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**ECOLOGY OF A DECLINING GREAT PLAINS FISH, *FUNDULUS*
SCIADICUS, IN THE MISSOURI OZARKS**

by

GREGORY TRAVIS THOMPSON

A THESIS

Presented to the Graduate Faculty of the

MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

IN

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ABSTRACT

Anthropogenic habitat disturbances are of growing concern due to their impacts on native biota, especially in freshwater ecosystems. Damming, channelization, urbanization, wetland draining, and non-native fish introductions all play large roles in habitat homogeneity, fragmentation, and species competition. This has negative effects on native fish and invertebrate species. In the Midwestern United States, the plains topminnow (*Fundulus sciadicus*) has been declining across its range, to the point of becoming a species of special concern in Missouri. This is possibly due to a combination of the above anthropogenic habitat disturbances. To better understand the ecology of the plains topminnow in Missouri, this study examined its distribution within the state from 1930-2010 and its diet, size distribution, and habitat preferences in two Ozark populations. I also examined their competitive interactions with the non-native western mosquitofish (*Gambusia affinis*) in a laboratory experiment. My findings indicate little to no change in plains topminnow populations in Missouri since 1930, while western mosquitofish distribution have grown by over 520% on a county scale. Plains topminnow diets from two Ozark sites indicated a broad range of prey items, including Diptera (larvae and adult), Coleoptera, Gastropoda, and a number of other invertebrate taxa. Size distribution in one study site indicated a healthy population with high recruitment of sexually mature adults and 4 age classes. Habitat data showed a significant correlation of topminnow presence with moderate to dense algae and macrophytes and no significant correlation with dissolved oxygen or temperature. My competition experiment revealed high adult topminnow mortality in the presence of *G. affinis* through intraspecific competition. *G. affinis* was also often killed by a territorial topminnow male.

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1. INTRODUCTION

It has been commonly cited that a mass extinction is looming. Research has focused primarily on the loss of terrestrial species in tropical rain forests, mainly due to the perception of their high peril (Reid, 1997; Ricciardi and Rasmussen 2001). Less attention has been given to the loss of fish species in freshwater ecosystems, despite several studies that demonstrate a growing number of freshwater extinctions (Ricciardi and Rasmussen 2001). Recent studies suggest that freshwater fishes of America have been disappearing at alarming rates. From 1898 to 2006, 57 taxa became extinct, and the rate of loss has increased by 25% since 1989. The highest extinction rates of North American freshwater fish started after 1950, which have been 7.5 taxa per decade. Compared to the background rate of one extinction every 3 million years, the extinction rate of North American fishes has increased by nearly 900 times (Burkhead 2012). This substantial rise can largely be attributed to the activity of humans.

Human impacts are the largest cause of freshwater fish extinctions in North America because of several major factors (Richter et al., 1997; Ricciardi and Rasmussen, 2001). Anthropogenic disturbances to habitat such as stream impoundments, riparian vegetation alteration, water quality, and introduction of non-native species can all have major impacts on stream ecosystems (Fischer and Paukert 2013).

1.1 OBJECTIVES

In the present study, the main goal was to collect general information on the plains topminnow in two small Missouri Ozark populations. After discussing this species with Missouri Department of Conservation Fisheries Biologist, Nick Gironde, it was

realized just how little data has been collected on the plains topminnow in the state of Missouri. To date, most studies have been conducted in the largest population stronghold of plains topminnow in Nebraska. The latitudinal gradient from southern Missouri to central Nebraska may induce ecological differences between these topminnow populations in the two states in terms of their ecology, such as spawning periods and food preferences. Further research revealed the topic of western mosquitofish invasion and their negative effects on various topminnow species in the United States. This raised the question as to whether this species could be affecting native plains topminnow populations in Missouri. The goal of this thesis was to address the following in regards to two small plains topminnow populations within the Mill Creek watershed near Newburg, Missouri: 1) habitat preferences within a spring-fed pond and another spring expanding into a small wetland due to beaver damming; 2) preferred food habits using non-lethal gut-content sampling methods; and 3) size distribution to check on recruitment and population viability. From these findings, there were expected to be differences in prey selection between the opportunistic wetland-dwelling population and a population essentially locked in the spring-fed pond. Most likely, prey items in Dewitt Pond would be pond-dwelling species, while a diet rich in stream invertebrates would be seen in the wetland population due to its immediate proximity to a spring channel. There was expected to be little difference in habitat use between the two populations, as their preferred habitat is prevalent in both study sites. No comparison of size distribution was made between the two sites, as birth and death rates are likely very similar. Only one site was used for this aspect of the study.

The second goal was to cast light on the possible negative effects placed on Missouri plains topminnows by western mosquitofish using the two following objectives: 1) analyze plains topminnow and western mosquitofish sampling data collected by the MDC to search for a relation between distribution declines and expansions of each species, respectively; and 2) conduct a laboratory experiment examining competition between the two species under food restriction using adult and juvenile plains topminnow treatments. From these objectives, there was expected to be an increase in western mosquitofish distribution throughout Missouri, while simultaneously plains topminnow distribution decreased. From the competition experiment it was predicted there would be no significant effects from mixed treatments of adult topminnows due to western mosquitofish aggression. However, it was hypothesized that the juvenile treatments would show significant effects from both direct aggression of western mosquitofish and their ability to outcompete for limited food resources.

These topics are of interest to the Missouri Department of Conservation and will be helpful in determining future conservation efforts for plains topminnows within the state, and possibly elsewhere. There is no primary literature available of such studies conducted for the plains topminnow in Missouri. Because so few studies have observed direct effects of western mosquitofish on plains topminnows, this study may provide information that should be considered by state and federal agencies when introducing mosquitofish to new environments as a biological control agent for mosquitoes.

2. REVIEW OF LITERATURE

In recent years, the plains topminnow has declined in overall distribution and local abundance throughout its historical range (Fischer and Paukert 2008; Pasbrig et al. 2012, Schumann et al. 2014). Changes to physical habitat through agriculture and development are thought to be key factors in their loss, by introducing several types of anthropogenic disturbances. Damming of streams creates habitat fragmentation and homogenization within those fragments (Katano et al. 2006), blocking fish migration routes (Keefer et al. 2010; Tentelier & Piou 2011; Keefer et al. 2012). Channelization eliminates stream margins and riparian wetlands, which provide critical habitat for plains topminnows (Pflieger et al. 1997; Haas 2005; Fischer and Paukert 2008). Stream channelization is often a result of agricultural development to fulfill irrigation needs and reduce flood occurrence. It is also a result of urbanization (Hupp 1992; Raborn & Schramm 2003). As land is developed for housing and businesses, wetlands are often drained and levees added to stream banks. The resulting channelization and loss of natural flood schemes negatively affects channel morphology and reduces habitat heterogeneity (Oscoz et al. 2005; Gorney et al. 2012). These disturbances provide opportunities for non-native, generalist fish species introduction and trigger migration of native species to more preferable habitat (Gorney et al. 2012). Plains topminnows often migrate in response to changes in physical habitat. This is important for re-establishment of local populations, but is likely hindered by the above anthropogenic disturbances (Fischer and Paukert 2008; Schumann et al. 2014). To understand how damming, channelization, wetland draining, and introduction of non-native fish affect native fishes

such as the plains topminnow, it is important to examine their ecological and biological consequences.

2.1. DAMS

Stream impoundments, such as dams, can greatly alter fish community structure through habitat fragmentation. Although dams provide many benefits to society, including hydropower, flood control, and recreational opportunities, they can have dramatic effects on fish communities (Katano, et al. 2006). Globally, more than 45,000 dams have been constructed in the 20th century, causing immediate disturbances to habitat by altering water flux, sediment transport, and temperature regimes (Poff et al. 2007). Water levels are often drastically changed by dams, creating static, lentic upstream habitats and increasing habitat homogeneity downstream (Katano et al. 2006; Ligon, et al. 1995). The shift from moving to still water often favors non-native generalist species over native specialists (Katano et al. 2006). Dams also retain sediment, reducing downstream nutrient availability and sediment transport. Changes in sedimentation negatively affect downstream habitat by reducing substrate availability important for fish nesting habitats and refuge (Vorosmarty et al. 2003). Many species, such as the endangered Niangua darter (*Etheostoma nianguae*), require gravel substrate and lithophilic conditions for reproduction (Mattingly et al. 2003). Sedimentation can eliminate these conditions, especially in stream reaches that suffer high amounts of erosion during large dam releases. Sedimentation affects turbidity and deposition rates, having negative effects on periphyton and macrophyte growth due to a lack of light penetration and variations in substrate composition. Aquatic invertebrates are impacted directly through changes caused by sedimentation, often affecting fish foraging behavior

and causing community shifts on multiple trophic levels (Vorosmarty et al. 2003; Katano et al. 2006; Mims & Olden 2013).

Perhaps the most apparent problem presented by dams is the blocking of migration routes for diadromous fish (Keefer et al. 2012; Keefer, Taylor, Garletts, Gauthier, Pierce, & Caudill 2010; Tentelier & Piou 2011). Many studies have examined the effects of both large and small dam structures on fish migration, coming to the conclusion that many species of anadromous and catadromous species are negatively affected by dams of many sizes (Thoni, Holcomb, Nichols & Gangloff 2014). For instance, Oregon's Willamette River basin contains dams that are on tributaries rather than the main river stem. A majority of these dams have no form of fish passage, blocking the migration of spawning Chinook salmon. Many of the salmon that do pass are at great risk of physical mutilation as they navigate through turbines and deep-water regulating outlets. Blocking of Chinook salmon migration routes combined with over-harvesting resulted in the spring Chinook run of the Willamette basin being classified as threatened on the Endangered Species List in 1999 (Keefer et al. 2012). Keefer et al. (2012) observed a mean of 26% (range 8-59%) mortality in juvenile salmon as they attempted to migrate downstream from spawning habitat to the ocean. A similar study was conducted by Norrgard et al. (2012) in which the effects of dams on downstream Atlantic salmon smolt migration were studied. Smolts were tagged with acoustic transmitters and tracked as they passed 37 receivers on a 180 km long river segment. 16% of the smolts survived the entire route and 8% died on unregulated river segments. 76% died on regulated stream segments during hydroelectric power passages. Norrgard et al. (2012) also found that the fish traveled 83% slower in dam-regulated waters.

Studies like these provide evidence that dams are a major hindrance to the river continuum. All of these disturbances can result in loss of species diversity and create more favorable habitat for non-native, generalist species, often resulting in community shifts on several trophic levels (Poff et al. 2007; Norrgard et al. 2012; Keefer et al. 2012). In areas of the U.S. with large salmon fisheries, measures are being taken to assist in the upstream migration of adult salmon and downstream migration of salmon smolts. These measures include loading fish onto trucks and transporting them above dams and fish ladders that allow fish to slowly move their way over or around river dams (Gowans et al. 1999; Volpato et al. 2009; Kynard et al. 2011). While such methods are becoming popular for economically important species in the U.S, more research is needed to test their effectiveness on other fish.

2.2. CHANNELIZATION

One of the most widespread human impacts on lotic ecosystems is channelization (straightening of a stream channel) (Lau, Lauer, & Weinman 2006; Lepori, Palm, Brannas, and Malmqvist 2005; Laeser, Baxter & Fausch 2005; Oscoz et al. 2005). Channelization has several uses to society, including flood control, navigation, land drainage, and irrigation (Hupp, 1992; Raborn & Schramm 2003; Horsak et al. 2009). However, when a stream is channelized, several aspects of its morphology are changed both vertically and horizontally. The stream is changed as substrate is removed and bank structures are put in place, often increasing water velocity and changing the direction of flow (Raborn and Schramm 2003). Water is then unable to extend into the floodplain until a much higher flow is reached, causing channel scouring and debris removal as unnaturally high water velocities occur after significant rainfall events. As a result,

refuge, nesting habit, and food sources become unavailable to specialized native biota, allowing non-native species to fill those niches (Gorney et al. 2012). Physical structures important for shelter to fishes and invertebrates, such as rocks and downed trees, are often moved or swept away by heavy currents or removed by humans during the channelization process. In addition to these negative effects, increased sedimentation and turbidity are often a result of channelization (Oscoz et al. 2005). As vegetation and natural structures are removed from stream banks, erosion intensifies, releasing nutrients into the water that are normally taken up by the roots of trees and smaller vegetation in the riparian zone. An increase in nutrient load, temperature, and sediment add to the homogenization of habitat in a channelized stream, making it unsuitable for naturally occurring species and causing declines in biodiversity downstream of and throughout channelized segments (Gorney et al. 2005, Smiley and Dibble 2005).

Fish diversity normally increases when habitat is more variable. Stream channelization leads to a lack of sinuosity, pool-riffle sequences, and woody deposits, which in turn lead to declines in fish diversity in streams and rivers. Lau et al. (2006) compared fish assemblages of 20 channelized and 20 natural streams in central Indiana to examine differences in species diversity. Their results demonstrated significantly higher fish diversity in streams containing natural riffle-pool sequences and sinuosity. Channelized streams had significantly lower species diversity and were generally lacking species sensitive to subtle changes in water quality. Similar results were found in another study conducted in the Larraun River of Northern Spain (Oscoz et al. 2005). This study compared fish diversity of a 500 meter diverted stream segment to upstream and downstream sites. The construction of a highway required diversion of the river. This

involved several stream alterations, including dredging, channelization, river straightening, bank stabilization, and vegetation removal. Oscoz et al. (2005) calculated density, biomass, length distribution, and diversity among the three stretches of river. Diversity and biomass were greater in the upper and lower sites than within the channelized section. Biomass was approximately 10 times lower within the channelized stretch and there was a significant decrease in the number of European minnows, *Phoxinus phoxinus*, greater than 150mm in length. This study provides evidence that the entirety of a stream does not need to be altered by channelization to have negative effects on fish populations. It illustrates the likelihood of relatively short segments of habitat homogenization having potential to induce somewhat of a cascade effect on fish diversity in a stream as more sections are channelized over time.

The growing concern of declining fish diversity due to channelization has led to numerous studies on the topic. They provide ample evidence that great care and consideration of stream habitat should be considered when channelization may occur due to human activities. When considering channelization, the chance of full biodiversity recovery should be evaluated to measure the benefits against the consequences. Studies have shown evidence that it can take 5-52 years for fish biodiversity in a channelized stream to return to historical levels (Raborn and Schramm, 2003).

Recovery of these streams is dependent on a conceptualized biological and ecological hierarchy (Allen and Hoekstra, 1992). Poff (1997) proposed a framework that required species be described in terms of their functional relationships to forces of habitat selection based on their scale of influence and process rates. He described these forces as hierarchal filters occurring at various landscape scales, ranging from microhabitats to

watersheds or river basins (Figure 2.1). Large-scale filters control expression of biotic potential at lower scales. In order to join a local community, organisms in a regional pool must contain the appropriate species traits, or functional attributes to pass through the filters. Species must first pass the physico-chemical habitat filters, after which biotic interactions will determine a species niche within a community. Smiley and Dibble (2005) developed a simplified hierarchy model of the one proposed by Poff (1997), in which the 'nested' filters of climate and landuse are excluded and the 'non-nested' filters of channel form, instream habitat, and stream communities are considered (Figure 2.1).

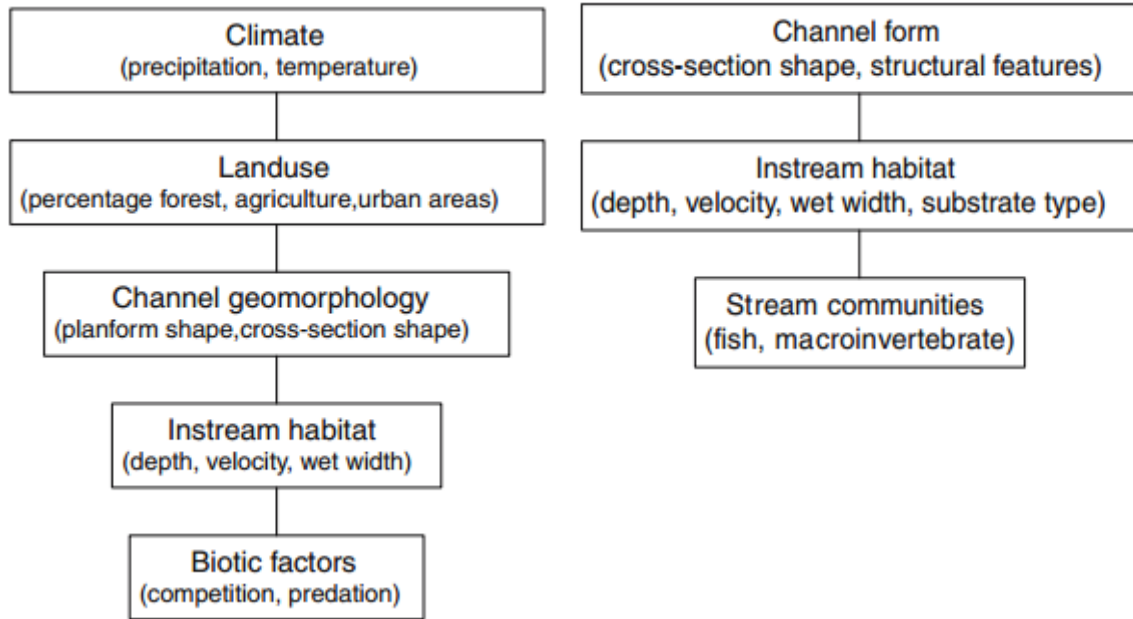


Figure 2.1. Hierarchy of Factors Affecting Fish Diversity. Left) Poff (1997) presents a hierarchy of biotic and abiotic factors affecting fish diversity dependent on the species ability to survive the ‘filters’ of each large and small scale habitat variable. Right) Simplified hierarchy presented by Smiley and Dibble (2005); main factors of fish diversity in recovering channelized streams.

In stream restoration, alteration of channel form and instream habitat are the two key factors critical to fish and invertebrate community recovery (Lepori et al. 2005). When replacing habitat heterogeneity, it is also important to consider the target species for recovery. Lepori et al. (2005) tested the recovery efforts in channelized streams of northern Sweden. They found restoration to have little or no effect when the restoration scheme was not designed specifically to facilitate particular species. The goal to perform these tasks in a manner that facilitates population or community growth arises from the paradigm in stream ecology that abiotic factors affect stream communities more than biotic factors. Many restoration projects have focused strictly on instream habitat with the

thought that this is adequate to achieve a full recovery, considering channel form a secondary factor. Channel form is many times only considered for location selection to place instream habitat and for engineering habitat structures. However, the shape of the channel and instream habitat are often dependent on one another, as channel form often determines habitat availability (Smiley and Dibble, 2005). The study by Smiley and Dibble (2005) illustrates the plausibility of this concept very well. The goal of their study was to examine the relationships of the three 'non-nested filters' with one another throughout three different channel types. They found positive correlations between channel form and instream habitat, but species richness, evenness, and abundance correlated more with instream habitat. Species composition, though, correlated more with channel form than with instream habitat. Overall, they were able to conclude that channel form, habitat structure, and fish communities are hierarchically related. Studies such as this present evidence to be considered when conducting recovery efforts of channelized streams and can be tools for conservation of endangered freshwater fish species.

2.3. URBANIZATION

Urban areas are an ever-increasing feature across the earth's landscape, leading to the study of ecological impacts imparted on freshwater streams that flow through them (Paul & Meyer 2001; Roy et al. 2007). Urban streams are important ecosystems, as they provide relatively complex habitats for diverse biota, are carriers of water and the materials in that water, and are important social and cultural focal points for human inhabitants (Walsh et al. 2005). Over the past two decades, the realization of harmful ecological impacts in these important stream systems has come to surface, resulting in numerous studies examining the many aspects in which urban streams are negatively

affected. These effects have been given the term “urban stream syndrome” (Paul & Meyer 2001; Walsh et al. 2005). The symptoms of urban stream syndrome seen most consistently throughout the world are flashier hydrographs, higher concentrations of nutrients and contaminants, reduced biotic richness with dominance by tolerant species, and changed channel morphology and stability. The degrees of these changes, however, vary throughout the landscape and are dependent upon the cities in which they are in.

Perhaps the most influential changes to urbanized streams stem from changes in the hydrology of the watershed. Roads and other impervious surfaces result in greater direct runoff into streams (Walsh et al. 2005). Evidence of this can be seen in hydrographs recorded from stage-discharge data collected by agencies such as the U.S. Geological Survey. The increased runoff causes shorter lag time to peaks of events. The intensity of the energy coming from these ‘flashy’ events causes major changes to the stream channel, such as scouring and erosion of the banks. It also results in a more homogenized channel, with less debris and fewer pool-riffle segments. The increase in small, flash flood events can affect the channel more so than larger, less frequent events that would normally account for changes in channel morphology and debris build-up. The continual loss in channel heterogeneity by a higher frequency of more intense small events results in an overall decrease in species diversity (Walsh et al. 2005).

As buildings and residential areas are constructed alongside streams, riparian habitat is destroyed. Trees and plants that once provided coarse particulate organic matter (CPOM) are removed, disrupting natural food webs and nutrient input (Hutmacher et al. 2014). This is particularly important in headwaters, where allochthonous CPOM is broken down by shredding invertebrates. In headwaters, the Production : Respiration

ratio is less than 1, meaning respiration outweighs primary production (Straka, Syrovatka, & Helesic 2012). Much of the finer, broken down organic matter is lost to the shredders, but what is left either gets biodegraded by bacteria or makes its way downstream, providing N and P for the productive “mid-reaches” of a stream, where production exceeds respiration and invertebrate communities shift to grazers and collectors (Gomi, Sidle & Richardson 2002; Goncalves & Callisto 2013). Losing input of CPOM in upper stream reaches depletes essential nutrients for downstream production, leading to a bottom-up trophic cascade (Gomi et al. 2013; Callisto and Graca 2013). As invertebrate communities shift to adjust for organic input changes, many fish communities must change throughout the stream to fulfill the new niche localities. This often results in a loss of native biota, providing more opportunities for non-native species invasion and causing a decrease in diversity (Callisto and Graca 2013).

In addition to a loss of CPOM, urbanization often results in concentrated inputs of nitrogen and phosphorus, leading to larger and more frequent algal blooms (Busse, Simpson & Cooper 2006). These effects, though, have been studied with inconsistent results depending on the size of the stream and its proximity to impervious surfaces and inputs of N and P, both direct and indirect. Increased algal biomass may often be counteracted by the changes in stream morphology and hydrology in urbanized streams (Hession et al. 2003a). Increased flow disturbance, turbidity, and depth may present too much variability to allow for excess growth of filamentous algae (Busse et al. 2006). In southern California, USA, Busse et al. (2006) found strong correlations between algal biomass and proximity to impervious surfaces. Stream pools adjacent to and immediately downstream of impervious surfaces were found to have extremely high concentrations of

algae (>300 mg chlorophyll $a \cdot m^{-2}$). Floating macro-algae in pools was strongly correlated with light availability, while benthic algae elsewhere in the study streams were related to phosphorus availability. However, other studies have found no correlation between algal biomass and impervious surfaces. Streams in Georgia, USA, were found to not show statistically strong evidence supporting increased algal biomass in relation to urbanized reaches (Hession et al. 2003a). Such inconsistencies are likely due to several variables, including nutrient limitation, channel morphology, stream discharge, intense flood events, and the amount and type of urbanization occurring near the stream.

2.4. WETLAND DESTRUCTION

In recent years, concern for wetland conservation has increased substantially (Gibbs 2000; Zedler 2000; Brinson and Malvarez 2002; Driver et al. 2009; McCauley et al. 2013). Types of wetlands connected to permanent waters vary widely (i.e. oxbows, freshwater marshes, river floodplains) across nearly all continents, sustaining a wide range of plant and animal species, including several fishes (McCauley et al. 2013). Many fishes are limited to permanent waters for the entirety of their life-cycle, while others have the ability to use wetlands as spawning, nursery, and feeding areas (Driver et al. 2009). Welcomme (1979) classified wetland using fish into two groups based off of their behavioral responses to flood regimes: 1) those that migrate to and from the main channel and avoid environmental fluctuations in wetlands. These fish use wetland habitat in an opportunistic fashion, resorting to the main river channel for refuge and feeding during low-water periods. 2) fish that are able to survive the widely fluctuating conditions of wetlands, including periods of drought.

As with many aquatic habitats, wetlands are subject to various types of anthropogenic change (Brinson & Malvarez 2002; Mateos-Moreno et al. 2012). With the constant increase of the global human population has come the destruction of wetlands for human use. Their fertility has been exploited for agricultural purposes, especially in the Midwestern US where their soils have been made arable by clearing and draining. Also, they are frequently destroyed as urbanization spreads, resulting in their filling and being disconnected from permanent aquatic habitat by levees (Brinson & Malvarez 2002). This disconnection of rivers from floodplain wetlands prevents natural inputs of water, sediments, and organisms (Cucherousset, Carpentier & Paillison 2008; Driver et al. 2009; Gibbs 2001). Changes as such undeniably alter the functioning of fish communities and modify their composition. As a result, tolerant non-native species are often able to encroach on these areas, displacing native fish communities (Cucherousset et al. 2008; Sargent & Galat 2002). Throughout the U.S., wetland loss has affected a disproportionate number of endangered and threatened fish species that are wetland obligates (Gibbs, 2001).

When Europeans first colonized the U.S., wetlands comprised approximately 9% of what is now the continental USA. Since the 1700's, roughly 50% of wetlands in the lower 48 have been converted to some other form of land use (Dahl 1990; Dahl and Allord 1996). Of those left over, 75% are privately owned and 25% make up protected federal wetlands. Over the past 20 years, federal regulations have slowed the destruction of wetlands in the U.S., and focus on restoration has increased in an attempt to regain wetland habitat that has not been permanently lost to anthropogenic changes, such as urbanization. Once a wetland has been urbanized, it is almost certain it has been

perpetually lost. Wetlands affected by less dramatic changes, however, can often times be recovered and restored (Dahl 1990; Dahl and Allord 1996).

Wetland recovery is conditional depending upon the topography, hydrology, nutrient input, and land development of the surrounding area (Schmitz, 2012). Schmitz summarized data collected by Mateos-Moreno et al. (2012) to describe findings of 124 restoration ecology studies conducted to illustrate wetland recovery systematics. A synthesis of these studies revealed it is possible for the biotic and physical functions of wetland ecosystems to recover entirely and quickly when topography, soil permeability, and surface and ground water flows are restored to past levels. Vertebrate species are generally able to recover to previous abundance and compositions within approximately 5 years. Large invertebrates tend to have a longer recovery time, often never reaching absolute reference numbers despite recovery efforts. Plant communities took as long as 30 years to return to their reference states, most likely due to competition and slow-overtaking of pioneer species communities that colonized the area prior to recovery efforts. Nutrient cycling was the aspect found to have the longest recovery time. In most studies it took 50-100 years for nutrient cycles to return to pre-disturbance levels.

The recovery times for the many aspects of wetland restoration emphasized in these studies are similar to those of secondary succession, and support the likelihood that many of the world's wetlands may someday be restored to natural states (Mateos-Moreno et al. 2012). Although wetlands lost to urbanization may be permanently lost, many wetlands lost to agricultural processes and other human needs may one day be restored to their natural states. This could provide supporting habitat for numerous endangered and threatened fish species endemic to parts of the world such as the Midwestern United

States. Properly restored wetlands would also offer suitable habitat for many other organisms such as birds, mammals, reptiles, amphibians, and invertebrates. Since a large share of wetland habitat is privately owned in the U.S., it is important that landowners understand the ecological importance of these ecosystems and how they can assist in their recovery (Moreno-Mateos et al. 2012; Schmitz 2012; Zedler 2000). State conservation departments, such as the Missouri Department of Conservation, provide decades of knowledge and experience to the general public via their website and one-on-one assistance with questions and concerns regarding wetland areas (mdc.mo.gov).

2.5. NON-NATIVE FISH INTRODUCTIONS

Perhaps one of the most concerning anthropogenic disturbances to freshwater ecosystems is the introduction of non-native fish species into new habitats (Cangelosi 2002; Gido et al. 2004; Gozlan et al. 2010; Britton et al. 2011; Smith et al. 2014). Introductions of non-native species have nearly doubled compared to numbers three decades ago, leading to a major public concern to prevent risks of further introductions (Gozlan et al. 2010).

Once in an ecosystem, there is always the risk of a non-native species thriving and causing detrimental circumstances to native fish and habitat (Gozlan et al. 2010). Non-native species affect environments through several different mechanisms, including predation (Meffe 1985), competition (Meffe 1985; Smith et al. 2014), hybridization (Britton et al. 2011), habitat modification, and disease (Almeida & Grossman 2012). Though not all introduced species have negative impacts, it is important for future conservationists to understand the risk factors and implications associated with introduced species and the regions they may inhabit (Schlaepfer, Sax & Olden 2011).

Despite the risks associated, fish are one of the most widely introduced groups of aquatic organisms in the world due to demands of society and aquaculture. Aquaculture alone accounts for over 50% of introduced species world-wide, followed by ornamental fish (21%), sport fishing (12%), and fisheries (7%) (Gozlan et al. 2010). Gozlan (2008) reported that at least 624 non-native species have been introduced into areas outside their ranges throughout the globe. While not all introduced species will survive a new environment, roughly 10% that do have been documented to make adverse changes to their new habitats. Almeida and Grossman (2012), conducted a survey of literature yielding 153 papers regarding interactions between native and introduced species with the objective to pin-point the most common mechanism of introduced species disruptions. Predation accounted for 34%, while competition made up about 32% of species interactions discussed in journal articles from 1999-2010.

The direct and indirect mechanisms in which introduced species affect native biota through predation and competition can be complex. Introduced fish species present new biological interactions to ecosystems that can destabilize trophic levels, disrupting native communities and food webs (Almeida & Grossman 2012). Interspecific competition observed between freshwater fishes can generally be described in two types: 1) exploitative competition, where one species is more efficient at exploiting valuable resources than another, and 2) interference competition, when one species behaviorally excludes another from a limited resource (Britton et al. 2010). Between native and non-native fishes, these forms of competition are compounded by anthropogenic disturbances. After aquatic habitat alteration has taken place via channelization, urbanization, damming, or wetland destruction, many native fish species are left more vulnerable to

exploitative and interference competition with non-native fish species (Britton et al. 2010; Almeida & Grossman 2012).

Over time, the physiological tolerances and requirements of native fish species have become very specific to the biological circumstances of the environments they inhabit (Smith et al. 2014). Physical habitat disturbances by humans rapidly alter these circumstances by changing hydrology, nutrient cycling, refuge, and food availability. In turn, trophic levels are disrupted, leading to changes in aquatic community structure and the specialized adaptations no longer benefit the species exhibiting them (Leprieur 2009). The inherent broad physiological tolerances of many introduced species allow them to slowly dominate these altered habitats (Cucherousset et al. 2012). The loss of habitat heterogeneity plays a role in this, by exposing refuge-requiring species to predators and increasing competition with species needing similar food and cover resources. This reduces native fish fitness by affecting growth rates and reproductive success, further enhancing the ability of more aggressive, generalist species to displace native fish (Cucherousset et al. 2011).

The effects of non-native introductions have been documented world-wide. Among the global regions affected by non-native fish introductions, the Great Plains of the U.S. are particularly at risk to introduced species. Conversion of aquatic habitat for agricultural and urbanization uses threaten many species in the Great Plains. Stream channelization and destruction of floodplains and wetlands has resulted in losses of fishes endemic to the Great Plains, such as the plains topminnow, *Fundulus sciadicus* (Fischer & Paukert 2008; Pasbrig, Koupal, Schainost, & Hoback 2012).

3. PLAINS TOPMINNOW (*Fundulus sciadicus*)

3.1. SPECIES DESCRIPTION

The plains topminnow, *Fundulus sciadicus*, is one of seven *Fundulus* species found in Missouri (Figure 3.1). The *Fundulidae* genus, also known as the killifishes, is comprised of a total 35 species in North America (Pflieger et al. 1997; Haas 2005). They share many morphological features that distinguish them from other species of minnows, allowing them to be well adapted to their way of life. These species tend to feed on insects and other small invertebrates by gliding just below the water surface. The plains topminnow's body is configured to suit these habits. The head and forward part of the back are broad and flat, with the mouth tilted upwards, opening at the upper surface of the head. A deep groove exists between the upper jaw and the snout, allowing for sizable jaw extension (Pflieger et al. 1997).

The plains topminnow is an olive-brown color (Pflieger et al. 1997), having no bars, streaks, or stripes on the side of the head or body (Haas 2005). The dorsal fin is situated toward the posterior end of the fish, above the anal fin. A side view in sunlight enhances the visibility of bronze reflections and faint blue-green crosshatching on their sides. Plains topminnows have a distinguishing golden stripe along their back, extending from the front of the dorsal fin to a point about halfway to the tip of the snout. On females and immature individuals, fins are plain or have a slight yellowish color. Breeding males have an orange-red color starting pale at the base of their fins, then intensifying toward the outer edges. Adults range in size from approximately 3.8 cm to 6.4 cm, reaching a maximum of about 7.1 cm (Pflieger et al. 1997; Haas 2005).



Figure 3.1. Male (top) and female *Fundulus sciadicus*

3.2. FOOD PREFERENCES

The food habits of plains topminnows have not been extensively studied. Thus far, their diet has been found to consist primarily of invertebrates. Stribley and Stasiak (1982) collected samples of plains topminnows (N=346) from 1979-1981 to examine age classes and primary diet. These topminnows were mostly carnivorous, feeding on variety of invertebrates, including ostracod crustaceans, snails of the genus *Physa*, and larval forms of dipteran insects belonging to Chironomidae and Simuliidae. More studies need to be conducted in this field to better understand the ecology of plains topminnows and how they may be affected by anthropogenic changes and non-native species introductions.

3.3. REPRODUCTION

Few studies have examined the breeding habits of the plains topminnow, and most of them are based on data collected in Nebraska. Spawning throughout their range

takes place in spring and summer. In Nebraska, spawning occurs from late March to late July, likely depending on water temperatures (Kinney and Lynch 1991). There have been no studies of plains topminnow reproduction in Missouri to confirm a spawning period. However, Pflieger et al. (1997) suggested the main onset likely occurs in May and June. Kaufmann and Lynch (1991) suggested that breeding takes place when water temperatures are between 18° – 22°C. Higher temperatures result in the regression of the ovaries and egg deposition ceases (Kaufmann and Lynch 1991; Rahel and Thel 2004).

Courtship behavior in an aquarium was observed and described by Kaufmann and Lynch (1991), with plentiful vegetation and gravel substrate. Male plains topminnow fins reportedly became bright red with black borders after acclimation to the aquarium water. The males began to engage in combat over territory, after which a few males had succeeded in exerting their dominance throughout the aquarium. Combative behavior consisted of two males lining up head to tail, circling one another, and biting the others dorsal fin if possible. Male-female courtship began when a male enlarged his gular region and lowered his jaw. Afterwards, the female would approach the male and the two would align head to tail and begin wiggling vigorously. Egg deposition was witnessed at this time, but eggs were found entangled in filamentous algae only after this behavior was observed (Kaufmann and Lynch 1991; Rahel and Thel 2004).

3.4. RANGE

Fundulus sciadicus is an endemic Great Plains fish whose distribution and abundance appears to be declining within its home range. *F. sciadicus* is found in two main disjunct populations. One population is found in central Nebraska, where the majority of the population occurs and spreads into South Dakota, Minnesota, Colorado,

Wyoming, Kansas, and Iowa. The other is in southwest Missouri and extends into the northeast corner of Oklahoma. Recent studies have demonstrated a 70% decline in plains topminnow populations throughout the majority of its range (Pasbrig et al. 2012). This decline has resulted in its extirpation in Kansas, Iowa, and possibly Oklahoma, being listed as threatened in Minnesota and South Dakota, and its listing as a species of concern in Missouri, Colorado, and Wyoming. In Nebraska, it is listed as a Tier 1 At-Risk species, which means it is a target for conservation efforts (Li et al. 2009). In Missouri, the plains topminnow is known to occur along the northern and western margins of the Ozarks from the Lost and Shoal creeks in southwestern Missouri to Missouri river tributaries in Callaway and Warren counties. Southwestern populations in Missouri are well isolated from the main body of the species in the central part of the state. It once populated the Osage Basin above Bagnell Dam, but is now thought to have disappeared from this area (Pflieger et al. 1997).

Plains topminnows inhabit shallow, still waters of stream margins, pools, and backwaters. They are invariably associated with submerged or floating vegetation, which provides refuge from predatory fish and avian predators, as well as access to a variety of aquatic invertebrates. The types of habitat they prefer are extremely vulnerable to destruction and fragmentation through wetland draining, stream channelization and diversion, urbanization, and impoundments. It is thought that these anthropogenic changes may play key roles in their recent declines. Many streams and rivers home to plains topminnows occur in a non-equilibrium state, resulting in sporadic disappearances of topminnow populations, requiring recolonization (Schumann et al. 2014). The study conducted by Schumann et al. (2014) examined the dispersal of plains topminnows over

large areas as a means of recolonizing sporadically lost and reappearing habitats in the plains of Nebraska. Life-history traits supporting large-scale dispersal throughout a watershed are crucial for many non-game plains fishes and are poorly understood. Schumann et al. (2014) marked plains topminnows with visible implant elastomer (VIE) tags and released them in two 3,000 m stream reaches. Their results demonstrate the ability of plains topminnows to migrate significantly greater distances throughout reaches than similar species in response to available habitat. Such findings show that plains topminnows have the innate ability to repopulate stream reaches and adapt to the fluctuating hydrology of Great Plains streams. Anthropogenic disturbances such as dams disable the recolonizing abilities of plains topminnows and may not allow them to repopulate stream reaches where they have become extirpated.

In addition to physical habitat alteration, a number of non-native species have been introduced to the plains topminnows home-range, leading to concerns of populations losses due to competitive interactions. Introductions of non-native fish to the Midwestern U.S. have caused concern for shifts in fish assemblages, particularly for native species that are threatened or of conservation concern. From 2003-2005, Fischer and Paukert (2008) conducted a large-scale study in Nebraska examining changes in fish assemblages. Plains topminnows were absent at over 75% of the historical sites visited, suggesting declines within its most secure range. This loss is parallel to losses of other native Great Plains species such as the federally endangered Topeka shiner and Nebraska-endangered northern redbelly dace. Historical sites for these species showed a shift toward generalist species (i.e. green sunfish, creek chub, and common carp). Other introduced species found were brown trout, northern pike, smallmouth bass, and western

mosquitofish. Among these, green sunfish, creek chub, and common carp were found most highly associated with plains topminnow absence. The shift toward generalist species and a loss of native diversity found in this study is consistent with habitat homogenization due to human impacts.

Though Fischer and Paukert (2008) did not attempt to correlate the introduction of western mosquitofish with range reduction of the plains topminnow, biologists have become concerned that western mosquitofish may have the ability to out-compete and displace plains topminnows from their native habitat (Kaufmann and Lynch 1991; Brinkman 1994; Rahel and Thel 2004; Haas 2005). The habitat requirements of plains topminnows and western mosquitofish are very similar. They are both within the same order and share morphological traits, feeding habits, and size (Haas 2005). Kaufmann and Lynch (1991) proposed western mosquitofish may be the cause of plains topminnow losses in Nebraska through competitive exclusion.

4. WESTERN MOSQUITOFISH (*Gambusia affinis*)

4.1. SPECIES DESCRIPTION

The western mosquitofish, *Gambusia affinis* is the only live-bearing fish species found in Missouri (Figure 4.1). It belongs to family Poeciliidae in the order Cyprinodontiformes (Pflieger et al. 1997). Its morphological features strongly resemble those of the plains topminnow, making them well adapted to living in shallow, static water in oxbows, sloughs, lakes and stream margins. Western mosquitofish are a short, stout species with a strongly upturned mouth and rounded tail. They are somewhat deeper bodied than the “cigar-shaped” plains topminnow. Their sides are plain with no bars, stripes, or streaks. They usually have a dark, V-shaped bar beneath their eyes. The dorsal fin of western mosquitofish is situated slightly farther back than that of plains topminnows. The base of the anal fin of males is situated entirely ahead of the dorsal fin and nearly entirely ahead on females and immature individuals. Western mosquitofish have fairly plain, yellowish-brown sides with dark scale edges forming a pattern of crosshatchings. Their dorsal and tail fins often have 2-3 rows of small black specks (Pflieger et al. 1997; Haas et al. 2003; Haas 2005; Pyke 2008).



<http://hbs.bishopmuseum.org/>

Figure 4.1. Male (top) and female *Gambusia affinis*

4.2. RANGE

Western mosquitofish have often been mistakenly known for their ability to control mosquito populations in small ponds, lakes, and other types of static aquatic systems (Pflieger et al. 1997; Haas 2005; mdc.mo.gov). Due to this misconception, they have been stocked countless times by humans for mosquito control throughout much of the U.S. and most other parts of the world (Haas 2005; Pyke 2008). The natural distribution of the western mosquitofish lies in the Mississippi River drainage basin east of the Rocky Mountains and west of the Appalachian Mountains and stretched as far north as southern Illinois. Today, they are among the most widespread species of fish in the world. Because of their use as a biological control agent for mosquitos, western mosquitofish now inhabit most of the U.S., parts of southern Canada, and as far south as Mexico (Pyke 2008).

4.3. HABITAT TOLERANCES

The dispersion of the western mosquitofish is not completely due to human introductions for mosquito control or use as baitfish (Haas 2005); their aggressive

behavior, generalist nature, and quick reproductive capabilities allow them to survive and expand their populations even after small introductions (Haas et al. 2003; Haas 2005). They are known to occur in water with temperatures from 0-45°C, salinities from 1 to 41 ppt, pH from 4.5 to 9, dissolved oxygen from approximately 1 to 11 mg/l, and turbidity of 3 to 275 JTU (Jackson Turbidity Units). They are able to inhabit a broad selection of ecosystems, including undisturbed swamps, lakes, and streams to highly disturbed water bodies near urban areas where water quality becomes extremely poor due to pollution and algae growth. Though western mosquitofish are able to survive such harsh conditions, they prefer warm water that is still or slow moving (Pyke 2008). Such habitat is also preferred by a number of topminnow species, including the plains topminnow (Pflieger 1997; Kaufmann and Lynch 2001; Haas et al. 2003; Haas 2005; Fischer and Paukert 2008; Schumann et al. 2008).

4.4. FOOD HABITS

Despite their name, western mosquitofish are not proficient mosquito killers. In fact, there is no primary literature demonstrating or supporting this misapprehension. Mosquitofish feed on a variety of invertebrates, including insects, spiders, small crustaceans, rotifers, and snails (Pflieger et al. 1997). They are also known to feed on young fish including their own, fish eggs, zooplankton, and algae (Haas 2005), although the consumption of algae may be due to accidental ingestion while feeding on invertebrates (Pflieger et al. 1997). Though mosquitos and their larvae may be a part of the mosquitofish diet, they cannot survive in the absence of other prey items (Haas et al. 2003; Haas 2005).

4.5. REPRODUCTION

Western mosquitofish reproduction takes place throughout the summer for a period of about 10 to 15 weeks in Missouri (Pflieger et al. 1997). Males pursue and court females almost continuously during this time period and impregnate females via internal fertilization. The sperm is transferred by a groove on the male anal fin, where special muscle help direct the fin forward and to the side during mating. Once inside the female, the sperm are reserved in a living state within a special pouch. One fertilization may be enough for several successive broods, in which a few or even several hundred young will be birthed after 21-28 days (Pflieger et al. 1997; Haas 2005). It is not uncommon for individuals born early in the breeding season to reach sexual maturity and reproduce before the breeding season is over due to their rapid growth. Many western mosquitofish die within their first summer, but some survive and reproduce for a second summer, after which very few survive (Pflieger et al. 1997; Haas et al. 2003).

4.6. PROBLEMS ASSOCIATED WITH *G. affinis* INTRODUCTION

The main contributors to the ability of western mosquitofish to invade new habitats are all due to their general biology. Their outstanding ability to reproduce at high rates, aggressive nature, and high tolerance for a large span of habitats allow them to quickly inhabit, disperse, and recolonize very easily (Haas et al. 2003; Haas 2005; Pyke 2008; Cote et al. 2010). Because of their affinity for similar habitat to that of various species of topminnows, they have been associated with a number of topminnow declines throughout the United States and may affect several other species (Meffe 1985; Haas et al. 2003; Haas 2005; Goldsworthy and Betolli 2006; Laha and Mattingly 2007; Schleier

et al. 2008; Sutton and Zeiber 2009; Hedrick and Hurt 2012; Westhoff, Watts, and Mattingly 2013).

Laha and Mattingly (2007) conducted an ex-situ study examining the effects of western mosquitofish on the well-being of the threatened Barren's topminnow of Tennessee. Their study demonstrated the predation of western mosquitofish on juvenile topminnows. 0% survival was seen in young topminnows <16 mm total length (TL), while 25% survival was seen in topminnows 20-30 mm TL, most of which succumbed to injuries inflicted by mosquitofish after the 24 hour study period. Adult topminnow survival was 100%, but a number of adults sustained injury from aggressive mosquitofish. From this study, Laha and Mattingly (2007) concluded that aggression and predation on young topminnows may be the primary mechanisms by which western mosquitofish affect the Barren's topminnow. These findings are consistent with observations made by Westhoff et al. (2013) in a study determining the efficacy of artificial refuge to enhance survival of young Barren's topminnow in the presence of western mosquitofish. After 20 days of exposure to large mosquitofish, topminnow survival was 13% with artificial refuge present and 7% with no refuge.

In another study, Sutton and Zeiber (2009) performed a mesocosm experiment to examine changes in abundance, biomass, and wet weight per individual of the Northern starhead topminnow in Indiana after coexisting with western mosquitofish for 110 days under varying treatments of each species, with three replicates per treatment. Control treatments contained 30 topminnows or 30 mosquitofish, while experimental treatments were made up of 1) 20 topminnows/10 mosquitofish, 2) 15 individuals of each species, and 3) 10 topminnows/20 mosquitofish. Their results showed a significant decrease of

northern starhead topminnow abundance, biomass, and wet weight per individual in the presence of western mosquitofish. Moreover, western mosquitofish demonstrated successful reproduction throughout the study. The food provided for the duration of the study consisted of zooplankton and other invertebrates contained within the pond water and algae added to each treatment at the beginning of the study. The authors speculated there was not enough food to sustain both northern starhead topminnows and western mosquitofish for the entirety of the study, indicating the possibility of western mosquitofish outcompeting northern starhead topminnows for the same food sources.

In Nebraska, the western mosquitofish has been implicated in declines of the plains topminnow from its historical distributions since its introduction in 1972 (Kaufmann and Lynch 1991; Haas et al. 2003; Haas 2005). The first intentional release was into the Republican River in 1975. A large unintentional release occurred in 1983 when ponds of a fish farm containing an estimated 200,000 western mosquitofish overflowed and released an unknown number of the fish to Nebraska's Platte River. Countless other introductions have taken place through "bait bucket" dumping. They are now found throughout the Platte and Republican River drainages and are spreading at an approximate rate of 10 km/ year upstream and 20 km/ year downstream. In addition to these quickly growing populations, Haas (2005) found evidence of western mosquitofish adaptations to the cold winters of Nebraska. In a laboratory experiment, Haas (2005) found that approximately 40% of wild-caught western mosquitofish from Nebraska rivers could withstand temperatures of 4°C for long periods of time, while 0% of individuals from their native range survived. This shows the ability of the western mosquitofish to quickly adapt to new environments, enhancing their ability to become invasive. In

Missouri, it may be expected that western mosquitofish would have similar negative effects on the native plains topminnow populations. However, there is no evidence to support this. Since the 1930's, western mosquitofish have become quite common throughout the state. Meanwhile, declines in plains topminnow populations have been noticed, resulting in the plains topminnow being considered a Species of Special Concern in Missouri.

5. METHODS AND MATERIALS

5.1. DESCRIPTION OF SITES

Two sites for plains topminnow collection and observations within Missouri were selected for this study based on recommendations of MDC Fisheries Biologist, Nick Girondo (Figure 5.1). Site 1 lies approximately 10 km south of Newburg within Mark Twain National Forest. It is a small pond (~0.82 hectares), referred to as Dewitt Pond (Figure 5.2). It is fed by a large spring (Wilkins Spring) on the south end that discharges a fairly constant flow of water throughout the year, thus maintaining a consistent depth in the pond at all times. Nick Girondo reported a large, apparently secure population of plains topminnows during fish surveys in 2012 (Nick Girondo, personal communication). The outflow from Dewitt Pond drains into Mill Creek 310 m to the north.

Site 2 is Elm Spring, located 790 m downstream of the Mill Creek/Dewitt Pond drainage confluence (Figure 5.3). Elm Spring originates just off the shoulder of County Road AA, where it runs underneath through a culvert pipe, then follows the road for approximately 110 m and makes a 90 degree right turn and drains into an old field on Bohigian Conservation Area. The spring has an obvious channel across the field that is blocked by two beaver dams, forming two pools, with one containing plains topminnows. This pool spreads into the field, forming a small wetland habitat. Site 2 also supports an apparently strong population of plains topminnows.



Figure 5.1. Site 1 and 2 proximity and layout. Plains topminnow sampling sites used for diet, size distribution, habitat analysis, and specimens for the competition experiment.



Figure 5.2. Dewitt Pond (Site 1).



Figure 5.3. Elm Spring wetland (Site 2).

5.2. HABITAT ANALYSIS

Habitat analysis was conducted within Dewitt Pond from March to May for a total of five site visits. Dewitt Pond offers an excellent opportunity to examine habitat preferences of plains topminnows within one small area having a variety of habitat variables. The entirety of the pond contains a consistent low/medium density of submerged macrophytes, while algal density varies greatly depending on wind directions. Environmental factors measured during each site visit were dissolved oxygen (mg/l) and temperature (°C) with a YSI 85 oxygen, conductivity, salinity, and temperature meter. Algae and macrophytes providing cover were classified into three densities (1=zero/light, 2=Moderate, and 3=Heavy) depending on the amount of open water visible within gaps between algae clumps. Plains topminnow presence/absence was noted at each of the 8 sites.

5.3. FISH COLLECTION

Plains topminnows were collected for several scientific measurements (detailed below). Fish were captured using two methods, dip-netting and seining. After the first sampling using the dip-netting method, it was realized that there may be a bias toward the capture of mostly juvenile fish rather than a mixture of juveniles and adults. For this reason, dip-netting was ceased at the end of the spawning season in July and seining was used for the majority of remaining samples in order to capture a broad range of age classes. Two techniques of seining were used based on the amount of filamentous algae at each location. With little algae present, a two person method was used in which one stands on the shore holding an end of the seine, while the other pulled the seine tight at a 90° angle to the shoreline and made one sweep, catching any fish within the 90° arc. If dense algae was present, it hindered the ability to make a proper 90° sweep. In such cases one end of the seine was attached upright on the shoreline using a 40 cm section of reinforcement steel. The seine would then be stretched to full length along the shoreline and a 45° sweep was made into the water, removing a majority of the algae. The end in the water would then be shoved upright into the sediment if I was alone, or held by another person assisting. The sediment in the swept section was then allowed to settle until the water was again clear and topminnows began to move back into the area. One person would then walk approximately 50 m down the shoreline on the open end of the seine and essentially corral any fish within this section into the 45° arc. The free end of the seine would then be pulled back to shore. This method tended to be very successful at collecting high numbers of fish and a broad range of age classes.

5.4. NON-LETHAL GUT CONTENT SAMPLING

Gut content samples were collected four times from June to October of 2014 to examine plains topminnow diets within the two sites (N=30 for per site). Non-lethal methods described by Kamler and Pope (2001) were used to obtain these samples as a means to prevent unnecessary topminnow deaths (Figure 5.4). Studies have examined the effectiveness of using a syringe to flush the gut content of small fish, demonstrating relatively good success (Kamler and Pope 2001). The method of inserting a small tube into the anus of the fish and pushing water through with a hypodermic needle forces gut content out of a fish's mouth. I used a similar method in this study, in which a 3 cc syringe was modified by removing the hypodermic needle and replacing it with a micropipette tip held and sealed onto the syringe with parafilm. This eliminated the need for inserting a tube into the fish and also allowed the syringe to be used for gastric lavage, another common technique employed in fish gut content sampling (Kamler and Pope 2001). Gastric lavage involves pumping water into a fish's stomach using a syringe or similar tool, which causes stomach content to be flushed out of the mouth. Flushing through the anus (Figure 5.4) appeared to result in larger amounts of content removal, but the technique failed ~40% of the time, in which case I would attempt gastric lavage. If no gut contents were removed with three attempts of each technique, the fish was released. Gut content samples were examined under a dissecting microscope and categorized based on invertebrate keys.



Figure 5.4. Flushing gut content from an adult plains topminnow.

5.5. SIZE DISTRIBUTION

Plains topminnows were measured a total of five times from June to late September. Approximately 40 specimens per sample (Total N = 194) were measured in Dewitt pond to interpret a size distribution for this population. Individuals were captured using the dip-netting and/or seining methods and measured with a plastic ruler to the nearest millimeter. Each fish was held in a small puddle of water in one hand and measured from the outer edge of the bottom jaw to the longest reach of the tail. Once measured, they were released unharmed. In order to avoid the possibility of catching the same fish for this study, each sampling took place on a different section of shoreline.

5.6. STATEWIDE DISTRIBUTION IN MISSOURI

This aspect of my study required historical data from the Missouri Conservation Department. Doug Novinger, MDC Aquatic Systems Resource Scientist, provided annual MDC fish sampling data beginning in 1930 and ending in 2006 for the plains topminnow. Sampling data for the western mosquitofish began in 1931 and ended in 2012. These data included the stream name, county, date, and method of capture, as well as sampling effort in hours and minutes. ArcGIS shapefiles of HUC 12 (Hydrologic Unit Code 12) data were included with this response to my inquiry. HUC 12 data shows hydrologic units at the sub-watershed level, covering an average of 103.6 km². As HUC's decrease (i.e. 10, 8, 6, etc.) in ranking, they increase in total surface area covered. By overlapping HUC 12 data for the plains topminnow and western mosquitofish, it is possible to see sub-watershed areas where the two species may be found within the same streams. This could identify current problems where plains topminnows are experiencing competition with western mosquitofish and target areas for future research of the interactions between these two species. ArcMap 10.1 software was used to upload the shapefiles and analyze the output. The HUC 12 output was also used to see increases and decreases in distributions of each species.

5.7. COMPETITION EXPERIMENT

To test for the effects of competition between plains topminnows and western mosquitofish, a 36 day laboratory trial was conducted. This required a Wildlife Collection permit issued by the MDC in which a set number (N=30) of plains topminnows was allotted. Eight 37.9 L tanks were filled with dechlorinated tap water. A shallow gravel system and ammonia filter was installed in each tank to ensure no die-off

due to ammonia build-up since such a small sample of plains topminnows was allowed. 30 plains topminnows (15 juveniles/15 adults) were collected from Dewitt Pond and placed in a holding tank. 30 randomly selected western mosquitofish were collected from Towell Lake on Little Prairie Conservation Area, 8.9 km northeast of Rolla, and held in a separate tank. Both species were fed frozen brine shrimp and left in holding tanks for acclimation to the new food source and laboratory conditions for 14 days.

On the day of initial stocking and placement into treatment and control groups, total length (TL) and total biomass (TBM) were measured for each species in their respective treatments. Follow-up measurements were made midway through the experiment and then 36 days from the beginning. Treatment groups consisted of the following:

-4 adult topminnows and 4 random mosquitofish (3 replicates)

-4 juvenile topminnows and 4 random mosquitofish (3 replicates)

-6 plains topminnows (3 adults and 3 juveniles) (1 replicate)

-6 western mosquitofish (1 replicate)

Each tank received frozen brine shrimp once daily through a home-made slow drip system for the entirety of the study to simulate prey falling onto the water surface. Tank water remained at room temperature throughout the study (20-25°C) depending on the variable air temperature within the lab.

Fish aggression was monitored by examining fish for evidence of fin nipping. Fin nipping was categorized into three levels: 1) edges of the tail and dorsal fin frayed, small

patches of scales missing between dorsal fin and tail; 2) 1/4 – 1/3 of tail and dorsal fins missing, most scales between dorsal and caudal fins missing, fish beginning to show signs of stress (i.e. isolation, constant swimming motion, rapid gill undulation); 3) 50%-100% of tail and dorsal fins missing, fish generally dead or near death on bottom of tank, nearly all scales missing from the front of the dorsal fin to beginning of the caudal fin.

6. RESULTS

6.1. SIZE DISTRIBUTION

Topminnows that were collected in Dewitt Pond ranged in size from 1.7 cm to 7.0 cm. The overall size distribution was relatively normal when illustrated with a histogram (Figure 6.1). Fish lengths 1.6-3.0 cm were considered young of the year (Age 0), Age 1 = 3.1 - 4.5 cm, Age 2 = 4.6 – 6.0 cm, and Age 3 = 6.1 – 7.0 cm.

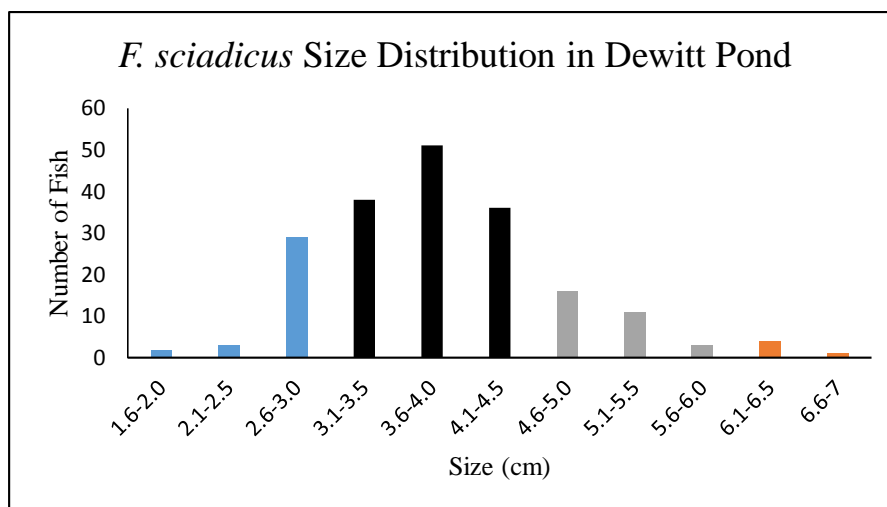


Figure 6.1. *F. sciadicus* Size Distribution in Dewitt Pond. Size distribution for 194 *F. sciadicus* specimens measured in Dewitt Pond. Blue = young of the year; black = 1 year; gray = 2 year; orange = 3 year and above.

Stribley and Stasiak (1982) found plains topminnows to reach sexual maturity at age 1. 64% of the topminnows measured for this study were classified as approximately one year. The normal distribution and high number of young, sexually mature adults in

Dewitt Pond signifies strong recruitment, which is important to sustaining a healthy population. Spawning was witnessed from mid-May to late June. Males began showing breeding colors in late April, then signs of aggression in early May. The majority of topminnows less than 3.0 cm were captured after approximately one month following the spawning witnessed in May and June.

6.2. GUT CONTENTS

Gut content samples showed a wide array of prey items of similar content from both Dewitt Pond and Elm Spring populations (Figure 6.2). The most prevalent gut items were filamentous algae (26%), juvenile gastropods (17%), adult Diptera specimens (9%), and Coleoptera (8.5%). Corixidae, the Hemipteran water boatman, was only found in Dewitt Pond, most likely because this has been an established pond-like habitat for many years. Overall, the diets reflected a broad range of invertebrate life stages from various species to include, benthic, pelagic, and airborne dwellers such as adult Diptera. A diet including adult Diptera is consistent with the thought that plains topminnows often feed from the water surface.

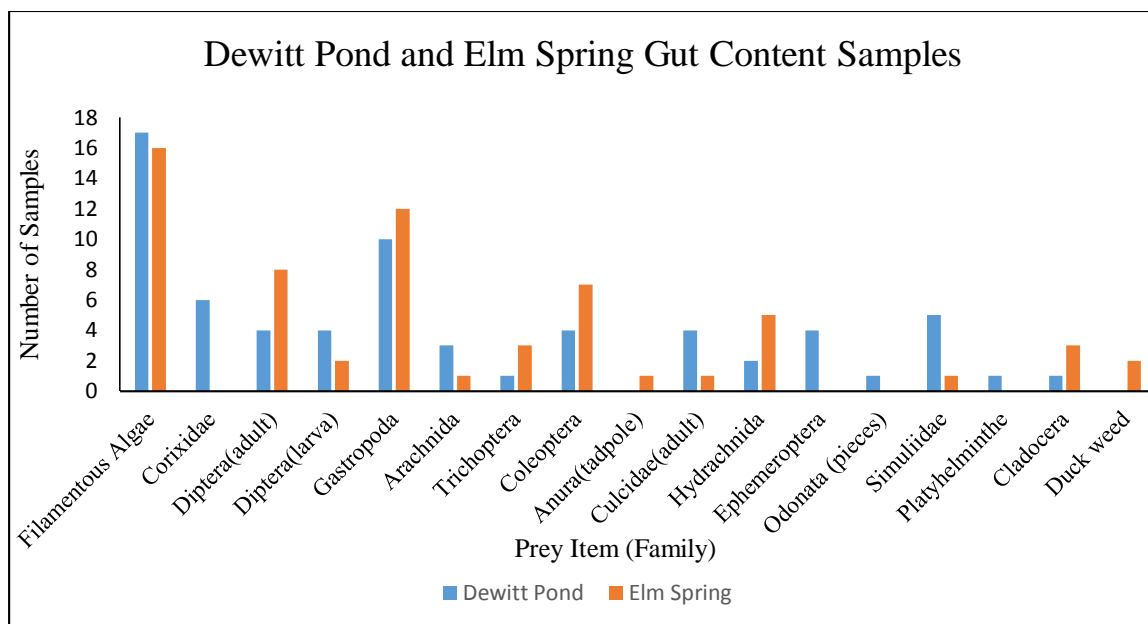


Figure 6.2. Dewitt Pond and Elm Spring Gut Content Samples

6.3. HABITAT ANALYSIS

A binary logistic regression model was used to relate the presence/absence of *F. sciadicus* to dissolved oxygen (DO mg/L), temperature (T °C), and cover at sites within Dewitt Pond. Moderate and heavy cover were grouped together in order to be used as a binary variable. Temperature of Dewitt Pond sites ranged from 11.3 °C to 23.6 °C over the course of my samplings. The pond is fed by Wilkins Spring, which has a constant temperature of about 13 °C. However, certain areas along the edges of the pond are secluded from the rest of pond, and heat up to over 20 °C. DO ranged from 0.17 mg/L to 17.6 mg/L, and was influenced by the amount of macrophytes and algae in the area. Supersaturated DO values were commonly reported during the mid-day samplings because of high rates of photosynthesis by the primary producers.

The test for all slopes showed a significant result ($p\text{-value} = <0.001$), indicating a significant relationship between topminnow presence and at least one variable.

Temperature and DO did not show a significant effect on topminnow presence ($p = 0.304$ and $p = 0.074$, respectively) (Figure 6.3). Cover had a significant effect on plains topminnow presence at each site ($p < 0.001$) (Figure 6.4).

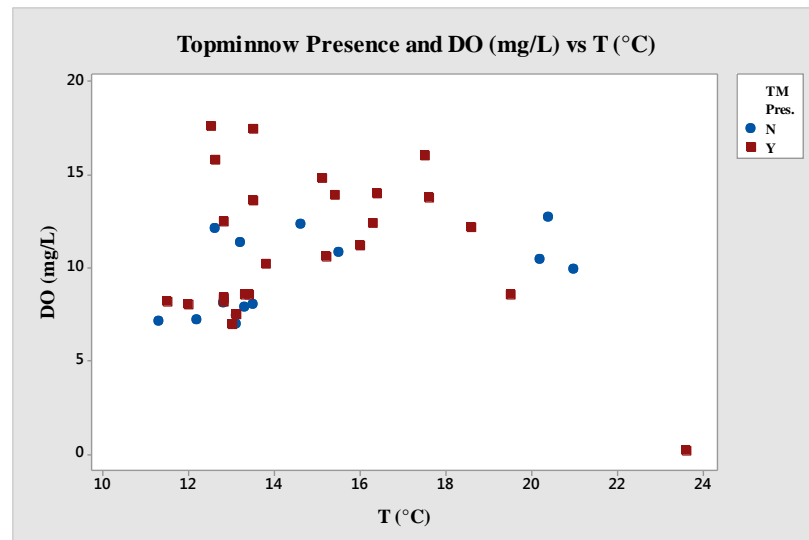


Figure 6.3. Topminnow Presence and DO (mg/L) vs T (°C) Scatterplot showing regression of topminnow presence with DO and T variables. Y and N = present and not present, respectively. “Yes” R-sq. = 0.042. “No” R-sq. = 0.260. No significant relationship was observed with either variable.

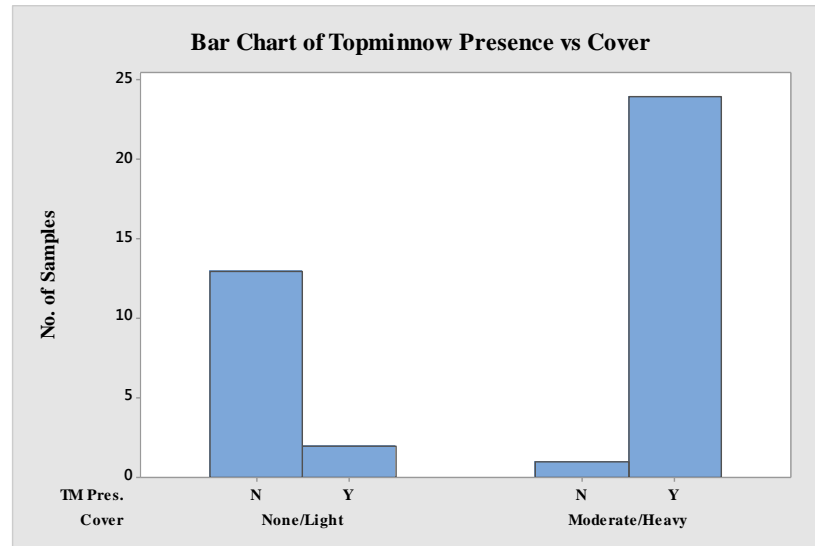


Figure 6.4. Bar chart of Topminnow Presence vs Cover. Presence of plains topminnow in relation to plant cover.

6.4. STATEWIDE DISTRIBUTION

Fish community data provided by the Missouri Department of Conservation was analyzed to observe changes in plains topminnow and western mosquitofish populations throughout their Missouri range from 1930-2010. Also, samples including plains topminnows and western mosquitofish within the same sampling location were extracted and analyzed for a significant difference in numbers of each species collected in MDC samplings. An overlay was constructed with ArcMap 10.1 to illustrate distribution changes for each species per county in 20 year segments. From 1930 – 1950, *F. sciadicus* was found in 14 counties, 12 in mid/south central region of the state and two in the southwest corner. *G. affinis* was found in 15 counties during this time period ranging from the southeast corner to north of St. Louis, MO. Most counties inhabited by *G. affinis* during this time period bordered the Mississippi River (Figure 6.5). 1950-1970 showed a

29% decrease in the number of counties where *F. sciadicus* were found (N=10). *G. affinis* distribution increased by 47% on the county level for a total of 22 counties inhabited, including one with *F. sciadicus* presence (Figure 6.6). From 1970-1990, *F. sciadicus* was found in 9 counties. *G. affinis* was found in 50 counties, three of which were shared with *F. sciadicus* (Figure 6.7). The 1990-2010 time period showed a slight increase of counties with *F. sciadicus* going back up to 14 counties. However, a substantial increase of *G. affinis* was found with it being present in 79 counties (527% increase), including all 14 counties inhabited by *F. sciadicus* (Figure 6.8). HUC 12 data overlays were made showing plains topminnow and western mosquitofish distribution on a sub-watershed basis in Missouri. The two species were found to share a total of 31 HUC 12 units in south-central and southeast Missouri (Figure 6.9).

For samples sharing *F. sciadicus* and *G. affinis*, counts of each species were converted to decimal form by dividing the number of each species by the combined total present of both species. This accounted for outliers and made for a more fulfilling statistical analysis. A two-sample t-test was used to analyze differences in percentage between sample means. There was a significantly high mosquitofish : topminnow ratio ($p < 0.001$), showing that western mosquitofish greatly outnumber plains topminnows when the two species were found together (Figure 6.10).

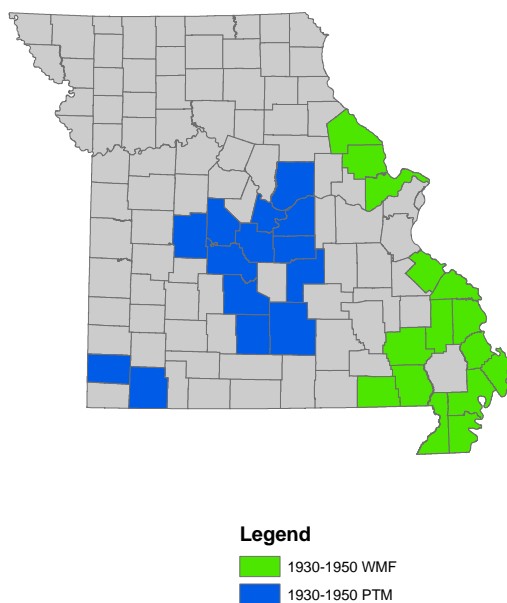


Figure 6.5. Plains topminnow and mosquitofish dist. (1930-1950). Plains topminnow (PTM) and western mosquitofish distribution per county from 1930-1950.

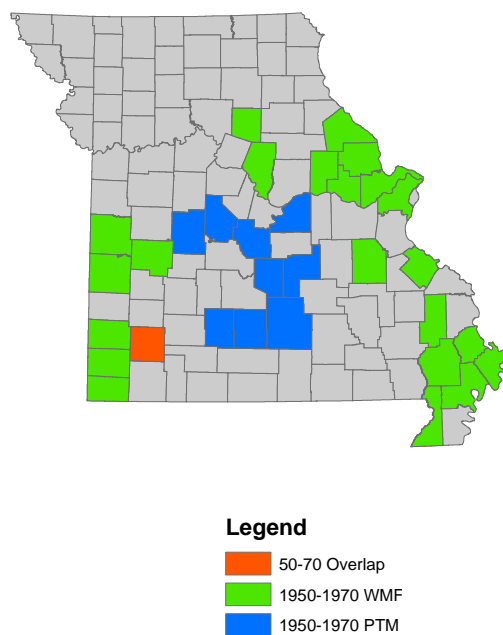


Figure 6.6. Plains topminnow and mosquitofish dist. (1950-1970) Plains topminnow (PTM) and western mosquitofish distribution per county from 1950 – 1970 with overlap.

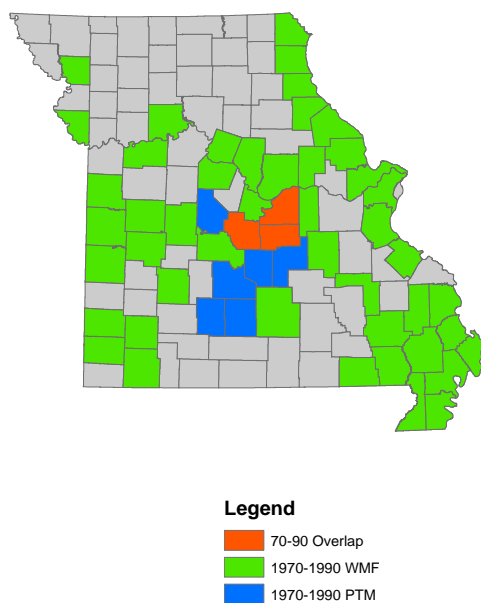


Figure 6.7. Plains topminnow and mosquitofish dist. (1970-1990) Plains topminnow (PTM) and western mosquitofish distribution per county from 1970-1990 with overlap.

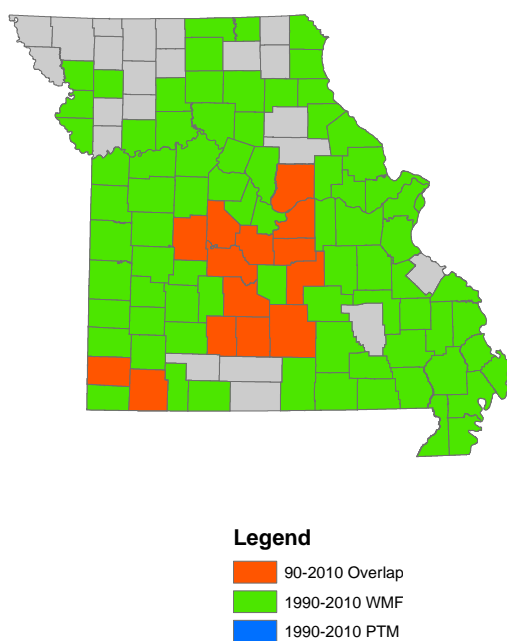


Figure 6.8. Plains topminnow and mosquitofish dist. (1990-2010) Plains topminnow (PTM) and western mosquitofish distribution per county from 1990-2010.

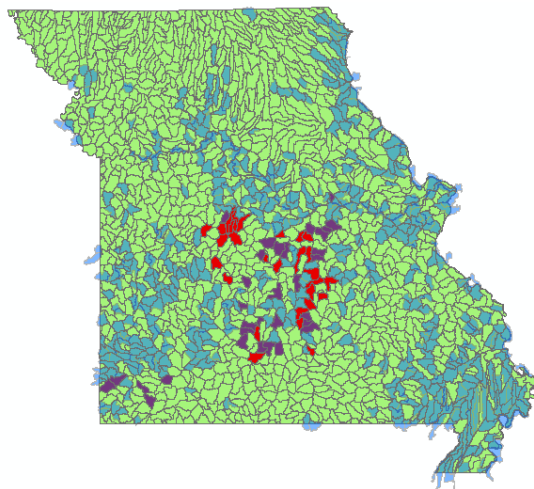


Figure 6.9. Hydrologic Unit code (HUC) 12 overlays. Green = HUC, Blue = Western mosquitofish, Red = Plains topminnow, and Purple = overlap of both species on a sub-watershed basis.

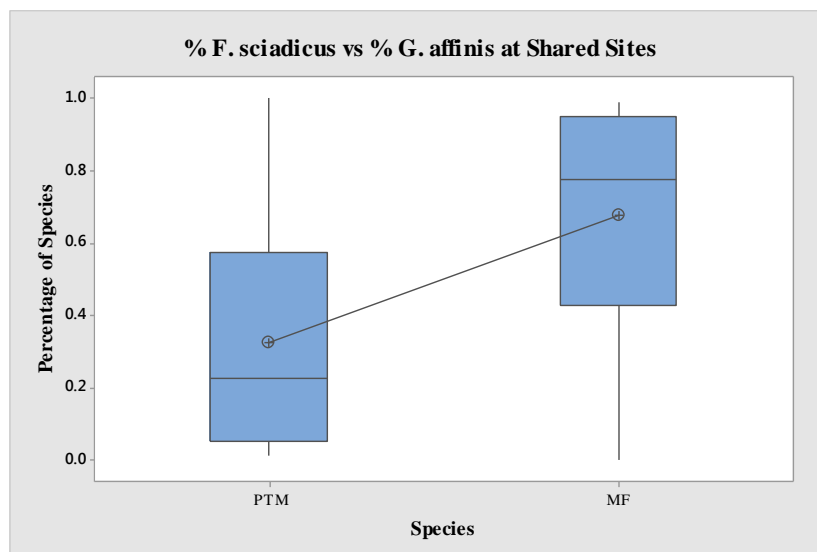


Figure 6.10. Percent plains topminnow (PTM) vs mosquitofish. (MF) at shared sites. Totals for each site were combined, then number of each species divided by combined total. PTM mean = 33% of total per site; MF mean = 67% of total per site.

6.5. COMPETITION EXPERIMENT

There was substantial mortality during the 36 day competition experiment in lab microcosms. Topminnow mortality was more prevalent in adult treatments (75%) than juvenile treatments (33%). Western mosquitofish mortality was also higher in adult topminnow treatments (67%) than juvenile topminnow treatments (0%). Signs of fin-nipping were monitored daily. Fish showing class 1 signs of fin-nipping would often remain at this level for 2-4 days. When class 2 fin-nipping was observed, those fish rarely survived 2 more days before sustaining class 3 injuries, resulting in death (Figure 6.11). Plains topminnows were targeted first by territorial male topminnows, followed by the mosquitofish as topminnows became unavailable to attack (Figure 6.12). Aggression was seen only in one juvenile tank, where one male topminnow had reached sexual maturity and was placed in a juvenile treatment by mistake. There was no such behavior observed in juvenile tanks containing the correct age group of young of the year topminnows. The topminnow control tank had a mixture of three adults and three juveniles, but no aggression was observed. One juvenile died in the topminnow control due to stress from handling. Adult treatments saw high aggression and mortality (83% topminnow, 78% mosquitofish). One male topminnow in two adult replicates became highly territorial and demonstrated both intra and interspecific aggression. A male in the remaining adult treatment only showed intraspecific aggression, killing all other plains topminnows, but not harming the four western mosquitofish. This observation demonstrates that the introduction of western mosquitofish was triggering extreme male territoriality in two adult treatments where all other fish were killed by one dominant male.



Figure 6.11. Evidence of Aggression. Plains topminnow (left) and western mosquitofish killed by fin-nipping.

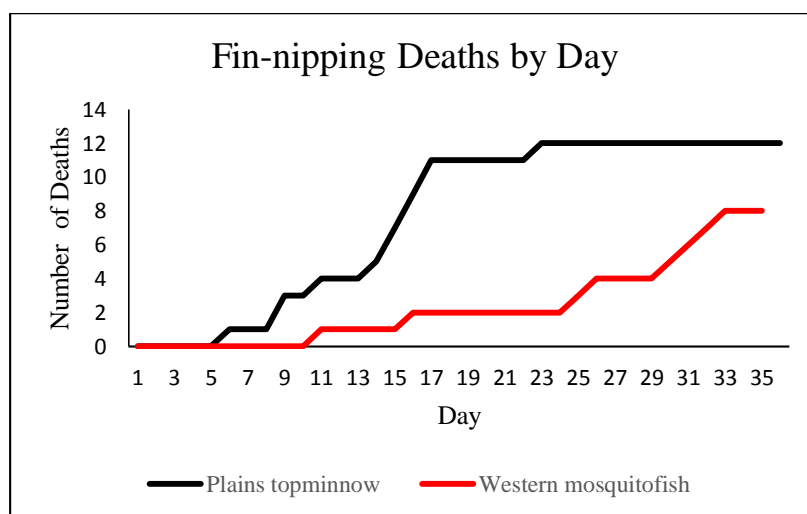


Figure 6.12. Fin-nipping Deaths by Day. Number of plains topminnow and western mosquitofish fin-nipping deaths as they occurred throughout the 35 day experiment.

Paired t-tests were used to analyze the differences in TL and TBM for *F. sciadicus* and *G. affinis* before and after the 34 day experiment. When grouped together, juvenile and adult topminnow treatments combined did not have a significant decrease in TL at the end of the experiment (p-value = 0.015, 99% CI). There was also no significance in the amount of TBM lost for this grouping (p-value = 0.052, 99% CI) (Figure A-1). When juvenile and adult topminnow groups were separated and a new paired t-test run for each, there was no significant difference between juvenile TL (p-value = 0.275, 99% CI) or TBM (p-value = 0.560, 99% CI) (Figure A-2). Adults did show significant loss in TL (p-value = 0.001, 99% CI) and TBM (p-value = 0.005, 99% CI) (Figure A-3). Control differences are reported in percent increase/decrease due to a lack of data. The topminnow control showed a -5.3% and -37% percent change in TL and TBM, respectively.

Western mosquitofish were randomly assigned to each treatment and not categorized as adults vs juveniles, so paired t-tests were only run on the overall sample of this species. There were no significant differences in mosquitofish TL (p-value = 0.272, 99% CI) and TBM (p-value = 0.473) between treatments (Figure A-4). The mosquitofish control showed an overall increase in TL and TBM, 20% and 45%, respectively. The body mass of the surviving fish from the treatments all increased during the 36 day experiment (Figure 6.13). Juvenile topminnows increased body mass by 35%, adult topminnows by 21%, and western mosquitofish by 20%. These differences among the fish in the treatments were not significant ($F = 1.5$, $P = 0.27$).

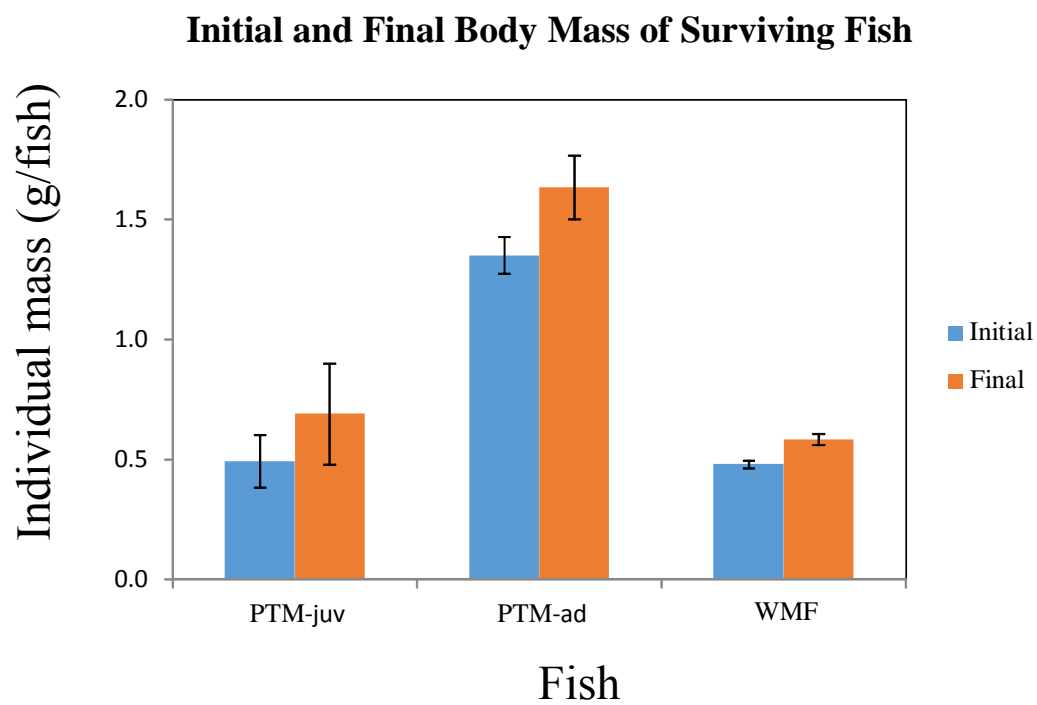


Figure 6.13. Initial and Final Body Mass of Surviving Fish. Initial and final mean body mass per individual surviving fish for the 36 day experiment.

7. DISCUSSION

In recent years, declines in native Great Plains fish species have become apparent. Among these declining species is the plains topminnow, which has declined within its two main populations centered in central Nebraska and south-central Missouri (Rahel and Thel 2004; Haas 2005; Fischer and Paukert 2008). In Missouri, *F. sciadicus* is a species of special concern (Fischer and Paukert 2008), but few studies have examined the ecology of these populations. In Nebraska, it is thought that anthropogenic disturbances such as stream impoundments, channelization, and non-native fish introductions are the key factors in plains topminnow declines. The aggressive western mosquitofish is thought to be associated with plains topminnow declines in Nebraska, as they show great overlap in habitat and feeding preferences (Pflieger et al. 1997; Haas et al. 2003; Rahel and Thel 2004; Haas 2005). Western mosquitofish are able to reproduce much faster than the plains topminnow and have been known to feed on juvenile topminnows (Pflieger et al. 1997; Haas 2005). Studies have found western mosquitofish to impose serious threats on *Fundulus* species, such as the Barren's topminnow of Tennessee and Sonoran topminnow of the southwestern United States (Laha and Mattingly 2007; Hedrick and Hurt 2012). Due to declines in plains topminnow populations and the need for basic ecology data on this species, the present study outlines food requirements, habitat preferences, and size distribution of a small plains topminnow population located in the Missouri Ozarks. And overall analysis of statewide MDC sampling data was also used to associate the invasion of western mosquitofish with changes in plains topminnow populations throughout several Missouri counties and watersheds. Finally, a competition experiment was

conducted to examine the possible effects of western mosquitofish on adult and juvenile plains topminnows selected from a small population in Phelps County, Missouri.

7.1. HABITAT ANALYSIS

My small-scale habitat analysis of Dewitt Pond backed up previous evidence that *F. sciadicus* is highly dependent on refuge provided by macrophytes, algae, and woody debris. The various habitat types provided within Dewitt Pond allowed me to examine plains topminnow presence/absence in relation to dissolved oxygen, temperature, and cover. Plains topminnows were present across a wide range of dissolved oxygen and temperature, showing no significant relationship between topminnow presence and the two variables. However, plains topminnows showed a highly significant presence when the amount of vegetative cover was moderate or high. Density of the cover affected both DO and T in Dewitt Pond by decreasing DO due to an increase in T and organism respiration. This relationship had no effect on topminnow presence, though, as long as plenty of cover was available. Water depth was noted during each environmental sampling, but was not used a variable, as it was obvious that the topminnows ranged quite evenly over a wide range of depths in Dewitt Pond (~0.02– 1.50 m) depending on the amount of cover available.

Although environmental factors were not measured at the Elm Spring site, very similar relationships were apparent upon each visit. Woody debris and aquatic plants were moderately dense in both the wetland section of this site and the outskirts of the main channel. Both sections of the site contained dense numbers of *F. sciadicus*, but they were never present within the channel itself. Since plains topminnows move out of shallow areas in the winter and into deep pools (Pflieger et al. 1997), they will most

likely move out of the wetland section and use the channel as winter refuge. This movement to deeper water does not take place in Dewitt Pond. However, I have visited this site several times during extremely cold periods of winter and found large numbers of plains topminnows in shallow (<4 cm) water that remains unfrozen due to the input of warmer water from Wilkins Spring.

7.2. GUT CONTENTS

Topminnow diet at the two sites showed an interesting array of prey items, fairly consistent with past findings from other studies (Stribley and Stasiak 1982), but including a broader range of invertebrate families and one amphibian larva (tadpole) most likely belonging to *Hylidae* (tree frogs). Stribley and Stasiak (1982) found that topminnows sampled in Nebraska fed primarily on Ostracod crustaceans, snails of genus *Physa*, and larval forms of Chironimidae and Simuliidae. The large amount of filamentous algae in fish from both sites could most likely be attributed to accidental ingestion when feeding on juvenile gastropods, which made up 17% of their overall diet. Pfieger et al. (1997) also noted large amounts of filamentous algae found in plains topminnow stomachs for similar reasons. Young gastropods attached to filamentous algae throughout the entirety of the water column were common throughout all areas of Dewitt Pond and Elm Spring. Topminnows were commonly observed in small groups (4-7) feeding on small snails from clumps of filamentous algae. The methods of non-lethal gut content sampling used seemed to remove what one would think of as a majority of what would logically fit in one of these fish's stomachs, but there was no way to determine the successful removal rate unless fish were killed and gut contents were surgically removed, which was not done. Topminnows containing large amounts of juvenile gastropods were difficult to

remove all contents from, as the snails would plug the esophagus and make removal difficult. In these cases, most snails were removed to unclog the esophagus before release, but there is a high likelihood that other prey items were present in the gut.

7.3. SIZE DISTRIBUTION

The size distribution section of my study was quite successful in sampling the population as a whole. The normal distribution illustrated by the histogram (Figure 6.1) shows successful sampling of a broad range of topminnow sizes. Once divided into age classes using size as the determining factor, it was apparent that recruitment in the Dewitt Pond population is high. This signifies that there is little stress on this population through competition with other species present, such as the Ozark minnow (*Notropis nubilus*) and least darter (*Etheostoma microperca*). Although the size-age relation results found by Stribley and Stasiak (1982) were used as a reference for this study, environmental factors such as water temperature and food availability may result in some error. The only species of predatory fish found in Dewitt Pond were green sunfish (*Lepomis cyanellus*) (personal observation). However, this fish was present in small numbers and were normally seen in water outside of preferred habitat of plains topminnows throughout the pond. They most likely have little effect on plains topminnows through predation. River otters and blue herons frequent Dewitt Pond, but they most likely feed on the large numbers of crayfish, which are easier prey.

Some factors affecting sample consistency while collecting size distribution data were the amount of macrophytes and algae present, and the size mesh of the seine. Dense algae and aquatic plants made it difficult in many instances to use the seine at its maximum capacity (4.5 meter length x 1 meter height), resulting in fewer topminnow

captures and requiring multiple attempts at different sites around the pond. The seine mesh size was 0.64 centimeters, which was large enough to allow passage of newly hatched topminnow fry, first seen in late June, but not captured until August and later.

7.4. STATEWIDE DISTRIBUTION

F. sciadicus populations appear to be relatively stable in Missouri when examined on a county scale. Since 1930, however, they have become exposed to a rapidly increasing population of *G. affinis* throughout the state. This raises concern for possible competition between the two species, as *G. affinis* has demonstrated its ability to displace native topminnow species through competitive interactions. Their aggressive behavior and rapid reproduction (Haas et al. 2005) allow them to successfully outnumber *F. sciadicus* in some habitats where both species are found. In my study, evidence of this was seen in the data provided by the Missouri Department of Conservation. Significantly higher numbers of *G. affinis* in *F. sciadicus* habitat indicates some sort of competition between the two species in Missouri in which *G. affinis* comes out as the victor. Understanding the mechanisms driving this competition could be crucial for *F. sciadicus* survival in Missouri and other states where the two species occur together. The results from this study demonstrate the ability of western mosquitofish populations to spread quickly and successfully. Their relatively rapid spread throughout Missouri is consistent with findings in Nebraska, where Haas (2005) noted the western mosquitofish populations spreading at a rate of 10 km/year upstream and 20 km per year downstream in the Platte and Republican Rivers. According to my findings when comparing local abundance of plains topminnows in the presence of western mosquitofish, topminnow presence is significantly less. This may be due to diet overlap (Stribley and Stasiak 1982;

Pflieger et al. 1997), competition (Haas 2005), or predation by mosquitofish on juvenile plains topminnows (Laha and Mattingly 2007).

The statewide distribution of both species over the last 80 years demonstrates a high successful introduction/invasion of western mosquitofish into the native habitat of the plains topminnow. The MDC data used for this portion of the study was contingent on the amount of effort of each sampling. Many samples were collected using drag seines, but dip-netting, electrofishing, and rotenone were also techniques used for data collection. Each technique has varying success when collecting fish samples, so the number of fish captured is likely relative to the type of sampling used and the length of sampling time. Sampling effort may be the cause for the decline in the number of counties found to have plains topminnows from 1950-1990, and not an actual loss in populations. It is also unknown whether or not the exact same locations were used for each sampling. This could be a factor causing an apparent decline in historic plains topminnow populations for the 1950-1990 collection period.

One concern when examining the results of this aspect of my study was the lack of plains topminnow dispersal from historical sites. Perhaps stream impoundments such as dams and poorly implemented road crossings hinder topminnow migration when current habitat becomes undesirable. Schumann et al (2014) discuss migration requirements of plains topminnows as a mechanism of re-establishing historic populations and colonizing new sites as they appear and disappear with ever-changing aquatic environments. According to their findings, plains topminnow distribution should have increased throughout Missouri since the 1930's. This indicates underlying

mechanisms causing resistance of plains topminnow migration and colonization from existing populations within the state that will require further attention in the future.

Fischer and Paukert (2008) indicated the negative effects of stream impoundments, siltation, and non-native fish introductions on plains topminnows in Nebraska. They found plains topminnows to be absent at 75% of the historical sites they visited. This decline is paralleled by losses of other native Great Plains fish, such as the federally endangered Topeka shiner and Nebraska endangered northern redbelly dace. Fischer and Paukert (2008) found non-native generalist species (i.e. creek chub, green sunfish, and common carp) to be most associated with plains topminnow absence. Other introduced species such as the brown trout, smallmouth bass, and western mosquitofish were also associated with plains topminnow absence. The introduction of non-native species (Fischer and Paukert 2008) combined with changes to surface and groundwater flow, and growth of urban development in the Great Plains (Rahel and Thel 2004) are the greatest threats presented to plains topminnows in Missouri. These aspects may be the driving force preventing further distribution of Missouri plains topminnows, which was apparent in this study.

7.5. COMPETITION EXPERIMENT

The laboratory experiment testing competition between plains topminnows and western mosquitofish showed unexpected results. I hypothesized that juvenile topminnows would be more affected than adults by mosquitofish presence. However, it was adult topminnows that experienced higher mortality. Throughout the experiment, the fish were observed daily for signs of fin-nipping and starvation. In two of the adult topminnow replicates, one male topminnow became highly territorial, killing all other

fish in the tank by fin-nipping to a degree leading to death. In the other adult topminnow replicate, one male became very territorial, but only killed the other topminnows, leaving the mosquitofish unharmed. The survival of mosquitofish in this adult treatment may be explained by the unfamiliarity of western mosquitofish to the dominant topminnow. The unfamiliar species may have caused some sort of fear in the male topminnow, causing him to shy away. Also, after killing all other topminnows, competition within the tank declined, possibly causing the male to be secure with the remaining fish. If aggression was due to residual spawning behaviors, it is likely the male topminnow may have only felt the need to kill other topminnows as part of their territorial spawning behavior. The loss of adult plains topminnows in the competition experiment due to western mosquitofish presence shows that male plains topminnows may become very territorial when experiencing competition with this non-native fish. This follows a comment by Pflieger et al (1997), in which he noted the difficulty of housing plains topminnows with other species within one aquarium. Male plains topminnows naturally have an aggressive and territorial disposition during the spawning season (Pflieger et al. 1997; Haas et al 2003). Though this study was conducted post-spawning, it may be likely there were still some residual spawning behaviors in effect, causing some male topminnows to engage in territorial behavior normally seen during the main portion of the spawning season.

Haas et al (2003) found large plains topminnows, presumably adults, to show higher survivorship compared to juvenile topminnows when mixed with western mosquitofish in aquaria. Their study also found the western mosquitofish to be more aggressive than the plains topminnow, resulting in higher interspecific competition. This is contradictory to observations in my study, which found topminnow mortality to occur

almost strictly from intraspecific competition. Such contradicting results may stem from genetic differences between topminnows used in the Haas et al (2003) study compared to those used in this study. O'Hare (1985) found plains topminnow populations in Missouri and Nebraska to be genetically different. Missouri populations had also diverged from one another within the state. Also, the topminnows in this study were exposed to only a few fish species in Dewitt Pond before their capture and have co-inhabited with only those species for many generations. Exposure to an unfamiliar species of such similar attributes and habits as the plains topminnow may have triggered a response mechanism not yet witnessed in any other plains topminnow competition experiments.

7.6. IMPLICATIONS

The results of this study present important ecological and habitat preference data for *Fundulus sciadicus* in Missouri, a topic for which very little information has been collected. Most other studies involving *F. sciadicus* have taken place within the larger disjunct portion of their population in Nebraska, which provides important data, nonetheless, but in a region geographically different from the Missouri Ozarks.

Since *F. sciadicus* is a species of special concern in Missouri, conservation efforts may be needed in the near future to help protect the second largest disjunct population of this species. The rapid spread of *G. affinis* throughout the plains topminnow habitat in Missouri since 1930, combined with the findings of my competition experiment, indicate the likelihood that *G. affinis* could be displacing *F. sciadicus* in several historical topminnow sites. It should be noted that *G. affinis* and *F. sciadicus* prefer very similar habitats, based on where the fish were collected in my study (*F. sciadicus* from the Mill Creek watershed, *G. affinis* from Little Prairie Conservation Area). Both species prefer

vegetative cover along the littoral zone of lakes and ponds. Fortunately, the Mill Creek watershed sampling sites did not have *G. affinis* yet, and efforts should be focused on keeping them from invading.

It may be necessary to recreate new habitat for plains topminnow reintroductions throughout Missouri to maintain healthy populations of this species. Creating isolated habitats on conservation land could limit the possibility of mosquitofish infestation through immigration. The food and habitat data in this thesis provides important information for designing new habitat and getting it established to a point where *F. sciadicus* could thrive. The size distribution data could be used as a key for monitoring population health throughout a 3-4 year period to ensure successful recruitment of young to sexually mature adults and beyond.

7.7 FUTURE RESEARCH

My research is hopefully just the beginning of more *F. sciadicus* studies to come in Missouri. Since anthropogenic changes, such as damming, channelization, wetland draining, and introduction of non-native species, have been shown to have negative effects on plains topminnow populations in Nebraska, it is necessary to examine these factors for Missouri and other states. The lack of plains topminnow dispersal throughout Missouri should be addressed. This could likely be due to the several anthropogenic disturbances highlighted in this thesis. Perhaps a study similar to that of Schumann et al (2014) should be conducted in Missouri watersheds containing plains topminnows to examine their migration and colonization patterns in the state. It is apparent that something is hindering these abilities and it should be brought to light in order to keep healthy populations of plains topminnows in their current habitats.

To examine the true effects of western mosquitofish on plains topminnows in Missouri, it may be necessary to examine interactions of these species in a natural environment, such as an MDC sampling site containing both. Also, migration capabilities should be examined for western mosquitofish in Missouri. Their exponential population growth since 1930 demonstrates just how quickly they can invade new habitat. Rapid reproduction and an ability to survive a broad range of environmental conditions are surely factors in this, but whether their spread is due to natural migration or artificial stocking is unknown. However, it is likely a result of both. Haas (2005) noted western mosquitofish introductions through “bait bucket” dumping, a possible problem occurring throughout Missouri. The use of western mosquitofish as a biological mosquito control method could compound the “bait bucket” issue, although western mosquitofish provide little mosquito control abilities compared to native topminnow species. Awareness of this issue should be considered in Missouri to prevent the possible loss of the plains topminnows in one of its last remaining population strongholds.

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VITA

Gregory Travis Thompson was born in Creve Coeur, Missouri. He graduated high school in 2005 and began studying at Missouri Valley College. After his first semester, Travis joined the United States Marine Corps Reserves where he served for six years. After initial training was completed, he started college again at the University of Central Missouri until his deployment to Iraq in 2009 as a turret gunner. After returning home, he started school again at the University of New Orleans in August 2010 to be closer to the woman who is now his wife. Here, he worked under Dr. Thomas Soniat as an undergraduate laboratory assistant doing research on eastern oyster parasites collected from the Gulf of Mexico. In January 2011, he and his wife moved to Kansas City, Missouri, where Travis began school at the University of Missouri-Kansas City. While attending school at UMKC and working at Lowes, he assisted in small mammal research with Dr. Aaron Reed and job shadowed regional fish and wildlife biologists with the Kansas Department of Wildlife and Parks. After receiving his B.A. in Biology in 2012 from UMKC, Travis began his graduate studies under the advising of Dr. Dev Niyogi at the Missouri University of Science and Technology in Rolla, Missouri. After graduating in December 2014, he began work full-time as a Hydrologic Technician at the U.S. Geological Survey.