The Effects of Land Use and Climate Change on Playa Wetlands and Their Invertebrate Communities.

by

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# Table of Contents

ACKNOWLEDGMENTS ........................................................................................................... II

TABLE OF CONTENTS ......................................................................................................... IV

ABSTRACT ................................................................................................................................. VI

LIST OF TABLES ....................................................................................................................... IX

LIST OF FIGURES ..................................................................................................................... X

CHAPTER 1:.............................................................................................................................. 1

INTRODUCTION ....................................................................................................................... 1

   Literature Cited: ................................................................................................................... 5

CHAPTER 2:.............................................................................................................................. 9

CLASSIFYING LAND-USE CHANGES AND EFFECTS ON PLAYA
WETLAND INUNDATION ON THE SOUTHERN HIGH PLAINS USING
REMOTE-SENSING TECHNIQUES ......................................................................................... 9

   Introduction: ...................................................................................................................... 9

   Methods: .......................................................................................................................... 14

      Study Area: .................................................................................................................. 14

      Approach: .................................................................................................................... 15

   Results: .......................................................................................................................... 19

   Discussion: ...................................................................................................................... 20

   Literature Cited: .............................................................................................................. 28

CHAPTER 3:............................................................................................................................ 53
LIFE HISTORY AND LAB HUSBANDRY OF ENALLAGMA CIVILE
(HAGEN) (INSECTA, ODONATA, COENAGRIONIDAE) .......................... 53

Introduction: ........................................................................................................... 53
Untapped Potential as a Model Organism: .............................................................. 53
Life History Review: ................................................................................................. 56
Lab Husbandry: ........................................................................................................ 63
Experimental Results and Discussion: ................................................................. 68
Conclusions: ............................................................................................................. 72
Literature Cited: ....................................................................................................... 73

CHAPTER 4: ............................................................................................................ 94

EFFECTS OF WATER TEMPERATURE UNDER PROJECTED CLIMATE
CHANGE ON THE DEVELOPMENT AND SURVIVAL OF THE FAMILIAR
BLUET DAMSELFLY (ENALLAGMA CIVILE) ....................................................... 94

Introduction: ............................................................................................................. 94
Methods: ................................................................................................................... 99
Results: ................................................................................................................... 102
Discussion: .............................................................................................................. 104
Literature Cited: ..................................................................................................... 111

CHAPTER 5: .......................................................................................................... 128

CONCLUSION ........................................................................................................... 128
Abstract

Climate and land-use changes are the primary threats to playa wetlands and their invertebrate communities. Playas are ephemeral, depressional wetlands that are the primary form of surface water in the Southern High Plains of North America, an area that has experienced recent land-use changes that may affect playas. I used remotely sensed imagery to assess changes in land use in five categories (agriculture, rangeland/grassland, fallow, developed, and water) and playa inundation in Texas on six dates during the late growing season over 23 years. A decrease in the number of wet playas was observed over that span, and significant differences among land uses were found between and within years around dry and wet playas. Mean patch size and overall area of rangeland/grassland increased over time, possibly due in part to conservation efforts in the area. Other land-use types consequently decreased, but agriculture remained one of the dominant land-use types throughout. Because playas are crucial habitats, these changes have likely affected regional biodiversity.

Playa-associated biodiversity is largely comprised of birds, amphibians, and invertebrates. The Familiar Bluet (Hexapoda: Odonata, Coenagrionidae, Enallagma civile) was selected as a model organism to study the effects of environmental changes on playa invertebrates. Odonates are good model organisms to study ecological and evolutionary concepts because of their amphibious life history, which makes them sensitive to both aquatic and terrestrial environmental changes. Enallagma civile is a habitat generalist with a widespread distribution throughout the New World that has
been underutilized in research. I summarized its life history and described lab husbandry techniques in an overview of the species as a potential model organism for studies on environmental subjects like climate change effects on elevated water temperature.

Current climate change predictions estimate increased air temperatures across the Southern High Plains, putting many organisms at risk from environmental changes affecting nymph and adult life stages. Increased air temperatures can lead to elevated water temperatures, but experiments are lacking on responses in terms of development or survival. *Enallagma civile* was used to examine these effects. Eggs were collected and reared under four water temperature regimes (26, 32, 38, and 41°C). Nymph body measurements after molts, development rate, and deaths were recorded daily. Nymphs in the two hotter treatments were smaller and had lower survivorship whereas individuals in the cooler temperatures generally survived to adulthood and were larger. Individuals reared at 32°C emerged the quickest, going from egg to adult in 26 days. Elevated temperatures can thus be both advantageous and detrimental, causing concern for aquatic invertebrates in the future.

In conclusion, these studies have demonstrated how land-use and climate changes are threats to playa wetlands and biota. With rangeland/grassland increasing over time, the frequency of playa wetland inundation may continue to decrease due to interactions between land use and overland water flow during precipitation events. With decreases in playa inundation frequencies and effects of climate change, playa invertebrate communities are threatened due to infrequent standing water and elevated
water temperatures. By understanding how land-use and projected climate changes are currently effecting playa wetlands, it will allow for better comprehension and management of current and future alterations.
List of Tables

2.1: Class- and landscape-scale metrics calculated in FRAGSTATS 4.2.1, with each identified as to whether it quantifies landscape composition, configuration, or connectivity. See McGarigal (2015) for details on how each metric is calculated. ................................................................. 37

2.2: Composition of land-use types across the landscape by year and total percent change over 23 years. ........................................ 38

2.3: Playa basin dry to wet ratio by land-use classification and year. .................................................................................................... 39

3.1: Reproductive information for *E. civile* collected on five dates from 2013-2014. ............................................................... 85

3.2: Survivorship and time until hatching of *E. civile* eggs from 2014 experiments. ................................................................. 86

4.1: Reproductive information for *E. civile* from summer 2014 ........................................................................................................... 119

4.2: Survivorship and time until hatching of *E. civile* eggs from 2014 experiments. ................................................................. 120
List of Figures


2.1: Map of the study region (black polygon): Landsat 5 Thematic Mapper (TM) WRS-2 P030/R036. Playa locations are denoted by blue dots and the Ogallala Aquifer is shown as the gray-shaded region. ................................. 40

2.2: Yearly total precipitation amounts recorded from Amarillo International Airport. Precipitation average (black line) of 51.3 cm (1900 – 2016) and study years (blue bars) are denoted. ................................. 41

2.3: Average monthly precipitation amounts (1900 – 2016) recorded from Amarillo International Airport. ................................. 42

2.4: Land use classification for 11 September 1986. ................................. 43

2.5: Land use classification for 30 September 1987. ................................. 44

2.6: Land use classification for 8 October 1996. ................................. 45

2.7: Land use classification for 25 September 1997. ................................. 46

2.8: Land use classification for 5 September 2007. ................................. 47

2.9: Land use classification for 22 August 2008................................. 48
2.11: Histogram displaying the number of wet playas during each study year. ................................................................. 50

2.12: A) Percent of landscape, B) number of patches, and C) mean patch area for each of the five land-use classes: green solid – agriculture, light brown dash – rangeland/grassland, dark brown dots – fallow, black bar and dots – developed, blue large dash – water ...................... 51

2.13: Dominant land uses surrounding wet and dry playas on study dates from 1986 – 2008. Chi-squared analysis performed each year comparing land use classes, with numbers in parentheses indicating degrees of freedom and sample size. ................................................................. 52

3.1: A. Adult E. civile female. B. Adult E. civile male. C. E. civile nymph. ................................................................. 87

3.2: Distribution of Enallagma civile. Shaded areas represent states, provinces, and countries where the species has been found. This does not mean that the species is found throughout the entire shaded area. ................................................................. 88

3.3: A. Male and female E. civile in wheel position. B. E. civile pairs in tandem with females oviposting in floating vegetation................................................................. 89

3.4: Mae Simmons Park, Lubbock, Texas (Pictometry International Corp., 2015). ................................................................. 90

3.5: A. Oviposition chambers. B. Experimental rearing chambers. C. Rearing containers with dowel rod and netting to enclose emerging adults................................................................. 91

3.6: Experimental tanks heating and cooling patterns over a 24 hour period. Temperatures recorded using a HOBO temperature logger. ................................................................. 92

3.7: A. Eggs after they were laid. B. Eggs with eye spots. C. Eggs with eye spots and legs apparent. D. Stadia 8
nymph. E. Stadia 6 nymph with developed trachea. F. Stadia 2-4, wing pads flat with veination present. G. Stadia 0-1, wing pads with rolled orientation. 

4.1: Egg hatch rate by temperature treatment ($\chi^2_{3} = 228.04$, $p < 0.0001$). Columns denoted with the same horizontal bar and letter were not significantly different (Tukey’s HSD $p > 0.05$). 

4.2: Mean number of days for eggs to hatch by temperature treatment ($F_3 = 38.50$, $p < 0.0001$). Columns denoted with the same letter were not significantly different (Tukey’s HSD $p > 0.05$). 

4.3: Survival curves for E. civile nymphs at four water temperatures (Wilcoxon $\chi^2_{3} = 209.45$, $p < 0.0001$). 

4.4: Percent emergence of adult damselflies by temperature treatment. Columns denoted with the same horizontal bar and letter were not significantly different (Tukey’s HSD $p > 0.05$). 

4.5: Length of time from egg hatching to adult emergence and abundance by temperature treatments of E. civile. Earliest emergence amongst all temperature treatments occurred at day 26, whereas the last occurred at day 71. Individuals in the 41°C treatment did not survive to emergence so no data are shown for that temperature. 

4.6: Adults had smaller body lengths at hotter temperatures. Individuals in the 41°C treatment did not survive so no measurements could be taken. Boxes denoted with the same letter were not significantly different (Tukey’s HSD $p > 0.05$). Lines in each of the boxes represent the 25th, median, and 75th percentiles. The error bars (if present) represent the 10th and 90th percentiles (38°C is missing error bars due to sample size of 2). Dots represent outliers within the data.
4.7: Adult had smaller head width at hotter temperatures. Individuals in the 41°C treatment did not survive so no measurements could be taken. Boxes denoted with the same letter were not significantly different (Tukey’s HSD p > 0.05). Lines in each of the boxes represent the 25th, median, and 75th percentiles. The error bars (if present) represent the 10th and 90th percentiles (38°C is missing error bars due to sample size of 2). Dots represent outliers within the data. .......................................................... 127
Chapter 1:

Introduction

Freshwater wetlands are important habitat resources throughout much of the world, especially in arid and semi-arid areas. Wetlands are also sensitive to anthropogenic activities such as land-use change and climate change. The freshwater wetlands of the southern and central Great Plains of North America—playas (Figure 1.1)—are ecologically irreplaceable but have been subjected to recent, ongoing, and projected future land-use and climate influences. My dissertation research focused on describing how past and current land-use history affects playa inundation and how future projected warming may affect a model playa-associated organism. I provide a review of the life history of that organism and discuss lab husbandry of it in the hopes that it may become more commonly used as a model organism for other ecological studies.

Playa wetlands are “shallow, depressional recharge wetlands formed by a combination of wind, wave, and dissolution processes with each wetland existing in its own watershed” (Smith, 2003). Playas only receive water from precipitation and overland runoff (chiefly during the rainy season from April through September in the Southern High Plains; NOAA, http://w2.weather.gov/climate/xmacis.php?wfo=ama) and lose water by evaporation, evapotranspiration, and groundwater recharge (Smith, 2003). Playas are temporary wetlands, dry for much of the year with negative water
balances and varying lengths of hydroperiod (Smith 2003). They are typically less than 2 m in depth (Guthrey & Bryant, 1982; Smith, 2003) and cover an average area of 6.3 hectares, with 87% smaller than 12 hectares (Guthrey & Bryant, 1982). Under the Cowardin et al. (1979) wetland classification system, playas are classified as palustrine, lacustrine littoral, or lacustrine limnetic. The majority of playas are palustrine wetlands, followed by lacustrine littoral and lacustrine limnetic wetlands (Smith, 2003). Palustrine wetlands are nontidal wetlands possessing woody plants or persistent emergent vegetation covering more than 30% of the basin; any wetlands with less than 30% emergent vegetation must be smaller than 8 hectares and less than 2 m deep to be classified as palustrine (Cowardin, Carter, Golet, & LaRoe, 1979; Smith, 2003). Lacustrine wetlands are larger than 8 meters but cannot have more than 30% of the basin covered in emergent vegetation. This category of wetlands can be further divided into two classes: littoral and limnetic. Lacustrine littoral are under 2 m deep with no persistent vegetation, whereas lacustrine limnetic have water depths greater than 2 m (Cowardin et al., 1979; Smith, 2003).

Current estimations of playa numbers vary depending on classification system. The Playa Lakes Joint Venture dataset enumerates 89,798 playa basins throughout the Great Plains (PLJV, 2018). The Southern High Plains region of the Great Plains contains the highest density of playas in North America (Fish, Atkinson, Mollhagen, Shanks, Brenton, 1998; Howard et al., 2003; Johnson et al., 2012); the Playa Wetlands Database enumerates 21,893 playa within this region (Mulligan, Barbato, & Seshadri, 2014).
The Southern High Plains is semi-arid and can experience extreme temperatures, strong winds, and low moisture levels. As the predominant source of surface freshwater, playas are centers of biodiversity in the Southern High Plains (Bolen et al., 1989; Haukos & Smith, 1994; Smith, 2003), and an important ecological parameter of playa wetlands is hydroperiod (Anderson & Smith, 2004; Ghioca & Smith, 2008; Collins, Heintzman, Starr, Wright, Henebry, & McIntyre, 2014). Playa hydroperiod can be days to months in length; an examination of 8,404 playas found on the Southern High Plains over a four-year period (2008-2011) found the average minimum hydroperiod was 55.5 days and the maximum was 141.2 days (Collins et al., 2014). Playa faunal communities are dominated by aquatic, semi-aquatic, and terrestrial invertebrates, amphibians, and birds, with different taxa favoring different hydroperiod lengths (Hall, Willig, Moorhead, Sites, Fish, & Mollhagen, 2004; Smith, Haukos, & Prather, 2004; Bagwell, 2012).

Like most wetlands around the world, playas and their associated wildlife are threatened by human activities within their watershed, particularly land-use change (Brinson & Malverez, 2002). Since settlement, agricultural land use has dominated the Southern High Plains but cultivation practices have changed over time, causing landscape alterations. Such alterations can affect playa hydroperiod and a playa’s ability to hold water through draining, ditching, and creation of erosional sediments that fill in playa basins (Ghioca and Smith, 2008; Collins et al., 2014; Starr et al., 2016).
Climate change is another threat to playa wetlands. Current predictions for the Great Plains include an increase in mean air temperature by upwards of 7.2°C by 2090 (USGCRP, 2009), with decreases in annual precipitation and changes in precipitation intensity and timing (Matthews, 2008; USGCRP, 2009). Land-use and climate changes can alter playa hydperiod, which can then cause changes to playa biodiversity.

To examine the effects of land-use and climate changes on playa wetlands and their invertebrate communities, I employed multiple approaches. In Chapter 2, I use remote sensing techniques to classify land use by utilizing Landsat images on six dates that spanned 23 years to evaluate the effects of land-use types on playa inundation. Chapter 3 provides a review of the life history and lab husbandry techniques for the Familiar Bluet (*Enallagma civile*), which was a model organism subsequently used in Chapter 4. In Chapter 4, a lab experiment was performed to determine the effects of elevated water temperatures on the development and survival of *E. civile*, based on climate change projections for the Southern High Plains. Finally, Chapter 5 provides an overall summation of the conclusions of the preceding chapters. Together, these chapters provide an assessment of the effects of land use and climate change on playa wetlands and their invertebrate communities.
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Joint Venture region. Technical Publication #T-9-813, Department of Natural Resources Management, Texas Tech University, Lubbock, TX.


Figure 1.1: Map of the playa wetland locations within the Great Plains of the United States. Playa wetland locations (PLJV, 2018) are denoted by blue dots, red outline denotes the Southern High Plains (EPA, US Level III Ecoregions shapefile, https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states) and the Ogallala Aquifer (Texas Water Development Board Major Aquifers shapefile, http://www.twdb.texas.gov/mapping/gisdata/doc/major_aquifers.zip) is shown as the gray-shaded region.)
Chapter 2:  

Classifying land-use changes and effects on playa wetland inundation on the Southern High Plains using remote-sensing techniques  

Introduction:  

Anthropogenic activities affect biodiversity by altering the distribution of resources, creating heterogeneous and dynamic landscapes. Among these activities, land-use change stemming from land conversion (particularly for agriculture and urbanization) is currently considered the most important factor affecting the abundance, distribution, and extinction risk of organisms around the globe (Vitousek, Mooney, Lubchenco, & Melillo, 1997). Of all the resources being affected by land-use change, freshwater wetlands are among the most important yet imperiled habitats on Earth, providing unsubstitutable resources but also being highly vulnerable to alterations to their watersheds that can affect water quality, frequency of inundation, and hydroperiod (Brinson & Malverez, 2002). In the U. S., it has been estimated that ~50% of wetlands have been lost entirely to land conversion in the past 200 years (Dahl, 2000). Such effects would be particularly hard-felt in arid and semi-arid areas, where water is already a limited resource.  

The Great Plains of North America is a key agricultural region yet is also home to millions of freshwater wetlands. The Great Plains supplies most of the nation’s wheat, corn, and cotton, as well as numerous other crops (USDA, 2007), but the
conversion of its grasslands to row-crop agricultural land use has greatly impacted its wetlands (Wright & Wimberly, 2013). As a result, about 98% of the freshwater wetlands in the U. S. that have been lost in recent years have been ones from the Great Plains (Dahl, 2011). Although there have been some studies on how land-use change has affected wetlands in the northern Great Plains (Voldseth, Johnson, Gilmanov, Guntenspergen, & Millett, 2007; Wright & Wimberly, 2013), there is a lack of concomitant studies for southern Great Plains landscapes.

The Southern High Plains portion of the Great Plains was one of the last areas of the United States to be settled, with relatively recent land-use change and, thus, relatively recent effects on wetlands. Also known as the Llano Estacado or the “staked plains” (Sabin & Holliday, 1995; Leatherwood, 2010), the Southern High Plains consists of a high mesa bounded by the Canadian River in the north, the Caprock escarpment in the east, and the Mescalero escarpment in the west; the south lacks a distinct boundary and joins the Edwards Plateau (Leatherwood, 2010). The two main forms of land use are tillage agriculture, most of which is subsidized with irrigation (Dennehy, Litke, & McMahon, 2002), and rangelands/grasslands, which consist of prairie remnants (very few) as well as grazed pastures and former cropland reclaimed to grassland under the United States Department of Agriculture’s Conservation Reserve Program (CRP), a voluntary set-aside program that began in 1985 (Gray, Smith, & Levya, 2004). The two largest cities within the region today are Lubbock (population 252,506; United States Census Bureau, 2016) and Amarillo (population 199,582; United States Census Bureau, 2016), Texas (Figure 2.1). Amarillo was first
settled around 1887 and incorporated in 1899 (Anderson, 2010); Lubbock was settled in 1890 and incorporated in 1909 (Graves, 2010).

The Southern High Plains is semi-arid with seasonal precipitation. Mean annual precipitation (1900-2016) is 51.3 cm in Amarillo (NOAA, http://w2.weather.gov/climate/xmacis.php?wfo=ama), with most of the precipitation occurring from May to September. A semi-arid climate and lack of standing (aboveground) freshwater delayed settlement until the Ogallala (High Plains) Aquifer was discovered at the turn of the 20th century (Diffendal Jr., 2011) (Figure 2.1). Shortly thereafter, the grasslands were plowed to plant crops (irrigated from groundwater extracted via windmills), but lack of soil conservation efforts coupled with severe drought led to the Dust Bowl (Worster, 2010).

Starting in the 1940s, the use of the gasoline-powered pumps allowed farmers to access the Ogallala Aquifer and grow cotton, corn, wheat, sorghum, and other irrigated crops (Nieswiadomy, 1988; Leatherwood, 2010). During the 1940’s and 1950’s, use of irrigation throughout the Southern High Plains increased, accounting for over half of Texas’ irrigated farmland, but the type of irrigation changed over time (Musick, Pringle, Harman, & Stewart, 1990; Bloodworth & Gillet, 2010). In 1958, 89% of the land was irrigated by gravity-feed methods (e.g. graded furrows) and 11% was irrigated using sprinklers (Nieswiadomy, 1988; Colaizzi, Gowda, Marek, & Porter, 2009). Sprinkler irrigation, largely in the form of center pivots, increased steadily throughout the 1980’s and more rapidly throughout the 1990’s (Colaizzi et al., 2009). Subsurface drip irrigation was first used in the mid-1980’s but has increased
since the early 2000’s (Henggeler, 1995; Colaizzi et al., 2009). Aquifer depletion has caused a decrease in irrigated cropland as well as a shift to dryland agriculture in some portions of the Southern High Plains (Heintzman, Starr, Mulligan, Barbato, & McIntyre, 2017). Irrigation in the Southern High Plains peaked in its extent in the mid-1970’s but has since declined in acreage and is now primarily center-pivot (Colaizzi et al., 2009). In its peak year (1974), >2.25 million hectares of the Southern High Plains were irrigated croplands, declining to 1.62 million ha by 1989 (Musick et al., 1990).

Another milestone in the history of the Southern High Plains is the development of the Conservation Reserve Program (CRP), established in 1985 to protect erodible land and minimize agricultural overproduction on marginal land. The CRP is the largest private-land conservation program in the United States. Landowners voluntarily enroll sensitive land into 10-15 year contracts, removing the selected land from agricultural production and instead planting species to help improve environmental health. The goals of the program are to improve water quality, prevent soil erosion, and reduce loss of wildlife habitat (USDA, 2018).

Embedded within this agriculture-rangeland mosaic are playa wetlands, with the Southern High Plains containing the highest concentration of playas in the Great Plains (Sabin & Holliday, 1995; McIntyre, Collins, Heintzman, Starr, & van Gestel, 2018). These wetlands are foci for biodiversity in this otherwise semi-arid region and as such are of considerable conservation value (Bolen et al., 1989; Haukos & Smith, 1994). Playas rely on precipitation and runoff (including irrigation tailwater) to become filled with water, which makes these depressional wetlands sensitive to
changes in the surrounding landscape (Smith, 2003; Smith, Haukos, McMurry, LaGrange, & Willis, 2011). These changes can affect a playa’s ability to hold water and the length of its hydroperiod, both important ecological parameters (Ghioca and Smith, 2008; Collins, Heintzman, Starr, Wright, Henebry, & McIntyre, 2014). For example, certain types of land uses may impede or help playa wetlands stay inundated (Cariveau, Pavlacky Jr., Bishop, & LaGrange, 2011; Bartuszevige, Pavlacky Jr., Burris, & Herbener, 2012; Collins et al., 2014), whereas sedimentation, drainage, and other forms of disturbance associated with land-use changes have contributed to an estimated 17-70% loss of playas (Johnson, Haukos, Smith, & McMurray, 2012; Ruiz et al., 2014; Starr, Heintzman, Mulligan, Barbato, & McIntyre, 2016; estimation differences due to different methods).

Playas are the only natural physical connection to the Ogallala Aquifer, making them the primary source of aquifer recharge (Bolen et al., 1989; Gurdak & Roe, 2009). There is thus a complex relationship among land use, irrigation, the Ogallala Aquifer, and playa wetlands. However, there is limited knowledge about how land use affects the inundation of playas. Given the importance of playas as well as the fact that that ~90% of playas in Texas occur within cropland-dominated watersheds (Smith, 2003), as well as changes in irrigation practices in recent decades from flood-row irrigation to center-pivot and drip irrigation (Musick et al., 1988; Nieswiadomy, 1988; Colaizzi et al., 2008) and establishment of the Conservation Reserve Program, there is a need to examine the relationships between landscape change and playa inundation in recent time.
Within the past 40 years, remote-sensing techniques have allowed for the ability to examine past landscape composition. In the Southern High Plains, land-use changes over recent extended periods of time have not been well-studied, nor associated with playa characteristics. Therefore, I used remote-sensing techniques to document land-use change and coincident effects on the playa wetlands in the Southern High Plains, with three study objectives. The first objective was to quantify land-use changes in the Southern High Plains, an area with known, extensive land conversion, on six dates during a 23-year span. The second objective was to quantify how many playas are characterized by wet or dry state on each date examined, to determine whether playa inundation has changed in frequency over recent time. Finally, the third objective was to determine how inundation state was related to land use surrounding playa basins.

Methods:

Study Area:

In west Texas, USA, land-use change and playa inundation in a time series of Landsat 5 Thematic Mapper (TM) WRS-2 path 030/row 036 images (hereafter, scene 30/36; Figure 2.1) was examined. The extent of scene 30/36 is approximately 185 × 185 km (34,225 km²) and contains the region with the highest density of playas in North America (Fish, Atkinson, Mollhagen, Shanks, Brenton, 1998; Howard et al.,
Landsat 5 was operational from 1984 to 2011 and has collected hundreds of images of scene 30/36 at a 16-day interval with 30-m resolution.

**Approach:**

Six years with above-average precipitation were chosen to examine changes in the land use and inundation of playa wetlands over a 23-year span: 11 September 1986, 30 September 1987, 8 October 1996, 25 September 1997, 5 September 2007, and 22 August 2008. Images were acquired from the United States Geological Survey (USGS) Global Visualization Viewer (GloVis) (http://glovis.usgs.gov). Only high-quality, cloud-free images within relatively these wet years were examined. Rainfall data for the focal scene were obtained from the National Weather Service’s Forecast Office at the Amarillo International Airport (NOAA, http://w2.weather.gov/climate/xmacis.php?wfo=ama; Figures 2.2 & 2.3); data were collected from this location since Amarillo is the most populated location within scene 30/36 and has the longest-term weather data (Figure 2.1). Relatively wet years (i.e., with annual precipitation amounts above the long-term average of 51.3 cm; Figure 2.2) were first selected, and then images from August 15\textsuperscript{th} to October 15\textsuperscript{th} within each of those years were selected. This span represents the peak annual growing season as well as the time when playas have a high likelihood of holding water due to most precipitation within the region occurring from June through September (Figure 2.3). The images were stacked and a
clip boundary (the area examined within each image) was standardized to the central 172 km × 179 km (30,788 km²) portion because of wobble in the satellite path.

Land use of each image was then classified as one of five mutually exclusive classes on a pixel-by-pixel basis at a 30-m resolution: agriculture, rangeland/grassland, fallow, developed, and water. Classifications were conducted using ERDAS Imagine 2016 (Hexagon Geospatial, Norcross, GA, USA). Images were first brought into ERDAS and clipped to the 172 km × 179 km clip boundary. A digital map of the Ogallala Aquifer boundary (Texas Water Development Board Major Aquifers shapefile, http://www.twdb.texas.gov/mapping/gisdata/doc/major_aquifers.zip) was used to subset the scene even further, leaving a study area of 19,996.37 km² containing 7,874 playas. Upon closer examination of the 2007 image, small clouds were found along the northeastern edge of the scene. These clouds were manually removed to reduce classification error and resulted in a loss of 1,321 km² and 18 playas on that date. Roads were extracted from each image using 2016 TIGER road data (https://www.census.gov/geo/maps-data/data/tiger.html) to reduce any error from dirt roads being classified as fallow land. Unsupervised classification was then performed on the remaining data of each image under the following parameters: 60 classes, maximum of 20 iterations, and a convergence of 0.97. Each of the 60 classes was then classified as one of the 5 land-use types.

Agriculture land use consisted of the active crops across the landscape. Rangeland/grassland land use consisted of grass-dominated land (including CRP, grazed and ungrazed pastures, and prairie remnants). Fallow land use consisted of
areas were crops had been harvested, defoliated, or did not grow. Developed land use consisted of all the roads, parking lots, houses, and other forms of anthropogenic development. Water land use consisted of reservoirs, rivers, ponds, lakes, and playa wetlands.

To check the accuracy of my classification protocol, the 2008 classified image was checked against 2008 Texas Orthoimagery Program 50 cm NC/CIR aerial photographs (https://TNRIS.org). Orthoimagery was downloaded for randomly selected regions of the study area encompassing all five land-use classes. Accuracy checks were conducted using 50 random points (10 points/class). A 91% classification accuracy was found for the 2008 image. Upon examining the classified images, it was found that the eastern edges of the images were incorrectly classified. These falsely classified areas consisted of fallow rock outcroppings on the Caprock Escarpment with bright reflectance that were initially classified as water. These areas were identified and selected as areas of interests and then manually reclassified as fallow land.

Remaining image processing was conducted in ArcGIS 10.4.1 (ESRI, Redlands, CA, USA). Classified images were fully assembled and the road layer was added back using raster math to create a landscape image for each date (Figures 2.4 – 2.10). Once images were fully constructed, playa basins were then extracted from each image, using the Playas and Wetlands Database (Mulligan, Barbato, & Seshadri, 2014) and characterized as wet or dry based on the presence of water land-use within each basin.
To classify the dominant land-use types around each playa basin, 100 m ring buffers were created. The land-use raster layers were then resampled down to 10 m resolution from their native 30 m resolution to perform zonal statistics. Each year’s image was divided into 5 layers (one layer per land-use type). Zonal statistics as table analyses were performed in ArcMap on each layer to calculate the number of cells per land-use type within the 100 m buffer around each playa basin. From this, each basin was classified as wet or dry and the dominant land-use type within its immediate watershed determined. A subset of 50 playas was randomly selected to examine changes in land use surrounding playas over time.

A Pearson chi-squared ($\chi^2$) analysis in SAS 9.4 (SAS Institute, Cary, NC, USA) was conducted to compare land-use composition around wet and dry playas across all years. Playa wet basin to dry basin ratio was calculated for each land-use across all years to compare playa likelihood to be dry based on surrounding land-use. FRAGSTATS version 4.2.1 (McGarigal, Cushman, & Ene, 2012) was used to examine land-use patterns over time; a suite of metrics for each land-use class as well as for the landscape as a whole was calculated for each date (Table 2.1). These metrics (out of >100 metrics that FRAGSTATS can generate) were selected to provide a thorough assessment of landscape composition, configuration, and connectivity via relatively unrelated metrics, in a quantitative assessment of land-use change over time in the Southern High Plains.
Results:

Over the 23-year focal time span, changes in land use were observed across the study region (Table 2.2, Figures 2.4 – 2.10). However, only percent cover on the landscape, number of patches of the five land-use types, and mean patch area illustrated changes over time; the other metrics were relatively invariant over the focal time span. Rangeland/grassland was the only land-use class that gained coverage (17.34%) between 1986 and 2008, while agriculture, fallow, developed, and water classes lost coverage (-7.36%, -8.33%, -0.29%, and -1.36%, respectively) (Figure 2.12 A). Rangeland/grassland was the dominant land-use type in scene 30/36, followed by agriculture, fallow, developed, and water. The number of patches decreased over time for all land-use classes (Figure 2.12 B). Mean patch area showed that rangeland/grassland and developed classes increased over time, whereas agriculture, fallow, and water decreased in mean patch area over time (Figure 2.12 C). From 1986 to 2008, 28% of playas did not change in their dominant land use classification. Most of these “stationary” playas were surrounded by rangeland/grassland (only one was in a cropland-dominated watershed). Most playas experienced between 1-5 changes in the dominant land-use types around them, with an average of 2.75 changes over 23 years. These playas that experienced the most change tended to be classified as agriculture, rangeland/grassland, or fallow.

A decrease in water over 23 years was seen, which relates to an observed decrease in the number of wet playa basins over time (Figure 2.11). Of the original
20

7,874 playa basins found within the study region, only a maximum of 4,707 basins were found wet (59.8%, in 1986), with a minimum of 1,125 basins wet (14.3%, in 2008). A 45.5% decrease in wet playas within the study region was observed between 1986 and 2008.

There were significant differences in wet and dry playa land-use classifications across all years and among individual years (Figure 2.13; All years: $\chi^2 = 999.62, p < 0.0001$; 1986: $\chi^2 = 261.87, p < 0.0001$; 1987: $\chi^2 = 171.65, p < 0.0001$; 1996: $\chi^2 = 126.80, p < 0.0001$; 1997: $\chi^2 = 118.62, p < 0.0001$; 2007: $\chi^2 = 190.34, p < 0.0001$; 2008: $\chi^2 = 171.33, p < 0.0001$). The only year when wet playas dominated each class was 1986; dry playas dominated in all other years. An increasing trend in dry playas was observed over time, with the dominant surrounding land use being rangeland/grassland, followed by agriculture and fallow land uses. The likelihood of playa basins being dry increased over time. Rangeland/grassland playas had the highest likelihood to be dry followed by fallow, agriculture, and developed (Table 2.3).

**Discussion:**

Changes to the landscape in terms of composition and configuration of five land-use types were observed during this study (Figures 2.4 – 2.10). Increases in rangeland/grassland and decreases in agriculture, fallow, developed, and water were observed, indicating that some land that was once agriculture or fallow was converted...
to rangeland/grassland over the time span of 1986 to 2008. This change may be due in part to the rising cost of irrigating crops (Colaizzi et al., 2009) and the diminishing sustainability of irrigation (Scanlon et al., 2012). The Southern High Plains as a whole has experienced a decrease in total irrigated land over the 23-year time span (Colaizzi et al., 2009), which was also seen in my focal area. Along with an increase in rangeland/grassland coverage seen over time, increases in the mean patch area and deceases in the number of patches were observed. These patterns indicate that rangeland/grassland patches are becoming bigger over time from the coalescence of multiple, smaller patches.

A decrease in developed land was observed that may have been related to urban growth, although pixels in urban areas dominated by green vegetation can cause under-estimation of developed land use. Roads were standardized across all years using TIGER data.

Variation in the amounts of fallow land varied between dates and years. Images taken later in September and early October may have higher amounts of fallow due to defoliation and harvesting of agricultural crops. One of the dominant crops farmed throughout the region is cotton (Allen et al., 2005; Leatherwood, 2010). Cotton fields are sprayed with a defoliant before harvesting, which causes the green vegetation to die (Anderson et al., 2013). A cotton field’s signal thus changes from green to brown in a relatively short span, potentially causing the classification to change from agriculture to fallow from one year to another.
Of the changes that occurred to landscape composition between 1986 and 2008, the most concerning was the decrease noted in the water class by 1.36% over the 23 years. This is particularly of concern because this decrease occurred even though the focal years were ones where annual precipitation was above the 51.3 cm long-term average, which implies that some component of the landscape must be causing this change. The decrease in water across the landscape also directly correlates with the decrease in number of wet playas observed (Figure 2.11). Of the focal years, 1986 was unique because a large precipitation event happened shortly before the satellite captured the image. During that year, 4,707 playa basins out of the 7,874 total basins were observed wet, the most of any date. But even on this wettest year of the study, 40.2% of the playas did not contain water. By 2008, only 1,125 playa basins were wet out of 7,874 total playa basins, equating to a loss of 85.7%. I thus found a loss in functioning playas ranging from 40.2% to 85.7%. Other studies that have examined playa losses have found similar results ranging from 17% to 85.7% (Johnson, 2011; Johnson et al., 2012; Collins et al., 2014; Starr et al., 2016; McIntyre et al., 2018). Differences in estimates are due to different regions surveyed and different methods used.

The minimum calculated loss estimate of playa basins that I obtained lies within the range of that found in other studies (Johnson et al., 2012; Collins et al., 2014; Starr et al., 2016; McIntyre et al., 2018), whereas my maximum playa loss estimation exceeded all but one study. Only Johnson’s (2011) estimated loss of 85.7% is comparable to my maximum playa loss estimation. Johnson’s estimate was based on
using aerial surveys to document tillage within a comparatively small number of playas within her study area, whereas mine is from satellite imagery. Using remote-sensing techniques allows for examination of larger areas over longer time spans than is possible by other means, but Landsat images are only available every 16 days, meaning that they are non-continuous data that may be even more intermittent if images are not usable due to clouds obstructing views of the ground. My calculations of playa loss may be skewed because my study only examined well-spaced snapshots in time in focal years that were chosen based on above-average annual precipitation amounts, so there is a possibility that my maximum playa loss is an over-estimation.

Above-average precipitation years were chosen to minimize potential effects of drought or climate change on the study so that observed changes would be more strongly related to land-use change. When comparing the dominant land uses surrounding playa basins, 1986 stood out because it was the only year where each land-use category contained more wet than dry playas. The other five years examined all had more dry playas than wet playas across all land-use types. The landscape-scale trend of increasing rangeland/grassland land use was also observed at the 100-m buffer scale around the playa basins, with more playas being classified as surrounded by rangeland/grassland land use over time. Fallow and agriculture-dominated playa basins were associated with dry playa status.

When the dominant land use of a subset of playas was tracked over time, only 28% of the playas did not change in the 23-year period. This indicates that the landscape is dynamic and that most playas undergo changes in their watersheds. The
area surrounding playas changed land-use type on average 2.75 times over 23 years. Playas that kept the same dominant land use were primarily rangeland/grassland playas (except for one agriculture-dominated playa). When the locations of these “stationary” playas was examined, they were located in the northern portion of the study region. The northern portion of the study region was dominated by rangeland/grassland land use and was more stable over time. The southern portion of the study region is dominated by agriculture and fluctuates in land-use designations from actively growing cropland to fallow as discussed above (Figures 2.04 – 2.09).

Studies have shown that the type of land use surrounding a playa can dictate the likelihood that a playa will fill, rate of water loss, and hydroperiod (Tsai, Venne, McMurry, & Smith, 2007). Previous studies have found that playas surrounded by cropland were inundated more often than those surrounded by grassland (Cariveau et al., 2011; Bartuszevige et al., 2012), although Collins et al. (2014) found contradictory patterns whereby grassland playas were wet more often than were agricultural ones. My study shows that playas surrounded by grassland were more likely to be dry, followed by fallow and then agricultural land uses. During 2008, the year with the fewest wet playas, those surrounded by rangeland were twice as likely to be dry than those surrounded by agriculture. Grassland may be perceived as being more “natural,” but grassland vegetation can impede overland runoff and absorb precipitation before the water can reach playa basins (Van der Kamp, Stolte, & Clark, 1999; Van der Kamp, Hayashi, & Gallén, 2003; Voldseth et al., 2007; Voldseth, Johnson, Guntenspergen, Gilmanov, & Millett, 2009; Bartuszevige et al., 2012), whereas
agricultural furrows can allow more water to reach playa basins (Van der Kamp et al., 1999; Van der Kamp et al., 2003; Voldseth et al., 2007; Voldseth et al., 2009) and impede water flow (Tsai et al., 2007; Tsai, Venne, McMurry, & Smith, 2010; Cariveau et al., 2011; Collins et al., 2014; Daniel, Smith, Haukos, Johnson, & McMurry, 2014), although they also deliver more sediments as well (Daniel et al., 2014). Conservation Reserve Program (CRP) grasslands within the Southern High Plains are primarily made up of large, clumping, nonnative grass species that can especially impede the flow of water to playa basins (O’Connell, Johnson, Smith, McMurry, & Haukos, 2012). The preponderance of rangeland/turfgrass in my study region may account for why so few playa basins held water on any given date, and the increase in rangeland/turfgrass seen over my focal span may also help explain why there was a decrease in the number of wet playas seen over time.

Agricultural land use can also affect playas in other ways. Sediments derived from cultivation have been deemed the primary threat to playa wetlands (Smith et al., 2011; Starr et al., 2016). Even playas that do not have cultivated watersheds may be affected because of the volume of sediments produced elsewhere that can be carried via overland flow to them (Smith, 2003; Starr et al., 2016). For example, Starr et al. (2016) found that 64% of playas did not hold water, most likely due to sedimentation that compromised playa functionality, and of the playas that did hold water, turbidity from suspended sediments was found in 30.5 to 70.5% of them, regardless of their surrounding land-use type (Starr et al., 2016).
My study, like other studies, showed that playa loss due to land-use change (which includes sedimentation from agricultural practices) is of major concern (Johnson, 2011; Johnson et al., 2012; Collins et al., 2014; Starr et al., 2016; McIntyre et al., 2018). My study has shown that playas are being lost as a result of surrounding land use, and that playas surrounded by rangeland/grassland are two times more likely to be dry than playas surrounded by agricultural land use. Although my study did not explicitly focus on playa hydroperiod, it is known that nearby land use can influence playa hydroperiod (Tsai et al., 2007; Collins et al., 2014). Because playa wetlands are centers of biodiversity and because hydroperiod is strongly associated with species occurrence and abundance (Anderson & Smith, 2004; Ghioca & Smith, 2008), the land-use changes that I observed may eventually change biotic community composition over time. These changes could be due to loss of functioning playa wetlands or changes in hydroperiods due to surrounding land use. With playa hydroperiods being shortened or non-existent in some cases, amphibians (Venne, Tsai, Cox, Smith, & McMurry, 2012), birds (Johnson et al., 2010), and invertebrates (Starr & McIntyre, unpublished data) that live in playa wetlands during their wet phase may not be as common or may be extirpated from the region.

One thing to keep in mind is even though rangeland/grassland land use increased in my study region over my focal time span, the landscape has much less grassland than was present around 1900, when the region was dominated by shortgrass prairies with sparse areas of human development and agriculture. Grasslands were influenced by grazing and episodic fire, creating a landscape different from the one
that we see today. Playas likewise functioned differently then, experiencing minimal
sediment accrual, absence of anthropogenic structures like ditches or wells, and
absence of non-native plant species, all features that currently affect playa inundation.
Even with recent expansion of rangeland/grassland cover, we are never going to see
the landscape as it existed in the past. Unfortunately the damage that has been done to
the landscape cannot be undone, but we can move forward knowing how we are
influencing the landscape and how we can help protect habitats of concern. Playas and
other wetlands are of great conservation concern and are at risk due to land-use
changes (Semlitch & Bodie, 1998). Current models are predicting that 75% of playas
in Texas and 90% within the Great Plains will be infilled by sediments by 2100, with
sedimentation occurring primarily due to projected land-use changes (Burris &
Skagen, 2013). Management and conservation plans that consider the land uses
surrounding playa wetlands, including plans that manipulate land use in such a way as
to facilitate water reaching playa basins while minimizing sedimentation, will be
necessary to help re-establish natural ecosystem functions throughout the Southern
High Plains.
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Table 2.1: Class- and landscape-scale metrics calculated in FRAGSTATS 4.2.1, with each identified as to whether it quantifies landscape composition, configuration, or connectivity. See McGarigal (2015) for details on how each metric is calculated.

<table>
<thead>
<tr>
<th>Class Metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of Landscape (PLAND) – composition</td>
</tr>
<tr>
<td>Patch Area Mean (AREA_MN) – configuration</td>
</tr>
<tr>
<td>Patch Area Coefficient of Variation (AREA_CV) – configuration</td>
</tr>
<tr>
<td>Perimeter-Area Fractal Dimension (PAFRAC) – configuration</td>
</tr>
<tr>
<td>Perimeter-Area Ratio Mean (PARA_MN) – configuration</td>
</tr>
<tr>
<td>Shape Index (SHAPE_MN) – configuration</td>
</tr>
<tr>
<td>Related Circumscribing Circle (CIRCLE_MN) – configuration</td>
</tr>
<tr>
<td>Number of Patches (NP) – configuration</td>
</tr>
<tr>
<td>Patch Density (PD) – configuration</td>
</tr>
<tr>
<td>Aggregation Index (AI) – connectivity</td>
</tr>
<tr>
<td>Clumpiness (CLUMPY) – connectivity</td>
</tr>
<tr>
<td>Normalized Landscape Shape Index (NLSI) – configuration</td>
</tr>
<tr>
<td>Patch Cohesion Index (COHESION) – connectivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Landscape Metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Edge (TE) – configuration</td>
</tr>
<tr>
<td>Edge Density (ED) – configuration</td>
</tr>
<tr>
<td>Contagion (CONTAG) – connectivity</td>
</tr>
<tr>
<td>Patch Richness (PR) – composition</td>
</tr>
</tbody>
</table>
Table 2.2: Composition of land-use types across the landscape by year and total percent change over 23 years.

<table>
<thead>
<tr>
<th>Land use</th>
<th>% of Landscape</th>
<th>% Change from 1986 to 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agriculture</td>
<td>31.07</td>
<td>35.96</td>
</tr>
<tr>
<td>Rangeland/Grassland</td>
<td>31.68</td>
<td>35.88</td>
</tr>
<tr>
<td>Fallow</td>
<td>29.43</td>
<td>20.75</td>
</tr>
<tr>
<td>Developed</td>
<td>6.04</td>
<td>6.1</td>
</tr>
<tr>
<td>Water</td>
<td>1.78</td>
<td>1.31</td>
</tr>
</tbody>
</table>
Table 2.3: Playa basin dry to wet ratio by land-use classification and year.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Agriculture</td>
<td>0.47</td>
<td>1.65</td>
<td>1.92</td>
<td>2.16</td>
<td>2.08</td>
<td>3.74</td>
</tr>
<tr>
<td>Rangeland/Grassland</td>
<td>0.86</td>
<td>1.66</td>
<td>1.57</td>
<td>1.92</td>
<td>3.44</td>
<td>7.72</td>
</tr>
<tr>
<td>Fallow</td>
<td>0.90</td>
<td>2.11</td>
<td>3.48</td>
<td>2.98</td>
<td>7.25</td>
<td>6.75</td>
</tr>
<tr>
<td>Developed</td>
<td>0.57</td>
<td>1.34</td>
<td>1.13</td>
<td>2.00</td>
<td>1.53</td>
<td>1.93</td>
</tr>
<tr>
<td>Water</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 2.1: Map of the study region (black polygon): Landsat 5 Thematic Mapper (TM) WRS-2 P030/R036. Playa locations are denoted by blue dots and the Ogallala Aquifer is shown as the gray-shaded region.
Figure 2.2: Yearly total precipitation amounts recorded from Amarillo International Airport. Precipitation average (black line) of 51.3 cm (1900 – 2016) and study years (blue bars) are denoted.
Figure 2.3: Average monthly precipitation amounts (1900 – 2016) recorded from Amarillo International Airport.
Figure 2.4: Land use classification for 11 September 1986.
Figure 2.5: Land use classification for 30 September 1987.
Figure 2.6: Land use classification for 8 October 1996.
Figure 2.7: Land use classification for 25 September 1997.
Figure 2.8: Land use classification for 5 September 2007.
Figure 2.9: Land use classification for 22 August 2008.
Figure 2.10: Land-use change progression from 1986 to 2008 for a focal area near Plainview, Texas.
Figure 2.11: Histogram displaying the number of wet playas during each study year.
Figure 2.12: A) Percent of landscape, B) number of patches, and C) mean patch area for each of the five land-use classes: green solid – agriculture, light brown dash – rangeland/grassland, dark brown dots – fallow, black bar and dots – developed, blue large dash – water.
Figure 2.13: Dominant land uses surrounding wet and dry playas on study dates from 1986 – 2008. Chi-squared analysis performed each year comparing land use classes, with numbers in parentheses indicating degrees of freedom and sample size.
Chapter 3:

Life History and Lab Husbandry of *Enallagma civile* (Hagen) (Insecta, Odonata, Coenagrionidae)

Introduction:

The Familiar Bluet (*Enallagma civile*) is one of the most widely distributed damselflies throughout North America, found in many different habitat types (Westfall & May, 1996; Westfall & May, 2006; Paulson & Dunkle, 2012). The wide distribution and habitat breadth of *E. civile* make it a potentially good model organism with which to study the effects of climate and anthropogenic landscape changes. Despite its commonness, *E. civile* has been underutilized and underrepresented in research studies, so we know relatively little about its life history. Gaining more information may be hampered from a lack of information on lab husbandry of this species. I will discuss the underutilized potential of *E. civile* as a model organism and then will summarize the life history and husbandry of *E. civile* from the literature and my own lab studies.

Untapped Potential as a Model Organism:

Odonates (Insecta, Odonata: dragonflies and damselflies) in general have been deemed good model organisms to study both ecological and evolutionary concepts
because of their amphibious life history, which makes them sensitive to both aquatic and terrestrial environmental changes, as well as their having a relatively short lifespan, being ectothermic, and occupying a high trophic position at all life-stages (Cordoba-Aguilar, 2008). As such, odonates have been used in a variety of ecological and evolutionary biology studies to examine effects of landscape change (e.g. Reece & McIntyre, 2009b; Kutcher & Bried, 2014) and climate change (e.g. Hassall, Thompson, French, & Harvey, 2007; Dingemanse & Kalkman, 2008; Richter, Suhling, Muller, & Kern, 2008; Hassall, 2015). Many of these studies focused on odonates as a collective (assemblage-level responses), yet different odonate species in the same assemblage may exhibit different patterns in abundance or occurrence as a function of the environment (McPeek, 1990; Bried & Ervin, 2006; Reece & McIntyre, 2009a). Therefore, focusing on individual species rather than on entire communities may be more useful for some studies.

Selecting which species to use as a focal organism is not necessarily straightforward, however. The focal species should be one that can represent broad rather than species-specific or idiosyncratic response patterns. Because dragonflies tend to be larger and more vagile (indeed, several species are migratory) than damselflies, they can pose logistical difficulties in their use in such studies. Furthermore, damselflies tend to be more abundant and speciose than dragonflies, and as a group they may be more sensitive to environmental conditions than dragonflies (Bried, McIntyre, Dzialowski, & Davis, 2015). A widespread and abundant damselfly species would have more evolutionary potential to respond to a changing environment than would a rare species because local populations may have the genetic potential to respond to changes in the environment.
whereas a restricted species may be limited in its genetic potential and when subjected to changes, could be extirpated (Williams, Shoo, Isaac, Hoffmann, & Langham, 2008). The Familiar Bluet damselfly (*Enallagma civile*, Coenagrionidae) is one such candidate of a good model organism.

Despite *E. civile* being one of the most abundant odonates in North America, this species is underutilized, not having been the focus of many studies. Consequently, relatively little is known about this species compared to some others, and much of what is known is anecdotal. Of the studies that have been conducted on this species, a majority are more than 15 years old. This lack of utilization of *E. civile* could be in part due to a lack of information on its lab husbandry. Many of the studies where *E. civile* is mentioned are field-based, with only a few lab-based studies. Many of these field studies focused on odonates as bioindicators (Catling, 2005), including assessments of flight, mating, and feeding of adults (Bick & Bick, 1963; Schaefer, Barth, & White, 1996); mate selection by color morph (Miller & Finke, 1999; Miller & Fincke, 2004; Finke, Fargevieille, & Schultz, 2007); phylogeny (Brown, McPeek, & May, 2000; May, 2002); reproduction (Hornuff, 1968); effects of pH (Blinn & Sanderson, 1989); nymph gut contents (Breene, Sweet, & Olson, 1990); nymph density (Miura, Takahashi, & Stewart, 1990); parasites (Richardson & Janovy, 1990); reproduction and life history (Braccia, Voshell, & Christman, 2007; Reece, 2009); coloration (Charles & Robinson, 1981; Prum, Cole, & Torres, 2004); and melatonin production (Tilden, Anderson, & Hutchison, 1994). Lab-based studies have focused on predation (Miura & Takahashi, 1988; Flynn & Moon, 2011); life history (Booker, 2002; Reece, 2009); adult female fitness (Baker, 2011; Baker
& McIntyre, 2013); color morphs (Finke, Fargevieille, & Schultz, 2007); morphology of external genitalia (Hornuff, 1968); acidic pH effects on predation rate and survivorship of nymphs (Gorham & Vodopich, 1992); alkaline pH and prey availability effects on nymph survivorship (Reece, 2009); and effects of water temperature on growth and survival (Chapter 4). Many of these lab studies used wild-caught nymphs, with very few of them using nymphs raised from eggs. Furthermore, life history studies on this species have not followed individuals from egg to adult. Here, I review the life history of E. civile as well as explain husbandry techniques used to raise E. civile in the lab from egg to adult. The purpose of this chapter is to provide a comprehensive collection of what has been published about E. civile, supplemented with personal observations.

Life History Review:

Enallagma civile was first described as Agrion civile by Hermann A. Hagen in 1861. Enallagma civile is also known under the synonyms Agrion canadense (Provancher, 1876), Enallagma civile plebeium (de Selys Longchamps, 1876), and Enallagma civile race? simile (de Selys Longchamps, 1876). As one of 47 species of the genus Enallagma, E. civile is a member of the family Coenagrionidae, collectively known as the pond damselflies or the narrow-winged damselflies (Schorr & Paulson, 2016). The type series for the species was collected from “Maryland; Matamoras (Matamoros), Mexico; New York; Pecos River, Texas; and Washington” (presumably Washington D. C. rather than Washington state, where it has not been found) (Hagen,
Nymphs of *E. civile* were first described by Needham and Cockerell (1903) from specimens from the Arroyo Pecos near Las Vegas, New Mexico. Dichotomous keys for adults and nymphs can be found within Westfall and May (1996, 2006).

Adult males and females show sexual dimorphism and dichromatism. Males are bright blue and black in coloration whereas females are polymorphic: they can display andromorphic coloration that is similar to but paler than males’, or a heteromorphic coloration ranging from brown and black to olive and black (Lam, 2004; Paulson, 2009; Paulson, 2011; Fig. 3.1 A & B). The thorax is striped, as is typical of bluets. Males have distinctive genitalia, large blue postocular eyespots, and abdominal color patterns. The top of the female’s abdominal segments are predominantly black in coloration, but females require examination of a suite of traits in hand to distinguish them from other, similar species (Westfall & May, 1996; Lam, 2004; Westfall & May, 2006; Paulson, 2009; Paulson, 2011). Males and females are also polymorphic in terms of their head color pattern. Three phenotypic classes of post-ocular spots exist (Johnson, 1964). Adult *E. civile* typically range in total body size 28-39 mm in length (Lam, 2004; Paulson, 2009; Abbott, 2011b; Paulson, 2011) and hindwing length of 16-21 mm (Paulson, 2009; Paulson, 2011).

Species that are similar to *E. civile* depend on the location within the species’ distribution. Similar species include the Alkali Bluet (*Enallagma clausum*), Arroyo Bluet (*Enallagma praevarum*), Boreal Bluet (*Enallagma boreale*), Hagen’s Bluet (*Enallagma hageni*), Marsh Bluet (*Enallagma ebrium*), Northern Bluet (*Enallagma annexum*), River Bluet (*Enallagma anna*), and Vernal Bluet (*Enallagma vernale*) (Paulson, 2009 & 2011).
E. civile has been found to hybridize with the Tule Bluet (Enallagma carunculatum) (Williamson, 1906; Leong & Hafernik, 1992; Corbet, 1999; Paulson, 2009) and River Bluet (Enallagma anna) (Donnelly, 2000).

Nymphs (also called naiads or larvae) have long, slender bodies. The head has well-developed eyes and is wider than the thorax and abdomen. The thorax is very short with long, thin legs and prominent wing pads present during the final few stadia (instars). The abdomen is slender with three prominent caudal gills present at the posterior end. Nymphs are transparent to slightly white or opaque in color during early stadia and can be light brown, green, dark brown, or tan in color as they reach their last stadia, possibly from dietary items being visible within the gut, sediment deposits on the exoskeleton, or algal growth on the exoskeleton (Fig. 3.1 C). Nymphs undergo a series of molts, which are numbered in decreasing order (e.g. for a species with 12 stadia, the numbering is from F–11 for the first/earliest and F–0 for the final instar). During the final stadium, the nymph emerges from the water, climbing onto vegetation or built structures such as bridge abutments or piers, and ecloses; the newly emerged adult is called a teneral and needs several more hours to gain the ability to fly. Even after it can fly, the teneral needs another 1-2 days to reach mature coloration and full maturity.

The distribution of E. civile is large, but populations are often localized in specific regions (Paulson, 2011). The distribution in the north extends into southern regions of Canada, including the provinces of British Columbia, Manitoba, New Brunswick, Newfoundland, Nova Scotia, Ontario, Prince Edward Island, Quebec, and Saskatchewan (Catling, 1996; Westfall & May, 1996; Lam, 2004; Westfall & May, 2006; Paulson,
2011) (Fig. 3.2). The distribution extends southward through the continental United States in every state except Idaho and Washington. Idaho seems to be under-surveyed and possibly contains *Enallagma civile* based on collection points in Oregon and Montana at the same latitude. The distribution of *E. civile* has not likely spread into Washington state based on current collection points within the region (Westfall & May, 1996; Lam, 2004; Westfall & May, 2006; Beaton, 2007; Paulson, 2009; DuBois, 2010; Garrison, von Ellenrieder, & Louton, 2010; Abbott, 2011b; Paulson, 2011). *Enallagma civile* was introduced to Hawaii in the 1930’s (Daigle, 2000; Paulson, 2010). Within Texas, *E. civile* has been documented in 234 of the 254 counties (Abbott, 2006-2016; Abbott, 2011a; http://www.odonatacentral.org as of May 8, 2018). The 20 Texas counties that lack records of *E. civile* are most likely due to lack of sampling and documenting rather than a lack of suitable habitat being present. The species’ range continues south through Mexico and Central America (Gonzalez-Soriano & Novelo-Gutierrez, 1991; Westfall & May, 1996; Esquivel, 2005; Westfall & May, 2006). The southernmost extent of the distribution of *E. civile* extends into South America in the higher elevations within Venezuela and Columbia (Paulson 2009; Garrison et al., 2010; Paulson, 2011).

*Enallagma civile* is also found in the Antilles in the countries of Cuba, Dominican Republic, Haiti, Jamaica, Puerto Rico, and in the Bahamas (Westfall & May, 1996; Esquivel, 2005; Westfall & May, 2006).

*Enallagma civile* can be found in high abundances in localized populations (Paulson, 2011). Local populations of adults can vary from the thousands to only a few individuals around a water source (personal observation). Nymphs can also often be
found in high densities. Miura et al. (1990) found that nymph density at the rice fields of their study site averaged 813.7 individuals per m² with a minimum of 230 individuals per m² and a maximum of 1,150 individuals per m².

Little is known about *E. civile*’s dispersal ability. Bick & Bick (1963) observed an adult male *E. civile* 137 meters away from the nearest water. I personally have observed several individuals 643-965 meters away from the closest water source. When examining other species of the genus *Enallagma*, *E. cyathigerum* has been documented 0.6 km (Conrad, Willson, Harvey, Thomas, & Sherratt, 1999) and 2.7 km (Moore, 1954) from source populations, and *Enallagma* sp. were found 1 km from a source population (McPeek, 1989).

In the southern part of its range in North America, *E. civile*’s flight season is all year, whereas in the northern extent of its range the flight season is from May to October (Paulson, 2009; Paulson, 2011). Its flight season is listed as yearlong within Texas (Abbott, 2011a; Abbott, 2011b), but this is mainly true for the southernmost counties, with the northern counties experiencing a flight season from May through November (personal observation). *Enallagma civile*’s conservation status worldwide is demonstrably secure, even though within some portions of its range, it can be rare (Abbott, 2011b). Within Texas the conservation status of the species is also demonstrably secure within the state, but it may be rare in parts of its distribution (Abbott, 2011a).

*Enallagma civile* is considered a habitat generalist (Butler & deMaynadier, 2008; Ball-Daerow, M’Gonigle, & Resh, 2014), which likely contributes to its abundance and wide distribution. The species requires intermediate floating plant richness, intermediate
to broad emergent zone width, and intermediate to coarse substrate granularity, and exhibits intermediate to high tolerance to local disturbance and limited to intermediate tolerance to emergent zone length (Butler & deMaynadier, 2008). Its habitats include ponds, lakes, open marshes, slow streams, and margins of rivers with emergent vegetation (Paulson, 2009; Abbott, 2011b; Paulson, 2011). The species also occurs near coastlines in brackish waters (Lam, 2004; Abbott, 2011b; Paulson, 2011). Enallagma civile is often found in early successional habitats (Paulson, 2011) and is adapted at colonizing temporary aquatic habitats and newly created aquatic habitats (including human-made habitats) (Voshell & Simmons, 1978; Westfall & May, 1996; Lam, 2004; Westfall & May, 2006).

The mean life expectancy of adults is approximately 3.3 days (Bick & Bick, 1963), but this most likely is an underestimation due to the sampling and estimation techniques used in that study and the difficulty of tracking individual adults throughout their life in a natural setting. Studies on other members of the Coenagrionidae found that the average lifespan of adult damselflies of the species Ischnura elegans was 15-16 days for males and 7-11 days for females in a laboratory setting (Van Gossum, Sanchez, & Rivera, 2003). Ischnura gemia male lifespan was 12.1-13.2 days and female lifespan was 12.3-13.2 days in a natural setting (Hafernik & Garrison, 1986). Therefore, it is not unreasonable to estimate that E. civile adults have a longer lifespan than that documented by Bick and Bick (1963).

During E. civile’s adult lifespan, individuals can be seen along the water’s edge and throughout the surrounding upland habitats foraging for food. Typically, the largest
abundances of *E. civile* are seen in late morning to midday along the water’s edge, with males outnumbering females (Bick & Bick, 1963; Baker, 2011; Baker & McIntyre, 2013). These aggregations tend to occur at oviposition sites, with females possibly being attracted to other females ovipositing (Moss, 1992). Males are typically nonterritorial but will occasionally exhibit territoriality by patrolling the water’s edge for females (Bick & Bick, 1963).

Once a female is spotted, a male will seize a female with its cerci and paraprocts. Typically, the pair will then fly to a perch, where copulation takes place while in a wheel position (Fig. 3.3 A). Copulation can last from 11-44 minutes, with an average of 18.7 minutes being reported (Bick & Bick, 1963). Oviposition takes place next, with the pair staying in tandem. Exploratory flights may be made, with the female testing substrate for suitability while the male continues to hold on to the female. Females will then lay eggs endophytically in plants and floating debris. The debris can be at the water’s surface or below the surface (Bick & Bick, 1963; personal observation) (Fig. 3.3 B). Females may also climb down submerged material, fully submerging themselves while they lay eggs underwater, still in tandem (Bick & Bick, 1963; Moss, 1992). Oviposition can last anywhere from 98-199 minutes, averaging 142.1 minutes (Moss, 1992). Females have been documented to lay between 1-1,047 eggs at a time, with an average of 250 eggs per clutch (Zehringer et al., 1962; Moss, 1992; Baker, 2011; Baker & McIntyre, 2013; Chapter 4). Once oviposition is complete, the pair will separate and the female will typically leave the water’s edge, while the male will typically go back to the water’s edge to patrol. As a
result, there is typically a male-biased sex ratio near water, a pattern that is common in many damselfly species (Stoks, 2001; Torres-Cambas & Fonseca-Rodríguez, 2011).

Relative to what is known about the adults, very little work has been done on the life history of *E. civile* eggs and nymphs. Baker (2011) and Baker and McIntyre (2013) found that *E. civile* egg sizes can vary between females and within clutches. Egg length varied from 0.6-0.9 mm, with an average length of 0.76 mm. Eggs took 11.9 ± 0.2 days to hatch, with a range of 10-34 days (98% hatched within 10-14 days) when incubated at 27°C, with a mean (± standard error) hatching success rate of 81.1 ± 0.0% (Baker, 2011; Baker & McIntyre, 2013). Miura and Takahashi (1988) state that *E. civile* has 12 stadia, and *E. vernale* has been reported to have 14 stadia (Corbet, 1999). Booker (2002) found the developmental time of *E. civile* nymphs to be 21 days at a field site in Texas; however, this has not been replicated in the lab. Reece (2009) attempted to raise *E. civile* nymphs in the laboratory to adulthood but terminated the experiment after 55 days with no emergence by that time (possibly due to factors relating to water chemistry and/or a cage effect, discussed below).

**Lab Husbandry:**

Very little information of how to raise *E. civile* from egg to adults in the lab environment was found in the literature, making this a significant challenge. Most studies that have worked with *E. civile* in the lab acquired nymphs from the wild and kept them in plastic cups at room temperature. The following husbandry techniques were developed
in order to test the effects of elevated water temperature on *E. civile* nymphs, which will be covered in Chapter 4.

Other species of damselflies have been studied in the laboratory setting and can provide some guidance about rearing of *E. civile*. For example, *Lestes viridis* has been used to study photoperiod and hydroperiod effects on life history (De Block & Stoks, 2004), growth under stress (De Block & Stoks, 2008), and effects of time constraints and predation on immune defense, energy storage, and life history traits (Stoks, De Block, Slos, Van Doorslaer, & Rolff, 2006). *Lestes sponsa* (Stoks, De Block, & McPeek, 2005), *Ischnura graellsi*, *I. pumilio*, *Coenagrion scitulum*, and *Enallagma cyathigerum* have been used to study the effects of sex and age on adult survivorship (Cordero, 1994); *Pyrrhosoma nympha* was used in a study of ecological energetics (Lawton, 1971); and *I. elegans* was used to study effects of temperature on functional response and life history of nymphs (Thompson, 1978a) and prey size selection (Thompson, 1978b). Nymphs of *Ischnura* and *Enallagma* have also been used to study growth/predation tradeoffs (McPeek, Grace, & Richardson, 2001).

Building from this body of knowledge from the literature, I conducted several field and lab studies from 2013-2014 that used *Enallagma civile* as a model organism. All damselflies used in these studies were collected from Canyon Lake 5 (Lake Mae Simmons) in Mae Simmons Park in Lubbock, Texas, on 18 July 2013, 31 July 2013, 17 June 2014, 3 July 2014, and 30 July 2014 (Fig. 3.4) on sunny days between 1030-1230 h. Canyon Lake 5 is an impoundment of the North Fork of the Double Mountain Fork of the Brazos River and is part of Lubbock's wastewater treatment system. Large segments of
the riparian zone are grass that is periodically mowed to the water’s edge; other portions are covered by large patches of cattails (*Typha*) and a variety of aquatic reeds. Along the open patches of the riparian zone, there is limited emergent vegetation, but broken *Typha* stalks and algal mats collect near the bank. This site has a high density of *E. civile*, is readily accessible, and is publicly available (Baker, 2011; Baker & McIntyre, 2013).

Adult male and female pairs were collected with aerial insect nets while the insects were in the wheel position. Females were retained and placed into clear specimen envelopes and stored in a shaded container before being brought back to the lab, whereas males were released at the site of capture.

Females were brought back to the laboratory at Texas Tech University and placed into oviposition chambers. The laboratory was kept at approximately 22°C and lights were kept on a 13.5 hour photoperiod (maintained by outlet timers) based on average daylight period in the Southern High Plains from the May to October breeding season (United States Naval Observatory, 2015). Oviposition chambers consisted of a 0.95 L glass mason jar containing ~2.5 cm of water from the site of collection. An oviposition apparatus, which consisted of a dowel (bamboo skewer) wrapped in paper towels, was placed at a 45-degree angle inside the jar, emerging from the water (Fig. 3.5 A). The oviposition apparatus simulated a stem that *E. civile* could oviposit into and also provided a perch for the female while in the chamber. This procedure has been used to obtain eggs for other studies (Reece, 2009; Baker, 2011; Baker & McIntyre, 2013). Eggs were collected after 48 hours, pooled, counted, and reared under designated water temperatures (details in Chapter 4). Rearing containers consisted of glass jars (6 cm diameter, 5.5 cm
tall, 120 ml volume) filled with artificial freshwater. The moderately hard water was based on a recipe by Weir and Salice (2012). Moderately hard water consisted of CaSO₄ (3 g), KCl (0.2 g), MgSO₄ (3 g), and NaHCO₃ (4.9 g) dissolved in 50 L deionized water. Moderately hard water was used instead of collecting water from Mae Simmons Park in order to keep consistency throughout the entire experiment due to natural water could contain other chemical that could add variation to the experiment that could not be controlled for. The moderately hard water became a crucial part of the experimental setup. Tap water was unsuitable, as it has a high natural mineral concentration in this region (i.e., is "hard" water) and also has a high natural and supplemented fluorine concentration. Trials using reverse osmosis-filtered tap water were not as successful in terms of survival as were trials using the moderately hard water, which mimicked natural water chemistry in this region of Texas. Using reverse-osmosis filtered tap water is likely the reason why the nymphs in the studies conducted by Reece (2009) suffered high mortality and prolonged development, with extremely low emergence.

Eggs were reared in chambers consisting of 10-gallon aquaria with a FLUVAL E100 (Rolf C. Hagen (USA) Corp., Mansfield, MA, USA) aquarium heater set to the desired lower temperatures (max temperature, 34°C) or in water baths capable of generating temperatures higher than 34°C (Fig. 3.5 B). Aquarium heaters were placed centrally within each of the tanks. A clear acrylic stand was placed over the heaters inside each aquarium to hold the containers at a standardized height. This height was also maintained within the water baths. Aquaria and water baths were filled to the same level with deionized water and the rearing containers placed within them, with open tops to all;
the deionized water came 4-5 cm up the rearing containers within the tanks and water baths. Onset HOBO® (www.onsetcomp.com, Bourne, MA, USA) water temperature loggers measured water temperature in 5-minute intervals. Glass thermometers were also placed at the level of the nymphs to visually monitor temperatures. Air stones were mounted within each experimental system to help circulate water to maintain temperature homogeneity. Heaters and water baths were on for 13.5 hour periods to simulate the sun heating up the water. These periods simulated gradual heating and cooling similar to natural conditions (Fig. 3.6). Differences in HOBO logger recorded temperatures and observations from glass thermometers were observed, likely due to thermometers being at different levels in the tank. Temperatures were reported from levels at which damsels occurred.

Once the eggs hatched, each nymph was housed in an individual glass jar (6 cm diameter, 5.5 cm tall, 120 ml volume) with moderately hard water, so that no specimens were lost to cannibalism. Eggs and nymphs were observed daily while conducting water changes, where 3 ml plastic pipettes were used to remove water and waste and to add moderately hard water with food. Nymphs were fed ad libitum daily (based on stadium: as body size increased, more food was supplied, consisting of a mixture of copepods, Daphnia sp., ostracods, and other live food that was size-appropriate). Prey populations were maintained in separate lab aquaria. Observations of nymph mortality and ecdyses (molting) were also made at water changes. If individuals had molted, total body length (excluding the external gills) and head capsule width were measured, and the shed exuvia was removed. Removal for measurement was done using a plastic 3 ml pipette with the
tip cut off. At the F–0 stadium, nymphs have well-developed wing pads and are approximately 10 mm from the front of the prothorax to the rear tip of the abdomen minus the caudal gills. Once a nymph’s body length reached 10 mm, a bamboo dowel was placed inside each rearing jar to give the nymph a way to leave the water and undergo ecdysis into the teneral adult stage. A mesh emergence trap was placed over each jar to enclose emerged adults (Fig. 3.5 C). Teneral adults were given 24 hours for their exoskeleton to harden and then they were measured for head capsule width and total body length.

**Experimental Results and Discussion:**

Two issues from a trial experiment in 2013 arose in the experimental setup, which were corrected in 2014. First, it was suspected that the water used in 2013 was not appropriate for nymph rearing. The 2013 experimental setup used reverse osmosis-filtered water, which lacked the minerals needed for the nymphs to grow properly. I subsequently changed the water in 2014 to the moderately hard water recipe. Another factor that contributed to poor trial survivorship in 2013 was the small size of containers that had been used to house the nymphs individually (6 ml glass vials). These smaller containers may have created a “cage effect” in the nymphs, limiting their growth and affecting their survival. Because of this, I increased the size of the containers used to house the nymphs in 2014, but this limited the number of nymphs that could be housed at one time, which led me to rearing three sequential cohorts throughout summer 2014 to
keep the sample size as high as possible. Due to the problems mentioned above, the only data presented from 2013 will be from the females collected from the field and the number of eggs laid by those females.

Over the course of two summers (2013-2014), 112 females were collected from the field and brought back to the lab. Of those females, only 31 (27.7%) laid eggs in the lab. On average, 157.8 eggs were laid per female, but a few females laid large quantities of eggs from each collection date. Of the females that laid eggs, the lowest number was 1 egg and the highest number was 549 eggs (Table 3.1). Baker and McIntyre (2013) found similar results, where 27.3% of the 561 females they collected laid eggs. During their study, the range of eggs laid had a higher maximum of 1,047 eggs, although my range of eggs laid fell within their observed range. Their average was higher, with 250 eggs laid per female, but in comparison they sampled five times more females than I did.

A random subsample of 48 eggs from 6 females revealed that the mean egg length was 0.86 mm and mean width was 0.18 mm. Eggs have an oblong shape, similar to a grain of rice. The end that the female first inserts into the oviposition material, which I am referring to as the attachment point. This end has a sharp point, whereas the opposite end is rounded. Eggs are predominantly opaque white in color with a light brown color at the attachment point end when first laid (Fig. 3.7 A). On day two, the eggs start to change color at the attachment point from tan to orange. The opaque white outer material of the egg starts to become clear, allowing the inner material to be seen, which starts to have a speckled appearance. By days four and five, the inside material starts to pull away from the clear shell of the egg. Two black dots, which are the eyes, start to appear near the
attachment point (Fig. 3.7 B). By day seven, some eggs start to hatch. In some eggs, legs start to become visible (Fig. 3.7 C). By days ten and eleven, most eggs have hatched.

Throughout this process, those eggs that stayed opaque white in color did not hatch. Time to hatch was temperature-dependent; see Chapter 4 for details. Eggs raised at 26°C had a survival rate of 62.1% and took on average 11.5 days with a standard deviation of ± 5.25 days. The quickest egg hatched in 7 days and the longest took 37 days (Table 3.2).

When the nymphs hatched, their average size was 1.09 mm total body length (not including caudal gills) and 0.35 mm head width (Fig. 3.7 D). Nymphs were followed through development and measured after each molt. In contrast to findings from Miura and Takahashi (1988), who found that *E. civile* had 12 stadia, *E. civile* nymphs in my study had 10 to 11 stadia. This difference could possibly be due to some molts being missed during observation periods, different populations being sampled, or different lab conditions that affected development. As the nymphs developed and molted, certain characteristics started to change. The body continued to get longer and the head wider, with trachea becoming visible (Fig. 3.7 E). Eyes became larger and more developed. In the later stadia, wing pads started to develop. First the wing pads appeared flat along the thorax and abdomen (Fig. 3.7 F). Veins started to develop within the wing pads. Eventually the wing pads turned in orientation, giving a rolled-up appearance (Fig. 3.7 G). All four wings became visible at that time. At this point, the nymph body was typically more than 14 mm long and the head was around 3.5 mm wide as nymphs neared the final stadium. Next, nymphs would find an object to use to emerge from the water.

From the perch above the water, the nymph completed its last ecdysis, going from a
nymph to a teneral adult. The process of hatching from the egg to emergence as a teneral adult took an average of 41.1 days at 26°C (range: 33 to 52 days). When examining the total development time from egg to teneral adult, the average span was 52.6 days, which was more than double what Booker (2002) reported from a north Texas field site. This difference could be due to a difference in lab and field conditions, or to Booker (2002) making assumptions about observing a single cohort even though *E. civile* are multivoltine, with overlapping generations. Booker (2002) did not follow individuals but instead made a cohort-level inference.

Once the nymph emerged, the teneral pumped hemolymph throughout its body, elongating the abdomen and unfolding the wings. In this form the teneral adult’s cuticle is unhardened and tan in color, lacking mature adult coloration, and the wings have an iridescent sheen. The damselfly must wait for the cuticle to dry before it is able to fly, which usually takes several hours. After waiting 24 hours for the teneral damselflies to become adults, I found that none of them displayed their full adult coloration in the lab. Because of this, individuals were brought into the greenhouse in net cages to receive natural sunlight and were given fruit flies to eat for upwards of 48 hours. Some males became slightly blue in coloration but never close to the color of adults caught in the wild. Adults also did not seem to be interested in the fruit flies that were presented as food. Future studies are needed to determine how to rear adult *E. civile* to full coloration and how to maintain a stable adult population in the lab in order for multi-generation studies to be carried out.
Conclusions:

*Enallagma civile* has good potential as a model research organism for ecology and evolutionary biology. As a habitat generalist with a widespread distribution, studying this species could provide broad insights of the effects of environmental and/or climate change. Unfortunately, this species has been underutilized as a study subject, and there are many basic life-history traits that are still unknown for this species, including its lifespan (particularly variation in lifespan as functions of habitat, climate, and latitude), diet, and dispersal distance (including distance as a function of sex). I hope that by reviewing what is known about the life history of *E. civile* from the literature and by sharing husbandry techniques that I have found to work to raise this species in the laboratory and the life history details of the species that I learned during my research, it can be a more prominent model species in future research.
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Including English Name, Etymology, Type Locality, and Distribution. Originally
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Table 3.1: Reproductive information for *E. civile* collected on five dates from 2013-2014.

<table>
<thead>
<tr>
<th>Collection Dates</th>
<th>18-Jul-13</th>
<th>31-Jul-13</th>
<th>17-Jun-14</th>
<th>3-Jul-14</th>
<th>30-Jul-14</th>
<th>Total</th>
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<tbody>
<tr>
<td>Number of females collected</td>
<td>14</td>
<td>21</td>
<td>25</td>
<td>27</td>
<td>25</td>
<td>112</td>
</tr>
<tr>
<td>Number of females that laid eggs</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>Proportion of females that laid eggs (%)</td>
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<td>42.86</td>
<td>20.00</td>
<td>18.52</td>
<td>24.00</td>
<td>27.68</td>
</tr>
<tr>
<td>Total eggs laid</td>
<td>1196</td>
<td>1111</td>
<td>1021</td>
<td>478</td>
<td>903</td>
<td>4709</td>
</tr>
<tr>
<td>Average number of eggs laid per female ± SD</td>
<td>199.33 ± 1144.28</td>
<td>138.625 ± 122.46</td>
<td>204.8 ± 138.29</td>
<td>95.6 ± 80.81</td>
<td>150.5 ± 94.81</td>
<td>157.77 ± 114.70</td>
</tr>
<tr>
<td>Minimum number of eggs laid by a female</td>
<td>60</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Maximum number of eggs laid by a female</td>
<td>409</td>
<td>500</td>
<td>549</td>
<td>421</td>
<td>327</td>
<td>549</td>
</tr>
</tbody>
</table>
Table 3.2: Survivorship and time until hatching of *E. civile* eggs from 2014 experiments.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Total eggs</th>
<th>Total hatched</th>
<th>Percent hatched (%)</th>
<th>Days until eggs hatched</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>701</td>
<td>435</td>
<td>62.1</td>
<td>11.5 ± 5.25</td>
</tr>
</tbody>
</table>

Min. | Max.
--- | ---
7   | 37
Figure 3.1: A. Adult *E. civile* female. B. Adult *E. civile* male. C. *E. civile* nymph.
Figure 3.2: Distribution of *Enallagma civile*. Shaded areas represent states, provinces, and countries where the species has been found. This does not mean that the species is found throughout the entire shaded area.
Figure 3.3: A. Male and female *E. civile* in wheel position. B. *E. civile* pairs in tandem with females ovipositing in floating vegetation.
Figure 3.4: Mae Simmons Park, Lubbock, Texas (Pictometry International Corp., 2015).
Figure 3.5: A. Oviposition chambers. B. Experimental rearing chambers. C. Rearing containers with dowel rod and netting to enclose emerging adults.
Figure 3.6: Experimental tanks heating and cooling patterns over a 24 hour period. Temperatures recorded using a HOBO temperature logger.
Figure 3.7: A. Eggs after they were laid. B. Eggs with eye spots. C. Eggs with eye spots and legs apparent. D. Stadia 8 nymph. E. Stadia 6 nymph with developed trachea. F. Stadia 2-4, wing pads flat with veination present. G. Stadia 0-1, wing pads with rolled orientation.
Chapter 4:

Effects of water temperature under projected climate change on the development and survival of the Familiar Bluet Damselfly (*Enallagma civile*)

Introduction:

Current climate change predictions estimate increased mean air temperatures between 2 to 4°C above preindustrial baseline over the next century throughout much of North America (Romero-Lankao, 2014), with the Great Plains predicted to experience increases in temperatures across all seasons (Shafer et al., 2014). Current climate projections indicate the average air temperature in the Great Plains could increase by 1.4 to 7.2°C by 2090 under the mid-high (A2) emissions scenario (USGCRP, 2009). The Intergovernmental Panel on Climate Change (IPCC) 2014 report found that 2-4°C above baseline is very likely (Romero-Lankao, 2014), although Raupach et al. (2007) determined that the IPCC likely underestimated emissions growth, causing uncertainty in current climate predictions. Thus, even with continual updates to climate models, we may be underestimating potential changes in temperatures in this region as well as ecological consequences of these changes. In addition to increased air temperatures, annual precipitation in the southern Great Plains is expected to decrease by 20-30% (USGCRP, 2009), very hot days (above 32.22°C) are expected to increase in occurrence, and the number of days below freezing is anticipated to decrease by 2090 (USGCRP, 2009;
Rainwater, Hayhoe, & Baake, 2010). Along with decreases in the overall amount of precipitation, its timing, intensity, and form are also expected to change (Matthews, 2008; USGCRP, 2009). Ecosystems and organisms of the Great Plains that are sensitive to changes in temperature and precipitation regimes should be particularly affected by these shifts. For example, wetlands are acknowledged as being at particularly great risk from climate change (Erwin, 2009). Wetlands of the southern Great Plains and their biotic communities should be acutely affected by changes in water temperature as well as by diminished habitat availability (due to lower water levels and decreased hydroperiod stemming from temperature-driven increases in evaporation and limited rainfall). There are over 80,000 wetlands in the southern Great Plains, known as playas, that should therefore experience negative climate change effects (Covich et al., 1997).

Playas are depressional recharge wetlands formed by wind, waves, and dissolution processes (Smith, 2003). Their water is gained from precipitation and runoff and is lost by evaporation, evapotranspiration, and aquifer recharge. These processes cause playas to be temporary in nature, with varying hydroperiods (Collins, Heintzman, Starr, Wright, Henebry, & McIntyre, 2014). Due to the wet and dry periods a playa experiences, playas are centers of biodiversity within the landscape (Smith, 2003). Playa faunal communities consist largely of aquatic, semi-aquatic, and terrestrial invertebrates, as well as less-numerous animals such as amphibians and birds. Aquatic invertebrates are greatly affected by water parameters including temperature and chemical composition and so are likely to experience changes in ontogeny, survival, fitness, and/or behavior as a result of climate change. For example, specifically in reference to playa wetlands with
their shallow basins, increased water temperature could reach upper lethal limits of some invertebrate species. If lethal temperatures are reached, playa wetlands could lose those members of their invertebrate community that cannot survive within projected environmental conditions. Without a mechanistic understanding of how climate change drives biotic responses, we will be unable to predict how populations and communities will fare in the future.

Water temperature has been shown to affect the distribution, physiology, and morphology of various aquatic invertebrates (e.g. Bhowmik & Schafer, 2015). Of the aquatic invertebrates, odonates (Hexapoda: Odonata, dragonflies and damselflies) are considered a flagship group of wetland indicators (Moore, 1997). With aquatic nymphs and aerial adults, these amphibious predators provide a “fine grained” assessment of environmental condition and habitat quality (New, 2009), especially as indicators of landscape and climate changes (Samways, 2008; Ott, 2010). Odonates may also serve as umbrella species for overall wetland conservation and as surrogates for broader freshwater biodiversity, although they have been underutilized as such (Bried & Samways, 2015). Because of their life history and trophic position, odonates are also potential sentinels of climate change (Collins & McIntyre, 2017).

Odonates are known to be able to withstand higher upper thermal tolerances than can other aquatic invertebrates (Stewart, Close, Cook, & Davies, 2013). Current knowledge of nymph growth implies that odonates are relatively warm-adapted (Suhling, Suhling, & Richter 2015) and are of tropical evolutionary origin (Pritchard, Harder, & Mutch, 1996). But even so, climate change has been implicated in northward range shifts
in some dragonfly species (Hickling, Roy, Hill, & Thomas, 2005; Ott, 2010) and in advancing the timing of emergence in others (Hassall, Thompson, French, & Harvey 2007; Dingemanse & Kalkman, 2008; Richter, Suhling, Mueller, & Kern, 2008). Various studies that have examined how odonates are affected by temperature can be used to formulate questions about how a warming climate may affect other life history traits in odonates. For example, studies have documented increased voltinism of dragonflies in Europe with higher temperatures (Braune, Richter, Sondgerath, & Suhling, 2008; Flenner, Richter, & Suhling, 2010). Similarly, Sternberg (1995) found that for the dragonfly species Somatochlora alpestris and S. artica, development rate increased with higher ambient temperature. Egg hatching in various dragonfly species also has been shown to increase with temperature (Zehring, Alexander, & Montgomery, 1962), as has the phenology of adult emergence (McCauley, Hammond, Frances, & Mabry, 2015).

In terms of mechanistic studies on how temperature increases affect life-history traits in odonates, there has been a greater focus on damselflies than on dragonflies. Because damselflies are generally smaller in size than are dragonflies, they should be even more sensitive to changes in ambient conditions, and because they are easier to rear in captivity than are dragonflies, damselflies have been the primary foci of lab studies on temperature effects. As ectotherms, nymph odonates may exhibit accelerated growth with warming, but it is possible that other costs will be incurred (such as smaller adult body size and thence costs to fitness and survival; Sokolovska, Rowe, & Johansson, 2000; McCauley et al., 2015) or that upper critical temperatures will be reached, thereby lowering survival rates. For example, when Ischnura elegans nymphs were reared at
higher temperatures, a physiological cost of rapid growth caused reduced cold resistance (Stoks & De Block, 2011). Similarly, increased temperatures accelerated development, resulting in a smaller body size between stadia in *Lestes sponsa, Coenagrion puella*, and *Ischnura elegans* (Pickup & Thompson, 1990). *Argia vivida’s* temperature preferendum was shown to be 28°C, its critical thermal maximum (temperature where an organism responds with unorganized locomotion) was 39.1-41.0°C, and its upper lethal temperature (temperature where death occurs) was 44.4-46.0°C (Leggott & Pritchard, 1986). In contrast, studies on other odonate species found a relatively cooler 41.9°C to be the upper thermal tolerance (Stewart, et al., 2013).

These limited studies focused on European damselflies; no similar studies have been conducted in the Great Plains, or indeed on any New World species. There is thus an important knowledge gap that needs to be filled. *Enallagma civile* (the Familiar Bluet, Coenagrionidae) is one of the most abundant and widespread playa odonate species in the southern Great Plains (Reece, 2009; Reece & McIntyre, 2009), but little is known about the temperature tolerances of this species and how it will fare under projected climate change. Due to its wide distribution, abundance throughout North America, and it being a habitat generalist (Butler & deMaynadier, 2008; Ball-Daerow, M’Gonigle, & Resh, 2014; see Chapter 3 for further details), this species should serve as a good model organism for examining how climate change-induced warming may affect life history traits pertaining to fitness and survival. Moreover, as one of the most abundant species at playas, changes to its abundance or distribution will have ripple effects throughout the food web.
My objectives were to rear *E. civile* under warmer conditions predicted for the southern Great Plains to quantify the survival of eggs and nymphs, number of stadia, adult morphology, number of days for eggs to hatch, and number of days it took to go from egg to adult in water temperatures ranging from current levels to future elevated levels. I hypothesized there would be a decrease in survival of both eggs and nymphs with increases in water temperature due to upper lethal temperatures being reached. Development duration (both in terms of number of nymph stadia as well as time to adult emergence) was also predicted to exhibit a threshold response: development should decrease initially with temperature (due to boosted metabolism under warmer conditions) until a lethal temperature is reached. Adult body size should likewise decrease as a result of accelerated growth rates.

**Methods:**

Adult female damselflies used in this study were collected from Canyon Lake 5 in Mae Simmons Park in Lubbock, Texas, on 17 June 2014, 3 July 2014, and 30 July 2014, on sunny days, between 1030-1230 h. Adult females were captured in copula as described in Chapter 3. Females were brought back to the laboratory at Texas Tech University and placed into oviposition chambers as described in Chapter 3. Eggs were collected after 48 hours, pooled (to eliminate any potential maternal effect), counted, and reared under 26, 32, 38, or 41°C average water temperature (while allowing for natural diel heating and cooling cycles). These temperatures were chosen to represent a range of
current average water temperature in playas (mean = 26.2°C, median = 25.9°C; N. E. McIntyre, unpublished data) to projected future playa water temperatures. On average, streams increase by 0.6-0.8°C in water temperature per each 1°C increase in air temperature (Morrill, Bales, & Conklin, 2005), but there are no corresponding extrapolations of air temperature to water temperature for lentic wetlands like playas. Because playas are so shallow, with a large surface area of non-flowing water, assuming that a 1°C increase in air temperature will correspond to a 1°C increase in playa water temperature is reasonable. The maximum playa water temperature recorded over a 10-year span (2006-2016, N. E. McIntyre, unpublished data) was 36.5°C, so with a projected 1.4-7.2°C increase in average air temperature by 2090 (USGCRP, 2009), a temperature of 41°C is therefore quite possible.

Eggs were reared in chambers within 10-gallon aquaria with a FLUVAL E100 (Rolf C. Hagen (USA) Corp., Mansfield, MA, USA) aquarium heater (capable of generating a maximum temperature of 34°C) for the lower-temperature treatments (26 and 32°C) or in water baths capable of generating temperatures higher than 34°C (for the 38 and 41°C treatments). Aquarium heaters, water baths, and lights were on for 13.5 hour periods (light:dark of 13.5:11.5 hours) to simulate diel water heating and cooling patterns and seasonal photoperiod (United States Naval Observatory, 2013). The full experimental chamber design is described in Chapter 3. Because there were multiple tanks or water baths for each temperature treatment, the nymph-rearing jars were subject to potential pseudoreplication from tank effects. In addition, odonates are known to experience different development rates with time of year (with a positive response to photoperiod;
Lutz, 1968), meaning that the three cohorts could potentially have expressed different
growth responses simply as a result of time of year. Therefore, I used an analysis of
variance (ANOVA) to test for possible tank or cohort effects; no significant differences
were found between experimental tanks of the same temperature or between cohorts (F =
1.68, p = 0.1380), so blocking by tank or cohort was unnecessary in further statistical
analyses. Given that the eggs within each cohort were pooled before being randomly
assigned to treatment, and given that the cohorts were collected within 44 days of each
other, it is not surprising that there were no potentially confounding effects of tank or
cohort detected.

Eggs were monitored daily until hatching. Once the eggs hatched, nymphs were
housed individually within glass jars filled with moderately hard water (formulated from
the recipe in Weir & Salice, 2012). Eggs and nymphs were observed daily while
conducting water changes to aid in waste removal and to add artificial water with food.
Nymphs were fed *ad libitum* daily (based on stadium: as body size increased, more food
was supplied, consisting of a mixture of copepods, *Daphnia* spp., ostracods, and other
live foods that were size-appropriate). Prey populations were maintained in separate lab
aquaria. Observations of nymph mortality and ecdysis (molting) were also made at water
changes. If individuals had molted, total body length (excluding the external gills) and
head capsule width were measured, and the shed exuvia were removed. Removal for
measurement was done using a plastic 3 ml pipette with the tip cut off. Once a nymph’s
body length reached 10 mm, a bamboo dowel was placed inside each rearing jar to give
the nymph a way to leave the water and undergo ecdysis into the teneral stage, and a
mesh emergence trap was placed over each jar to enclose emerged adults. Teneral adults were given 24 hours for their exoskeleton to harden and then were measured for final adult head capsule width and total body length (see Chapter 3).

Results across treatments were statistically compared using Kaplan-Meier survival analyses on survival curves of nymphs. A Wilcoxon chi-square test was conducted to test for differences in survival percentages across temperatures. Logistic regression with a Wald chi-square test was used for binomial (lived/died) count data, which included egg and nymph survival. General linear model ANOVAs with Tukey’s HSD post-hoc tests of means were used to examine effects of temperature treatment on nymph body measurements and days until eggs hatched (SAS 9.4, Cary, NC, USA).

Results:

Seventy-seven female E. civile were caught over three collection periods (17 June 2014, 3 July 2014, 30 July 2014), referred to as cohorts 1-3 (Table 4.1). Sixteen of the 77 females (20.7%) were successful in laying eggs in the lab. In total, 2,402 eggs were laid. Egg quantities varied from 1 to 549 eggs per female, with an average (± standard error) of 150 ± 105 eggs. These data are comparable to numbers seen by Baker and McIntyre (2013) in a larger sample size of E. civile (where 27.3% of females laid eggs, with a mean ± standard error of 250 ± 18 and a range of 1-1,047 eggs) and by Zehring, Alexander, and Montgomery (1962) for various dragonfly species.
Enallagma civile eggs successfully hatched in all experimental water temperatures. However, significantly fewer eggs hatched at 41°C compared to 26°C, 32°C, and 38°C (Wald $\chi^2_3 = 228.04$, $p < 0.0001$; Fig. 4.1). The 41°C treatment had an egg survivorship of 25.08% whereas the other temperatures had similar survival rates of 60.22% to 65.53% across all cohorts (Table 4.2). Eggs reared in hotter temperatures hatched significantly more quickly than did eggs in relatively less-warm temperatures ($F_3 = 38.50$, $p < 0.0001$). Eggs took on average 9.8 days ($n = 1179$ eggs) among all temperature treatments. Eggs in colder temperature treatments (26 and 32 °C) took longer to hatch, averaging 11.5 days and 9.2 days, whereas those in warmer temperature treatments (38 and 41°C) hatched quicker, averaging 8.2 and 8.7 days (Table 4.2). The quickest an egg hatched was in 6 days and the longest it took an egg to hatch was 38 days, showing the variability in the length of this life stage. There was thus variation in hatching duration and success both within and between rearing water temperatures.

Nymphs reared in hotter temperatures had greater mortality than those from lower water temperatures. Nymphs in the 41°C treatment died off quickly (with 100% mortality by day 4), and 38°C nymph only had 15% survival after day 8 (Fig. 4.3). The relatively cooler water temperatures had higher overall survival, with 26°C having 38.58% and 32°C having 31.45% survival. The two warmer water temperatures had significantly lower survival rates, with 38°C having 2.78% survive whereas 41°C had 0% (Wilcoxon $\chi^2_3 = 209.45$, $p < 0.0001$; Fig. 4.3). Nymphs were found to go through 10-11 stadia, with no observable differences in number of developmental stages among temperatures. Number of days until emergence varied significantly by temperature ($F_3 = 36.51$, $p <$
The 32°C treatment had the earliest emergence (occurring at day 26 post-hatch), whereas the earliest emergence for the 26°C treatment was at day 33. Last emergence for 32°C happened on day 49, and for 26°C it occurred on day 52. The 38°C treatment had only two nymphs eclose as adults, with their emergences occurring relatively late, at days 56 and 71 (Fig. 4.5). The process of hatching from the egg to emergence as a teneral adult took an average of 39.5 days among all temperature treatments in the lab (range: 26 to 71 days). When examining the total development time from egg to teneral adult, the average span was 49.3 days, which was more than double what Booker (2002) reported.

Body length and head width significantly differed by temperature \( (F_2 = 26.44, p < 0.0001, \text{Fig. 4.6}; F_2 = 19.80, p < 0.0001, \text{Fig. 4.7};) \) 26°C and 32°C individuals grouped together and had larger body lengths and head widths, whereas 38°C individuals were significantly smaller in both body length and head width. Finally, 41°C was not included in this analysis because no individuals survived to adulthood. There was no significant difference in either body size measurement between sexes within treatments. Males tended to be slightly larger than females in both measurements, but there was enough overlap between the sexes to nullify any significant differences \( (p > 0.05) \).

**Discussion:**

Although increased environmental warming may provide some benefits to ectotherms by accelerating their development rate and decreasing time spent as immature
forms (Gillooly, Charnov, West, Savage, & Brown, 2002), including for some odonate species (Doi, 2008), there may be tradeoffs in terms of adult body size and fitness (Atkinson, 1994), particularly for aquatic species (Forster, Hirst, & Atkinson, 2012). In my study, elevated water temperatures were found to significantly affect the survivorship of *Enallagma civile* eggs and nymphs as well as final adult body size (length and head width) at emergence: an increase in temperature incurred a decrease in survival and size. These findings are worrisome because if elevated temperatures are affecting this model organism, elevated temperatures will likely affect other aquatic invertebrate species. My results lend support to the negative effects of elevated water temperature on the growth and survival of damselflies that have been well-documented in various European species, and in various dragonfly species. These effects occurred at temperatures higher than currently experienced: nymphs reared in experimental temperatures of 26°C (current average) and 32°C (lower than current maximum) did not exhibit any significant differences. Projected climate change in the southern Great Plains will therefore likely be manifested in fewer Familiar Bluets surviving to adulthood, with those that do survive being smaller. Although adult female body size in *E. civile* was not associated with fitness (Baker, 2011; Baker & McIntyre, 2013), a meta-analysis of various odonate species revealed overall significantly positive relationships between adult body size and fitness (Sokolovska, Rowe, & Johansson, 2000).

A warming experiment conducted in outdoor mesocosms on the dragonfly *Pachydiplax longipennis* showed that nymphs reared in cattle tanks with heaters providing 2.5°C and 5°C increases in temperature (reaching a maximum water
temperature of only 33°C) experienced lowered survival and eclosed ~3 weeks earlier than those reared at ambient temperatures, but there were no significant effects on adult body size in terms of thorax or wing measurements (McCauley et al., 2015). There thus appear to be some consistent trends across odonate species in response to even modest environmental warming.

Because *Enallagma civile* is a generalist, we may not be able to see subtle morphological and behavioral responses to elevated water temperatures that perhaps other, more specialized species may display because specialists are presumably more tightly coupled with the environment than are generalist species (Pandit, Kolasa, & Cottenie, 2009). When generalists like *E. civile* do show responses to environmental factors such as elevated water temperatures, we should be concerned for all other species of aquatic invertebrates living within the same aquatic environment. Species that are not able to adapt to changes to the environment will become extirpated. Changes to the food web can occur from these extirpations, causing other species that are able to withstand the environmental changes to be affected indirectly by climate change (through trophic and other biotic interactions).

Because females were captured while in tandem, we may have interrupted the mating behavior of the pair. Copulation of *E. civile* has three distinct stages: insemination, oviposition, and termination (Corbet, 1999). We are not sure which behavior stage females were in when caught, because they were caught in tandem. Oviposition could have already occurred, causing lower numbers of eggs to be laid (see Baker and McIntyre, 2013).
Water temperature affected the survivorship of *E. civile* eggs and the number of days for eggs to hatch. Hotter temperatures caused a lower survivorship of eggs. Eggs at 41°C had significantly lower survivorship than the rest of the temperatures. The 26°C, 32°C, and 38°C temperature treatments all had similar survival rates, indicating that water temperature does not affect egg survival until water temperature reaches levels above 38°C. The number of days for the eggs to hatch were fewer in hotter temperatures than in cooler temperatures: eggs reared at 38°C hatched 3.3 days faster than eggs at 26°C, and eggs at 41°C hatched 2.8 days quicker than eggs at 26°C. This demonstrates how hotter temperatures can accelerate the hatching rate of *E. civile* eggs.

Water temperature influenced survival of *E. civile* nymphs. Nymph survival to adulthood was significantly higher at the relatively cooler temperatures, with 38.58% of nymphs surviving at 26°C and 31.45% surviving at 32°C. Hotter temperatures had significantly lower nymph survival, with only 2.78% at 38°C and 0% at 41°C. This suggests that the upper lethal temperature for *Enallagma civile* nymphs is between 38 and 41°C. This finding is similar to other studies that have found other odonate species to have upper thermal tolerances of 41.9°C (Leggott & Pritchard, 1986; Stewart et al., 2013).

Adult emergence occurred earliest at 32°C (26 days) and 26°C (33 days), with emergence continuing until days 49 and 52, respectively (that is, eclosion began on day 26 in the 32°C treatment and continued until the last adult emerged at day 49). Of the two hotter temperature treatments (38°C and 41°C), only 38°C had adults emerge, which occurred at days 56 and 71; no adults emerged from the 41°C treatment. The only
previous known development length known for *E. civile* is 21 days from egg to adult in a
playa setting (Booker, 2002). Unfortunately these data were from an unpublished thesis
conducted at a single field site in Texas, and have not been able to be reproduced in the
lab. The accelerated phenology I observed in my study (a difference in 30 days between
the six degrees of difference in the 32°C and 38°C treatments) is consistent with findings
of Richter et al. (2008), who found adults emerged an average of 4.7 days later for every
1°C of warming. It is unknown whether this shift in adult emergence will have non-
optimal effects as the result of some kind of phenological mismatch.

My results demonstrate that hotter temperatures will affect the overall
survivorship of the *E. civile* by causing fewer individuals to emerge with hotter water
temperatures. The length of time (56 and 71 days) taken for individuals from the 38°C
treatment to emerge is particularly troubling. Other species of damselflies are known to
emerge after longer durations than the *E. civile* in my study: *Lestes disjunctus* emerges
after 70 days, and *Coenagrion resolutum* takes between 10-22 months to emerge
(Krishnaraj & Pritchard, 1995), although both have a more northerly distribution than
does *E. civile*. Fewer individuals could emerge in the future if time needed to emerge
exceeds playa hydroperiod length. Collins et al. (2013) found a minimal hydroperiod
length of 55.5 days, which both specimens from the 38°C treatment both exceeded this
length of time.

Adult morphology was also found to be influenced by water temperature. No
significant differences were found between 26°C and 32°C, whereas the two individuals
that emerged at 38°C were significantly smaller in terms of body length and head width.
This suggests that the individuals at hotter water temperature made tradeoffs in body size in order to be able to emerge.

Playa water temperatures up to 36.5°C have been documented in Texas (N. E. McIntyre, unpublished data). Under current climate projections, playa water temperatures of 41°C would be extreme depending on the relationship of air temperature to water temperature. Current estimations for streams show an increase 0.6-0.8°C in water temperature for each 1°C increase in air temperature (Morrill, Bales, & Conklin, 2005), but similar estimations for lentic wetlands like playas do not currently exist. Because playas are typically shallow, usually less than 2 m deep (Guthrey & Bryant, 1982; Smith, 2003), and have a large surface area to volume ratio, every 1°C increase in air temperature may increase playa water also by 1°C. Under a 1 to 1 air temperature to water temperature relationship, with projected air increase of up to 7.2°C (USGCRP, 2009), playa water temperatures of 41°C are possible. If relation is closer to 0.6-0.8°C per 1°C air temperature, then 41°C water temperatures would be an unlikely and extreme event under most climate projections. However, by testing this temperature it allowed me to see how this model organism responded to an extreme temperature. With the number of days with air temperatures above 37.8 °C expected to increase with climate change (Shafer et al., 2014), 41°C water temperature could become more of a possibility in the future.

Overall, my study shows that the model organism *E. civile* is susceptible to elevated water temperature. With predicted climate change, we will see a reduction in
aquatic invertebrates that are not tolerant of elevated water temperature, and/or
phenological and morphological changes.
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Table 4.1: Reproductive information for *E. civile* from summer 2014.

<table>
<thead>
<tr>
<th>Collection Dates</th>
<th>17-Jun-14</th>
<th>3-Jul-14</th>
<th>30-Jul-14</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of females collected</td>
<td>25</td>
<td>27</td>
<td>25</td>
<td>77</td>
</tr>
<tr>
<td>Number of females that laid eggs</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Proportion of females that laid eggs (%)</td>
<td>20.00</td>
<td>18.52</td>
<td>24.00</td>
<td>20.84</td>
</tr>
<tr>
<td>Total eggs laid</td>
<td>1021</td>
<td>478</td>
<td>903</td>
<td>2402</td>
</tr>
<tr>
<td>Average number of eggs laid per female ± SE</td>
<td>204.8 ± 27.66</td>
<td>95.6 ± 15.55</td>
<td>150.5 ± 18.96</td>
<td>150.30 ± 12.07</td>
</tr>
<tr>
<td>Minimum number of eggs laid by a female</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Maximum number of eggs laid by a female</td>
<td>549</td>
<td>421</td>
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</tr>
</tbody>
</table>
Table 4.2: Survivorship and time until hatching of *E. civile* eggs from 2014 experiments.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Total eggs</th>
<th>Total hatched</th>
<th>Percent hatched (%)</th>
<th>Days until eggs hatched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Avg. ± SD</td>
</tr>
<tr>
<td>26</td>
<td>701</td>
<td>435</td>
<td>62.1</td>
<td>11.5 ± 5.25</td>
</tr>
<tr>
<td>32</td>
<td>626</td>
<td>377</td>
<td>60.2</td>
<td>9.2 ± 4.71</td>
</tr>
<tr>
<td>38</td>
<td>322</td>
<td>211</td>
<td>65.5</td>
<td>8.2 ± 1.90</td>
</tr>
<tr>
<td>41</td>
<td>622</td>
<td>156</td>
<td>25.1</td>
<td>8.7 ± 1.73</td>
</tr>
</tbody>
</table>
Figure 4.1: Egg hatch rate by temperature treatment ($\chi^2 = 228.04, p < 0.0001$). Columns denoted with the same horizontal bar and letter were not significantly different (Tukey’s HSD $p > 0.05$).
Figure 4.2: Mean number of days for eggs to hatch by temperature treatment ($F_3 = 38.50, p < 0.0001$). Columns denoted with the same letter were not significantly different (Tukey’s HSD $p > 0.05$).
Figure 4.3: Survival curves for *E. civile* nymphs at four water temperatures (Wilcoxon $\chi^2_3 = 209.45$, p < 0.0001).
Figure 4.4: Percent emergence of adult damselflies by temperature treatment. Columns denoted with the same horizontal bar and letter were not significantly different (Tukey’s HSD p > 0.05).
Figure 4.5: Length of time from egg hatching to adult emergence and abundance by temperature treatments of *E. civile*. Earliest emergence amongst all temperature treatments occurred at day 26, whereas the last occurred at day 71. Individuals in the 41°C treatment did not survive to emergence so no data are shown for that temperature.
Figure 4.6: Adults had smaller body lengths at hotter temperatures. Individuals in the 41°C treatment did not survive so no measurements could be taken. Boxes denoted with the same letter were not significantly different (Tukey’s HSD p > 0.05). Lines in each of the boxes represent the 25\textsuperscript{th}, median, and 75\textsuperscript{th} percentiles. The error bars (if present) represent the 10\textsuperscript{th} and 90\textsuperscript{th} percentiles (38°C is missing error bars due to sample size of 2). Dots represent outliers within the data.
Figure 4.7: Adult had smaller head width at hotter temperatures. Individuals in the 41°C treatment did not survive so no measurements could be taken. Boxes denoted with the same letter were not significantly different (Tukey’s HSD p > 0.05). Lines in each of the boxes represent the 25th, median, and 75th percentiles. The error bars (if present) represent the 10th and 90th percentiles (38°C is missing error bars due to sample size of 2). Dots represent outliers within the data.
Chapter 5:

Conclusion

Climate and land-use changes are the predominant threats to playa wetlands of the Southern High Plains. This dissertation has demonstrated how both of these threats are affecting playa wetlands and their community of organisms. Chapter 1 introduced playa wetlands and the history of the Southern High Plains. Chapter 2 demonstrated the impact of land-use change over time by assessing changes in five land-use types over 23 years, quantifying how many playas were wet or dry on each date examined, and determining that playa inundation changed in frequency over time as a function of land use surrounding playa basins. Wet playas were observed to decrease over the 23-year study span, and significant differences in land uses were concurrently observed. Rangeland/grassland land-use increased in area, although patch size decreased over time, indicating coalescence of fewer but larger rangeland/grassland patches. Agricultural land-use was the second-most dominant land-use type. Because playas are crucial habitats, these changes have likely affected regional biodiversity.

Along with land-use changes, climate shifts will likely affect playas in future. For Chapter 3, the Familiar Bluet damselfly (Enallagma civile) was selected as a model organism to study the effects of environmental changes (land use and climate) on playa invertebrates. I documented life history characteristics and husbandry techniques for rearing E. civile in an evaluation of use of this species as a suitable model organism. Then in Chapter 4, I used this species to evaluate the effects of one
aspect of projected climate change (elevated temperature). I conducted a lab study that examined the effects of elevated water temperature on the survivorship and development of *Enallagma civile*. I reared *E. civile* under warmer conditions predicted for the Southern Great Plains to quantify: 1) survival of eggs and nymphs, 2) number of developmental stadia, 3) adult morphology, 4) number of days for eggs to hatch, and 5) number of days it took to go from egg to adult. Eggs were collected and reared under four temperatures: 26, 32, 38, and 41°C. Eggs were found to survive the best in the three colder temperatures. Nymphs in the two hotter treatments were smaller and had lower survivorship, whereas individuals in the cooler temperatures generally survived to adulthood and were larger. *Enallagma civile* was observed to have 10 to 11 stadia. Individuals reared at 32°C emerged the quickest, going from egg to adult in 26 days. Elevated temperatures can thus be both advantageous and detrimental, causing concern for aquatic invertebrates in the future.

In conclusion, these studies demonstrated how land-use and climate changes are threats to playa wetlands and biota. With rangeland/grassland increasing over time, the frequency of playa wetland inundation may continue to decrease due to interactions between land use and overland water flow during precipitation events. With decreases in playa inundation frequencies and effects of climate change, playa invertebrate communities are threatened due to infrequent standing water and elevated water temperatures. By understanding how land-use and projected climate changes are currently effecting playa wetlands, it will allow for better comprehension and management of current and future alterations.