McIntosh et al. 2017). As stream pools dry down, changes in physiology, phenotype, and behavior of stream organisms can result in changes to water quality and organic matter processing. Overall, many stream organisms have the potential to impact community assemblages and trophic structure via direct or indirect effects, but the magnitude of their influence will likely be dependent on environmental conditions which are extremely variable in intermittent stream systems.

## Research Objectives

This research follows a previous natural snapshot study that investigated the patterns of distribution and diversity of stream organisms at their maximal distribution. The purpose of this research experiment is to investigate (via manipulative treatments) the interactive effects of climate change (i.e., drought) and trophic structure on biomass, biodiversity, and nutrients in intermittent stream pools. Previous studies have investigated the effects of flooding or pool drying on stream organisms (Wilbur 1987; Franssen et al. 2006; Davey & Kelly 2007; Wilkins et al. 2019; Sarramejane et al. 2020). However, this study is novel because we are simulating recolonization events. While we are interested in how drying effects stream organisms, our study introduces the organisms after the mesocosms have dried and refilled to simulate recolonization. This allows us to investigate how the recolonization of vertebrate groups affect trophic interactions and community structure in intermittent stream pools.

Due to the seasonal patterns of drying and refilling of stream pools, local extirpation and subsequent recolonization of vertebrate groups occurs often (Dodds et al. 2004). As refilling and recolonization occurs, both amphibians and fish have the potential to influence the trophic interactions of the pools. Treatments will consist of a trophic structure variable (presence of larval amphibians, fish, or both) and a climate variable (drought or no drought). Specifically, this experiment will research how drying and refilling of pools affects growth rates and survivorship of larval amphibians and fish after recolonization of different (or combination of) vertebrate groups occurs. To investigate these effects, response variables related to trophic interactions and community assemblage (e.g., growth rates and survivorship of vertebrates, primary productivity, zooplankton diversity and biomass, and water column nutrients) will be compared across treatments. Our specific research questions are:

1. Does the drying and refilling of “pools” affect water quality, nutrients, and/or survival or growth of stream organisms?
2. Does the presence of fish, amphibians, or both affect water quality, nutrients, and/or survival or growth of stream organisms?
3. Does drying and refilling alter the effects of trophic level treatments on the response variables?

## Research Design and Methods

### *Study Site*

This experiment will be conducted at Wichita State University’s Ninnescah Biological Reserve in Sedgewick County, Kansas. The cattle tanks (mesocosms) will be set up in the southeast section of the site near the main building (Fig. 14; blue square). Soil will be excavated from an existing wetland on the study site (Fig. 14; red square) and capturing of *Lepomis cyanellus* and *Lithobates blairi* will occur at various locations throughout the wetlands and Ninnescah river via fyke nets and seining. All vertebrates collected from the study site will be released back to their capture location at time of metamorphosis or at the conclusion of the experiment.

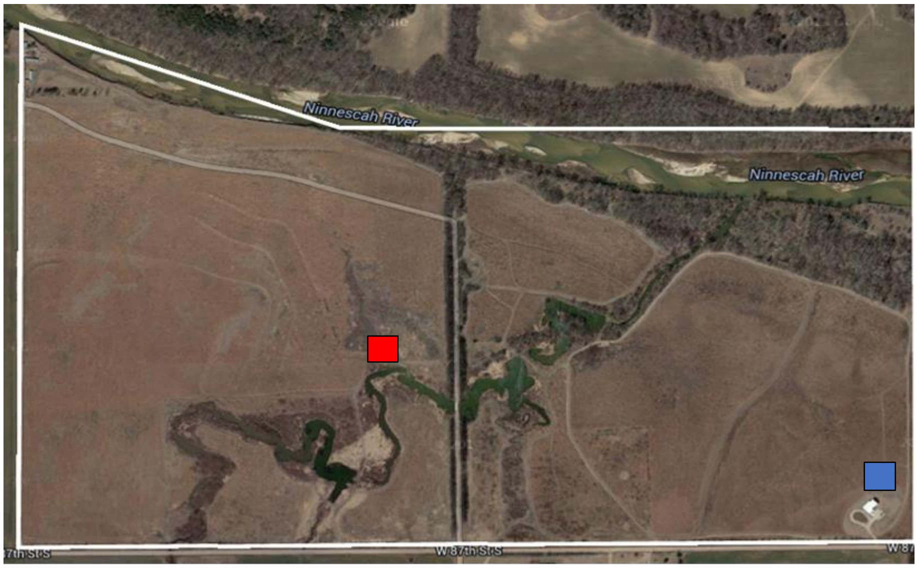


**Figure 14**. Location of cattle tank set up (blue square) and wetland where soil will be excavated (red square). The tanks will be set in an 8 x 8 grid in the southeast section of the field site.

*Experimental Setup*

This research will be conducted via mesocosms where one treatment will be applied to each tank. Mesocosms will be made of hard plastic 1,000 L cattle tanks (n = 64) that are set up in an 8 x 8 grid. The experiment will consist of 4 trophic structure treatments (1 – no vertebrate control, 2 – larval amphibians, 3 – fish, and 4 – larval amphibians and fish) crossed with 2 drying treatments (1 – remain filled, and 2 – dry and refill) for a total of 8 combination treatments replicated 8 times each, resulting in a total of 64 replicate mesocosms. The independent variables are trophic structure and drying treatments. The dependent (response) variables measured will be 1) fish growth [accumulated biomass], 2) larval amphibian growth rates, 3) emergent amphibian biomass, 4) zooplankton presence/absence and biomass, 5) primary productivity, 6) periphyton [chlorophyll a], and 7) nutrients [total nitrogen and phosphorus]. However, the focus of this experiment is the growth rates and survivorship of the vertebrates. The other response variables will be satellite data collected if available on an as needed basis and may or may not be included in analyses for the thesis chapter. Controlled variables will be the soil, water, and litter initially introduced into each tank. However, this will be an open system experiment (i.e., tanks will not be covered) and therefore, uncontrollable variables such as temperature, precipitation, and insect/animal activity exist.

Wetland soil (110 liters/tank; 7,040 liters total) will be excavated from a wetland located at the field site. Water introduced into the tanks (~750 liters/tank; 48,000 liters total) will be rainwater collected from a rainwater cistern located at the field site. Vegetative litter (225 grams/tank, 15,000 grams total) will be collected from areas surrounding wetlands located at the field site. The amount of vegetative litter introduced into each tank was determined by calculating the mean biomass of 4 vegetative litter samples taken from the wetland where soil was excavated from. The densities of green sunfish (3 individuals/tank; 96 individuals total) were determined using average densities of green sunfish (i.e., 2.35 individuals/$m^3$) that naturally occur in intermittent stream pools calculated from data collected at Youngmeyer Ranch during summer 2020 field season along with data from a previous mesocosm experiment (Huang & Sih 1991; Ward et al. in prep). The densities of Plains leopard frog tadpoles (50 individuals/tank; 1,600 individuals total) were determined similarly to green sunfish densities described previously along with data from an early mesocosm experiment by Wilber 1987.

Plains leopard frog egg masses (not individuals) will be collected from various wetlands at the field site and transferred to shallow plastic splash pools until they hatch. Once hatched, they will be held in the splash pool until enough tadpoles have hatched to distribute an even number of individuals to each tank with a larval amphibian treatment. However, due to the natural history of breeding frogs, collections of egg masses and hatching spurts will likely occur multiple times. Therefore, multiple introductions of tadpole individuals into the tanks will occur, but each time, the same number of tadpoles (and roughly the same biomasses) will be introduced to each tank. This process may take several weeks and will be strongly influenced by precipitation events. Plains leopard frogs remain tadpoles for 3 months on average before metamorphosing (Powell et al. 2016), therefore, there is little risk of tadpoles metamorphosing before fish are introduced. Once all tadpoles have been introduced into the tanks, juvenile green sunfish (n = 96) will be captured from the field site, have their initial biomasses recorded, and then 3 fish will be introduced into each tank assigned a fish treatment. This process is expected to occur within 2-3 days. The collective biomass of the 3 fish introduced into each tank will be standardized as much as is possible between tanks (i.e., striving for the same number of fish and same *biomass* of fish per tank).

Green sunfish (*Lepomis cyanellus*) and Plains leopard frogs (*Lithobates blairi*) were chosen for the fish and larval amphibian treatments because of their high local abundance in Kansas and at the field site (easy to find, identify, and capture), and their extremely wide geographic ranges allow the results of the study to be extrapolated across North America. Only juvenile green sunfish (< 9 cm standard length) will be used since this size group is most abundant in intermittent streams and most likely to disperse and recolonize pools that have recently dried and refilled (Berra & Gunning 1972; Thornbrugh & Gido 2010; Ward et al. in prep). The four trophic structure treatments were chosen because as intermittent stream pools dry and refill, they may be colonized by different vertebrate groups (Dodd et al. 2004). Also, although many amphibian species prefer ephemeral breeding habitats without fish present (Holbrook & Dorn 2016), members of the genus *Lithobates* have been well documented breeding in pools with sunfish (Smith et al. 2016). Therefore, since Plains leopard frogs and green sunfish do co-occur in nature, we chose to include a fourth treatment with a combination of these vertebrate groups.

### *Timeline*

Experimental set up began in November 2020. Wetland soil, water, and vegetative litter will be added to each of the 64 cattle tanks and then left for 4 weeks. Then, 32 tanks (randomly selected; Fig. 15) will be dried down and left dry for 4 weeks before being refilled. Then trophic structure treatments will be applied to 16 tanks each (8 that remained wet and 8 that had dried and refilled; randomly selected; Fig. 15). Sampling will begin May 2021 and end in September 2021 at which point the remaining organisms will be released back to their capture site, soil and water will be removed from the tanks, and the tanks will be removed from their grid setup and stacked. The entire establishment, duration, and removal of this experiment will last approximately one year.

| **Date** | **Activity** |
|---|---|
| November 2020 | Setup mesocosms in grid position |

| | |
|---|---|
| December 2020 | Excavate soil from Ninnescah wetland and place in mesocosms<br>Fill mesocosms with 2-4 inches of water |
| January 2021 | Collect 4 litter samples<br>Calculate amount of litter needed for each mesocosm<br>Collect litter and place into mesocosms<br>Develop protocols/standard operating procedures |
| February 2021 | Develop protocols/standard operating procedures<br>Finalize methods |
| March 2021 | Fill mesocosms with water from cistern |
| April 2021 | Dry down 32 mesocosms<br>Collect leopard frog egg masses from Ninnescah reserve (this will occur multiple times dependent upon precipitation events)<br>Hatch tadpoles in buckets or plastic splash pools |
| May 2021 | Refill mesocosms and distribute hatched tadpoles to tanks<br>Trap, collect, and distribute fish to tanks<br>Begin data collection |
| June 2021 | Continue data collection |
| July 2021 | Continue data collection |
| August 2021 | Continue data collection |
| September 2021 | Deconstruct mesocosms and release all remaining vertebrates |

### *Sampling and Protocols*

Survival and growth of vertebrates - Fish biomass will be measured before they are introduced into their mesocosms, weekly throughout the experiment, and at the conclusion of the experiment to determine fish growth. Fish and amphibian counts will be completed weekly throughout the experiment and all vertebrate deaths will be recorded to measure survivorship. Amphibian biomass measurements will occur weekly by randomly selecting and measuring the biomass of 10 larval amphibians per tank to determine amphibian growth. Furthermore, emergent amphibian biomass will be measured by the combining the biomasses of all emerging metamorphosed frogs per tank. Biomass for fish and larval amphibians will be measured via volumetric displacement.

Zooplankton presence/absence, density, and biomass – Zooplankton samples will be collected with a clear, vertical 3-inch diameter pipe where the pipe is inserted into the water to a specified depth, the rubber stopper is pulled to plug the bottom of the pipe, and the pipe is removed from the mesocosm. Water samples from this pipe will be removed, volumes of samples recorded, preserved using a 40% formalin-sucrose solution, and stored in a refrigerator (5° C) until sample analysis occurs. The formalin-sucrose solution will be made from mixing 60 grams of sucrose with 1000 mL of formalin (Haney & Hall 1973). Samples will be sent off for zooplankton presence/absence, density, and biomass analysis. The number of water samples for zooplankton analysis will be dependent upon costs of analysis and shipment and will be determined at a later date.

Primary productivity – Primary productivity will be determined by diel dissolved oxygen measurements. Each measurement of diel dissolved oxygen will be measured using a YSI meter with one measurement taken before dawn and another taken after dusk. This technique assumes that changes in oxygen concentrations reflect the difference between photosynthetic production and respiratory consumption (Staehr et al. 2010) Since production of oxygen from photosynthesis occurs only during the daylight and consumption of oxygen is the only metabolic process at night, gross primary production can be quantified by measuring temporal changes in dissolved oxygen throughout a 24-hour period (Staehr et al. 2010). Diel dissolved oxygen measurements will occur two times a week until the conclusion of the experiment.

Periphyton – Periphyton will be determined qualitatively via chlorophyll *a* analyses. We will create artificial substrates composed of either fiberglass or ceramics and place one in each tank at the beginning of the experiment. Samples will be collected by removing the artificial substrate, scraping off the periphyton from the substrate, preserving the sample with formalin, wrapping the sample bottle in aluminum foil, and storing the sample in a freezer. These samples will be sent off for analysis to Dr. Amy Burgin at The University of Kansas. The number of samples collected for periphyton analysis will be dependent upon costs of analysis and shipment and will be determined at a later date.

Nitrogen and Phosphorus Content – Nutrient contents of the water column (total nitrogen and total phosphorus) will be measured by collecting water samples and sending them off for analysis to Dr. Amy Burgin at The University of Kansas. The number of samples collected for nitrogen and phosphorus analysis will be dependent upon costs of analysis and shipment and will be determined at a later date.

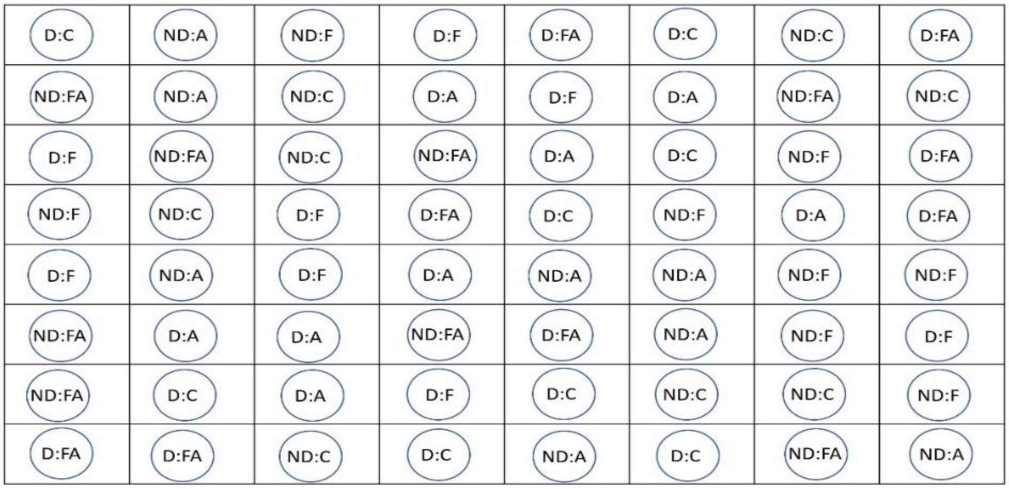
### *Materials Needed*

YSI unit, 64 cattle tanks, graduated cylinders, measuring cups, ruler, digital scale, plastic kid pools, 5-gal buckets, plastic cups, floatation devices, aquarium nets, seine, fyke net, bubblers, formalin, table sugar (sucrose), plastic bottles, 3-inch diameter pipe with rubber stopper, 70% alcohol solution, and water sample containers.

### *Experimental Design Considerations*

Several principles of experimental design such as replication, power, and randomization influenced the design of this experiment. Replication is incorporated into the design (n = 8 replicates) to ensure that observed differences are due to the applied treatment and not chance variation. Regarding power, a sensitivity analysis was conducted using sample size = 64, alpha = 0.05, and power = 0.80 that resulted in an effect size of 0.4274 (G*Power; Faul et al. 2007). At the conclusion of this experiment, this effect size will be used to conduct a post-hoc analysis when a null hypothesis is not rejected to determine power. Randomization is incorporated into this experiment by the treatments being randomly assigned to each cattle tank (Fig. 15). Pseudoreplication will be avoided by having a completely random design where no blocking or grouping of tanks occurs (Hulbert 1894).

This study is a mesocosm field experiment with controlled variables and experimental manipulation of treatments but occurs under natural field conditions. This type of experiment was chosen for increased regulatory control of variables and site matching compared to natural experiments; and because experiments investigating whole community level responses are impractical in lab experiments due to their lack of realism and generality (Diamond 1986). Cattle tanks were chosen as the mesocosms because they are comparable in size to intermittent stream pools. The volume (1 $m^3$) and depth (52 cm) of a cattle tank represents a smaller but deeper than average intermittent stream pool. However, intermittent stream pools are extremely variable in water volume and depth (i.e., range of 0.018-49.918 $m^3$ and 3-136 cm, respectively; Ward et al. in prep), and since these cattle tanks fall well within the range of stream pool parameters, they represent an appropriate spatial scale with which to conduct this experiment (Wiens et al. 1986). These aspects of experimental design have been incorporated to strive for precise and accurate results that are representative of natural conditions and can be extrapolated widely.



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D:C | ND:A | ND:F | D:F | D:FA | D:C | ND:C | D:FA |
| ND:FA | ND:A | ND:C | D:A | D:F | D:A | ND:FA | ND:C |
| D:F | ND:FA | ND:C | ND:FA | D:A | D:C | ND:F | D:FA |
| ND:F | ND:C | D:F | D:FA | D:C | ND:F | D:A | D:FA |
| D:F | ND:A | D:F | D:A | ND:A | ND:A | ND:F | ND:F |
| ND:FA | D:A | D:A | ND:FA | D:FA | ND:A | ND:F | D:F |
| ND:FA | D:C | D:A | D:F | D:C | ND:C | ND:C | ND:F |
| D:FA | D:FA | ND:C | D:C | ND:A | D:C | ND:FA | ND:A |

**Figure 15**. Experimental setup of mesocosm experiment consisting of 64 cattle tanks on an 8 x 8 grid where each circle represents a single tank with a treatment randomly assigned to it. D:C = drying:no vertebrate control; D:A = drying:amphibians; D:F = drying:fish; D:FA = drying:fish+amphibians; ND:C = not dried:no vertebrate control; ND:A = not dried:amphibians; ND:F = not dried:fish; ND:FA = not dried:fish+amphibians

*Permits*

This research will be conducted under a modification to Dr. Luhring's existing WSU IACUC #277a permit for working with vertebrates at Ninnescah (following a renewal) and a KS wildlife collection permit similar to the one issued to Dr. Luhring for 2020.

## Statistical Analyses

Differences between trophic level treatments, differences between drying regimes, and whether drying regimes alter the effects of trophic level treatments on the response variables

will be analyzed using a two-way ANOVA. This technique will determine if the means of the response variables of the 8 treatments are statistically different from each other. For these analyses, four assumptions are made: 1) each sample is taken from a normally distributed population, 2) each sample is independent of other samples, 3) variance of data is the same for all groups, and 4) dependent variables are continuous and independent variables (treatments) are categorical and independent. Differences between emergent biomass of larval amphibians will be analyzed with a t-test since only two groups will be compared (tadpoles only and tadpole + fish treatments). The same analysis will be completed for biomass production of fish between the fish only and tadpole + fish treatments.

## Expected Results

In general, we predict that drying and refilling of mescosms will have a significant impact on nutrient availability and survival and/or growth of stream organisms. We predict that both fish and amphibians will impact primary productivity, nutrients, and survival and growth of stream organisms through their impacts of predation, competition, consumption, and excretion. We also predict that drying and refilling of mesocosms will significantly alter the effects of trophic level treatments on the response variables (but the relationship of effect will depend on the response variable). For all response variables except amphibian and fish biomass production, the null hypothesis ($H_0$) is that there is no difference between the treatments and the control group. In other words, if $H_0$ is *not* rejected, then the response variables do not differ between the control group and the treatments. For analysis of fish biomass production (change in total fish biomass from the original stocking biomass), the null hypothesis will be no difference in fish biomass between the fish only treatment and the fish + tadpole treatment. Additionally, for larval amphibian biomass production, the null hypothesis will be no difference in amphibian biomass between the larval amphibian only treatment and the fish + tadpole treatment. Alternative hypotheses will be a statistical difference in response variables between the treatments and control group and these hypotheses are based on expected consumption, predation, and competition interactions between the fish, tadpoles, and the dependent variables. $H_1$ = biomass accumulation of fish and tadpoles will be significantly different in treatments where they occur alone compared to the fish + tadpole treatment (Fig. 16). $H_2$ = periphyton will be lower in the tadpole and fish + tadpole treatments (Fig. 17A). $H_3$ = zooplankton density will be lower in the fish and fish + tadpole treatments (Fig. 17B). These hypotheses (null and alternative) are falsifiable using an ANOVA or t-test. The figures below are only conceptual graphs of predictions generated from the hypotheses listed above.

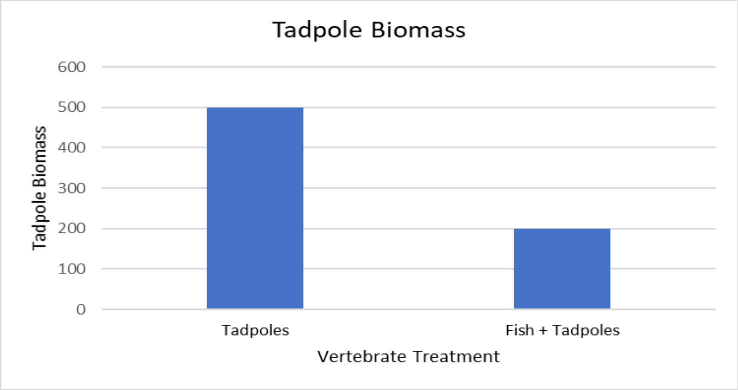


**Figure 16**. Predicted results of $H_1$ where tadpole biomass is expected to be higher in treatments where they occur without fish compared to the fish + tadpole vertebrate treatment.

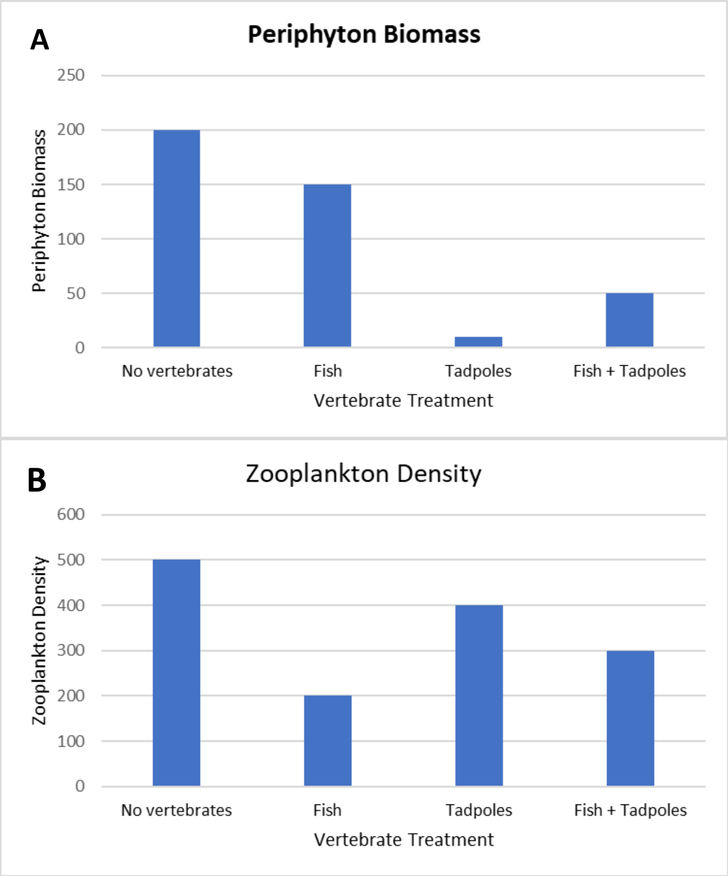


**Figure 17**. Predicted results of $H_2$ and $H_3$ where periphyton will be lower in treatments containing larval amphibians (**A**) and zooplankton will be lower in treatments containing fish (**B**).

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# IDENTIFYING FACTORS THAT INFLUENCE AQUATIC INVERTEBRATE USE OF TALLGRASS PRAIRIE STREAM POOLS, KANSAS

MASTER'S PROSPECTUS

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## INTRODUCTION

Non-perennial streams, with surface waters that temporarily stop flowing, make up 59% of watercourses within the United States (Goodrich et al. 2018, Busch et al. 2020). They are subject to variable drying and rewetting cycles, and the aquatic organisms that utilize these habitats must be adapted to their harsh conditions (Bertrand et al. 2013, Leigh et al. 2015, Busch et al. 2020). Aquatic invertebrates are a major contributor to the biological diversity and trophic complexity of these systems, with numerous strategies to survive their variable disturbance regimes (Datry et al. 2014, Hay et al. 2018). This includes some sensitive species, like endangered freshwater mussels. Climate change and anthropogenic impacts threaten to alter the natural disturbance regimes of all aquatic ecosystems (Hay et al. 2018). There is an expectation that the number of perennial streams reclassified as intermittent will increase in the future as they experience higher rates of drying, due to increased drought, water diversions, and impoundments (Larned et al. 2010, Datry et al. 2018, Majdi et al. 2020). For non-perennial streams that naturally experience variable drying and flooding cycles, these impacts could further limit the organisms able to utilize these systems (Datry et al. 2014, Hay et al. 2018).

Historically, stream ecologists have focused their studies on perennial systems (Larned et al. 2010, Leigh et al. 2015, Busch et al. 2020). With increased interest in understanding the role of non-perennial streams, a greater importance has been placed on correctly classifying and characterizing these stream types across disciplines to better inform policy makers (Leigh et al. 2015, Busch et al. 2020). Perennial streams have larger channels, more constant water flow and a riparian canopy cover. In general, non-perennial streams are smaller, consisting of headwaters that periodically dry producing isolated pools. Non-perennial systems are further classified as intermittent or ephemeral, differing in respect to long term flow patterns. Intermittent streams continue to gain water and have considerable groundwater inputs, while ephemeral streams lose water and are more influenced by precipitation inputs (Busch et al. 2020). These classifications can also exist along the length of an individual stream reach, with perennial headwaters draining into an intermittent middle reach, before connecting back to a more permanent perennial flow (Bertrand et al. 2013).

Tallgrass prairie streams are complex systems, with perennial and non-perennial streams, but many headwaters are classified as intermittent (Dodds et al. 2004). They exhibit variable annual discharge and frequent periods of short-term drought (Stagliano and Whiles 2002). Future impacts of a changing climate for Kansas grasslands include increased frequency of intense rainstorms and periods of prolonged drought that could directly influence drying and flooding regimes (Bertrand et al. 2013). Aquatic organisms within tallgrass prairie streams are more immediately impacted by consequences of human land use, including cattle ranching and alterations to natural streamflow. Much of tallgrass prairie within Kansas is utilized as pasture, with limited ability to be converted to agricultural land, lessening the impact of common anthropogenic disturbances to streams, such as pollution and increased nutrients from agricultural runoff (Dodds et al. 2004). While cattle grazing helps to mimic natural grazing patterns, increased stocking rates and the diversion of water to sustain the number of cattle can cause significant impacts. Cattle with access to streams can degrade banks and increase nutrient inputs. Within the tallgrass prairie, determining the types of streams, number of streams, and which parts of watersheds are essential to sustain natural assemblages of aquatic organisms is important for their future preservation (Dodds et al. 2004).

Aquatic invertebrates have developed complex life history strategies to survive within the variable nature of intermittent streams and are crucial to their ecological functioning. These invertebrates are commonly classified into two main groups, macroinvertebrates that are large enough to be visible to the naked eye, and meiofauna that are small and only identified by microscopic observation (Stubbington et al. 2020). Aquatic invertebrates play a central role within the aquatic trophic structure. Most small invertebrates consume primary producers and detritus, eventually becoming prey for larger bodied taxa including aquatic insects, crayfish, and fishes (Bertrand et al. 2013). Others are filter-feeders, whose feeding strategy benefits aquatic systems through water clarification. Aquatic invertebrates also provide transfer of materials between aquatic and terrestrial systems (Stubbington et al. 2020).

There has been limited study into the aquatic invertebrates of tallgrass prairie streams, with most research conducted at the Konza Biological Field Station in northeastern Kansas (Kansas State University) (Stagliano and Whiles 2002, Larson et al. 2013). Of these, non-insect invertebrate groups remain understudied (Dodds et al. 2004). Expanding the number of study locations that incorporate a diverse assemblage of aquatic invertebrates will contribute to our growing knowledge of prairie streams (Larson et al. 2013). Specifically, determining how invertebrate assemblages with different dispersal strategies are distributed within pools of varying hydrology and their ability to withstand drying in isolated pools can contribute to our understanding how invertebrates utilize these systems and how they respond to expected change in disturbance regimes (Gleason and Rooney 2017).

Study site

Both projects will be conducted at Youngmeyer Ranch, Elk County, Kansas (37.545022, -96.489850) (Figure 1), a 1902 ha (4700 acre) Wichita State University Biological Field Station, owned and managed by the Youngmeyer Trust (Figure 2). The site is located within the Flint Hills (Ecoregion Level IV) consisting of mostly natural tallgrass prairies, with oak-hickory forests along permanent stream reaches (Chapman et al. 2001). The site includes ephemeral, intermittent, and perennial streams as well as natural springs and artificial cattle ponds, allowing for to the exploration of aquatic organisms within a variety of aquatic habitats. The mean annual temperature is 13.7ºC and mean annual precipitation is 979 mm (Houseman et al. 2016). Prescribed burning and cattle grazing have been included in the management regime for the last 20 years. The area is composed of layers of hard limestone, soft shale, and chert (flint) with silty clay loam soils (Houseman et al. 2016). The physical geology of the area lies within the western edge of the Osage Cuestas (physiographic region) (Buchanan 2010), with the west side of the property sloping to the west and the east side consisting of steeper slopes of exposed limestone (escarpments) to the east.

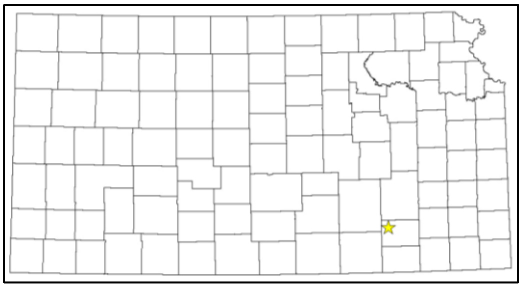

Figure 1. Map of counties within Kansas, USA. Youngmeyer Ranch (star) is located within the northwestern corner of Elk County.

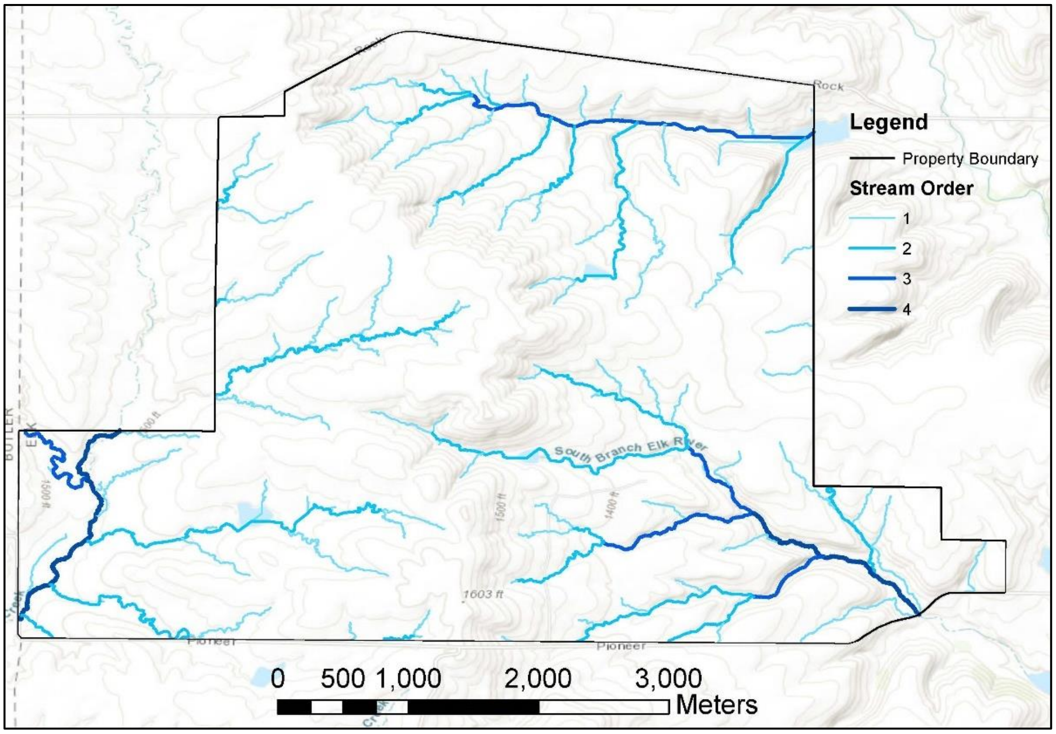

Figure 2. Map of Youngmeyer Ranch, Elk County, KS, showing the property boundary, streams, and elevation. Map created using ArcGIS software by ESRI.

## PROJECT ONE: FACTORS INFLUENCING MACROINVERTEBRATE DISTRUBTION WITHIN TALLGRASS PRAIRIE STREAM POOLS

### Macroinvertebrates

Freshwater macroinvertebrates vary greatly in size, dispersal strategies, and feeding roles. Macroinvertebrates common to tallgrass prairie streams include molluscs (e.g., snails and mussels), crustaceans (e.g., crayfish), and the larval and adult stages of aquatic insects. Snails and crayfish have abilities to survive short periods of stream drying, but must ultimately rely on flowing waters for dispersal (Dodds et al. 2004, Thorp and Covich 2010). Snails consume detritus and algae, scraping these resources off substrates, and are a common component of invertebrate assemblages across a variety freshwater habitat (Thorp and Covich 2010). Crayfish (Crustacea) differ from other macroinvertebrate groups due to their capacity to grow to large body sizes. They are omnivorous and most fishes cannot consume adult crayfishes, allowing them to become dominant macroconsumers. Snail abundance has been proposed to be influenced by predation of crayfish within tallgrass prairie streams (Stagliano and Whiles 2002).

Of all stream invertebrates, insects are one of the most well-studied due to their high abundance and diversity (Thorp and Covich 2010). Reflecting this diversity, aquatic insects serve as important food sources for both invertebrate and vertebrate predators, and are important predators themselves (Thorp and Covich 2010). Aquatic insects expected in tallgrass prairies include beetles (Coleoptera), true bugs (Hemiptera), larval damselflies and dragonflies (Odonata), larval mayflies (Ephemeroptera), and larval flies (Diptera). Aquatic insects were found to contribute 85% to the total community macroinvertebrate production within tallgrass prairie streams, with greatest contributions from larval flies and mayflies (Stagliano and Whiles 2002). Many of these insects have larval stages that exist solely within the water before emerging as terrestrial adults. Most mayflies are considered detritivores or herbivores and are an important food source for stream fishes (Thorp and Covich 2010). Their emergence is usually in large numbers with short adult stages that mate and quickly die. Odonates are important predators within freshwater, consuming other invertebrates and some vertebrates (e.g., tadpoles) (Thorp and Covich 2010). Both dragonflies and damselflies have longer lived adults that are also important predators within terrestrial systems. Macroinvertebrates contribute greatly to the diversity of aquatic organisms within intermittent streams and utilize these systems in different ways to survive their variable nature.

Generally, streams with increased channel width, water permanency, and connectivity offer increased habitat types and more predictable conditions that allows for increased invertebrate abundance and diversity. These generalizations have been shown to hold true even within an initially perennial headwater stream that shifted to an intermittent middle reach (Whiting et al. 2011). Although, this consensus is not concrete evidence that this is holds true for all tallgrass prairie streams. Some studies have shown that intermittent reaches can have higher invertebrate abundance of certain taxa when compared to nearby perennial reaches (Dodds et al. 2004). For example, crayfish have been shown to have higher abundance within isolated pools of intermittent streams when compared to nearby perennial reaches (Flinders and Magoulick 2003). Potential impacts of climate change could alter the normal disturbance regime of these systems by increasing connectivity to upstream reaches and creating longer periods with more isolated pools that could alter invertebrate assemblages. The creation of impoundments for cattle and fishing ponds alter the natural flow and function of a stream reach, with the added effects of cattle and fishes on invertebrate communities. With limited understanding of these systems, and their multiple potential impacts, exploration of the requirements needed for a variety of aquatic organism to utilize these systems is necessary for their ultimate preservation.

Within intermittent streams, large numbers of pools with and without flow can become important refuge for aquatic organisms. Most studies of stream macroinvertebrates sample riffles rather than pool habitats (Stagliano and Whiles 2002). Riffles are commonly sampled because they have been shown to have higher diversity, but more importantly what are deemed pollution intolerant “EPT” taxa (combined richness of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Tricoptera)) that allow for the assessment of water quality (Thorp and Covich 2010). These EPT taxa have been shown to have low abundance in headwater streams of tallgrass prairies (Fritz and Dodds 2004). The collection of large macroconsumers is underestimated when collections are made solely from riffle habitats. Moreover, isolated pools can exist as permanent refuge for many aquatic organisms within intermittent streams. Studies that incorporate the collection of macroinvertebrates within stream pools may provide a more realistic component of their distribution within intermittent streams that have limited connectivity.

The small sample size of most invertebrate collections limits the incorporation of large crayfish and their contribution to invertebrate assemblages has been likely been underestimated within tallgrass prairie streams (Stagliano and Whiles 2002, Whiting et al. 2011). A larger constrained volume search, like trashcan enclosures, would help to aid in collection of large macroinvertebrates, allows for calculation of the total volume of water sampled, and the simultaneous capture of amphibians and fishes. When collecting larger samples sizes for the simultaneous capture of invertebrates, amphibians, and fishes, it is expected that comparatively invertebrates would have a higher average abundance across all pools. For aquatic invertebrates collected, there would be a tradeoff between allowing for a larger sample size and the inclusion of larger organisms, and factors that would be incorporated within many invertebrate studies, including measuring the size of each individual and preservation of all invertebrates collected to be identified in the lab, rather than in the field.

### Factors that influence macroinvertebrate distribution

Within intermittent streams, physical factors have been shown to be more important in structuring invertebrate assemblages than biological factors (Bertrand et al. 2013). Physical factors that could influence the distribution of aquatic invertebrates include water permanency, connectivity to permanent refuge, and streambed composition. Biological factors of influence the distribution include instream or riparian vegetation and predatory fishes.

#### *Physical factors*

The length of the hydroperiod, the duration of surface water, of stream pools can determine the composition of aquatic invertebrate communities (Rolls et al. 2018). Hydroperiod is expected to be a function of pool volume and depth, as pool size increases, so does the potential to hold water over time. Streams with high water permanency are expected have higher invertebrate abundance and diversity (Dodds et al. 2004). A decrease in pool hydroperiod and volume would limit the amount of available space and resources, increasing interactions like predation and competition (Majdi et al. 2020).

Stream pools that are connected by flowing water or are more connected to perennial refuge allow for greater dispersal of many macroinvertebrates (Majdi et al. 2020). Dispersal strategies for most macroinvertebrates include moving to more permanent refuge or emerging as adults (aquatic insects). Concerning potential impacts of climate change, an increase in intense storms could cause flash floods that increase the (longitudinal) connectivity to upper reaches of the stream, allowing for rapid colonization by larger macroinvertebrates and fishes. Once colonized, these taxa can persist in isolated pools. Longer periods of drought decrease the connectivity to permanent refuge and increase the number of isolated pools (Larned et al. 2010).

The natural connectivity of stream pools can be disrupted through anthropogenic disturbance. The diversion of water for the creation of cattle ponds or fishing ponds influence aquatic invertebrates by disrupting natural flow patterns and creating large, unnatural bodies of water (Dodds et al. 2004). Fishing ponds that are created for recreational use and can have the added negative effect of being stocked with sport fishes. Creating unnatural, permanent refuge along a stream reach could increase the number of large predators able to colonize upstream pools. Studies incorporating multiple headwater streams with and without impoundments could expand on our knowledge of these disturbances in influencing aquatic invertebrate abundance.

#### *Biological factors*

Fishes are an important component of intermittent streams and can influence the distribution of other aquatic organisms. They are a major predator of aquatic invertebrates and have been shown to directly influence their distribution and abundance. For example, *Physa* snail use of open habitats decrease when sunfish are present, having been reported to hide within refugia and only leaving due to starvation (Thorp and Covich 2010). It has been suggested that predatory fishes can congregate in high abundance within pools during low flow periods, resulting in decreased invertebrate biomass and production (Whiting et al. 2011). Studies that incorporate simultaneous capture of invertebrates and fishes within intermittent stream pools could help to better determine the effects of these large predators on invertebrate distribution (Biggs et al. 2017).

## Specific aims

The specific aim of this project is to determine abiotic and biotic factors that influence the distribution of aquatic macroinvertebrates within intermittent stream pools of the tallgrass prairie. A field study will be conducted to test the hypotheses: 1) aquatic macroinvertebrate richness and abundance (density) increases with increasing pool hydroperiod, size, and connectivity to permanent refuge and 2) aquatic macroinvertebrate richness and abundance (density) decreases with fish presence (Figure 3). The influence of these factors on macroinvertebrate groups with differing dispersal abilities will also be assessed. Specifically, how these factors influence the distribution of snails, crayfish, mayflies, and odonates will be analyzed.

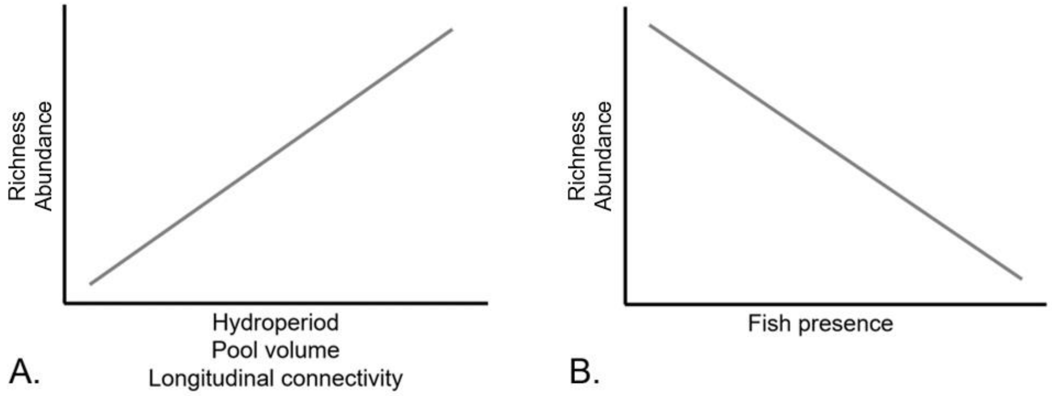

Figure 3. Hypotheses predictions for macroinvertebrate richness and abundance across (3A) pool hydroperiod, volume, and connectivity, and (3B) fish presence.

## Methods

### *Sample streams and pools selection*

All sample streams and pools were initally mapped using Google Earth Pro, utilized for its accessibility. Undergraduate students within the Aquatic Ecology lab (Spring 2020) were assigned weekly tutorials to assist in mapping sample sites. All streams across the study site were mapped in increments of 100 m. Seven headwater streams were then selected for sampling. These sample streams varied in water permanency, connectivity to permanent refuge, and the presence or absence of cattle ponds. All pools were then mapped for each 100 m segment. For each segment, two of the mapped pools were randomly selected for field sampling. A total of 153 pools were ultimately selected, of which 118 held water at the time of sampling (Figure 4).

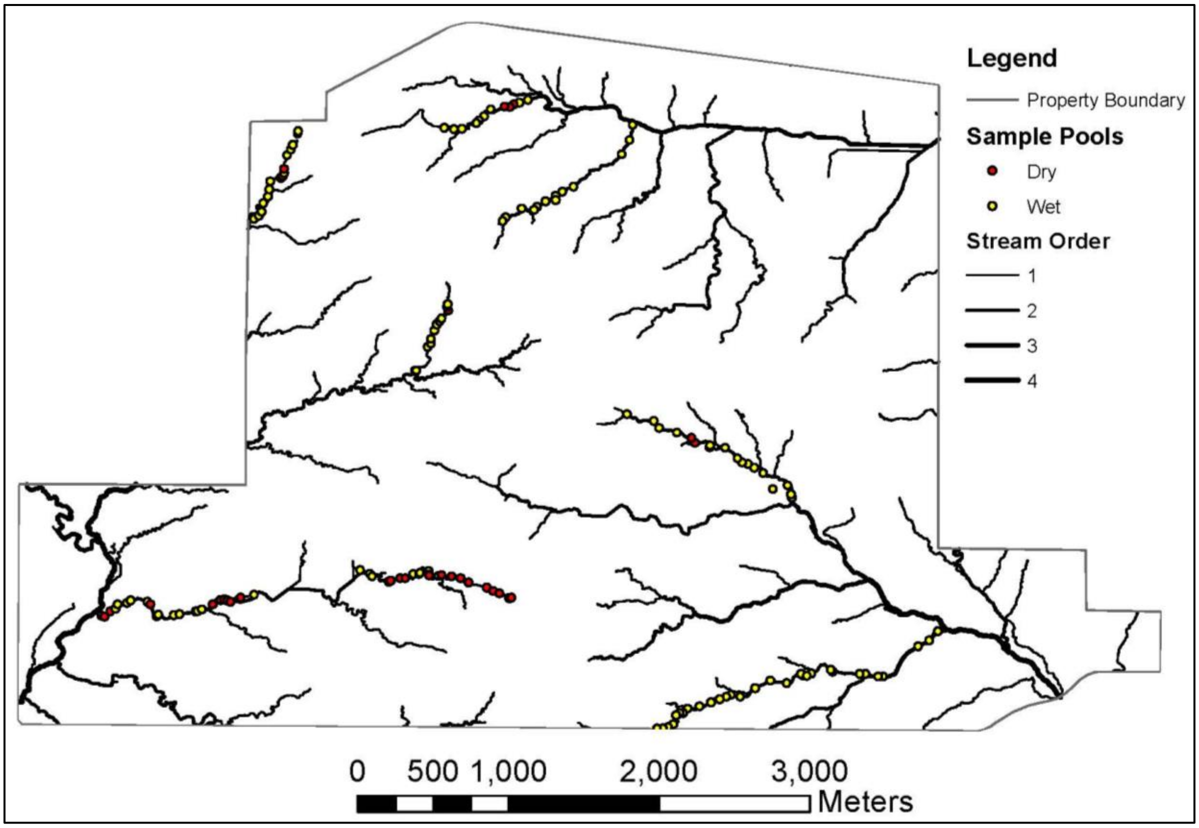

Figure 4. Youngmeyer Ranch, Elk County, KS. Sample pools are marked by whether they held water at the time of sampling (yellow circles) or were dry (red circles). Map created using ArcGIS software by ESRI.

*Sample collection*

A constrained volume search was conducted to collect study organisms simultaneously and allowed for the calculation of total pool volume sampled (May – June 2020). Trashcan enclosures were used, consisting of a plastic trashcan (51 x 51 x 65 cm) with the bottom removed, to conduct the constrained volume search. A transect is first placed along the length of the right bank of the pool from downstream to upstream and total length recorded. For pools greater than 14 m, enclosures are placed and sampled every 2 m along the transect. For pools less than 5 m, enclosures are placed and sampled every 1 m. Placement of the first enclosure is randomly selected to start within the middle or near either bank of the pool, and followed this pattern for the remaining placements (e.g., first enclosure placed in middle, second left, third right, fourth middle).

For each placement, a net (0.3 cm mesh) is used to sweep the contents of the trashcan to collect all organisms trapped within. After one sweep throughout the entirety of the enclosure, we remove all macroinvertebrates, amphibians, and fishes from the net and separate them into small pitchers and five-gallon buckets. Sweeps continue following this process and discontinue when three consecutive sweeps collect no organisms. We then measured and recorded the depth of the water within each enclosure to calculate the volume of water sampled. The entire process is then repeated for the next sampling point along the transect. Invertebrates collected were counted and identified in the field. All crayfish were categorized as less than or greater than 5 cm. As needed, representative specimens were collected and preserved in 75% ethanol for further identification (Merritt et al. 2008, Thorp and Covich 2010).

### *Pool volume*

To calculate total volume of each pool, we collected volume measurements at the time of sampling. A transect is placed through the middle of the stream pool, from downstream to upstream, and total length recorded. Measurements were taken every 1-2 m along this transect, dependent on pool length, starting and ending 0.25 m from the beginning and end of the pool. At each point along the transect, we measure the total width of the pool, the width from left wetted bank to the deepest point, and the depth of the deepest point. Two successive width and depth measurements are taken to the right and left mid-point from the deepest point to each wetted bank.

### *Site characteristics*

Site characteristics are collected for each pool at the time of sampling to determine potential factors of influence. This includes current weather conditions, bank and in-stream vegetation, in-stream sediment composition, in- and outflow, and canopy cover.

### *Data Analysis*

#### *Hydroperiod*

Hydroperiod indices ($H_i$), see Equation 1, were calculated from satellite images of 19 randomly selected stream reaches for a total 318 of randomly selected pools using Google Earth Pro. For each pool, the total number of observations ($O_i$) was determined using four years of satellite imagery (10/17/2010, 2/28/2012, 2/16/2013, and 11/5/2014) to then estimate the total number of observations with water present ($P_i$).

$$H_i = P_i / O_i \qquad (1)$$

#### *Connectivity*

Total stream wetted length to permanent refuge for each sample pool was measured using Google Earth Pro.

#### *Statistical analysis*

Dependent (response) variables include taxa richness and abundance (density), as well as these measurements for determined select taxa groups. Independent (predictor) variables include hydroperiod, pool volume, maximum depth, connectedness to permanent refuge, substrate composition, and fish presence. Model comparisons will be conducted for the determined predictor and response variables. Based on Akaike Information Criterion (AIC), model selection will determine the best model for each response variable. These comparisons will then be conducted for predetermined macroinvertebrate groups, differing in dispersal and life-history characteristics, to further evaluate macroinvertebrate utilization of intermittent stream pools.

## PROJECT TWO: RESPONSE OF MEIOFAUNA OF ISOLATED TALLGRASS PRAIRIE STREAM POOLS TO EXPERIMENTAL DRYING

### Meiofauna

Aquatic invertebrates have adapted to historical hydrology regimes through various dispersal and life history strategies (Hay et al. 2018). Macroinvertebrates rely heavily on aerial dispersal or physical movement to escape stream drying. Common small invertebrates (meiofauna), including microscopic worms, rotifers, and microcrustaceans, have developed life history strategies that make them able to withstand drying and immediately recolonize after drying events. Meiofauna are an often-overlooked aspect of invertebrate studies, especially for freshwater habitats (Majdi et al. 2020). Expected meiofauna of intermittent streams within the tallgrass prairie include nematodes (Nematoda), microturbellarians (Platyhelminthes), oligochaetes (Annelida), rotifers (Rotifera), and microcrustaceans (Crustacea: Ostracoda, Branchiopoda, Copepoda). More studies are needed to determine if their resistant abilities facilitate recolonization and re-establishment of trophic connections after drying and refilling of isolated pools (Majdi et al. 2020).

Resistant traits are those that allow individuals to resist drying (Dodds et al. 2004, Strachan et al. 2015). The production of resisting eggs or cysts is a common trait of meiofauna (Strachan et al. 2015). Many crustaceans, including the more explored cladocerans, are well-known for the development of resistant eggs. Other taxa have abilities to hibernate or aestivate for short periods of time in constructed resistant structures (Strachan et al. 2015). Some respond to the onset of disturbance through shifts in their reproduction and development (Verberk et al. 2008, Strachan et al. 2015). Nematodes can produce a resistant juvenile stage with a modified cuticle to resist drying (Strachan et al. 2015). Meiofauna, can also enter resistant stages at different periods of their life cycle (Strachan et al. 2015). When species can resist drying at an immature, juvenile, or adult stage, they could potentially have an advantage over other species when water returns (Horne 1993, Strachan et al. 2015). Species can have multiple traits and strategies employed at a time or at different stages of life that allow for them to better cope with variation in drying regimes (Strachan et al. 2015).

The abilities of many meiofaunal groups to survive drying has been well documented throughout the history aquatic ecology, but were not considered of great importance to the functioning and recolonization of aquatic systems (Stanley et al. 1994, Fritz and Dodds 2004, Chester and Robson 2011, Strachan et al. 2015, Hay et al. 2018). Dry sediments of intermittent aquatic habitats had mostly been deemed “biologically inactive” (Steward et al. 2012, Strachan et al. 2015). This has also been stated for meiofauna within tallgrass prairie streams (Fritz and Dodds 2002, Dodds et al. 2004). Recent studies have contrasted with this historical view, finding dry sediments to be a significant refuge with diverse invertebrate assemblages (Hay et al. 2018).

In a changing climate, it expected that increased drying times will cause a loss in connectivity to permanent refuge and an increase in the number of isolated pools. If these pools lose all surface water for long periods of time, then once refilled resistant taxa can quickly recolonize and aid in the reestablishment of ecological processes (Gaudes et al. 2010, Majdi et al. 2020). It is also reasonable to assume, as drought increases, species that are able to cope with these extremes may have the potential to become dominant with their major predators unable to survive and recolonize due to the harsh conditions (Altermatt et al. 2009, Strachan et al. 2015). Prolonged drought may limit the survival and colonization of isolated pools by actively dispersing macroinvertebrates. Short spates of increased flow from heavy rains may not create the conditions that would usually enable large macroconsumers to move upstream. Decreased lateral connectivity may decrease the number of aerial dispersals that would be potential first consumers of primary producers and meiofauna. Because of their potential to rapidly recolonize after drying disturbance, meiofauna may play an important role in restoring aquatic food webs and "kick-starting" ecosystem functions (Gaudes et al. 2010, Majdi et al. 2020).

While it is clear many meiofauna taxa can permit after drying, less is known about how different drying durations affect their ability to recolonize isolated pools (Robson et al. 2011, Strachan et al. 2015, Hay et al. 2018, Vargas et al. 2019). Tests of the flexibility of strategies used to survive desiccation in different environmental conditions have been limited (Strachan et al. 2015). It has been suggested that experimental manipulations (e.g., rehydration of sediment cores) would allow for the evaluation of the role of meiofauna groups (Hay et al. 2018, Majdi et al. 2020). Determining how these communities respond to drying disturbance within intermittent tallgrass prairie streams will help aid in preserving their biodiversity (Boersma et al. 2014).

### Rehydration experiments

Many studies concerned with the recolonization of meiofauna after dying disturbance utilize sediment rehydration and specifically focus on the role of the resistant egg bank. Most microcosm rehydration experiments are conducted on wetlands and lakes. Those that focus on streams or rivers have been conducted in dry or arid landscapes within the southwestern USA (Simovich and Hathaway 1997), Australia (Hay et al. 2018), and the Mediterranean (Majdi et al. 2020). Many of these systems have similar conditions to tallgrass prairie streams, including short hydroperiods, low connectivity, and limited riparian resources. Microcosms have been shown to be limited in their abilities to replicate the complexity of natural systems (Schindler 1998), but have been commonly used for rehydration experiments and are reported to reasonably approximate the functioning of inundated riverbeds (Jenkins and Boulton 2003, Jenkins and Boulton 2007, Hay et al. 2018). It was therefore important to incorporate as many natural processes as possible and limit the amount of initial manipulation, to specifically focus on our questions concerning meiofauna and their response to drying.

Experimental drying is difficult to study in the field setting (Jenkins and Boulton 2003, Hay et al. 2018) and most collect soil when the stream is dry for experimental rehydration (Stubbington et al. 2016, Maijdi et al. 2020). Sediments are usually collected in the field from the top layers of soil, homogenized, and divided across microcosms (Sternert et al. 2017). Few collect both water and sediment to allow for the assessment of the invertebrate assemblage before sediments dry. Even less collect the intact sediment core for each microcosm that would represent the true sediment structure.

Experimental rehydration studies vary greatly in their design. Microcosms have been housed in the lab under 12 h light, 12 h dark cycles (Sternert et al. 2017), within a temperature-controlled greenhouse (Hay et al. 2018), or outside (Stubbington et al. 2016). When placed outside, many utilize a shade cloth or mesh net as a cover to avoid contamination (Diez-Brantley et al. 2002, Boersma et al. 2014). These covers limit the amount of ecological realism by limiting light penetration, dispersal or colonization, and other inputs, but ultimately allows for some control of these influencing variables (Boersma et al. 2014).

Water used for rehydration includes filtered groundwater (Anderson and Smith 2004), well water (Bright and Bergey 2015). distiller water (Freiry et al. 2016), declorinated tap water (Stubbington et al. 2016), and deionized water (Hay et al. 2018). After rehydration treatments, the process for sampling invertebrates from microcosms have been conducted only once after a period of rehydration (Bright and Bergey 2015), a couple times a week for several weeks (Freiry et al. 2016), or on a scheme from 0, 7, 14 and 28+ days after rehydration (Ávila et al. 2015, Stubbington et al. 2016, Freiry et al. 2016, Sternert et al. 2017, Hays et al. 2018). It has been reported that after 30 days, there is an increase in algal growth and deteriorating water quality.

Collection of samples from microcosms have been completed through the use of small nets (Freiry et al. 2016, Sternert et al. 2017) to siphoning all surface water through a sieve (Hay et al. 2018). Samples have been immediately placed in ethanol or observed live. Preservation allows for a wait time before sample must be assessed, but many small invertebrates are not easily identifiable after preservation (Stubbington et al. 2016). While live samples must be immediately analyzed, more species can be recorded and can be more readily identified (Stubbington et al. 2016). Some then return all collected samples to microcosms after identification (Boulton and Lloyd 1992, Hay et al. 2018), while others discard samples. Other manipulations of the sample population, like initially removing large macroinvertebrates from microcosms, is conducted in order to decrease predation of study organisms (Stubbington et al. 2016). Microcosms are considered a closed system with increased biotic interactions, like predation, that may limit their ability to mimic natural processes (Boulton and Lloyd 1992, Hay et al. 2018). The experimental design will ultimately differ based on the research questions asked, but provide a means for the assessment of experimental drying and rehydration of meiofauna communities (Hay et al. 2018).

### Specific aims

The aim of this project is to determine the meiofauna assemblages and their abilities to recolonize after pool drying within isolated pools of intermittent tallgrass prairie stream. An experimental microcosm study will be conducted to test the hypotheses: (1) meiofauna richness and abundance (density) will be lower in drying treatments after refill than the fill treatment, and (2) meiofauna richness and abundance (density) will be lower after the longer drying treatment than the shorter drying treatment (Figure 5). Specifically, meiofauna groups with differing resistant traits and strategies to survive drying will be assessed, including nematodes, rotifers, and three microcrustacean groups, ostracods, cladocerans, and copepods.

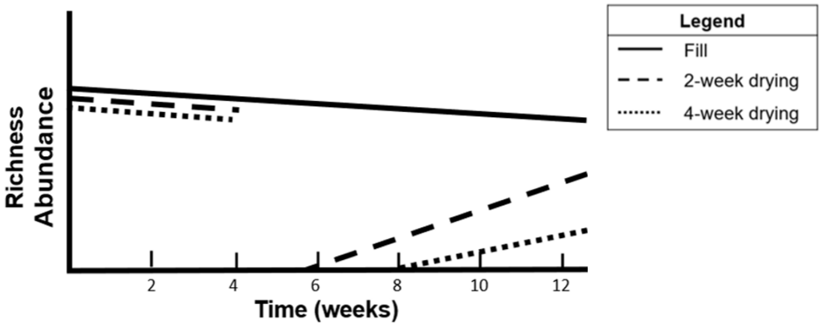

Figure 5. Hypotheses predictions for changes in total meiofauna taxa richness and abundance (density) over time for each treatment (fill, 2-week drying, 4-week drying).

## Methods

### *Sample streams and pools selection*

Ten isolated stream pools from four stream segments were randomly selected from Youngmeyer Ranch (Figure 6). Specifically, only pools holding water that consisted of sediments that allowed for the collection with a sediment corer were included.

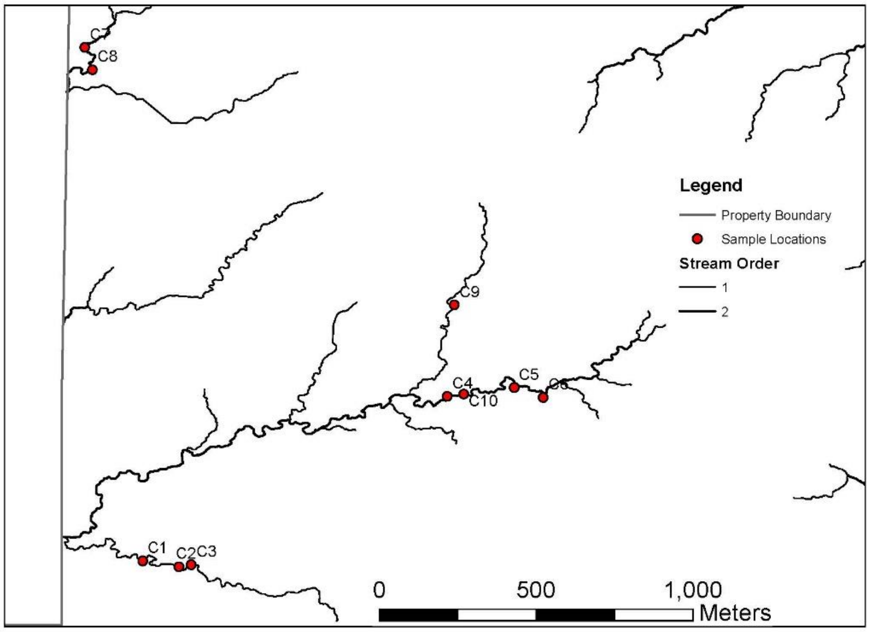

Figure 6. Map of the northwestern corner of Youngmeyer Ranch, Elk County, Kansas. Ten sample pools (red circles) are marked by their sample name. Map created using ArcGIS software by ESRI.

### *Microcosms*

A soil sleeve (30.48 cm tall, 7.68 cm diameter), consisting of a plastic cylinder that is open on both ends, is used to collect the contents of the microcosm in the field (August 2020) (Figure 7). The sleeve is pushed through the water and sediment column, until 10 cm of sediment core is collected, or bedrock is hit. Three cores per pool are collected, each randomly selected to be collected from the middle, right of left side of the pool. This provided the random selection of habitat characteristics of each pool. Site characteristics and pool volume were collected as described for Project 1.

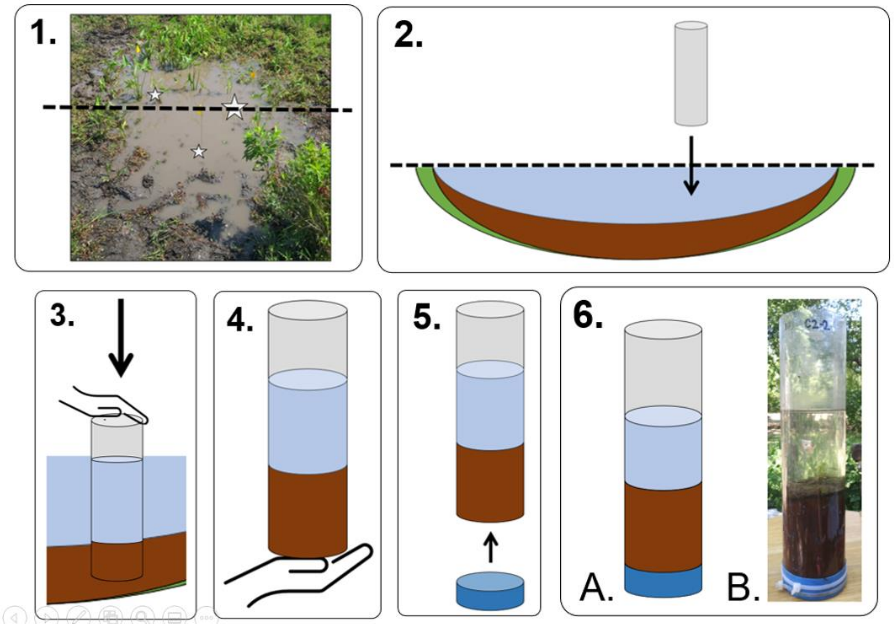

Figure 7. Microcosm field collection steps. 1. Determine sample points. Three sample points (white stars) are shown within the sample pool. 2. Hold sleeve above water, perpendicular to surface above sample point. Showing cross-section above pool. 3. Push sleeve through water and sediment column. 4. Remove sleeve. 5. Place cap on bottom of sleeve. 6. Complete microcosm. 6A. Model image of microcosm. 6B. Photo of microcosm.

After collection, microcosms are housed outside at an off-site "facility" (my backyard, Wichita, KS, 67203) (August – October 2020). Microcosms will therefore experience similar seasonal temperatures and photoperiod to the study site. The microcosms are placed within aboveground crates and confined by wooden stakes to hold them in place. After each sampling period, microcosms will be rotated ("snaked") throughout the structure to help control for any variation in light penetration. A fine mesh net will cover each microcosm to limit any aerial contamination. The crates are covered with a tarp that does not seal the microcosms but allows for protection during precipitation events. This cover will also be used at night to discourage animal mischief (racoons).

*Experimental treatments*

The three microcosms collected from each pool are randomly assigned one of three treatments: (1) a fill treatment, with microcosms receiving water additions throughout the duration of the study and not allowed to dry (fill-treatment), (2) allowed to naturally dry with a complete drying duration of two-weeks before refill (2-week dry treatment), and (3) allowed to naturally dry with a complete drying duration of four-weeks before refill (4-week dry treatment). The drying treatments were started once all surface water naturally dried. Water used for refill will consist of collected pool water from the study site that is filtered and autoclaved. This type of water was used to retain some of the natural properties, rather than using treated water or relying on rainwater collection. Dry treatments were eventually refilled with 100 ml of water and 50 ml on subsequent days as needed. Water was added to fill treatments throughout the study as needed.

### *Sample collection*

Water and a small amount of the surface sediment is collected from each microcosm three times a week (Monday, Wednesday, and Friday). For each sample, 10 ml of water and sediment is collected by pipette, excluding those undergoing drying treatments. 5 ml of the collected sample is then microscopically analyzed. All invertebrates are counted, identified (Thorp and Covich 2010), and the body size of microcrustaceans is measured. Collected samples are not added back to the microcosm. Characteristics and changes of each microcosm is recorded during sample collection, including sediment depth, water depth, and algae/macrophyte presence. Measured water depth of each microcosms before sample collection will allow for the calculation of volume sampled.

### *Statistical Analysis*

The independent (treatment) variables are the three treatments: fill, 2-week drying, and 4-week drying. The dependent (response) variables include taxa richness and abundance (density). Assurance that the data meet the test assumptions and required transformations will be performed before analysis. Analysis of variance (ANOVA) will be conducted to initially determine any differences between treatments or pools. Further analysis on differences before and after drying on specific taxa groups collected will be performed, with the possible inclusion of body size and life-stage changes for the three microcrustacean groups. The possible analysis on an interval scale of days before or after drying (e.g., 3, 6, 9, etc. days before and after refill) would provide equal sample sizes for comparisons and any significant results could provide insight into the immediate recolonization of isolated pools. The ultimate analysis will be dependent on the number of samples before and after drying completed, which is controlled by the eventual seasonal shift to winter. Freezing temperatures will freeze the water within the microcosms, rendering them unable to be sampled.

# TIMELINE

| **Project** | **Task** | **Semester** | **Year** | **Completed** |
|---|---|---|---|---|
| **General** | Prospectus defense | Fall | 2020 | |
| | Presentation for Biology Seminar 1 | Fall | 2020 | |
| | Presentation for Biology Seminar 2 | Spring | 2021 | |
| | Target completion date of thesis and defense | Spring | 2021 | |
| **Project 1** | Define question and analysis | Spring | 2020 | ☒ |
| | Finalize materials and methods | Summer | 2020 | ☒ |
| | Field sampling | Summer | 2020 | ☒ |
| | Data entry | Fall | 2020 | |
| | Analysis | Spring | 2021 | |
| **Project 2** | Define question and analysis | Spring | 2020 | ☒ |
| | Finalize materials and methods | Summer | 2020 | ☒ |
| | Microcosm setup | Fall | 2020 | ☒ |
| | Field sampling | Fall | 2020 | ☒ |
| | Microcosm treatments | Fall | 2020 | ☒ |
| | Data collection | Fall | 2020 | ☒ |
| | Data entry | Fall | 2020 | |

| | | | | |
|---|---|---|---|---|
| | Analysis | Spring | 2021 | |

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# Appendix III: Christine Streid Masters Prospectus

# IDENTIFYING FACTORS THAT INFLUENCE AQUATIC INVERTEBRATE USE OF TALLGRASS PRAIRIE STREAM POOLS, KANSAS

## MASTER'S PROSPECTUS

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Department of Biological Sciences
Wichita State University

Committee Members: Dr. Thomas Luhring, Dr. Gregory Houseman, Dr. Andrew Swindle

Submission Date: November 23, 2020

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# INTRODUCTION

Non-perennial streams, with surface waters that temporarily stop flowing, make up 59% of watercourses within the United States (Goodrich et al. 2018, Busch et al. 2020). They are subject to variable drying and rewetting cycles, and the aquatic organisms that utilize these habitats must be adapted to their harsh conditions (Bertrand et al. 2013, Leigh et al. 2015, Busch et al. 2020). Aquatic invertebrates are a major contributor to the biological diversity and trophic complexity of these systems, with numerous strategies to survive their variable disturbance regimes (Datry et al. 2014, Hay et al. 2018). This includes some sensitive species, like endangered freshwater mussels. Climate change and anthropogenic impacts threaten to alter the natural disturbance regimes of all aquatic ecosystems (Hay et al. 2018). There is an expectation that the number of perennial streams reclassified as intermittent will increase in the future as they experience higher rates of drying, due to increased drought, water diversions, and impoundments (Larned et al. 2010, Datry et al. 2018, Majdi et al. 2020).  For non-perennial streams that naturally experience variable drying and flooding cycles, these impacts could further limit the organisms able to utilize these systems (Datry et al. 2014, Hay et al. 2018).

Historically, stream ecologists have focused their studies on perennial systems (Larned et al. 2010, Leigh et al. 2015, Busch et al. 2020). With increased interest in understanding the role of non-perennial streams, a greater importance has been placed on correctly classifying and characterizing these stream types across disciplines to better inform policy makers (Leigh et al. 2015, Busch et al. 2020). Perennial streams have larger channels, more constant water flow and a riparian canopy cover. In general, non-perennial streams are smaller, consisting of headwaters that periodically dry producing isolated pools. Non-perennial systems are further classified as intermittent or ephemeral, differing in respect to long term flow patterns. Intermittent streams continue to gain water and have considerable groundwater inputs, while ephemeral streams lose water and are more influenced by precipitation inputs (Busch et al. 2020). These classifications can also exist along the length of an individual stream reach, with perennial headwaters draining into an intermittent middle reach, before connecting back to a more permanent perennial flow (Bertrand et al. 2013).

Tallgrass prairie streams are complex systems, with perennial and non-perennial streams, but many headwaters are classified as intermittent (Dodds et al. 2004). They exhibit variable annual discharge and frequent periods of short-term drought (Stagliano and Whiles 2002). Future impacts of a changing climate for Kansas grasslands include increased frequency of intense rainstorms and periods of prolonged drought that could directly influence drying and flooding regimes (Bertrand et al. 2013). Aquatic organisms within tallgrass prairie streams are more immediately impacted by consequences of human land use, including cattle ranching and alterations to natural streamflow. Much of tallgrass prairie within Kansas is utilized as pasture, with limited ability to be converted to agricultural land, lessening the impact of common anthropogenic disturbances to streams, such as pollution and increased nutrients from agricultural runoff (Dodds et al. 2004). While cattle grazing helps to mimic natural grazing patterns, increased stocking rates and the diversion of water to sustain the number of cattle can cause significant impacts. Cattle with access to streams can degrade banks and increase nutrient inputs. Within the tallgrass prairie, determining the types of streams, number of streams, and which parts of watersheds are essential to sustain natural assemblages of aquatic organisms is important for their future preservation (Dodds et al. 2004).

Aquatic invertebrates have developed complex life history strategies to survive within the variable nature of intermittent streams and are crucial to their ecological functioning. These invertebrates are commonly classified into two main groups, macroinvertebrates that are large enough to be visible to the naked eye, and meiofauna that are small and only identified by microscopic observation (Stubbington et al. 2020). Aquatic invertebrates play a central role within the aquatic trophic structure. Most small invertebrates consume primary producers and detritus, eventually becoming prey for larger bodied taxa including aquatic insects, crayfish, and fishes (Bertrand et al. 2013). Others are filter-feeders, whose feeding strategy benefits aquatic systems through water clarification. Aquatic invertebrates also provide transfer of materials between aquatic and terrestrial systems (Stubbington et al. 2020).

There has been limited study into the aquatic invertebrates of tallgrass prairie streams, with most research conducted at the Konza Biological Field Station in northeastern Kansas (Kansas State University) (Stagliano and Whiles 2002, Larson et al. 2013). Of these, non-insect invertebrate groups remain understudied (Dodds et al. 2004). Expanding the number of study locations that incorporate a diverse assemblage of aquatic invertebrates will contribute to our growing knowledge of prairie streams (Larson et al. 2013). Specifically, determining how invertebrate assemblages with different dispersal strategies are distributed within pools of varying hydrology and their ability to withstand drying in isolated pools can contribute to our understanding how invertebrates utilize these systems and how they respond to expected change in disturbance regimes (Gleason and Rooney 2017).

Study site

Both projects will be conducted at Youngmeyer Ranch, Elk County, Kansas (37.545022, -96.489850) (Figure 1), a 1902 ha (4700 acre) Wichita State University Biological Field Station, owned and managed by the Youngmeyer Trust (Figure 2). The site is located within the Flint Hills (Ecoregion Level IV) consisting of mostly natural tallgrass prairies, with oak-hickory forests along permanent stream reaches (Chapman et al. 2001). The site includes ephemeral, intermittent, and perennial streams as well as natural springs and artificial cattle ponds, allowing for to the exploration of aquatic organisms within a variety of aquatic habitats. The mean annual temperature is 13.7ºC and mean annual precipitation is 979 mm (Houseman et al. 2016). Prescribed burning and cattle grazing have been included in the management regime for the last 20 years. The area is composed of layers of hard limestone, soft shale, and chert (flint) with silty clay loam soils (Houseman et al. 2016). The physical geology of the area lies within the western edge of the Osage Cuestas (physiographic region) (Buchanan 2010), with the west side of the property sloping to the west and the east side consisting of steeper slopes of exposed limestone (escarpments) to the east.

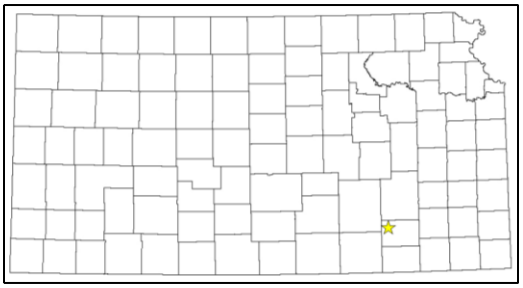

Figure 1. Map of counties within Kansas, USA. Youngmeyer Ranch (star) is located within the northwestern corner of Elk County.

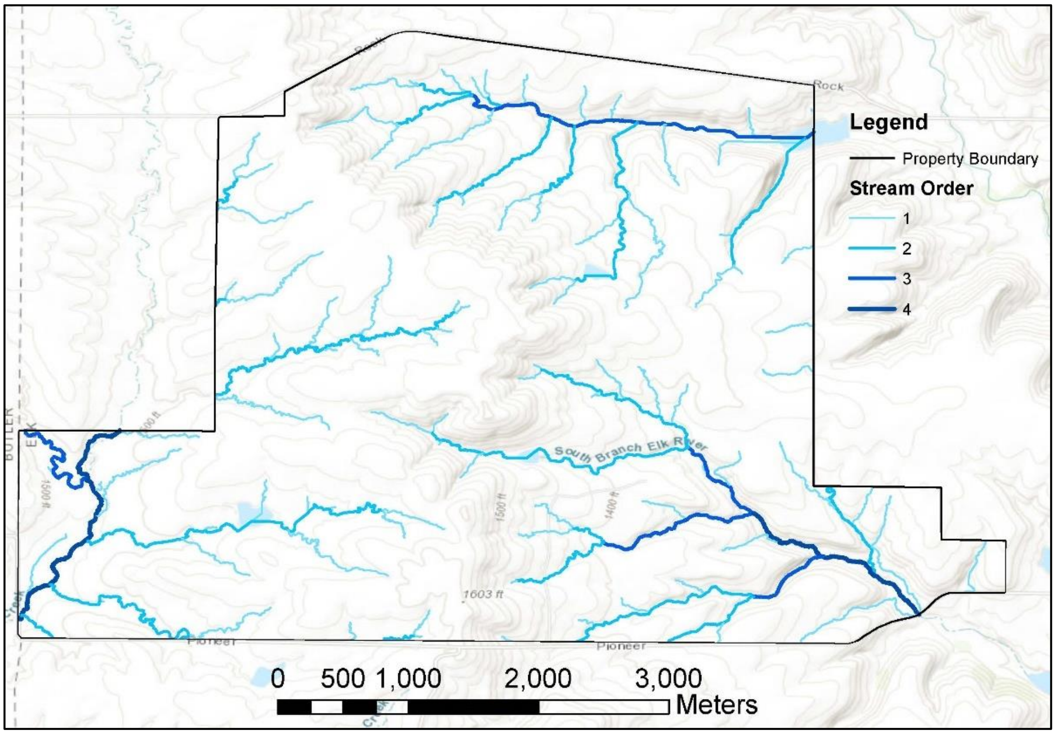

Figure 2. Map of Youngmeyer Ranch, Elk County, KS, showing the property boundary, streams, and elevation. Map created using ArcGIS software by ESRI.

## PROJECT ONE: FACTORS INFLUENCING MACROINVERTEBRATE DISTRUBTION WITHIN TALLGRASS PRAIRIE STREAM POOLS

### Macroinvertebrates

Freshwater macroinvertebrates vary greatly in size, dispersal strategies, and feeding roles. Macroinvertebrates common to tallgrass prairie streams include molluscs (e.g., snails and mussels), crustaceans (e.g., crayfish), and the larval and adult stages of aquatic insects. Snails and crayfish have abilities to survive short periods of stream drying, but must ultimately rely on flowing waters for dispersal (Dodds et al. 2004, Thorp and Covich 2010). Snails consume detritus and algae, scraping these resources off substrates, and are a common component of invertebrate assemblages across a variety freshwater habitat (Thorp and Covich 2010). Crayfish (Crustacea) differ from other macroinvertebrate groups due to their capacity to grow to large body sizes. They are omnivorous and most fishes cannot consume adult crayfishes, allowing them to become dominant macroconsumers. Snail abundance has been proposed to be influenced by predation of crayfish within tallgrass prairie streams (Stagliano and Whiles 2002).

Of all stream invertebrates, insects are one of the most well-studied due to their high abundance and diversity (Thorp and Covich 2010). Reflecting this diversity, aquatic insects serve as important food sources for both invertebrate and vertebrate predators, and are important predators themselves (Thorp and Covich 2010). Aquatic insects expected in tallgrass prairies include beetles (Coleoptera), true bugs (Hemiptera), larval damselflies and dragonflies (Odonata), larval mayflies (Ephemeroptera), and larval flies (Diptera). Aquatic insects were found to contribute 85% to the total community macroinvertebrate production within tallgrass prairie streams, with greatest contributions from larval flies and mayflies (Stagliano and Whiles 2002). Many of these insects have larval stages that exist solely within the water before emerging as terrestrial adults. Most mayflies are considered detritivores or herbivores and are an important food source for stream fishes (Thorp and Covich 2010). Their emergence is usually in large numbers with short adult stages that mate and quickly die. Odonates are important predators within freshwater, consuming other invertebrates and some vertebrates (e.g., tadpoles) (Thorp and Covich 2010). Both dragonflies and damselflies have longer lived adults that are also important predators within terrestrial systems. Macroinvertebrates contribute greatly to the diversity of aquatic organisms within intermittent streams and utilize these systems in different ways to survive their variable nature.

Generally, streams with increased channel width, water permanency, and connectivity offer increased habitat types and more predictable conditions that allows for increased invertebrate abundance and diversity. These generalizations have been shown to hold true even within an initially perennial headwater stream that shifted to an intermittent middle reach (Whiting et al. 2011). Although, this consensus is not concrete evidence that this is holds true for all tallgrass prairie streams. Some studies have shown that intermittent reaches can have higher invertebrate abundance of certain taxa when compared to nearby perennial reaches (Dodds et al. 2004). For example, crayfish have been shown to have higher abundance within isolated pools of intermittent streams when compared to nearby perennial reaches (Flinders and Magoulick 2003). Potential impacts of climate change could alter the normal disturbance regime of these systems by increasing connectivity to upstream reaches and creating longer periods with more isolated pools that could alter invertebrate assemblages. The creation of impoundments for cattle and fishing ponds alter the natural flow and function of a stream reach, with the added effects of cattle and fishes on invertebrate communities. With limited understanding of these systems, and their multiple potential impacts, exploration of the requirements needed for a variety of aquatic organism to utilize these systems is necessary for their ultimate preservation.

Within intermittent streams, large numbers of pools with and without flow can become important refuge for aquatic organisms. Most studies of stream macroinvertebrates sample riffles rather than pool habitats (Stagliano and Whiles 2002). Riffles are commonly sampled because they have been shown to have higher diversity, but more importantly what are deemed pollution intolerant “EPT” taxa (combined richness of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Tricoptera)) that allow for the assessment of water quality (Thorp and Covich 2010). These EPT taxa have been shown to have low abundance in headwater streams of tallgrass prairies (Fritz and Dodds 2004). The collection of large macroconsumers is underestimated when collections are made solely from riffle habitats. Moreover, isolated pools can exist as permanent refuge for many aquatic organisms within intermittent streams. Studies that incorporate the collection of macroinvertebrates within stream pools may provide a more realistic component of their distribution within intermittent streams that have limited connectivity.

The small sample size of most invertebrate collections limits the incorporation of large crayfish and their contribution to invertebrate assemblages has been likely been underestimated within tallgrass prairie streams (Stagliano and Whiles 2002, Whiting et al. 2011). A larger constrained volume search, like trashcan enclosures, would help to aid in collection of large macroinvertebrates, allows for calculation of the total volume of water sampled, and the simultaneous capture of amphibians and fishes. When collecting larger samples sizes for the simultaneous capture of invertebrates, amphibians, and fishes, it is expected that comparatively invertebrates would have a higher average abundance across all pools. For aquatic invertebrates collected, there would be a tradeoff between allowing for a larger sample size and the inclusion of larger organisms, and factors that would be incorporated within many invertebrate studies, including measuring the size of each individual and preservation of all invertebrates collected to be identified in the lab, rather than in the field.

## Factors that influence macroinvertebrate distribution

Within intermittent streams, physical factors have been shown to be more important in structuring invertebrate assemblages than biological factors (Bertrand et al. 2013). Physical factors that could influence the distribution of aquatic invertebrates include water permanency, connectivity to permanent refuge, and streambed composition. Biological factors of influence the distribution include instream or riparian vegetation and predatory fishes.

### *Physical factors*

The length of the hydroperiod, the duration of surface water, of stream pools can determine the composition of aquatic invertebrate communities (Rolls et al. 2018). Hydroperiod is expected to be a function of pool volume and depth, as pool size increases, so does the potential to hold water over time. Streams with high water permanency are expected have higher invertebrate abundance and diversity (Dodds et al. 2004). A decrease in pool hydroperiod and volume would limit the amount of available space and resources, increasing interactions like predation and competition (Majdi et al. 2020).

Stream pools that are connected by flowing water or are more connected to perennial refuge allow for greater dispersal of many macroinvertebrates (Majdi et al. 2020). Dispersal strategies for most macroinvertebrates include moving to more permanent refuge or emerging as adults (aquatic insects). Concerning potential impacts of climate change, an increase in intense storms could cause flash floods that increase the (longitudinal) connectivity to upper reaches of the stream, allowing for rapid colonization by larger macroinvertebrates and fishes. Once colonized, these taxa can persist in isolated pools. Longer periods of drought decrease the connectivity to permanent refuge and increase the number of isolated pools (Larned et al. 2010).

The natural connectivity of stream pools can be disrupted through anthropogenic disturbance. The diversion of water for the creation of cattle ponds or fishing ponds influence aquatic invertebrates by disrupting natural flow patterns and creating large, unnatural bodies of water (Dodds et al. 2004). Fishing ponds that are created for recreational use and can have the added negative effect of being stocked with sport fishes. Creating unnatural, permanent refuge along a stream reach could increase the number of large predators able to colonize upstream pools. Studies incorporating multiple headwater streams with and without impoundments could expand on our knowledge of these disturbances in influencing aquatic invertebrate abundance.

### *Biological factors*

Fishes are an important component of intermittent streams and can influence the distribution of other aquatic organisms. They are a major predator of aquatic invertebrates and have been shown to directly influence their distribution and abundance. For example, *Physa* snail use of open habitats decrease when sunfish are present, having been reported to hide within refugia and only leaving due to starvation (Thorp and Covich 2010). It has been suggested that predatory fishes can congregate in high abundance within pools during low flow periods, resulting in decreased invertebrate biomass and production (Whiting et al. 2011). Studies that incorporate simultaneous capture of invertebrates and fishes within intermittent stream pools could help to better determine the effects of these large predators on invertebrate distribution (Biggs et al. 2017).

## Specific aims

The specific aim of this project is to determine abiotic and biotic factors that influence the distribution of aquatic macroinvertebrates within intermittent stream pools of the tallgrass prairie. A field study will be conducted to test the hypotheses: 1) aquatic macroinvertebrate richness and abundance (density) increases with increasing pool hydroperiod, size, and connectivity to permanent refuge and 2) aquatic macroinvertebrate richness and abundance (density) decreases with fish presence (Figure 3). The influence of these factors on macroinvertebrate groups with differing dispersal abilities will also be assessed. Specifically, how these factors influence the distribution of snails, crayfish, mayflies, and odonates will be analyzed.

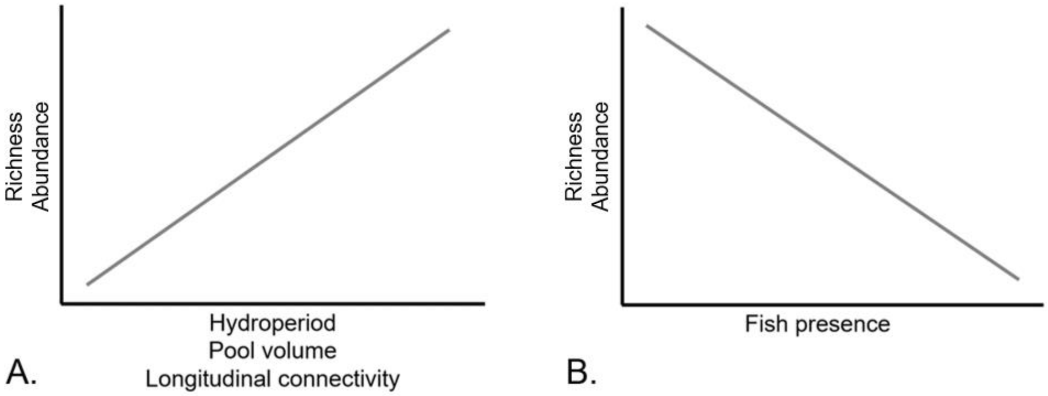

Figure 3. Hypotheses predictions for macroinvertebrate richness and abundance across (3A) pool hydroperiod, volume, and connectivity, and (3B) fish presence.

## Methods

### *Sample streams and pools selection*

All sample streams and pools were initally mapped using Google Earth Pro, utilized for its accessibility. Undergraduate students within the Aquatic Ecology lab (Spring 2020) were assigned weekly tutorials to assist in mapping sample sites. All streams across the study site were mapped in increments of 100 m. Seven headwater streams were then selected for sampling. These sample streams varied in water permanency, connectivity to permanent refuge, and the presence or absence of cattle ponds. All pools were then mapped for each 100 m segment. For each segment, two of the mapped pools were randomly selected for field sampling. A total of 153 pools were ultimately selected, of which 118 held water at the time of sampling (Figure 4).

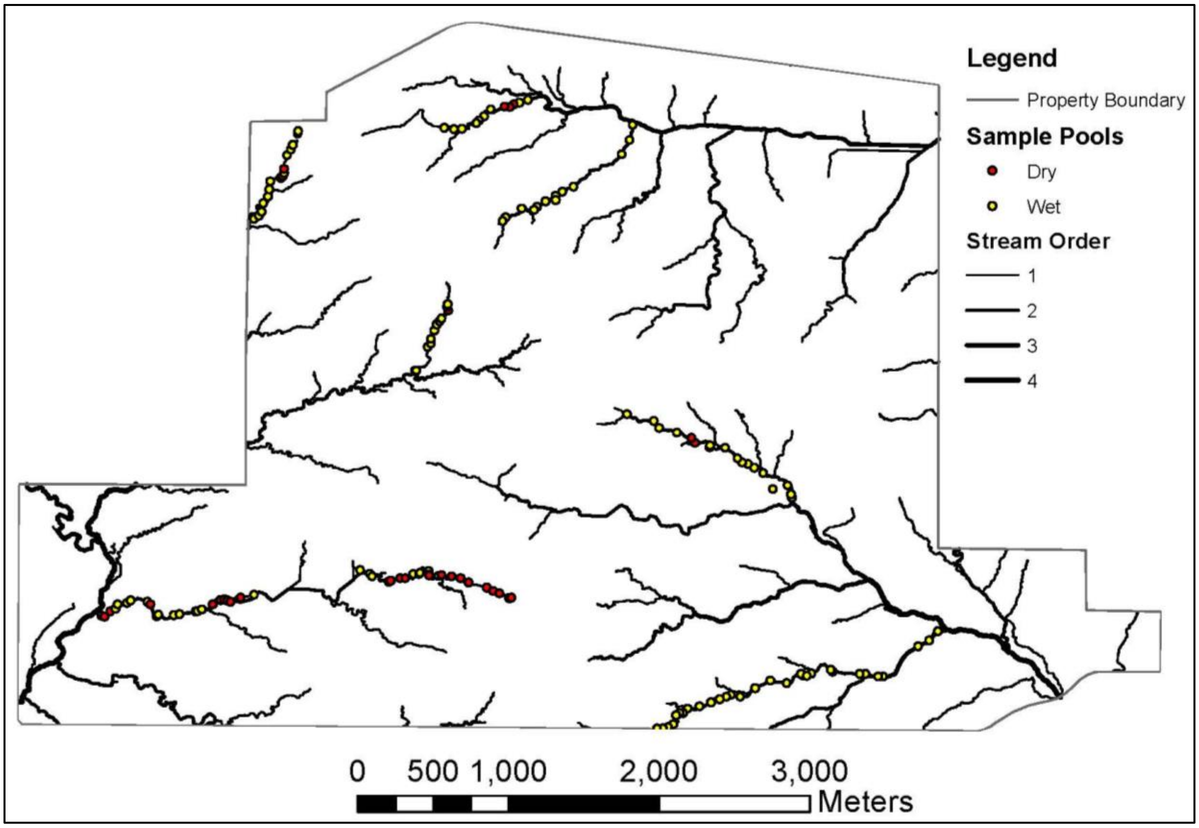

Figure 4. Youngmeyer Ranch, Elk County, KS. Sample pools are marked by whether they held water at the time of sampling (yellow circles) or were dry (red circles). Map created using ArcGIS software by ESRI.

*Sample collection*

A constrained volume search was conducted to collect study organisms simultaneously and allowed for the calculation of total pool volume sampled (May – June 2020). Trashcan enclosures were used, consisting of a plastic trashcan (51 x 51 x 65 cm) with the bottom removed, to conduct the constrained volume search. A transect is first placed along the length of the right bank of the pool from downstream to upstream and total length recorded. For pools greater than 14 m, enclosures are placed and sampled every 2 m along the transect. For pools less than 5 m, enclosures are placed and sampled every 1 m. Placement of the first enclosure is randomly selected to start within the middle or near either bank of the pool, and followed this pattern for the remaining placements (e.g., first enclosure placed in middle, second left, third right, fourth middle).

For each placement, a net (0.3 cm mesh) is used to sweep the contents of the trashcan to collect all organisms trapped within. After one sweep throughout the entirety of the enclosure, we remove all macroinvertebrates, amphibians, and fishes from the net and separate them into small pitchers and five-gallon buckets. Sweeps continue following this process and discontinue when three consecutive sweeps collect no organisms. We then measured and recorded the depth of the water within each enclosure to calculate the volume of water sampled. The entire process is then repeated for the next sampling point along the transect. Invertebrates collected were counted and identified in the field. All crayfish were categorized as less than or greater than 5 cm. As needed, representative specimens were collected and preserved in 75% ethanol for further identification (Merritt et al. 2008, Thorp and Covich 2010).

### *Pool volume*

To calculate total volume of each pool, we collected volume measurements at the time of sampling. A transect is placed through the middle of the stream pool, from downstream to upstream, and total length recorded. Measurements were taken every 1-2 m along this transect, dependent on pool length, starting and ending 0.25 m from the beginning and end of the pool. At each point along the transect, we measure the total width of the pool, the width from left wetted bank to the deepest point, and the depth of the deepest point. Two successive width and depth measurements are taken to the right and left mid-point from the deepest point to each wetted bank.

### Site characteristics

Site characteristics are collected for each pool at the time of sampling to determine potential factors of influence. This includes current weather conditions, bank and in-stream vegetation, in-stream sediment composition, in- and outflow, and canopy cover.

### Data Analysis

#### Hydroperiod

Hydroperiod indices ($H_i$), see Equation 1, were calculated from satellite images of 19 randomly selected stream reaches for a total 318 of randomly selected pools using Google Earth Pro. For each pool, the total number of observations ($O_i$) was determined using four years of satellite imagery (10/17/2010, 2/28/2012, 2/16/2013, and 11/5/2014) to then estimate the total number of observations with water present ($P_i$).

$$H_i = P_i / O_i \tag{1}$$

#### Connectivity

Total stream wetted length to permanent refuge for each sample pool was measured using Google Earth Pro.

#### Statistical analysis

Dependent (response) variables include taxa richness and abundance (density), as well as these measurements for determined select taxa groups. Independent (predictor) variables include hydroperiod, pool volume, maximum depth, connectedness to permanent refuge, substrate composition, and fish presence. Model comparisons will be conducted for the determined predictor and response variables. Based on Akaike Information Criterion (AIC), model selection will determine the best model for each response variable. These comparisons will then be conducted for predetermined macroinvertebrate groups, differing in dispersal and life-history characteristics, to further evaluate macroinvertebrate utilization of intermittent stream pools.

## PROJECT TWO: RESPONSE OF MEIOFAUNA OF ISOLATED TALLGRASS PRAIRIE STREAM POOLS TO EXPERIMENTAL DRYING

### Meiofauna

Aquatic invertebrates have adapted to historical hydrology regimes through various dispersal and life history strategies (Hay et al. 2018). Macroinvertebrates rely heavily on aerial dispersal or physical movement to escape stream drying. Common small invertebrates (meiofauna), including microscopic worms, rotifers, and microcrustaceans, have developed life history strategies that make them able to withstand drying and immediately recolonize after drying events. Meiofauna are an often-overlooked aspect of invertebrate studies, especially for freshwater habitats (Majdi et al. 2020). Expected meiofauna of intermittent streams within the tallgrass prairie include nematodes (Nematoda), microturbellarians (Platyhelminthes), oligochaetes (Annelida), rotifers (Rotifera), and microcrustaceans (Crustacea: Ostracoda, Branchiopoda, Copepoda). More studies are needed to determine if their resistant abilities facilitate recolonization and re-establishment of trophic connections after drying and refilling of isolated pools (Majdi et al. 2020).

Resistant traits are those that allow individuals to resist drying (Dodds et al. 2004, Strachan et al. 2015). The production of resisting eggs or cysts is a common trait of meiofauna (Strachan et al. 2015). Many crustaceans, including the more explored cladocerans, are well-known for the development of resistant eggs. Other taxa have abilities to hibernate or aestivate for short periods of time in constructed resistant structures (Strachan et al. 2015). Some respond to the onset of disturbance through shifts in their reproduction and development (Verberk et al. 2008, Strachan et al. 2015). Nematodes can produce a resistant juvenile stage with a modified cuticle to resist drying (Strachan et al. 2015). Meiofauna, can also enter resistant stages at different periods of their life cycle (Strachan et al. 2015). When species can resist drying at an immature, juvenile, or adult stage, they could potentially have an advantage over other species when water returns (Horne 1993, Strachan et al. 2015). Species can have multiple traits and strategies employed at a time or at different stages of life that allow for them to better cope with variation in drying regimes (Strachan et al. 2015).

The abilities of many meiofaunal groups to survive drying has been well documented throughout the history aquatic ecology, but were not considered of great importance to the functioning and recolonization of aquatic systems (Stanley et al. 1994, Fritz and Dodds 2004, Chester and Robson 2011, Strachan et al. 2015, Hay et al. 2018). Dry sediments of intermittent aquatic habitats had mostly been deemed “biologically inactive” (Steward et al. 2012, Strachan et al. 2015). This has also been stated for meiofauna within tallgrass prairie streams (Fritz and Dodds 2002, Dodds et al. 2004). Recent studies have contrasted with this historical view, finding dry sediments to be a significant refuge with diverse invertebrate assemblages (Hay et al. 2018).

In a changing climate, it expected that increased drying times will cause a loss in connectivity to permanent refuge and an increase in the number of isolated pools. If these pools lose all surface water for long periods of time, then once refilled resistant taxa can quickly recolonize and aid in the reestablishment of ecological processes (Gaudes et al. 2010, Majdi et al. 2020). It is also reasonable to assume, as drought increases, species that are able to cope with these extremes may have the potential to become dominant with their major predators unable to survive and recolonize due to the harsh conditions (Altermatt et al. 2009, Strachan et al. 2015). Prolonged drought may limit the survival and colonization of isolated pools by actively dispersing macroinvertebrates.  Short spates of increased flow from heavy rains may not create the conditions that would usually enable large macroconsumers to move upstream. Decreased lateral connectivity may decrease the number of aerial dispersals that would be potential first consumers of primary producers and meiofauna. Because of their potential to rapidly recolonize after drying disturbance, meiofauna may play an important role in restoring aquatic food webs and “kick-starting” ecosystem functions (Gaudes et al. 2010, Majdi et al. 2020).

While it is clear many meiofauna taxa can permit after drying, less is known about how different drying durations affect their ability to recolonize isolated pools (Robson et al. 2011, Strachan et al. 2015, Hay et al. 2018, Vargas et al. 2019). Tests of the flexibility of strategies used to survive desiccation in different environmental conditions have been limited (Strachan et al. 2015). It has been suggested that experimental manipulations (e.g., rehydration of sediment cores) would allow for the evaluation of the role of meiofauna groups (Hay et al. 2018, Majdi et al. 2020).

Determining how these communities respond to drying disturbance within intermittent tallgrass prairie streams will help aid in preserving their biodiversity (Boersma et al. 2014).

### Rehydration experiments

Many studies concerned with the recolonization of meiofauna after dying disturbance utilize sediment rehydration and specifically focus on the role of the resistant egg bank. Most microcosm rehydration experiments are conducted on wetlands and lakes. Those that focus on streams or rivers have been conducted in dry or arid landscapes within the southwestern USA (Simovich and Hathaway 1997), Australia (Hay et al. 2018), and the Mediterranean (Majdi et al. 2020). Many of these systems have similar conditions to tallgrass prairie streams, including short hydroperiods, low connectivity, and limited riparian resources. Microcosms have been shown to be limited in their abilities to replicate the complexity of natural systems (Schindler 1998), but have been commonly used for rehydration experiments and are reported to reasonably approximate the functioning of inundated riverbeds (Jenkins and Boulton 2003, Jenkins and Boulton 2007, Hay et al. 2018). It was therefore important to incorporate as many natural processes as possible and limit the amount of initial manipulation, to specifically focus on our questions concerning meiofauna and their response to drying.

Experimental drying is difficult to study in the field setting (Jenkins and Boulton 2003, Hay et al. 2018) and most collect soil when the stream is dry for experimental rehydration (Stubbington et al. 2016, Maijdi et al. 2020). Sediments are usually collected in the field from the top layers of soil, homogenized, and divided across microcosms

(Sternert et al. 2017). Few collect both water and sediment to allow for the assessment of the invertebrate assemblage before sediments dry. Even less collect the intact sediment core for each microcosm that would represent the true sediment structure.

Experimental rehydration studies vary greatly in their design. Microcosms have been housed in the lab under 12 h light, 12 h dark cycles (Sternert et al. 2017), within a temperature-controlled greenhouse (Hay et al. 2018), or outside (Stubbington et al. 2016). When placed outside, many utilize a shade cloth or mesh net as a cover to avoid contamination (Diez-Brantley et al. 2002, Boersma et al. 2014). These covers limit the amount of ecological realism by limiting light penetration, dispersal or colonization, and other inputs, but ultimately allows for some control of these influencing variables (Boersma et al. 2014).

Water used for rehydration includes filtered groundwater (Anderson and Smith 2004), well water (Bright and Bergey 2015). distiller water (Freiry et al. 2016), declorinated tap water (Stubbington et al. 2016), and deionized water (Hay et al. 2018). After rehydration treatments, the process for sampling invertebrates from microcosms have been conducted only once after a period of rehydration (Bright and Bergey 2015), a couple times a week for several weeks (Freiry et al. 2016), or on a scheme from 0, 7, 14 and 28+ days after rehydration (Ávila et al. 2015, Stubbington et al. 2016, Freiry et al. 2016, Sternert et al. 2017, Hays et al. 2018). It has been reported that after 30 days, there is an increase in algal growth and deteriorating water quality.

Collection of samples from microcosms have been completed through the use of small nets (Freiry et al. 2016, Sternert et al. 2017) to siphoning all surface water through a sieve (Hay et al. 2018). Samples have been immediately placed in ethanol or observed live. Preservation allows for a wait time before sample must be assessed, but many small invertebrates are not easily identifiable after preservation (Stubbington et al. 2016). While live samples must be immediately analyzed, more species can be recorded and can be more readily identified (Stubbington et al. 2016). Some then return all collected samples to microcosms after identification (Boulton and Lloyd 1992, Hay et al. 2018), while others discard samples. Other manipulations of the sample population, like initially removing large macroinvertebrates from microcosms, is conducted in order to decrease predation of study organisms (Stubbington et al. 2016). Microcosms are considered a closed system with increased biotic interactions, like predation, that may limit their ability to mimic natural processes (Boulton and Lloyd 1992, Hay et al. 2018). The experimental design will ultimately differ based on the research questions asked, but provide a means for the assessment of experimental drying and rehydration of meiofauna communities (Hay et al. 2018).

### Specific aims

The aim of this project is to determine the meiofauna assemblages and their abilities to recolonize after pool drying within isolated pools of intermittent tallgrass prairie stream. An experimental microcosm study will be conducted to test the hypotheses: (1) meiofauna richness and abundance (density) will be lower in drying treatments after refill than the fill treatment, and (2) meiofauna richness and abundance (density) will be lower after the longer drying treatment than the shorter drying treatment (Figure 5). Specifically, meiofauna groups with differing resistant traits and strategies to survive drying will be assessed, including nematodes, rotifers, and three microcrustacean groups, ostracods, cladocerans, and copepods.

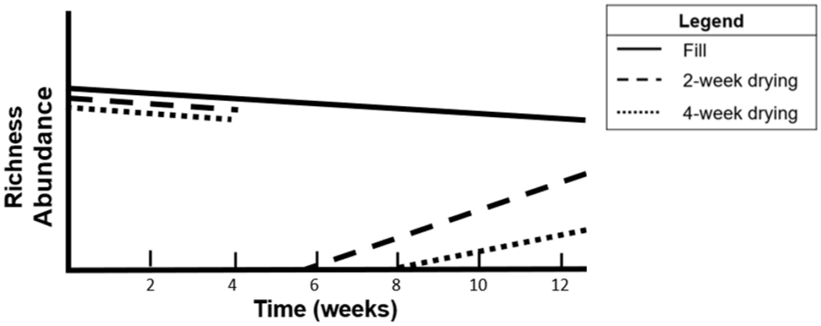

Figure 5. Hypotheses predictions for changes in total meiofauna taxa richness and abundance (density) over time for each treatment (fill, 2-week drying, 4-week drying).

## Methods

### *Sample streams and pools selection*

Ten isolated stream pools from four stream segments were randomly selected from Youngmeyer Ranch (Figure 6). Specifically, only pools holding water that consisted of sediments that allowed for the collection with a sediment corer were included.

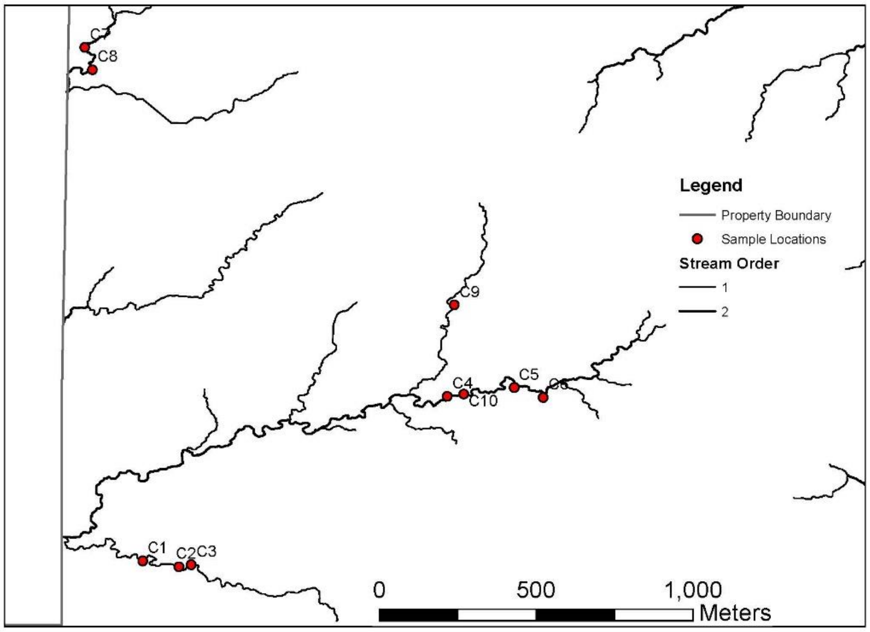

Figure 6. Map of the northwestern corner of Youngmeyer Ranch, Elk County, Kansas. Ten sample pools (red circles) are marked by their sample name. Map created using ArcGIS software by ESRI.

### *Microcosms*

A soil sleeve (30.48 cm tall, 7.68 cm diameter), consisting of a plastic cylinder that is open on both ends, is used to collect the contents of the microcosm in the field (August 2020) (Figure 7). The sleeve is pushed through the water and sediment column, until 10 cm of sediment core is collected, or bedrock is hit. Three cores per pool are collected, each randomly selected to be collected from the middle, right of left side of the pool. This provided the random selection of habitat characteristics of each pool. Site characteristics and pool volume were collected as described for Project 1.

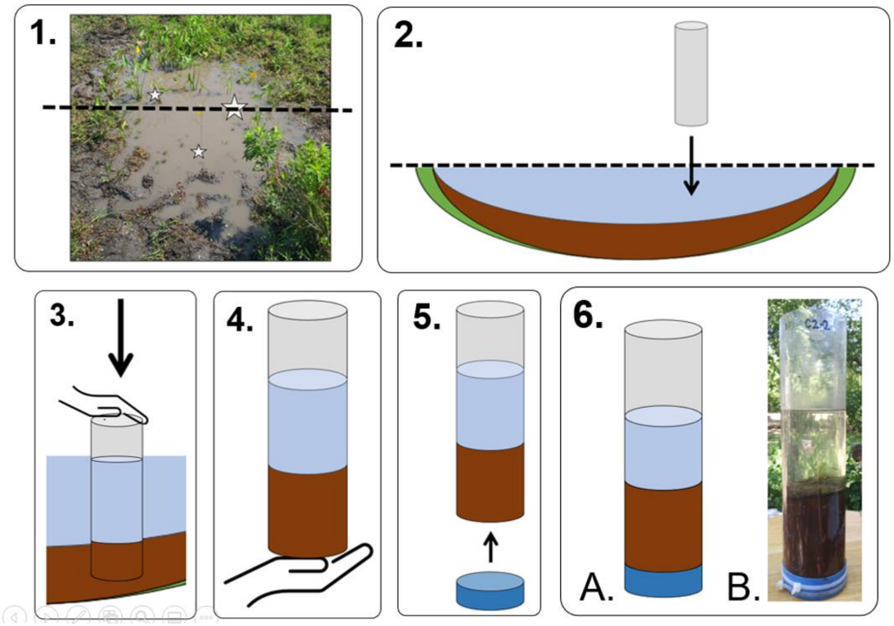

Figure 7. Microcosm field collection steps. 1. Determine sample points. Three sample points (white stars) are shown within the sample pool. 2. Hold sleeve above water, perpendicular to surface above sample point. Showing cross-section above pool. 3. Push sleeve through water and sediment column. 4. Remove sleeve. 5. Place cap on bottom of sleeve. 6. Complete microcosm. 6A. Model image of microcosm. 6B. Photo of microcosm.

After collection, microcosms are housed outside at an off-site “facility” (my backyard, Wichita, KS, 67203) (August – October 2020). Microcosms will therefore experience similar seasonal temperatures and photoperiod to the study site. The microcosms are placed within aboveground crates and confined by wooden stakes to hold them in place. After each sampling period, microcosms will be rotated (”snaked”) throughout the structure to help control for any variation in light penetration. A fine mesh net will cover each microcosm to limit any aerial contamination. The crates are covered with a tarp that does not seal the microcosms but allows for protection during precipitation events. This cover will also be used at night to discourage animal mischief (racoons).

### *Experimental treatments*

The three microcosms collected from each pool are randomly assigned one of three treatments: (1) a fill treatment, with microcosms receiving water additions throughout the duration of the study and not allowed to dry (fill-treatment), (2) allowed to naturally dry with a complete drying duration of two-weeks before refill (2-week dry treatment), and (3) allowed to naturally dry with a complete drying duration of four-weeks before refill (4-week dry treatment). The drying treatments were started once all surface water naturally dried. Water used for refill will consist of collected pool water from the study site that is filtered and autoclaved. This type of water was used to retain some of the natural properties, rather than using treated water or relying on rainwater collection. Dry treatments were eventually refilled with 100 ml of water and 50 ml on subsequent days as needed. Water was added to fill treatments throughout the study as needed.

### *Sample collection*

Water and a small amount of the surface sediment is collected from each microcosm three times a week (Monday, Wednesday, and Friday). For each sample, 10 ml of water and sediment is collected by pipette, excluding those undergoing drying treatments. 5 ml of the collected sample is then microscopically analyzed. All invertebrates are counted, identified (Thorp and Covich 2010), and the body size of microcrustaceans is measured. Collected samples are not added back to the microcosm. Characteristics and changes of each microcosm is recorded during sample collection, including sediment depth, water depth, and algae/macrophyte presence. Measured water depth of each microcosms before sample collection will allow for the calculation of volume sampled.

### *Statistical Analysis*

The independent (treatment) variables are the three treatments: fill, 2-week drying, and 4-week drying. The dependent (response) variables include taxa richness and abundance (density). Assurance that the data meet the test assumptions and required transformations will be performed before analysis. Analysis of variance (ANOVA) will be conducted to initially determine any differences between treatments or pools. Further analysis on differences before and after drying on specific taxa groups collected will be performed, with the possible inclusion of body size and life-stage changes for the three microcrustacean groups. The possible analysis on an interval scale of days before or after drying (e.g., 3, 6, 9, etc. days before and after refill) would provide equal sample sizes for comparisons and any significant results could provide insight into the immediate recolonization of isolated pools. The ultimate analysis will be dependent on the number of samples before and after drying completed, which is controlled by the eventual seasonal shift to winter. Freezing temperatures will freeze the water within the microcosms, rendering them unable to be sampled.

# TIMELINE

| **Project** | **Task** | **Semester** | **Year** | **Completed** |
|---|---|---|---|---|
| **General** | Prospectus defense | Fall | 2020 | |
| | Presentation for Biology Seminar 1 | Fall | 2020 | |
| | Presentation for Biology Seminar 2 | Spring | 2021 | |
| | Target completion date of thesis and defense | Spring | 2021 | |
| **Project 1** | Define question and analysis | Spring | 2020 | ☒ |
| | Finalize materials and methods | Summer | 2020 | ☒ |
| | Field sampling | Summer | 2020 | ☒ |
| | Data entry | Fall | 2020 | |
| | Analysis | Spring | 2021 | |
| **Project 2** | Define question and analysis | Spring | 2020 | ☒ |
| | Finalize materials and methods | Summer | 2020 | ☒ |
| | Microcosm setup | Fall | 2020 | ☒ |
| | Field sampling | Fall | 2020 | ☒ |
| | Microcosm treatments | Fall | 2020 | ☒ |
| | Data collection | Fall | 2020 | ☒ |
| | Data entry | Fall | 2020 | |
| | Analysis | Spring | 2021 | |

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