Downstream Transport of Zebra Mussel (*Dreissena polymorpha*) Environmental DNA and its Implications for Analysis

by

Ashley P. Whitehead, B.S.

A Thesis

In

Wildlife, Aquatic, and Wildlands Science and Management

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

Approved

Matthew A. Barnes, Ph.D.
Chair of Committee

Scott F. Collins, Ph.D.

Nathaniel Hall, Ph.D.

Mark Sheridan, Ph.D.
Dean of the Graduate School

May 2023

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Matthew Barnes, for providing this opportunity and for his support and guidance during these past two years at Texas Tech. I would also like to thank Dr. Scott Collins and Dr. Nathaniel Hall for serving on my committee and providing valuable insight on this project. A huge thank you to U.S. Fish and Wildlife Services for providing funding for my research.

A special thank you to my wonderful lab partner, Kaitlin Plate, for allowing me to drag her to multiple highway and road overpasses and sharing the struggles of navigating fieldwork. A big thank you to the Barnes lab members, both past and present, for their unwavering support and harsh constructive criticism, I would never have made it this far without them: Elif Can, Hayden Hays, Mark Johnson, Kristin Kabat, Madelyn Knauss, Elizabeth Roesler, and Sadie Roth.

I would like to thank my amazing parents and my wonderful sisters for the encouragement and support they have provided during my time at Texas Tech. Lastly, I would like to thank my partner, Cristian Gallardo, for his endless support and kind words as I navigated the world of graduate school and research.

TABLE OF CONTENTS

ABSTRACT

Collection and analysis of environmental DNA (eDNA; genetic material that organisms shed into their environment such as sloughed cells and other wastes) enables detection of organisms without direct observation, promoting earlier detection and more rapid response than conventional sampling methods. Although eDNA analysis has been applied extensively in lentic systems, there is a limited understanding of the ecology of eDNA in lotic systems. For example, flowing water may confound the relationship between eDNA concentration and target species biomass by influencing eDNA degradation, dilution, and resuspension. I aimed to quantify the effects of downstream transport on eDNA concentration, using invasive zebra mussels (*Dreissena polymorpha*) in Texas reservoirs as a case study. My objectives were 1) define the rate of eDNA decline during downstream transport and 2) evaluate how abiotic factors of the river affect eDNA concentration during transit downstream. I sampled eDNA at five sites at varying distances downstream from six zebra mussel "infested" Texas reservoir and one zebra mussel "eradicated" reservoir and used quantitative PCR to measure zebra mussel eDNA concentration. I also collected environmental parameters at each site, including water temperature (°C), turbidity (NTU), and specific conductance (µS/cm). Zebra mussel eDNA concentration varied between sites of the same lake and between different lakes. Two lakes significantly decreased in zebra mussel eDNA with increasing downstream distance whereas one lake significantly increased in zebra mussel eDNA. The "eradicated" lake had positive detection of zebra mussel eDNA. I also found that none of the abiotic factors significantly affected eDNA quantity while moving downstream, contrary to the literature. Understanding the dynamics of eDNA and flowing water will further enable the ability to accurately locate the source of organisms, including invasive species, in lotic systems.

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. INTRODUCTION

The advancement of genetic detection methods has benefitted conservation efforts by combating the decline in biodiversity and increased invasions from non-indigenous species through more sensitive and rapid detection. These advancements provide a way to enhance the evaluation of previously challenging-to-obtain biodiversity data (i.e., difficult study taxa due to low site fidelity or elusive and cryptic behaviors, or difficult sample location), making them crucial tools for conservation (Stewart 2019). One method of genetic detection, environmental DNA (eDNA) analysis, refers to the collection and analysis of genetic material (e.g., scales, dead cells, mucous) shed by organisms in bulk environmental samples such as water, soil, and even air (Barnes and Turner 2016, Johnson et al. 2019). Collection and analysis of eDNA enables detection of species without direct observation, promoting early detection and rapid response relative to conventional methods, aiding the advancement in conservation efforts and management (Beng and Corlett 2020). With its non-invasive approach, low cost, and high sensitivity (Beng and Corlett 2020), eDNA analysis has been widely used in detecting an array of rare, endangered, and invasive species including amphibians (Barata et al. 2021, Eiler et al. 2018, Secondi et al. 2016, Spear et al. 2015), fish (Klymus et al. 2015, Laramie et al. 2015, Robson et al. 2016), and invertebrates such as mussels, crayfish, and snails (Goldberg et al. 2013, Ikeda et al. 2016, Larson et al. 2017, Stoeckle et al. 2016, Xia et al. 2017, Yip et al. 2021).

Before being collected, numerous interactions between eDNA and its surrounding environment affect its origin, state, transport, and fate (i.e., the ecology of eDNA), influencing the inferences made on the results (Barnes and Turner 2016, Barnes et al. 2021). For example, certain factors can influence the amount of genetic material shed from an organism (i.e., origin). In bluegill sunfish (*Lepomis macrochirus* Rafinesque, 1810), juveniles shed more eDNA than adults per unit biomass, leading the authors to conclude changes in behavior and metabolism impact the rate of eDNA production (Maruyama et al. 2014). To provide important context for its collection and interpretation, the state of eDNA also needs to be considered. Turner et al. (2014) examined the particle size distribution of common carp (*Cyprinus carpio* Linnaeus, 1758) eDNA in small lakes and ponds using serial filtration of water samples. Though common carp was collected in all size fractions, the largest percentage of total common carp eDNA collected

was in the 1-10 µm size fraction. Likewise, the downstream transport of eDNA in lotic systems could be detected several hundred meters downstream or up to 20 kilometers downstream (Table 1.1), which could lead to challenges relating detecting eDNA to species presence in both space and time (Barnes and Turner 2016). Generally, when an organism sheds genetic material, it begins to degrade (i.e., rate) The degradation rate of eDNA is influenced by various factors including temperature, UV radiation, and microbial activity, and varies from being preserved for thousands and even millions of years (Anderson-Carpenter et al. 2011, Pedersen et al. 2013, Kjaer et al. 2022) to one to multiple days until it is no longer detectable (Barnes et al. 2014, Pilliod et al. 2014, Strickler et al. 2015, Seymour et al. 2018). Similar to understanding transport, better understanding of the fate of eDNA is a vital factor in identifying an organism's temporal and spatial proximity (Barnes and Turner 2016).

The analysis of eDNA has been highly successful in aquatic settings, with extensive research done in freshwater ecosystems. However, few studies have examined how eDNA is affected within lotic systems (i.e., rivers and streams) during downstream transport (Table 1). Rivers and streams are dynamic and complex systems with rapid turbid flowing water connected to slower calmer flowing water, which influences how materials are transported downstream (Newbold et al. 2005). Hence, the flow of a river can cause materials in the water to shift from the main flow to getting trapped in sediment (suspended or benthic) or biofilm (Battin et al. 2003, Newbold et al. 2005), or be biologically removed (Cushing et al. 1993). For example, organic matter is a largely studied component of stream ecology due to its influence of downstream consumer community structure and food web (Vannote et al. 1980, Minshall et al. 1983). The use of tracers has demonstrated that organic matter is continuously and rapidly being deposited into the streambed, preventing its use further downstream (Cushing et al. 1993, Minshall et al. 2000). Similarly, eDNA is not exempt from such a fate, however, the dynamic of eDNA in flowing water is not as widely researched. The complexity of lotic systems makes it difficult to study eDNA concentration as it moves downstream, possibly creating the main cause of rivers and other flowing waters to be understudied. Therefore, it is vital to comprehend the mechanics of eDNA in flowing water to enable its usage effectively in lotic systems.

Downstream transport is likely to affect the rate of degradation, dilution, and resuspension of eDNA, which can ultimately affect the detection of target species and

consequently, the inferences of results of eDNA-based study and conservation (Barnes and Turner 2016). To date, studies have demonstrated that eDNA can travel long distances downstream (Table 1.1), however, these studies are limited to utilizing eDNA to confirm presence or absence of a target species residing somewhere in the river system. Although prior research has proposed mechanisms for eDNA transit, so far only weak correlations between population density and downstream eDNA quantity have been observed (Jane et al. 2015, Spear et al. 2015). By learning more about how various downstream mechanisms impact eDNA quantity, researchers and managers may more accurately determine population abundance of a rare or invasive species and locate target species' general location to better implement conservation regulation.

Table 1.1 Studies demonstrating eDNA travel distance in lotic systems.

Author	Location	Taxa	Furthest eDNA Distance Detected	Notes
Balasingham et al. (2017)	Little River, Ontario, Canada	Atlantic salmon (*Salmo salar* Linnaeus, 1758)	0.96 km	Target species eDNA introduced at specific rate to system via water from barrels
Deiner & Altermatt (2014)	Glatt River, Zurich, Switzerland	Water flea (*Daphnia longispina* Müller, 1776) Swollen river mussel (*Unio tumidus* Retzius, 1788)	12.3 km 9.1 km	Target species reside in lake upstream from sample sites

Table 1.1 Continued

Jane et al. (2015)	Avery Brook, Massachusetts, USA Amethyst Brook, Massachusetts, USA	Brook trout (*Salvelinus fontinalis* Mitchill, 1814)	0.24 km	Target species caged and introduced to system
Pilliod et al. (2013)	Deadwood River, Idaho, USA	Idaho giant salamder (*Dicamptodon aterrimus* Cope, 1867)	0.05 km	Target species caged and introduced to system
Villacorta-Rath et al. (2021)	Wet Tropics of Queensland, Australia	Armoured mistfrog (*Litoria lorica* Davies and McDonald, 1979) Waterfall frog (*Litoria nannotis* Andersson, 1916)	23 km	Target species reside in stream
Wacker et al. (2019)	River Drakstelva, Trøndelag County, Norway	Freshwater pearl mussel (*Margaritifera margaritifera* Linnaeus, 1758)	1.7 km	Target species introduced to system
Wood et al. (2020)	Sunkhaze Stream, Maine, USA	Atlantic salmon (*Salmo salar* Linnaeus, 1758)	0.2 km	Target species caged and introduced to system

The detectability of eDNA is affected by water temperature and turbidity. Pilliod et al. (2013) observed that higher water temperature caused variation in eDNA degradation rates with no detection within one to two weeks of organism removal, which negatively influences the rate of eDNA detection. Notably, Pilliod et al. (2013) combined the effects of temperature and artificial sunlight, making it impossible to analyze their results separately. However, they argued their experiment simulated natural environmental conditions. Another factor affecting eDNA detection rate is turbidity. Stoeckle et al. (2021) concluded that turbidity was strongly inversely correlated with positive detection rate, where an increase in turbidity is linked to a decrease in positive PCR rates. Consequently, further understanding how temperature and turbidity interact with and influences eDNA quantity as it transports downstream will undoubtedly enable the development of eDNA as a tool that is more complete for managing conservation (Stewart 2019).

Benthic substrate and water flow rate also affect eDNA detection rate. Shogren et al. (2017) demonstrated that while moving downstream, the interaction with benthic substrates and other surfaces causes eDNA to be anomalously retained in the streambed, leading to a decrease of eDNA in the flowing water. They also determined different benthic substrates (i.e., sand and pea gravel) retained eDNA for different amounts of time, with finer benthic substrates delaying eDNA resuspension more than coarser substrates. Water flow has also displayed interference with eDNA detectability with the ability to rapidly transport and dilute eDNA, reducing the capability to accurately detect a source location (Stoeckle et al. 2017). Critically, Stoeckle et al. (2017) did not test whether higher or lower flow rates had a greater effect on eDNA detectability; they only tested whether water flow overall had an impact on eDNA detection versus the impact on eDNA detection with no water flow. Though these studies are essential in understanding how the interaction of downstream transport has on eDNA, they were limited to a laboratory setting, short duration enclosures in rivers, or in experimental streams, not under real-world conditions (excluding Stoeckle et al. 2021).

Understanding eDNA transport is imperative in accurately detecting a species' presence in both space (i.e., what was the distance between the species to the eDNA detection site) and time (i.e., when was the species present), enabling the improvement of conservation applications to draw sound conclusions (Barnes and Turner 2016). Therefore, for my thesis, I explored how

downstream transport affected the concentration of eDNA in seven different lakes and their respective rivers in central Texas with the invasive zebra mussels (*Dreissena polymorpha*) as a case study. The overall goal is to further understand how downstream transport affects eDNA concentration using zebra mussels as the target species. **To achieve this, I proposed two objectives: 1) describe the rate of eDNA change during downstream transport and 2) quantify the effects of abiotic factors (i.e., benthic substrate, water temperature, water flow, and turbidity) of the river has on eDNA quantity during downstream transport.** I tested this by collecting water samples from seven different Texas lakes with established zebra mussel populations and their respective outflow rivers. The water samples were extracted for DNA and using a species-specific quantitative PCR assay, I quantified zebra mussel eDNA concentration for each sample. I hypothesized 1) zebra mussel eDNA concentration would decline in a generalized pattern between downstream sites and 2) abiotic factors of the rivers would influence the rate of change of eDNA concentration as it flows downstream. A better understanding of how eDNA interacts with downstream transportation will be beneficial to conservation efforts with refined early detection methods and rapid response to invasive species in lotic systems.

CHAPTER II. METHODS

Study Species

Zebra mussels are a major conservation concern. An aquatic invasive species native to Eurasia, zebra mussels were first introduced to North America in 1988 in the Great Lakes, likely via ballast water (Hebert et al. 1991). They have since invaded inland waters across the United States, including at least 27 reservoirs in Texas. In addition to the zebra mussels' high fecundity and the ability of their free-swimming veliger life stage (i.e., larvae) to be rapidly dispersed by water currents, their successful invasion is also aided by uncleaned recreational vessels and natural and anthropogenic connectivity between waterways (de Ventura et al. 2016, Griffiths et al. 1991, Ram and McMahon 1996). Their high tolerance of varying physicochemical environments also enabled them to colonize a variety of aquatic ecosystems that differ in characteristics such as nutrients, temperature, and turbidity (Ram and McMahon, 1996). The presence of zebra mussels has caused significant ecological and economical harm, including displacement of native mussels (Ricciardi et al. 1998), increased water clarity (MacIsaac 1996), and major infrastructure damage to water-processing plants and hydroelectric power plants (Pimentel et al 2005). Prolific reproductive ability, rapid dispersal capability, and high physicochemical tolerance has enabled the zebra mussel to establish itself as one of the most notorious aquatic invasive species in North America.

Zebra mussels have since spread to at least six Texas river basins (i.e., Red, Trinity, Brazos, Colorado, Guadalupe, and San Antonio) since their first detected in Texas in 2009 on the Texas-Oklahoma border in Lake Texoma with no signs of slowing down (TPWD 2017). In 2016 alone, Texas Parks and Wildlife (TPWD) classified eight lakes previously unreported for zebra mussels as "infested", with an established thriving reproductive population (TPWD 2017). Barnes and Patiño (2019) predicted using a Maxent species distribution that a majority of north and east Texas contain suitable habitats for zebra mussels, increasing the urgency for prevention and management efforts.

Study Sites

The study sites included seven lakes in central Texas, six of which are classified by Texas Parks and Wildlife (TPWD) as “infested” (i.e., denoting a thriving and reproducing adult population) and one classified as “eradicated” (i.e., no detection of settled adult zebra mussels, larvae, or DNA) since January of 2021 (TPWD, 2021; Figure 2.1). As with all large lentic systems in Texas, all seven lakes are anthropogenic reservoirs. Of the seven lakes, four occur in the East Texas Gulf freshwater ecoregion: Belton Lake, Canyon Lake, Stillhouse Hollow Lake, and Lake Waco (eradicated). The other three lakes occupy the Sabine-Galveston freshwater ecoregion: Lake Bridgeport, Lake Lewisville, and Lake Worth. Freshwater ecoregions, similar to terrestrial ecoregions, are ecologically and geographically defined areas of freshwater biodiversity (FEOW, 2019a). Characteristics of each ecoregion tend to be unique in terms of the biodiversity of flora, fauna, and ecosystem. East Texas Gulf, for example, is largely defined by the watersheds of San Jacinto, Neches, Trinity, and Calcasieu rivers. It is dominated by wetlands, with salinities ranging from saline to freshwater. Sabine-Galveston is defined by watersheds of Matagorda Bay including the Brazos and Colorado rivers (FEOW, 2019b). Freshwater and intertidal marshes are dominated along the coast with Edwards Plateau defining the southwest of this ecoregion.

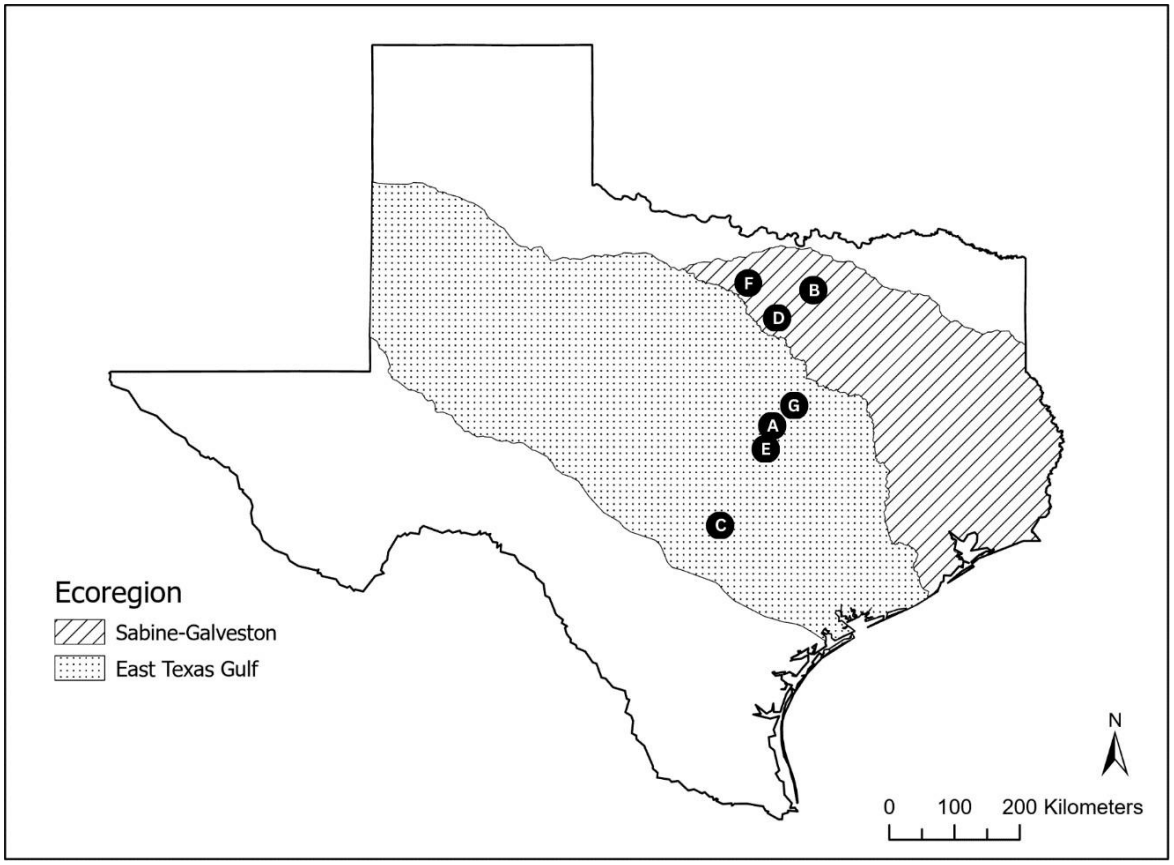

Figure 2.1 Seven lakes sampled in September of 2021 for zebra mussel eDNA in central Texas, 6 classifieds as "infested" and 1 "eradicated" by state management agency Texas Parks and Wildlife Department. A = Belton Lake; B = Lewisville Lake; C = Canyon Lake; D = Lake Worth; E = Stillhouse Hollow Lake; F = Lake Bridgeport; G = Lake Waco.

At each study site, I identified up to four downstream sampling locations on each lake's respective outlet rivers (i.e., Stillhouse Hollow Lake only has three downstream sites due to lack of accessibility before 30 river km) and one upstream site located as close to the dam as possible (Figure 2.2). The distances between sites varied between lakes due to limitations in accessibility (Table 2.1). Sites were chosen based on accessibility to the rivers via road crossings or other public access points (Figure 2.3). The distance from the farthest downstream site to the dam did not exceed 30 river km for any of the lakes and their rivers (Table 2.1).

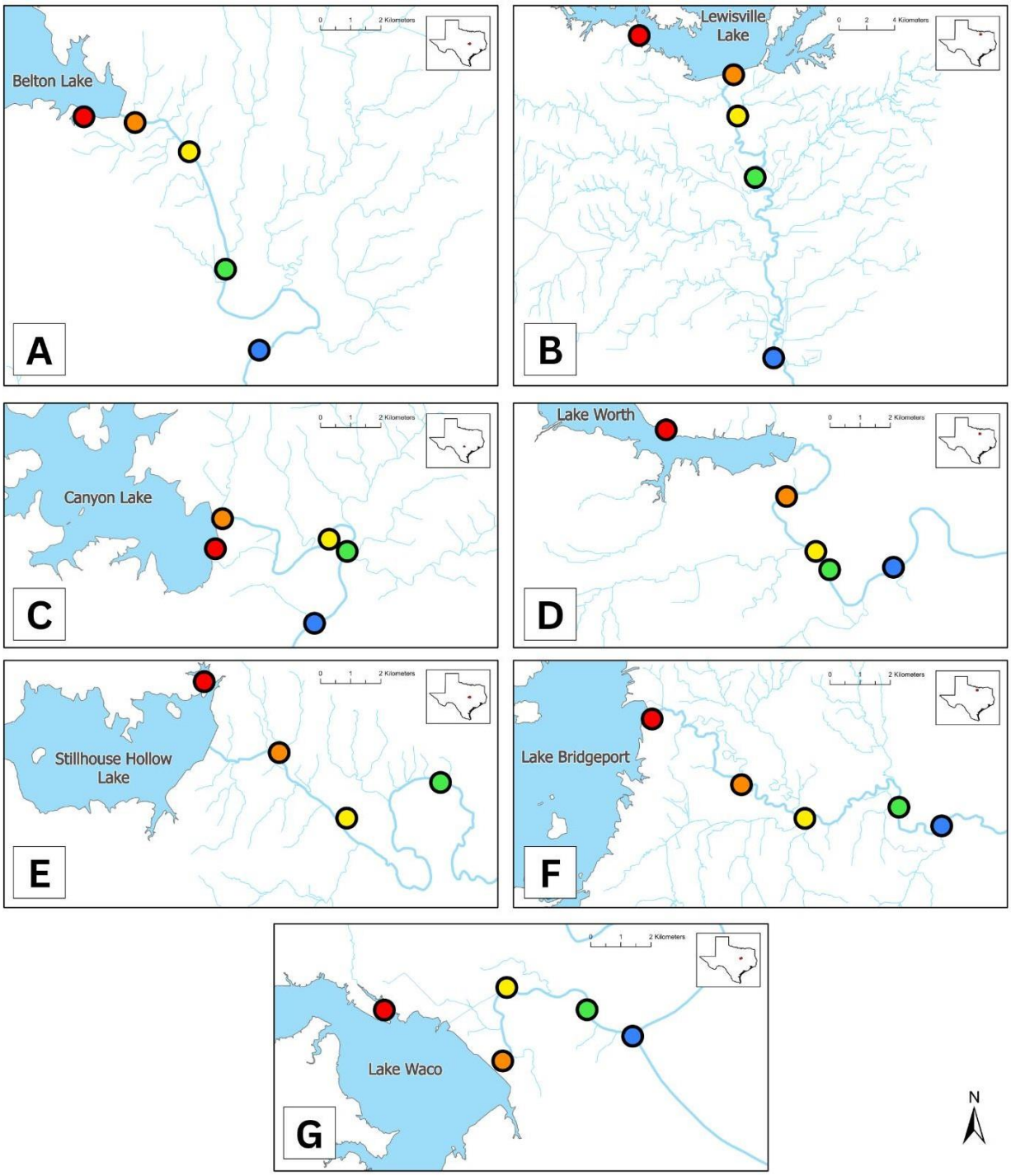

Figure 2.2 Seven sample lakes and their respective rivers. Each river has one dam site (red) and up to four downstream sites (i.e., Site 1 = orange; Site 2 = yellow; Site 3 = green; Site 4 = blue). Colors corresponding to river site will be constant throughout this thesis. The lettering corresponds to the letters in Figure 2.1.

Table 2.1 Sample sites of eDNA from the seven central Texas lakes and their respective rivers in September of 2021.

Lake Name	River	Site ID	USGS River Gauge ID	Coordinates	Distance from Dam Site (rkm)
Belton	Leon	Dam Site	08102500	31.105363, -97.484787	0.0
		Site 1		31.103899, -97.469524	0.4
		Site 2		31.096413, -97.453393	2.5
		Site 3		31.066411, -97.442555	6.0
		Site 4		31.045753, -97.432505	13.1
Bridgeport	West Fork Trinity	Dam Site	08042800	33.218517, -97.829723	0.0
		Site 1		33.202144, -97.803113	4.5
		Site 2		33.193740, -97.784156	7.5
		Site 3		33.196515, -97.756151	12.1
		Site 4		33.191868, -97.743402	14.3
Canyon	Guadalupe	Dam Site	08167800	29.862074, -98.197742	0.0
		Site 1		29.869845, -98.195596	0.2
		Site 2		29.864532, -98.163815	4.9
		Site 3		29.861421, -98.158451	6.5
		Site 4		29.842809, -98.168174	9.2
Lewisville	Elm Fork Trinity	Dam Site	08053000	33.089426, -97.025646	0.0
		Site 1		33.068137, -96.964443	0.2
		Site 2		33.045781, -96.961788	3.1
		Site 3		33.012334, -96.950539	9.7
		Site 4		32.914259, -96.938477	27.5

Table 2.1 Continued

Stillhouse Hollow	Lampasas	Dam Site	08104100	31.0391210, -97.533291	0.0
		Site 1		31.021019, -97.510898	1.9
		Site 2		31.004260, -97.490770	4.9
		Site 3		31.013382, -97.462781	13.6
Waco	Middle Bosque	Dam Site	08095300	31.6015480, -97.241404	0.0
		Site 1		31.582447, -97.195315	0.6
		Site 2		31.601100, -97.194088	2.6
		Site 3		31.595430, -97.170138	5.5
		Site 4		31.588710, -97.156584	7.0
Worth	West Fork Trinity	Dam Site	08045550	32.79586, -97.453405	0.0
		Site 1		32.779162, -97.417475	2.8
		Site 2		32.765400, -97.408761	5.2
		Site 3		32.760812, -97.404634	6.0
		Site 4		32.761408, -97.385616	9.0

Figure 2.3 Lewisville Lake sample site 4 underneath a highway overpass.

To account for potential colonies of zebra mussels downstream, I used Google Earth to extensively survey each lake's river for structures such as pillars from overpasses and bridges where zebra mussel might have colonized.

Sample Collection

Sample collection took place September 16-28, 2021. Water samples were collected from the furthest downstream site to the most upstream site to minimize the effect of activities at one site on conditions at another site. At Lewisville, the furthest downstream site (Site 4) was the last site sampled instead of sampled first due to complications with site accessibility. Clean gloves were worn at each site to prevent contamination between sites. At each site, three 500 mL surface water samples were collected in sterile (i.e., soaked in 10% bleach water for 10 minutes and air-dried overnight) Nalgene bottles from the bank of the rivers or a boat ramp on the lake and placed in a cooler with ice (Figure 2.4). Finally, on each sampling day, one 500mL bottle of distilled water was placed in the cooler with the samples to serve as a negative field control (i.e., "field blank").

Figure 2.4 Collecting eDNA samples under a road crossing over the river at Canyon Lake Site 3.

After water collection, water parameters, including temperature (°C), specific conductance (µS/cm), dissolved oxygen (%L), and turbidity (NTU), were measured using a YSI Pro2030 probe (YSI Inc., Yellow Springs, Ohio, USA) and Oakton Turbidimeter T-100 (Cole-Palmer, Vernon Hills, Illinois, USA) respectively. Discharge rates of the river were recorded from the United States Geological Survey website (see Table 1.1 for Gauge IDs), and the dominant substrate of the rivers (i.e., mud, sand, granule, pebble, cobble, and boulder; Table 2.2) were recorded based on visual observation.

Table 2.2 Different substrate types and their respective sizes (Valentine, 2019).

Substrate	Size (mm)
Mud	< 0.06
Sand	0.06 – 2
Granule	2 – 4
Pebble	4 – 64
Cobble	64 – 256
Boulder	> 256

Filtration equipment (e.g., funnels, forceps) were soaked in 10% bleach prior and after use to prevent contamination. The water samples were vacuum filtered within four hours of collection. Each sample was removed from the cooler and the outside of the bottle was rinsed with tap water to remove external contaminants. Each sample was filtered in a three-funnel manifold with three filtrations simultaneously through 1µm Whatman Nuclepore Track-Etch Membrane. All filters from the same sample were combined in a single 1.5mL plastic tube and stored in the freezer until DNA extraction.

DNA Extraction and qPCR

DNA extractions were performed in a laboratory specifically dedicated to DNA extraction (i.e., physically separated from post-PCR analyses). The bench and pipettes used were sterilized with 10% bleach before each extraction event. I extracted all samples using a modified CTAB-chloroform protocol from Turner et al. (2014). To lyse the cell and release the DNA as well as dissolve the filter, I added 500mL CTAB lysis buffer and 500 mL 24:1 chloroform:isoamyl alcohol to each sample. The tubes were vortexed and shaken until the filters had completely dissolved and centrifuged at 15,000 RPM for 15 minutes. I carefully pipetted 500 µL supernatant, avoiding the subnatant and intermediate material, and transferred the supernatant into a new and sterile 1.5mL tube. Equal to the volume of the supernatant pipetted, 500µL isopropanol was added as was a half volume (250µL) of 5M NaCl. This mixture was incubated at -20°C for an hour, then the tubes were centrifuged at 15,000 RPM for 15 minutes, and the

supernatant was carefully poured out, retaining the DNA pellet in the tube. I washed the DNA pellet with 150µL 70% ethanol and centrifuged it at 15,000 RPM for 5 minutes, discarding the supernatant after centrifuging. This step was repeated once more. To remove the residual ethanol, the tubes were dried on a heat block at 45°C under careful observation to prevent over-drying the DNA pellet. I resuspended the DNA pellet in 100µL low TE buffer and incubated the re-suspended DNA at room temperature for 10 minutes with occasional vortexing. The re-suspended DNA was stored at 4°C until further analysis.

I quantified the zebra mussel DNA in each sample with qPCR on a QuantStudio 3 Real-Time PCR System (Applied Biosystems, Foster City, California, USA). Setup occurred in an UV-treated laminar flow hood. A species-specific qPCR assay was applied, targeting the zebra mussel Cytochrome B gene (CytB; Gingera et al. 2017) for quantification. Each 20 µL qPCR reaction included 1µM each forward and reverse primers, 0.6µM hydrolysis probe, 1x PerfeCTa qPCR ToughMix (Quantabio, Beverly, MA, USA), and 4 µL template DNA. A four-point serial dilution of tissue-derived zebra mussel DNA was prepared using a Qubit fluorometer and broad range dsDNA sensitivity kit (Life Technologies, Grand Island, NY, USA) to enable absolute quantification. All samples ran with six technical replicates with duplicate reactions of each standard and non-template control. To test for inhibition, I performed a 10-fold dilution of my samples (Wiedbrauk et al 1995) then compared the average number of cycles required for the samples to exhibit sufficient fluorescence to confirm the presence of DNA (known as the cycle threshold and abbreviated to CT, determined with the default settings of QuantStudio 3 Real-Time PCR System) of the diluted sample to the undiluted sample to check if it decreased.

Statistical Analysis

Technical replicates with no amplification were assigned a zero concentration from sample detections that had at least one positive reaction when calculating average quantification (Ellison et al. 2006). All analyses were performed using R Statistical Software (v4.2.2; R Core Team, 2022). I conducted a Shapiro-Wilks test to confirm the normality of my environmental data. I used the function *cor* to determine whether any abiotic factors correlated with one another and *corrplot* to visualize the correlation matrix (correlogram) and determine if factors significantly correlated with one another. Specific conductance and turbidity were significantly

correlated (see Results); hence, I excluded turbidity from further analysis because specific conductance was measured on the same probe as the other abiotic parameters whereas turbidity was obtained on a different instrument.

Objective one: Rate of eDNA change during downstream transit

I used the *lm* function to characterize zebra mussel eDNA concentration as a function of downstream distance, creating an independent model for each lake. Linear regression was then used to assess the relationship between the slope of eDNA quantity from each lake (N = 6) and downstream distance using the *lm* function cumulatively instead of independently. Lake Waco's results were not comparable with the other lakes and were removed from further analyses.

Objective two: Effects of abiotic factors on eDNA quantity

To examine whether eDNA quantities were influenced by abiotic factors (i.e., temperature, water flow, specific conductance, and dissolved oxygen), substrate type, and distance, I used mixed effect modeling (MEM) with abiotic factors and substrate type as fixed effects and the lakes and distance were added as random effects. Mixed effects modeling was done using the *lmer* function in the lmerTest package (Kuznetsova et al. 2017). I employed backward elimination multiple regression to determine which abiotic factors or combination of abiotic factors significantly affect eDNA quantity by using the *lm* function and by removing the factor with the highest p-value until I had the best fit model. In addition, I implemented a principal component analysis (PCA) by combining all abiotic parameters including distance into one variable. Then I used linear regression to assess the relationship between eDNA quantity and PC1.

To assess the effects the two ecoregions (East Texas Gulf and Sabine-Galveston) have on eDNA quantity and abiotic factors, I used an Analysis of Covariance (ANCOVA) and Welch's t-test. I performed an ANCOVA using the *aov* function to determine if abiotic factors affect eDNA quantity at local sites, using freshwater ecoregion where the lakes reside in as the covariate. I used Welch's t-test to determine if the means of the abiotic factors differed between ecoregions with an adjusted p-value of 0.01 to account for multiple comparisons.

CHAPTER III. RESULTS

Field controls, extraction controls, and qPCR negative controls were all negative for zebra mussel eDNA. I conducted qPCR trials using diluted template DNA and compared the diluted sample' CT with the undiluted samples' CT. There was no decrease in CT values, and I determined there was no inhibition. Across all qPCR reactions performed, standard curve efficiencies were 95.76 ± 2.61% (mean ± 1 standard deviation) with R^2 averaging 0.988 ± 7.11, demonstrating a reliable capacity to measure zebra mussel eDNA. The Shapiro-Wilks test confirmed the normality of my data. Google Earth surveys also indicated no pattern of structure that could account for high quantities of zebra mussel eDNA.

Overall, I found zebra mussel eDNA at all sites of all six infested lakes and at two sites at Lake Waco where zebra mussels were thought to be eradicated (Figure 3.1). The quantity of eDNA ranged from 0 to 52.728 pg/mL per site. I found positive technical replicates ranging from 0 to 6 per sample. Notably, Belton Lake had the highest quantities of eDNA at downstream Site 2 with almost 80 pg/mL, Lewisville had the highest quantity of eDNA at Site 4, and Worth had almost the same highest quantity at sites 2 and 4. Lake Waco was also listed as "eradicated" by TPWD; however, I did detect zebra mussel eDNA in samples from its Dam Site and Site 1.

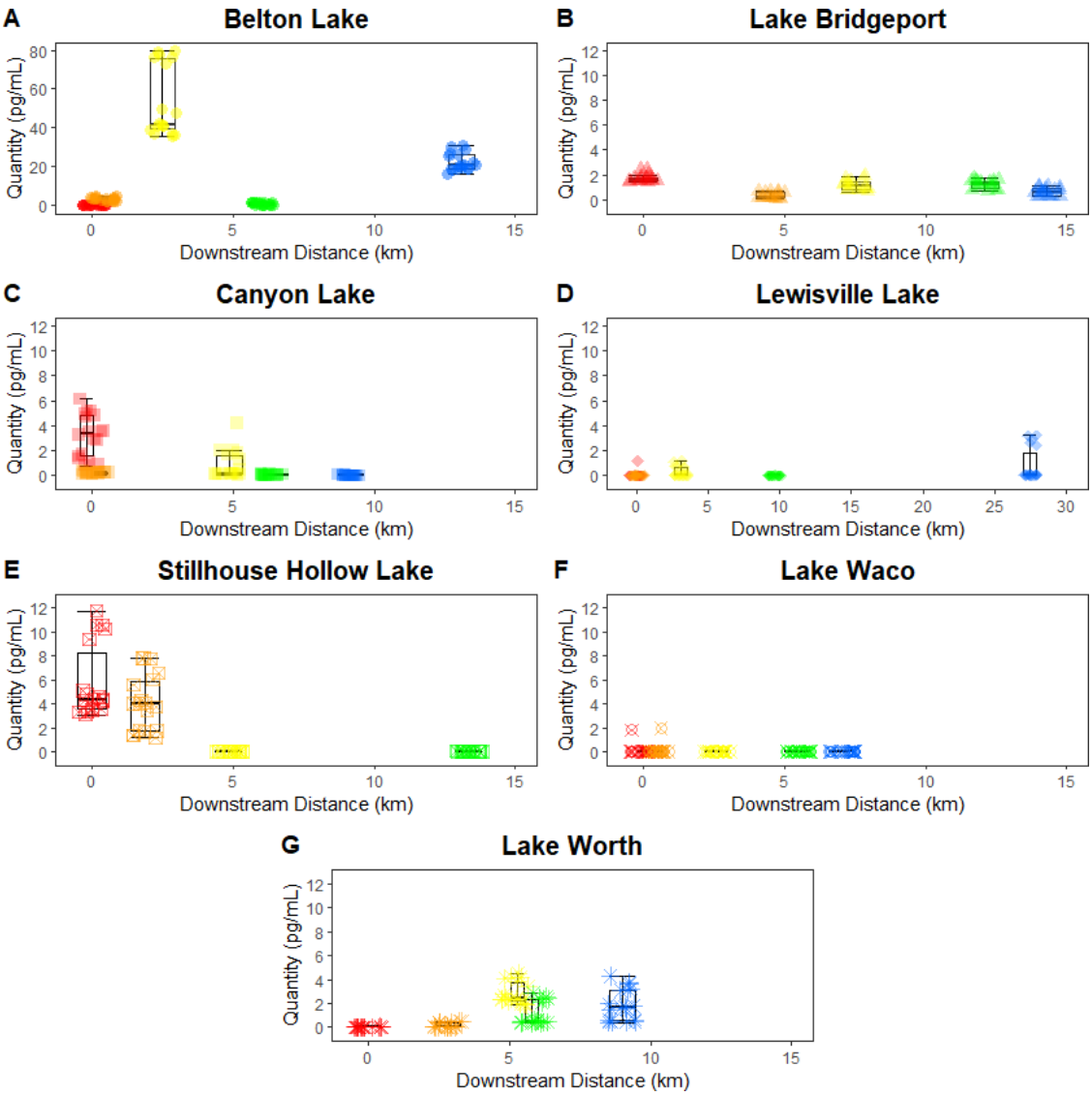

Figure 3.1 Quantity of zebra mussel eDNA at each downstream site of each lake. Colors correspond to downstream sites as shown in Figure 2.2. Note: The graph for Belton Lake (A) has a different scale on the y-axis than all other sites.

Objective one: Rate of eDNA change during downstream transit

There was no significant relationship between eDNA quantity and downstream distance when combining the data from all sites at all lakes ($p = 0.47$). However, I found a significant ($p < 0.05$) relationship between the slope of eDNA quantity and downstream distance at three out of the six lakes: Canyon, Stillhouse Hollow, and Worth (Figure 3.2). Canyon ($b = -0.207$; $p = 0.047$)

and Stillhouse (b = -0.401; p = 0.017) had a significant negative slope indicating the decrease in eDNA quantity is related to an increase of downstream distance; Worth had a significant positive slope (b = 0.249; p = 0.029) indicating the increase in eDNA quantity is related to an increase of downstream distance.

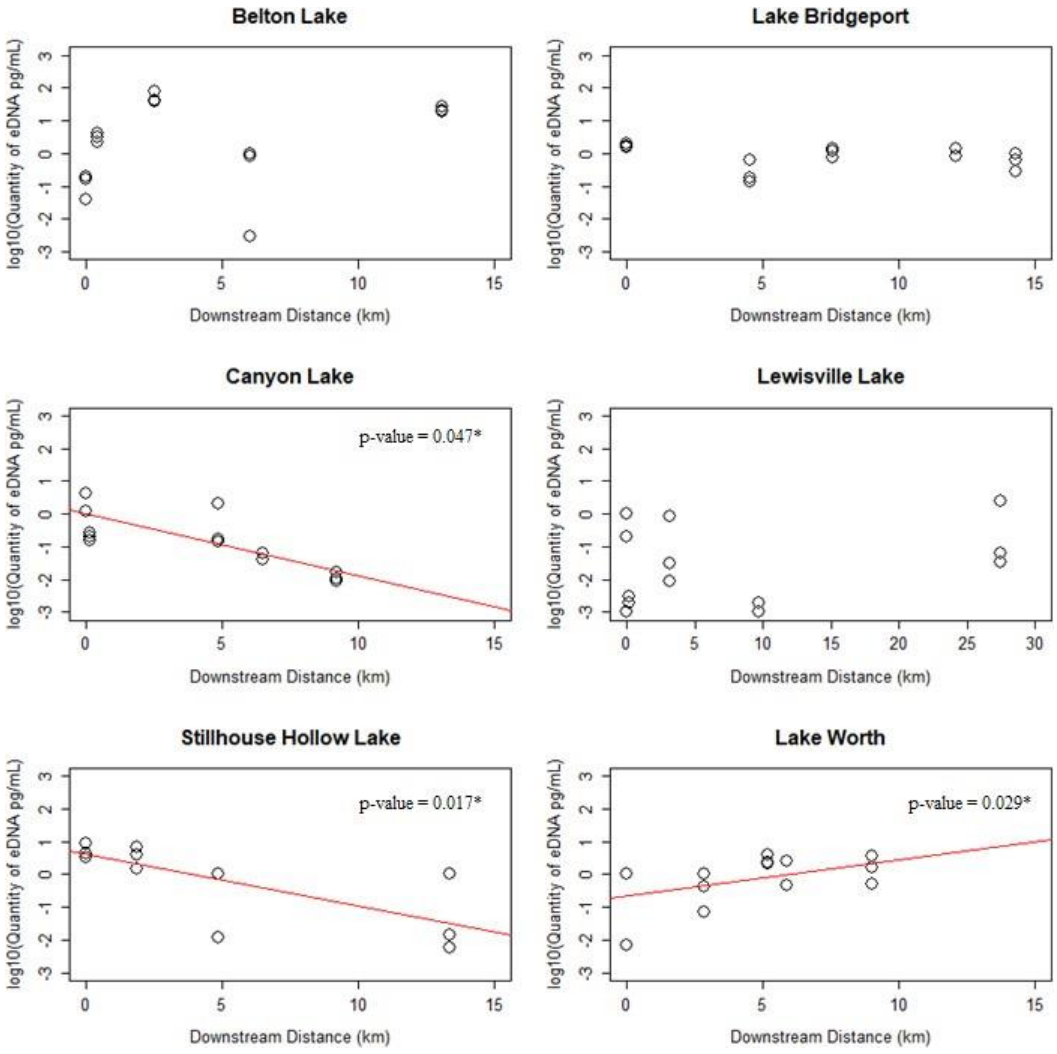

Figure 3.2 Linear regression visualised for each lake with the rate of eDNA decline or incline at each lake.

Objective two: Effects of abiotic factors on eDNA quantity

I wanted to determine if abiotic factors and the freshwater ecoregion the lakes reside in affect eDNA quantity. A significant positive relationship existed between specific conductance and turbidity ($p = 0.009$; Figure 3.3), so turbidity was excluded from further analyses. There was also no multicollinearity between the remaining abiotic factors.

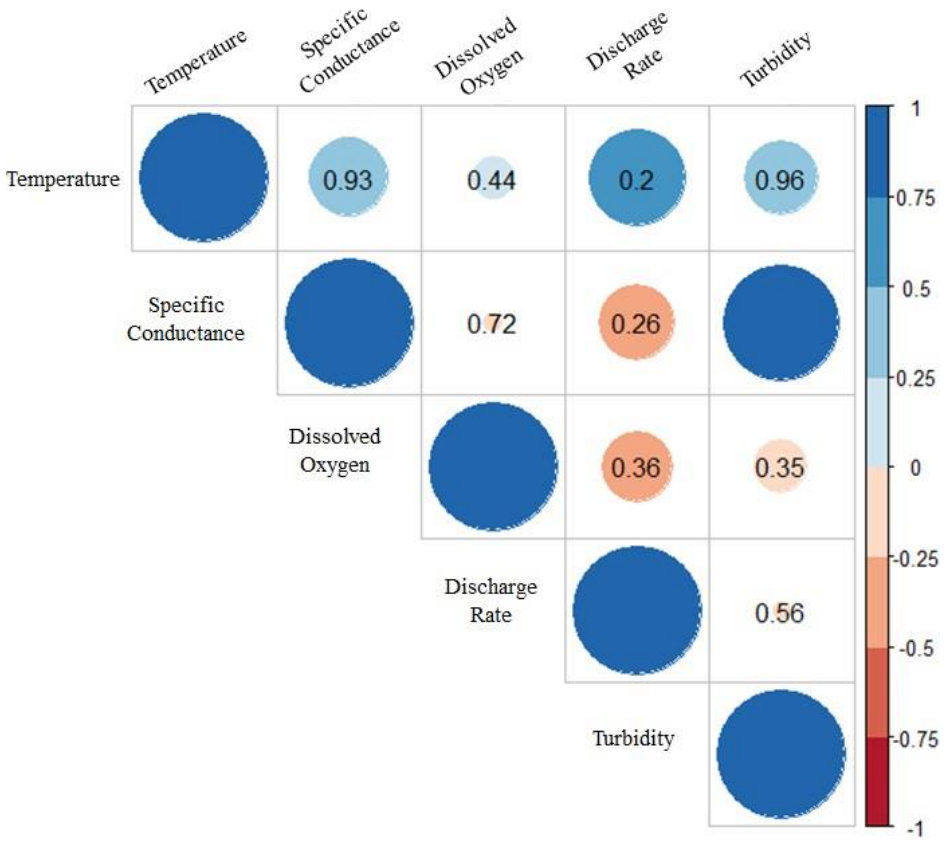

Figure 3.3 Correlogram visualizing correlation between abiotic factors.

Results from backward elimination multiple regression and mixed effects model determined there was no relationship between abiotic parameters and eDNA (Figure 3.4). The MEM also produced nothing significant (Table 3.1) with a marginal R^2 (i.e., variance explained by only fixed effect) of 3.27% and conditional R^2 (i.e., variance explained by both fixed and random effect) of 97.39%.

Table 3.1 Mixed-effects model produced p-value for each fixed factor.

Fixed effect	**p-value**
Temperature	0.602
Specific conductance	0.863
Dissolved oxygen	0.475
Discharge rate	0.354
Substrate	0.704

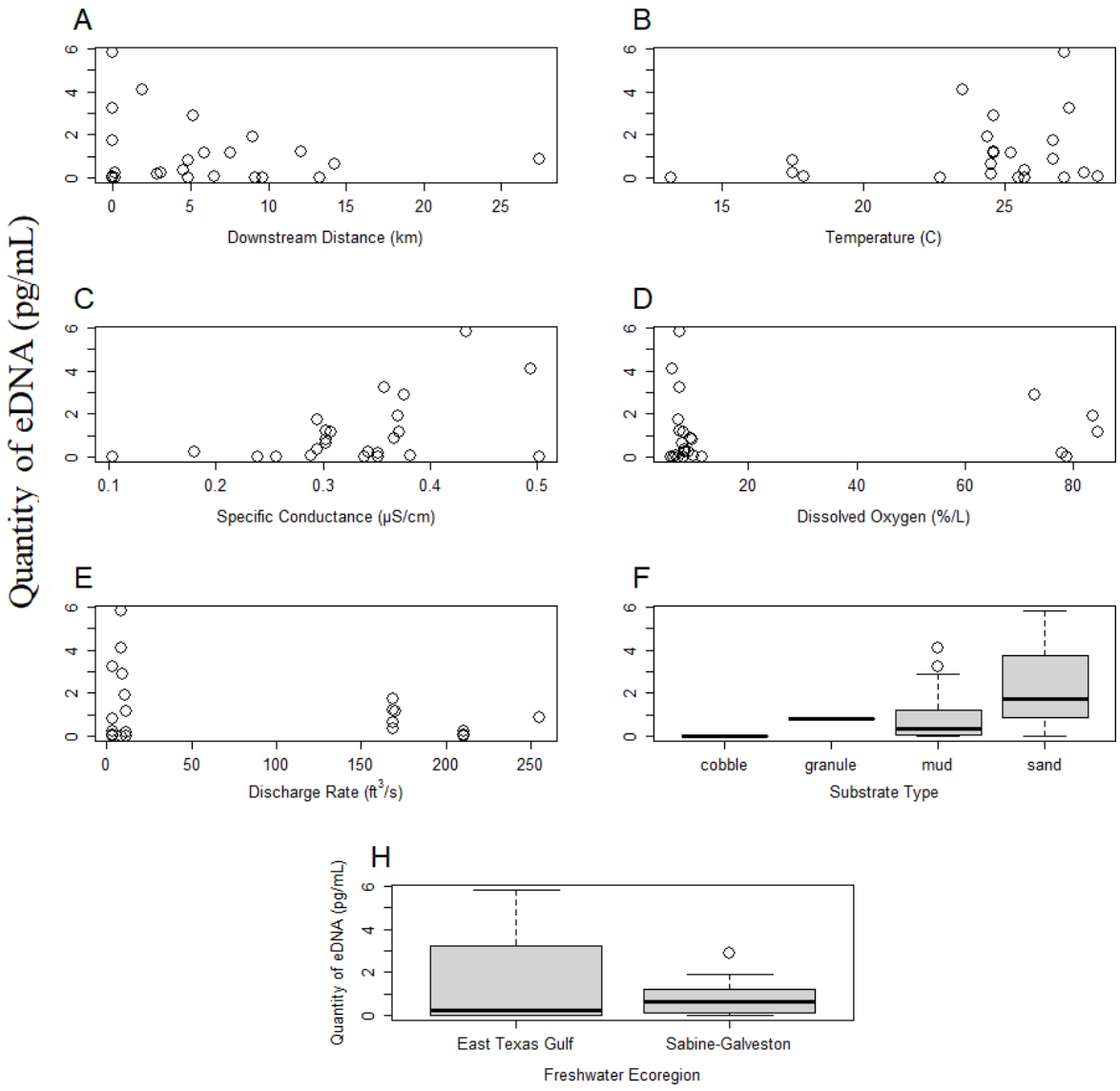

Figure 3.4. Visualization of the absence of relationship between abiotic factors including distance and ecoregions to eDNA quantity. Note: Belton Lake was removed from all graphs for visualization purposes; Belton was not removed from the analysis.

A biplot of the principal component analysis also showed no overall pattern between abiotic factors and eDNA quantity (Figure 3.5). The first principal component explained 32.82% of the variation and had a strong positive relationship with discharge rate, a positive relationship with temperature and downstream distance, and a negative relationship with specific conductance and dissolved oxygen. The second principal component, on the other hand, accounted for 25.75% of variation and had a slightly positive relationship with discharge rate, a

positive relationship with temperature and specific conductance, and a negative relationship with distance. Lakes Bridgeport and Canyon are grouped with a positive relationship with PC1 hence, the eDNA quantities could be tied to the positive relationship with temperature, discharge rate, and downstream distance.

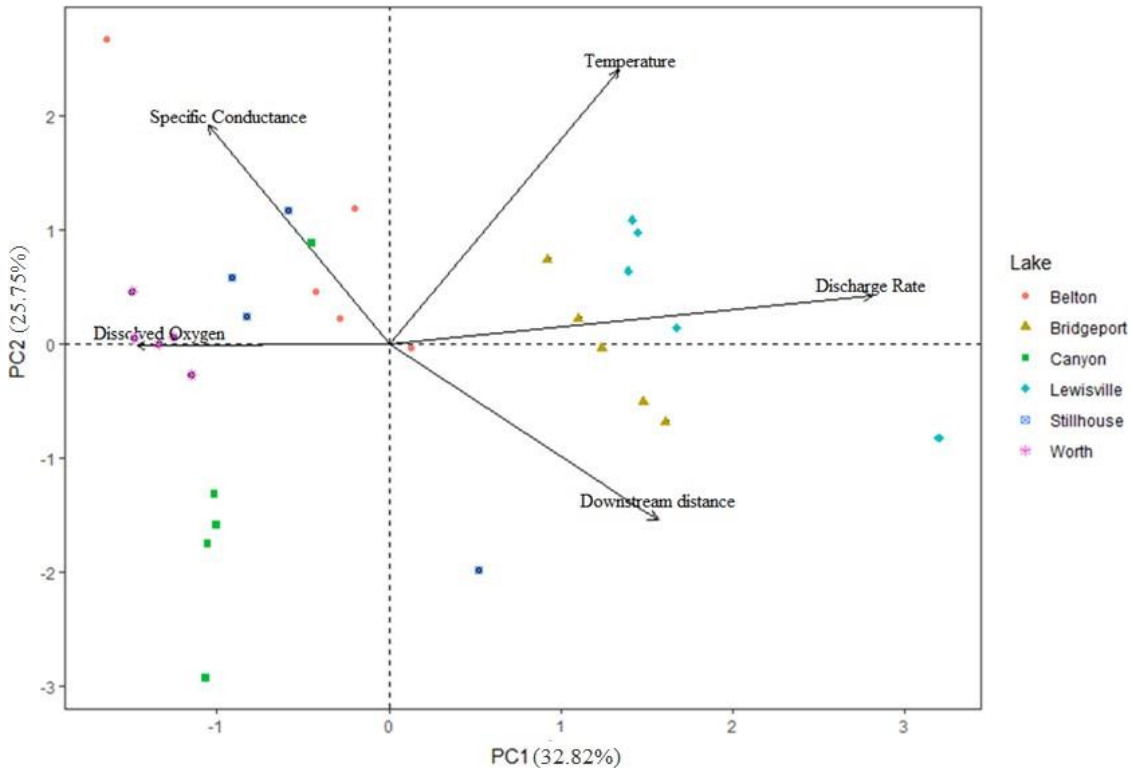

Figure 3.5 Biplot of components 1 and 2 from principal components analysis of abiotic parameter and distance and eDNA quantity. Each point represents a specific lake's downstream site.

To determine if freshwater ecoregion affected the eDNA quantities at each local site, I used ANCOVA with abiotic factors as independent variables and ecoregion as the covariate. The results indicate there is no significant difference between local sites due to abiotic factors, even while controlling for the different ecoregions the local sites reside in. Discharge rate was the only abiotic parameter to differ in means between the two ecoregions with an adjusted p-value of 0.01 (t-test p = 0.00014, Figure 3.6).

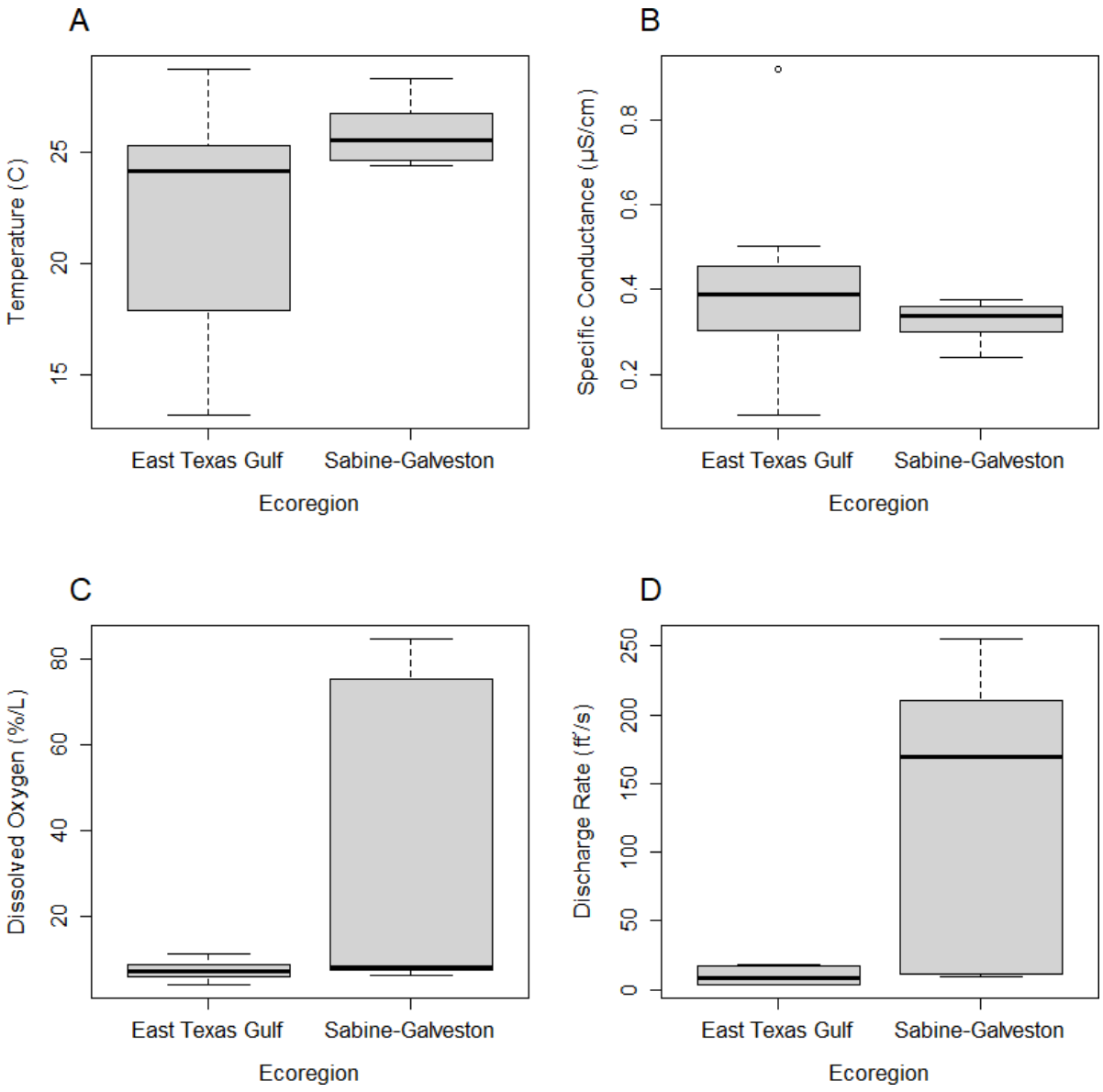

Figure 3.6 Visualization of the abiotic parameters between the two freshwater ecoregions.

CHAPTER IV. DISCUSSION

The collection and analysis of eDNA has aided conservation efforts and management. With the capacity to identify species without direct observation, it promotes early detection and rapid response. Despite the rapid advancement of eDNA-based techniques, the analysis of eDNA in lotic systems is still widely understudied. By using zebra mussels as a case study, I was able to quantify their eDNA at seven different lakes and their respective rivers. Additionally, I demonstrated that zebra mussel eDNA can move a considerable distance downstream of at least 27 rkm.

With increasing distance from the source populations, I hypothesized that the amount of DNA would continuously decrease while moving downstream. However, that is not what I observed. Of the six lakes included in my analysis (excluding Lake Waco), two lakes (Canyon and Stillhouse) had a significant negative slope, indicating eDNA quantity decreased as downstream distance increased, but one lake (Worth) had a significant positive slope, indicating eDNA quantity increased as downstream distance increased (Figure 3.2). On the other hand, Belton Lake had its highest quantity of eDNA at Site 2, Lewisville at Site 4, and Worth had almost equal quantities at sites 2 and 4. I had expected the dam sites to have the highest quantity of zebra mussel eDNA due to the close proximity to the source with a steady decrease of eDNA at the downstream sites. Further research should be conducted to ascertain whether the lakes would still have a positive or negative slope if sampled further downstream.

As previously mentioned, only two lakes (Canyon and Stillhouse) out of the six supported the hypothesis that increased downstream distance related to a decrease in zebra mussel eDNA. I had expected zebra mussel eDNA would gradually decline the further away it was from the source population. For example, Balasingham et al. (2017) assessed the sensitivity of residual eDNA (i.e., eDNA molecules persisting in the environment after the removal of the source) as it moved downstream and discovered a decrease in residual eDNA as the sample distance increased downstream of the eDNA source point. However, this was after the source was removed from the system. Additionally, it is not uncommon for eDNA to differ insignificantly or increase at downstream sites away from the source population. For example, Stoeckle et al. (2021) had hypothesized *Unio crassus* (Retzius, 1788), an endangered thick

shelled river mussel, eDNA detectability would decrease with increasing distance from the mussel source population due to rapid transportation downstream and dilution. Instead, the majority of streams, with the exception of one, had the strongest DNA evidence 100 m downstream from each source population instead of directly downstream. Furthermore, Wacker et al. (2019), while analyzing downstream transport in freshwater pearl mussel (*M. margaritifera*), saw no significant decrease in eDNA concentrations over the 1.7 rkm distance they sampled from.

Lake Worth, on the other hand, had a significant increase of eDNA as downstream distance increased. An explanation of this, including the higher quantities of eDNA at lakes Belton and Lewisville, is the potential of the retention of eDNA via benthic substrates (Jerde et al. 2016, Shogren et al. 2017). Jerde et al. (2016) concluded that finer substrates are more effective at eDNA retention than coarser substrates (i.e., sand will retain more eDNA than pebbles). Moreover, the authors also reported eDNA is not released from benthic substrates at a consistent rate but at a more unpredictable rate. Shogren et al. (2017) also came to similar conclusions. My results indicated no significant relationship between substrate type and eDNA quantity. However, a majority of benthic substrates found at my sample sites were mud, the finest substrate I recorded; hence it is possible that zebra mussel eDNA was highly retained at those sites. Furthermore, it is possible that at the time of sampling at these particular sites, zebra mussel eDNA was released from its substrate prison, increasing the amount of eDNA quantified at those sites. Additionally, I only recorded the *dominant* substrate I observed; benthic substrates are heterogenous, and it is possible that though a site had mud as the dominant substrate, pebbles and boulders could potentially be mixed in. I was also unable to record the substrate in between sites, hence the substrates in between my sites could also affect the quantity of eDNA detected.

Though I did not specifically record the riverine habitat type (i.e., riffle, pool, and run), it is possible these habitats could also affect eDNA quantity. For example, Preece et al. (2020) determined that they were more likely to capture *Gonidea angulata* (Lea, 1838) eDNA within riffles than pool or runs. Their explanation for this finding was the prevention of eDNA settling into the riverbed in riffle habitats due to influences of velocity and turbulence associated with these habitats. The slower and deeper moving water associated with pools and runs could have caused the eDNA to settle into deeper water or into the riverbed, preventing detection (Wilcox et

al. 2016). Additionally, slower flowing water is less likely to resuspend eDNA into the water column when eDNA is held within benthic substrates, making it more challenging to detect eDNA in pools and runs (Samson and Sassoubre 2017, Shrogren et al. 2017). Alternatively, there is a possibility water flow could dilute eDNA, also preventing eDNA detection (Jane et al. 2015). When analyzing the eDNA of caged brook trout (*Salvelinus fontinalis* Mitchill, 1814) downstream at various flows, Jane et al. (2015) found that eDNA counts were low both near and distant from the source at the highest flows, most likely as a result of DNA dilution. Future research needs to be conducted to better understand the interaction of riverine habitat type (i.e., riffles, pools, and runs), substrate type, and water flow and their combined effect on eDNA.

Pockets of zebra mussel colonies located at or upstream from sample sites may also influence zebra mussel eDNA quantity. Due to their ability to attach to hard substrates, zebra mussels could potentially attach to structures such as overpasses and bridges. However, utilizing Google Earth, I was unable to discern patterns of structures near the sample sites with high eDNA that could explain these results. On the other hand, Olson et al. (2018) had conducted surveys on Leon River (outflow river of Belton Lake) for both veligers and juvenile zebra mussel settlement between May 2015 to August 2016 during the zebra mussels' reproductive active and inactive seasons. They consistently found high levels of juvenile settlement at 2.5 rkm downstream, the same site (Site 2) where I had detected the highest quantity of zebra mussel eDNA (Figure 3.1). The authors also recorded the highest number of juvenile mussels at 13.1 rkm, which is the same site (Site 4) where I detected the second highest quantity of zebra mussel eDNA at Belton Lake. Though I had not conducted surveys to confirm the presence of zebra mussel settle at any sites, it is possible pockets of zebra mussel colonies at Belton Lake could be the cause of the high spike of eDNA at sites 2 and 4.

I also analyzed whether there was a relationship between eDNA quantity and abiotic factors in the two different ecoregions I sampled in: East Gulf and Sabine-Galveston. The ANCOVA, with ecoregion as the covariate, implied ecoregion had no relationship with eDNA quantity. The means of discharge rate were significantly different between the two ecoregions (Figure 3.6). The East Texas Gulf ecoregion has a discharge rate mean of 9.77 ft^3/s while Sabine-Galveston had a mean of 133.02 ft^3/s. Though there was no significant relationship between discharge rate and eDNA quantity, I still want to point out the difference in eDNA quantity mean

at each ecoregion. The East Gulf ecoregion had an eDNA quantity mean of 6.65 ± 14.49 pg/mL whereas Sabine-Galveston had a mean of 0.81 ± 0.85 pg/mL. There is a possibility the discharge rate of the river diluted the eDNA (Jane et al. 2015). Jane et al. (2015) discovered that DNA counts were low both close to and far from the source at the highest flows downstream when examining the impacts of various flows on caged brook trout, most likely as a result of DNA dilution. However, the high quantities of sites 2 and 4 at Belton Lake does increase the mean of eDNA at East Texas Gulf. Without the quantities of Belton, the mean of eDNA drops to 1.58 ± 2.21 pg/mL. Hence, it is unlikely discharge rate diluted eDNA in the Sabine-Galveston ecoregion.

Another noteworthy result was the presence of zebra mussel eDNA below Lake Waco at the Dam Site and Site 1. Though low in quantity, various blanks including field blanks, extraction blanks, and non-template controls were free of contamination, suggesting this is not a false positive. Zebra mussels were originally introduced to Lake Waco in September of 2014 at a localized area of a boat ramp and adjacent marina (TPWD 2021). TPWD's method for eradication was the installation of nearly an acre of plastic in the affected area (i.e., the boat ramp where zebra mussels were originally sighted and its surrounding areas), covering the shoreline and the bottom of the lake. The purpose of the plastic was to block oxygen to kill the mussels and prevent reproduction and spread to the rest of the lake. The plastic was removed five months after the installation in March 2015 and TPWD, after no detection of veligers, adult mussels, or DNA, labeled the lake "undetected/negative." However, it is possible they were introduced again due to recreational activities such as boats or from adult zebra mussels and/or veligers that slipped through the cracks in the original removal process. In-depth surveys utilizing both traditional surveys and eDNA methods should be implemented immediately to officially confirm the presence or absence of zebra mussels in Lake Waco, starting with the sites I sampled that were positive for zebra mussel eDNA. If present, the most appropriate method of removal should be put into action to prevent its spread to the rest of the lake. However, I was unable to determine the origin of the zebra mussel detection and it is possible that the zebra mussel eDNA could be from decomposing zebra mussels, highlighting the importance of further studying the ecology of eDNA.

Overall, it was unexpected that there was no relationship between abiotic factors and eDNA quantity. As mentioned previously, there were studies conducted that supported eDNA is

affected by certain parameters such as temperature, turbidity, substrate type, and water flow (Pilliod et al. 2013, Strickler et al. 2015, Jerde et al. 2016, Shogren et al. 2017, Stoeckle et al. 2017, Stoeckle et al. 2021). However, the main difference in my experimental design compared to these studies is the environment. A majority of these studies (excluding Stoeckle et al. 2021) are conducted in either a laboratory setting (Pilliod et al. 2013, Strickler et al. 2015, Stoeckle et al. 2017) or experimental streams (Jerde et al. 2016, Shogren et al. 2017. Additionally, of the studies listed in Table 1.1, 71% of the studies introduced the target species via short-duration exposure either though caging the species and removing them or by introducing their DNA to the stream for a period of time. Only 29% of the studies in Table 1.1 focused on a target species in their natural environment. If the studies were conducted in a natural environment, they were conducted in one stream as opposed to multiple. Quite expectedly, when moving into a field setting with multiple different locations to consider, ecology gets more complicated. Working with zebra mussels in their natural setting, I was unable to control the amount of time they were exposed in the stream and had to rely on the population upstream for the amount of eDNA shed. Additionally, my study was conducted in multiple uncontrollable flowing systems. I also potentially did not have the appropriate power to detect differences (N = 7). I would recommend sampling at more zebra mussel infested lakes to increase power to determine whether abiotic factors effect zebra mussel eDNA.

In conclusion, my study revealed no generalized pattern of eDNA quantity flowing downstream. Furthermore, abiotic factors did not significantly affect eDNA quantity during its downstream transport. Further understanding of the eDNA dynamic in lotic environments would be beneficial to use eDNA methodologies in such environments to better comprehend and manage the distribution of species of interest such as invasive species like zebra mussels. Future research should expand my study to a wider range of infested lakes to determine the effects abiotic factors have on zebra mussel eDNA quantity. Overall, my study adds to the unpredictable nature of eDNA and emphasizes the need for further studies to understand the dynamic between eDNA and lotic systems.

LITERATURE CITED

Anderson-Carpenter, L. L., McLachlan, J. S., Jackson, S. T., Kuch, M., Lumibao, C. Y., & Poinar, H. N. (2011). Ancient DNA from lake sediments: Bridging the gap between paleoecology and genetics. *BMC Evolutionary Biology*, *11*(1). https://doi.org/10.1186/1471-2148-11-30

Balasingham, K. D., Walter, R. P., & Heath, D. D. (2017). Residual eDNA detection sensitivity assessed by quantitative real-time PCR in a river ecosystem. *Molecular Ecology Resources*, *17*(3), 523–532. https://doi.org/10.1111/1755-0998.12598

Barata, I. M., Griffiths, R. A., Fogell, D. J., & Buxton, A. S. (2021). Comparison of eDNA and visual surveys for rare and cryptic bromeliad-dwelling frogs. *Herpetological Journal*, *31*(1), 1–9. https://doi.org/10.33256/hj31.1.19

Barnes, M. A., Klymus, K., & Yamanaka, H. (2021). Editorial: Environmental DNA Innovations for Conservation. In *Frontiers in Ecology and Evolution* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fevo.2021.785077

Barnes, M. A., & Patiño, R. (2020). Predicting suitable habitat for dreissenid mussel invasion in Texas based on climatic and lake physical characteristics. Management of Biological Invasions, *11*(1), 63–79. https://doi.org/10.3391/mbi.2020.11.1.05

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science and Technology*, *48*(3), 1819–1827. https://doi.org/10.1021/es404734p

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Battin, T. J., Kaplan, L. A., Newbold, J. D., & Hansen, C. M. E. (2003). Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature*, *426*(6965), 439–442. https://doi.org/10.1038/nature02152

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodiversity and Conservation*, *29*(7), 2089–2121. https://doi.org/10.1007/s10531-020-01980-0

Cushing, C. E., Minshall, G. W., Newbold, J. D., Andrews, D., Arunacha-Lam, M., Check, J., Dey, P., Koetsier, P., Lawrence, D., Mcintyre, M., Mihuc, J., Mihuc, T., Minshall, J., Minshall, S., Misner, D., Mladenka, G., Olenick, B., Robinson, C., & Watters, G. (1993). Transport dynamics of fine particulate organic matter in two Idaho streams. *Limnology and Oceanography*, *38*(6), 1–101. https://doi.org/10.4319/lo.1993.38.6.1101

de Ventura, L., Weissert, N., Tobias, R., Kopp, K., & Jokela, J. (2016). Overland transport of recreational boats as a spreading vector of zebra mussel *Dreissena polymorpha*. *Biological Invasions*, *18*(5), 1451–1466. https://doi.org/10.1007/s10530-016-1094-5

Deiner, K., & Altermatt, F. (2014). Transport distance of invertebrate environmental DNA in a natural river. *PLoS ONE*, *9*(2), e88786. https://doi.org/10.1371/journal.pone.0088786

Eiler, A., Löfgren, A., Hjerne, O., Nordén, S., & Saetre, P. (2018). Environmental DNA (eDNA) detects the pool frog (*Pelophylax lessonae*) at times when traditional monitoring methods are insensitive. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-23740-5

Freshwater Ecoregions of the World (FEOW). (2019a). *East Texas Gulf*. Retrieved February 9, 2023, from https://www.feow.org/ecoregions/details/140

Freshwater Ecoregions of the World (FEOW). (2019b). *Sabine – Galveston*. Retrieved February 9, 2023, from https://www.feow.org/ecoregions/details/141

Gingera, T. D., Bajno, R., Docker, M. F., & Reist, J. D. (2017). Environmental DNA as a detection tool for zebra mussels Dreissena polymorpha (Pallas, 1771) at the forefront of an invasion event in Lake Winnipeg, Manitoba, Canada. *Management of Biological Invasions*, *8*(3), 287–300. https://doi.org/10.3391/mbi.2017.8.3.03

Goldberg, C. S., Sepulveda, A., Ray, A., Baumgardt, J., & Waits, L. P. (2013). Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, *32*(3), 792–800. https://doi.org/10.1899/13-046.1

Griffiths, R., Schloesser, D., Leach, J., & Kovalak, W. (1991). Distribution and Dispersal of the Zebra Mussel (*Dreissena polymorpha*) in the Great Lakes Region. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(8), 1381–1388. https://doi.org/10.1139/f91-165

Hebert, P. D. N., Wilson, C. C., Murdoch, M. H., & Lazar, R. (1991). Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. *Canadian Journal of Zoology*, *69*(2), 405–409. https://doi.org/10.1139/z91-063

Ikeda, K., Tanaka, K., Kawai, T., & Negishi, J. N. (2016). Using environmental DNA to detect an endangered crayfish *Cambaroides japonicus* in streams. *Conservation Genetics Resources*, *8*(3), 231-234. https://doi.org/10.1007/s12686-016-0541-zS

Jane, S. F., Wilcox, T. M., Mckelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: EDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216–227. https://doi.org/10.1111/1755-0998.12285

Jerde, C. L., Olds, B. P., Shogren, A. J., Andruszkiewicz, E. A., Mahon, A. R., Bolster, D., & Tank, J. L. (2016). Influence of Stream Bottom Substrate on Retention and Transport of Vertebrate Environmental DNA. *Environmental Science and Technology, 50*(16), 8770–8779. https://doi.org/10.1021/acs.est.6b01761

Johnson, M. D., Cox, R. D., & Barnes, M. A. (2019). Analyzing airborne environmental DNA: A comparison of extraction methods, primer type, and trap type on the ability to detect airborne eDNA from terrestrial plant communities. *Environmental DNA, 1*(2), 176–185. https://doi.org/10.1002/edn3.19

Kjær, K.H., Winther Pedersen, M., De Sanctis, B. et al. (2022). A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature 612*, 283–291. https://doi.org/10.1038/s41586-022-05453-y

Klymus, K. E., Richter, C. A., Chapman, D. C., & Paukert, C. (2015). Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biological Conservation*, *183*, 77–84. https://doi.org/10.1016/j.biocon.2014.11.020

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software, 82*(13), 1-26. https://doi.org/10.18637/jss.v082.i13

Laramie, M. B., Pilliod, D. S., & Goldberg, C. S. (2015). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation, 183*, 29–37. https://doi.org/10.1016/j.biocon.2014.11.025

Larson, E. R., Renshaw, M. A., Gantz, C. A., Umek, J., Chandra, S., Lodge, D. M., & Egan, S. P. (2017). Environmental DNA (eDNA) detects the invasive crayfishes *Orconectes rusticus* and *Pacifastacus leniusculus* in large lakes of North America. *Hydrobiologia*, *800*(1), 173–185. https://doi.org/10.1007/s10750-017-3210-7

MacIsaac, H. J. (1996). Potential Abiotic and Biotic Impacts of Zebra Mussels on the Inland Waters of North America. *American Zoologist*, *36*(3), 287–299. https://doi.org/10.1093/icb/36.3.287

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The release rate of environmental DNA from juvenile and adult fish. *PloS one*, *9*(12), e114639. https://doi.org/10.1371/journal.pone.0114639

Minshall, G. W., Petersen, R. C., Cummins, K. W., Bott, T. L., Sedell, J. R., Cushing, C. E., & Vannote, R. L. (1983). Interbiome comparison of stream ecosystem dynamics. *Ecological monographs*, *53*(1), 1-25. https://doi.org/10.2307/1942585

Minshall, G. W., Thomas, S. A., Newbold, J. D., Monaghan, M. T., & Cushing, C. E. (2000). Physical factors influencing fine organic particle transport and deposition in streams. *Journal of the North American Benthological Society*, *19*(1), 1-16. https://doi.org/10.2307/1468278

Newbold, J. D., Thomas, S. A., Minshall, G. W., Cushing, C. E., & Georgian, T. (2005). Deposition, benthic residence, and resuspension of fine organic particles in a mountain stream. *Limnology and Oceanography*, *50*(5), 1571–1580. https://doi.org/10.4319/lo.2005.50.5.1571

Olson, J., Robertson, J. J., Swannack, T. M., McMahon, R. F., Nowlin, W. H., & Schwalb, A. N. (2018). Dispersal of zebra mussels (Dreissena polymorpha) downstream of an invaded reservoir. *Aquatic Invasions, 13*(2), 199-209. https://doi.org/10.3391/ai.2018.13.2.02

Pedersen, M. W., Ginolhac, A., Orlando, L., Olsen, J., Andersen, K., Holm, J., Funder, S., Willerslev, E., & Kjær, K. H. (2013). A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Reviews*, *75*, 161–168. https://doi.org/10.1016/j.quascirev.2013.06.006

Pilliod, D. S., Goldberg C. S., Arkle, R. S., & Waits, L. P. (2013). Factors influencing detections eDNA from a stream-dwelling amphibian. *Molecular Ecology, 14*(1), 109-116. https://doi.org/10.1111/1755-0998.12159

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, *52*(3 SPEC. ISS.), 273–288. https://doi.org/10.1016/j.ecolecon.2004.10.002

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ricciardi, A., Neves, R. J., & Rasmussen, J. B. (1998). Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology*, *67*(4), 613–619. https://doi.org/10.1046/j.1365-2656.1998.00220.x

Robson, H. L. A., Noble, T. H., Saunders, R. J., Robson, S. K. A., Burrows, D. W., & Jerry, D. R. (2016). Fine-tuning for the tropics: application of eDNA technology for invasive fish detection in tropical freshwater ecosystems. *Molecular Ecology Resources*, *16*(4), 922–932. https://doi.org/10.1111/1755-0998.12505

Secondi, J., Dejean, T., Valentini, A., Audebaud, B., & Miaud, C. (2016). Detection of a global aquatic invasive amphibian, *Xenopus laevis*, using environmental DNA. *Amphibia Reptilia*, *37*(1), 131–136. https://doi.org/10.1163/15685381-00003036

Seymour, M., Durance, I., Cosby, B. J., Ransom-Jones, E., Deiner, K., Ormerod, S. J., Colbourne, J. K., Wilgar, G., Carvalho, G. R., de Bruyn, M., Edwards, F., Emmett, B. A., Bik, H. M., & Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Communications Biology*, *1*(1). https://doi.org/10.1038/s42003-017-0005-3

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, Retention, and Resuspension. *Scientific Reports, 7*, 5065. https://doi.org/10.1038/s41598-017-05223-1

Spear, S. F., Groves, J. D., Williams, L. A., & Waits, L. P. (2015). Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. *Biological Conservation*, *183*, 38–45. https://doi.org/10.1016/j.biocon.2014.11.016

Stewart, K.A. (2019). Understanding the effects of biotic and abiotic factors on sources of aquatic environmental DNA. *Biodiversity Conservation 28*(5), 983–1001. https://doi.org/10.1007/s10531-019-01709-8

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation* 183, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038

Stoeckle, B. C., Kuehn, R., & Geist, J. (2016). Environmental DNA as a monitoring tool for the endangered freshwater pearl mussel (*Margaritifera margaritifera L.*): a substitute for classical monitoring approaches? *Aquatic Conservation: Marine and Freshwater Ecosystems, 26*(6), 1120–1129. https://doi.org/10.1002/aqc.2611

Stoeckle, B. C., Beggel, S., Cerwenka, A. F., Motivans, E., Kuehn, R., & Geist, J. (2017). A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. *PLoS ONE*, *12*(12), e0189119. https://doi.org/10.1371/journal.pone.0189119

Stoeckle, B. C., Beggel, S., Kuehn, R., & Geist, J. (2021). Influence of stream characteristics and population size on downstream transport of freshwater mollusk environmental DNA. *Freshwater Science*, *40*(1), 191–201. https://doi.org/10.1086/713015

Turner, C. R., Barnes, M. A., Xu, C. C., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution*, *5*(7), 676-684. https://doi.org/10.1111/2041-210X.12206

TPWD. (2017). Texas Parks and Wildlife Department. The Zebra Mussel Threat. https://tpwd.texas.gov/huntwild/wild/species/exotic/zebramusselmap.phtml (accessed 2022).

TPWD. (2021). Zebra Mussels Eradicated from Lake Waco in Central Texas. Texas Parks and Wildlife: News and Media. https://tpwd.texas.gov/newsmedia/releases/?req=20210121a

Valentine, P. C. (2019). *Sediment Classification and the Characterization, Identification, and Mapping of Geologic Substrates for the Glaciated Gulf of Maine Seabed and Other Terrains, Providing a Physical Framework for Ecological Research and Seabed Management* (Report Number 2019-5073). United States Geological Survey. https://www.usgs.gov/publications/sediment-classification-and-characterization-identification-and-mapping-geologic

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, *37*(1), 130-137. https://doi.org/10.1139/f80-017

Villacorta-Rath, C., Hoskin, C. J., Strugnell, J. M., & Burrows, D. (2021). Long distance (>20 km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations. *PeerJ*, *9*, e12013. https://doi.org/10.7717/peerj.12013

Wacker, S., Fossøy, F., Larsen, B. M., Brandsegg, H., Sivertsgård, R., & Karlsson, S. (2019). Downstream transport and seasonal variation in freshwater pearl mussel (*Margaritifera margaritifera*) eDNA concentration. *Environmental DNA*, *1*(1), 64–73. https://doi.org/10.1002/edn3.10

Wiedbrauk, D. L., Werner, J. C., & Drevon, A. M. (1995). Inhibition of PCR by aqueous and vitreous fluids. *Journal of clinical microbiology*, *33*(10), 2643-2646. https://doi.org/10.1128/jcm.33.10.2643-2646.1995

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., Whiteley, A. R., Lowe, W. H., & Schwartz, M. K. (2016). Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char *Salvelinus fontinalis*. *Biological Conservation*, *194*, 209-216. https://doi.org/10.1016/j.biocon.2015.12.023

Wood, Z. T., Erdman, B. F., York, G., Trial, J. G., & Kinnison, M. T. (2020). Experimental assessment of optimal lotic eDNA sampling and assay multiplexing for a critically endangered fish. *Environmental DNA*, *2*(4), 407–417. https://doi.org/10.1002/edn3.64

Xia, Z., Zhan, A., Gao, Y., Zhang, L., Haffner, G. D., & MacIsaac, H. J. (2018). Early detection of a highly invasive bivalve based on environmental DNA (eDNA). *Biological Invasions*, *20*(2), 437–447. https://doi.org/10.1007/s10530-017-1545-7

Yip, Z. T., Lim, C. S., Tay, Y. C., Tan, Y. H. J., Beng, S., Tun, K., Teo, S. L. M., & Huang, D. (2021). Environmental DNA detection of the invasive mussel *Mytella strigata* as a surveillance tool. *Management of Biological Invasions*, *12*(3), 578–598. https://doi.org/10.3391/MBI.2021.12.3.05