NORTHERN LEOAPRD FROGS IN NORTH DAKOTA: ASSESSING THE CONSERVATION STATUS OF A WIDESPREAD AMPHBIAN SPECIES

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DOCTOR OF PHILOSOPHY

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ABSTRACT

North Dakota's midcontinent location within the Prairie Pothole Region is widely known for the dense wetlands. These highly productive wetlands are mixed within an agricultural mosaic which places increased pressure on water quality and overall wetland persistence. These threats to wetlands affects other, more common species which are presumed to have healthy populations but lack statewide information. Such is the case with the northern leopard frog (*Lithobates pipiens*).

I examined genetic variation for 41 populations across the state. Genetic diversity was not correlated with latitude, but was negatively correlated with longitude. Along this genetic diversity gradient, there was a distinctive break near the 100th meridian, a historical boundary between the arid western United States and the wet eastern side. Further data exploration revealed wetland densities to be positively correlated with genetic diversity whereas precipitation and anthropogenic disturbance were not correlated with genetic diversity.

I also examined population genetic structure to identify conservation units. Strong population structuring was defined by the Missouri River, identifying the Western Badlands and Western Prairie conservation units. Further structuring of *L. pipiens* occurred within these two defined conservation units with rough correspondence to local watersheds. Additionally, I used approximate Bayesian computational analyses to evaluate coalescence times among the 10 defined units. The Western Prairie and Western Badlands unit shared common ancestry 13,600 to 18,100 generations ago. The coalescence times of the 6 populations within the Western Prairie unit varied from as recently as 588 generations to 10,900 generations, while populations within the Western Badlands unit varied as recently as 2,890 generations to 5,220 generations.

In addition to the northern leopard frog genetics research, I conducted research that considered how sampling biases may lead to inaccurate estimates aquatic invertebrate abundance. I present an assessment of potential biases associated with sampling a population of the amphipod *Gammarus lacustris* in the presence of Polymorphus spp. acanthocephalan parasites shown to increase positive phototaxis in their amphipod hosts. Results indicated that the highest captures of *G. lacustris* individuals were in benthic traps, however, parasitized individuals were captured most often in surface traps.

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CHAPTER 1. INTRODUCTION

1.1. Conservation of Amphibians

The global decline of amphibians has led to an increased interest in understanding amphibian dispersal and population structure across anthropogenically altered landscapes (Wake 1991; Blaustein et al. 1994; Collins and Storfer 2003; Storfer 2003; Stuart et al. 2004; Beebee and Griffiths 2005). For instance, amphibian dispersal processes can be disrupted by anthropogenically altered landscapes (Ricketts 2001; Berry et al. 2005). The resulting reduction in gene flow among populations affects the genetic structure of populations, potentially leading to loss of genetic diversity and/or increasing breeding, which can increase population extinction risk (e.g., Wauters et al. 1994; Stow et al. 2001; Keller and Largiadèr 2003; Burkey and Reed 2006).

Assessing these risks require tools for predicting the impacts of habitat alteration on movement among populations (Gustafson et al. 2001; Cushman 2006). Fortunately, molecular tools can be used to describe patterns of genetic diversity and give potential insights into population demographic history (Frankham 1995). Such molecular tools are valuable to record genetic diversity within and among amphibian populations due to high natural variance in natural amphibian populations (Pechman and Wilbur 1994). In fact, molecular markers have been very useful for evaluating population structure and genetic health for a variety of rare amphibian species (e.g., Blank et al. 2013; Dias et al. 2014). These tools can also be used in studies of more common species such as the northern leopard frog to provide baseline information for future monitoring and sound information for conservation rankings.

Once, one of the most wide-ranging amphibians in North America (Figure 1.1), the northern leopard frog suffered range wide population declines beginning in the 1960's and

1970's (Smith 2003; Rorabaugh 2005), due largely to overharvest. During the early 1970's, 9 to 22 million northern leopard frogs were harvested annually in the U.S. and Canada for educational and research purposes alone causing drop in numbers of approximately 50% in the U.S. (Gibbs et al. 1971; Merrell 1977). The specific reasons for continued declines of northern leopard frogs to date are not well known but may be due to a number of natural and anthropogenic factors such as global climate change, disease, habitat loss and fragmentation, habitat alteration, reduced water quality, and the introduction of exotic predators (Smith and Keinath, 2004).

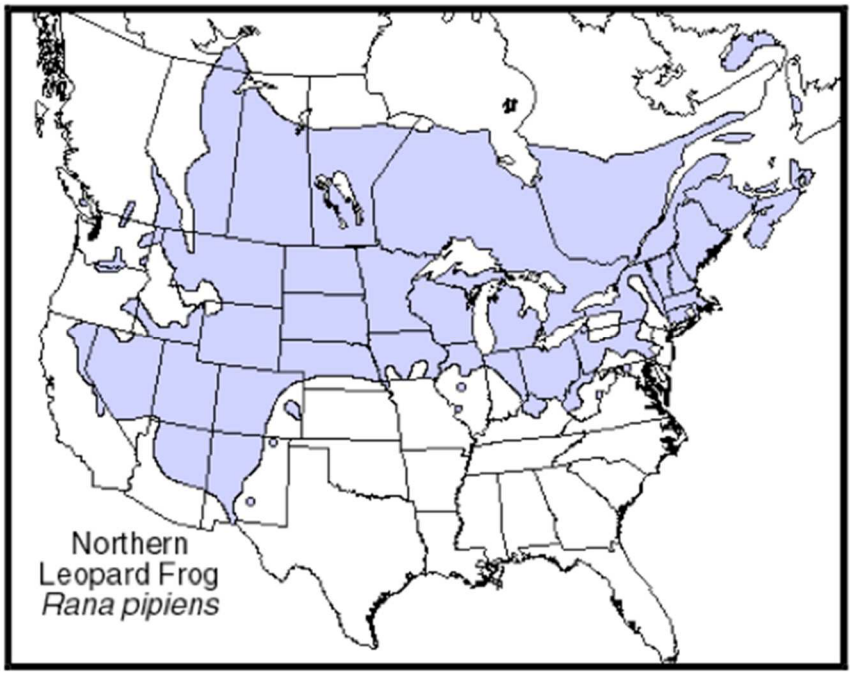

Figure 1.1. Northern leopard frog range across North America (photo adapted from USFWS 2009a petition).

Because these threats vary geographically, it is important to clarify how genetic variation is structured in northern leopard frogs. In fact, northern leopard have been recently proposed as two distinct genetic populations: the *western* population and the *eastern* population (Figure 1.2)(USFWS 2009a). The recognition of these *distinct population segments* was based on

significant differences in mt-DNA haplotype frequencies between populations east and west of the Mississippi River (Hoffman and Blouin 2004; O'Donnell and Mock (2012). The *western* population occurs in 18 states west of the Mississippi River and south of the international boundary between the U.S. and Canada. The *eastern* population consists of all U.S. states east of the Mississippi River as well as all the Canadian provinces in which the northern leopard frog is found. These two proposed *distinct population segments* gained recent attention as the *western* population was petitioned for protection under the Endangered Species Act. The petition was based on range-wide status assessment which showed that the *western population* had declined in 13 out of the 18 western U.S. states (NatureServe 2014). Only one state, North Dakota, has not established a conservation ranking for northern leopard frogs but all populations west of North Dakota were considered at risk while populations in states to the east were considered secure (Figure 1.3; NatureServe 2014).

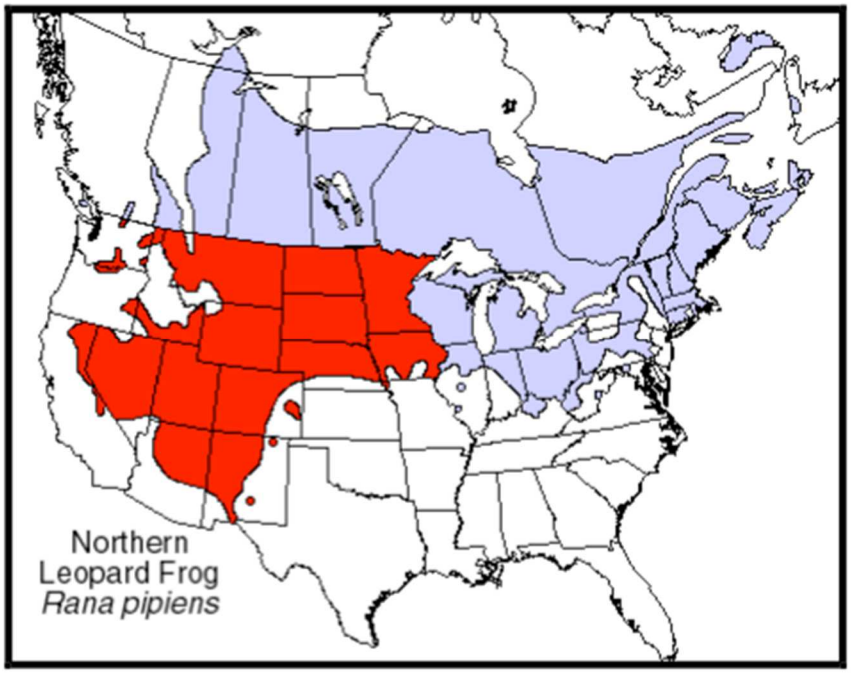

Figure 1.2. Proposed genetically different northern leopard frog populations in North America. Red region indicates proposed western population and blue region indicates eastern population (photo adapted from USFWS 2009a petition).

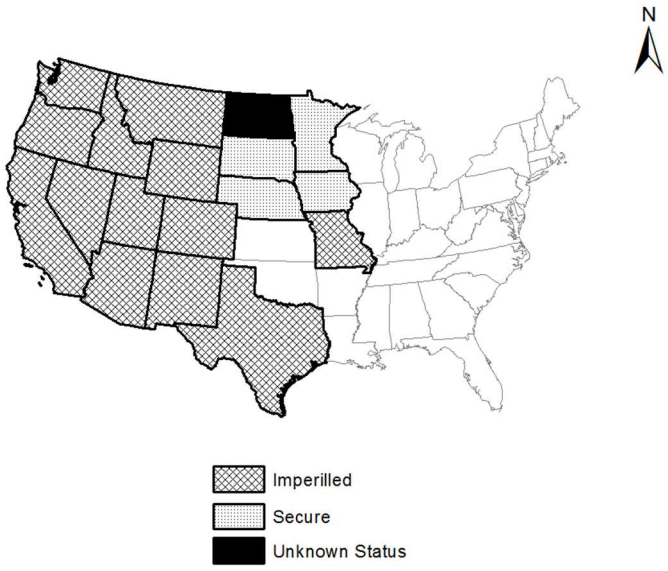

Figure 1.3. NatureServe conservation status of *L. pipiens* among western population.

1.2. Understanding Northern Leopard Frog Status in North Dakota

1.2.1. North Dakota's Landscape

Northern leopard frog distribution and history in North Dakota is likely tied to the spatial and temporal patterns of glaciation during the Pleistocene. Previous genetic work indicates that northern leopard frogs recolonized northern portions of their current range from differing southern refugia outside of the last glacial maximum (Hoffman and Blouin 2004; Wilson et al. 2008). During the last glacial maximum, the northern and eastern parts of North Dakota were covered with ice, while the southwestern portion of North Dakota was ice-free. As glacial ice melted, the associated run-off produced the Missouri River (Bluemle 1972). Thus, it would appear that the Missouri River would have acted as a potential barrier to gene flow and thus has played a role in structuring northern leopard frog genetic diversity in North Dakota.

1.2.2 North Dakota's Climate and Northern Leopard Frog Populations

North Dakota northern leopard frog populations are exposed to annual climatic extremes with summer temperatures exceeding 40°C and winter temperatures nearly reaching -40°C (Jensen 1998). Northern leopard frog persistence in this extreme environment is tied to their use of a mosaic of permanent and temporary wetlands. Northern leopard frogs overwinter by settling at the bottom of wetlands, and only survive if the wetlands do not completely freeze or experience winterkill conditions due to deep snow (Cory 1952, Dole 1965, Merrell and Rodell 1968). In North Dakota, winter conditions can produce ice thickness of up to a meter deep so it is important for *L. pipiens* to overwinter in deeper wetlands (Barica 1979; Mushet et al. 2013). Therefore, northern leopard frog population persistence requires a mosaic of wetlands with sufficient depth for overwintering, and wetland depth is tied to both precipitation and evapotranspiration (Mushet 2010).

Northern leopard frog populations of North Dakota are also impacted by drought to deluge cycle that can persist for 10 to 20 years, with most wetlands dessicating during drought cycles (Karl and Koscielny 1982; Karl and Riebsame 1984; Diaz 1983; Diaz 1986). Once the deluge portion of a cycle starts, wetlands refill (van der Valk and Davis 1976; Euliss et al. 2004). Thus, habitat availability for northern leopard frogs contract and expand in response to drought and deluge periods, respectively (Mushet 2010). Further, drought events can have particularly drastic consequences on northern leopard frog populations in the western portion of the state where annual precipitation rates are less than 34cm compared to more than 62cm of average precipitation in the far eastern edge (PRISM Climate Group, PRISM 2014). Over the next century Karl et al. (2009) predicts that North Dakota will see dramatic annual precipitation changes with increases of nearly 40% or greater throughout the state due the high rate of climate

change. These annual precipitation changes, coupled with an anticipated increase in average temperatures of 4 °C by the year 2090 (Karl et al. 2009), will most likely alter historical wet/dry cycles and in turn have direct impacts on northern leopard frog populations due to potentially reduced hospitable wetlands.

1.2.3. Current Knowledge of Northern Leopard Frogs in North Dakota

The status of the leopard frog in North Dakota is not well known, but recent work by Mushet et al. (2013) suggests that large populations of this species persist in central North Dakota. These inferences are drawn from high levels of genetic diversity found within and among populations of northern leopard frogs, in Stutsman County, in the Prairie Coteau region of North Dakota. Such results were attributed to the spatial and temporal dynamics of deep, overwintering wetlands that are more often found within this region (Mushet et al. 2013). The genetic health and population structure of northern leopard frogs beyond Mushet et al.'s (2013) limited study area has not been characterized, yet the high spatial variation in precipitation and wetland densities across the state would suggest reduced genetic diversity for northern leopard frog populations in the drier portions of the state, particularly southwestern North Dakota. As stated earlier, northern leopard frog population status probably changes across North Dakota as populations to the east are secure while those to the west are considered at risk. Thus, providing insights on the status of North Dakota populations of northern leopard frogs will fill an important information gap and provide insights on environmental factors that impact northern leopard frog populations

1.4. Organization of Dissertation

This dissertation consists of five chapters including a general introduction, three chapters which report the results of original research I conducted for this dissertation, as well as a fifth

chapter on conclusions. Chapter two discusses the use of molecular markers to evaluate the spatial patterns in genetic diversity of northern leopard frog populations. This chapter highlights a longitudinal gradient of genetic diversity that decreases from east to west. Furthermore, this chapter identifies and discusses a unique break in genetic diversity at the 100th meridian, a historical location that separates the arid western United States from the wetter eastern half. I also report a high correlation of genetic diversity with wetland densities.

Chapter three focuses on the population genetic structure of northern leopard frog populations throughout North Dakota. The results identify a clear break of population structure on either side of the Missouri River, suggesting a barrier to gene flow. I discuss these findings in the context of conservation units.

Chapter four explores how sampling methodology may be biased when assessing aquatic communities within wetlands where/when parasites may alter the behavior of invertebrate hosts thus changing their spatial and temporal distribution in the wetland. The results, and follow-on simulations, identify that parasitic infection may affect the reliability of invertebrate abundance estimates. Finally, chapter five summarizes and synthesizes major findings of the three major chapters.

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CHAPTER 2. GENETIC VARIATION FOR NORTHERN LEOPARD FROG POPULATIONS BEYOND THE HUNDREDTH MERIDIAN[1]

2.1. Abstract

Conservation efforts commonly target rare and declining species, with less effort directed towards impacts on common species. However, even common species may rapidly decline as evidenced by recent declines of numerous previously common amphibian species, but detecting declines in amphibians can be challenging due to naturally great demographic variation. Here, we use genetic markers to explore the status of the northern leopard frog (*Lithobates pipiens*) in North Dakota. Populations of this historically common species are stable in areas east of North Dakota, but have declined in areas to the west. Using genetic tools, we found expected heterozygosity (H_E) and average number of alleles (N_A) were both significantly lower for populations west of the 100th meridian in North Dakota compared to eastern populations within the state. We used multiple regression analyses to evaluate correlations of landscape attributes with the genetic metrics, H_E and N_A, and a model selection approach to compare seven a priori landscape level models. Model parameters included: 1) disturbance area, 2) wetland density and 3) average annual precipitation. Wetland density was positively correlated with genetic diversity and found to be the most important explanatory variable. This finding is consistent with northern leopard frog use of multiple wetland types, including deep wetlands as overwinter refugia, during its lifecycle. Our exploration of northern leopard frog genetic diversity in North Dakota revealed that eastern populations are relatively stable within the state, but that populations within

[1] The material in this chapter was co-authored by Justin D. L. Fisher and Craig A. Stockwell. Justin D. L. Fisher had the primary responsibility for collecting samples in the field as well as processing samples in the laboratory. Justin D. L. Fisher was the primary developer of the conclusions described here within. Justin D. L. Fisher also drafted and revised all previous versions of this chapter. Craig A. Stockwell served as a proofreader and supplied constructive comments for an improved chapter. This chapter has been submitted to *Conservation Genetics*.

the state west of the 100th meridian are of higher conservation concern. Our findings illustrate the value of genetic markers in assessing species conservation status, particularly in light of potential changing climatic conditions.

2.2. Introduction

Rare and declining species are widely recognized as targets for conservation investment (Gaston and Fuller 2008); however, anthropogenic threats have also impacted common species (Gaston and Fuller 2007). Within the past few centuries, major drivers of extinction (Diamond 1984) have reduced the abundance and range sizes of many previously common species (Gaston 2010). In some cases, this has led to high levels of threat or even extinction (Lindenmayer et al. 2011). In this vein, even common amphibian species have declined during the last few decades (Wake 1991, Luoma 1997; Blaustein et al. 1994; see also Adams et al. 2013). Such declines of common amphibians are noteworthy and include species such as boreal chorus frog (*Pseudacris triseriata maculata*; Lemmon et al. 2007, McMenamin et al. 2008), Columbia spotted frog (*Rana luteiventris*; Funk et al. 2005, McMenamin et al. 2008), boreal toad (*Bufo boreas*; Drost and Fellers 1996, McMenamin et la. 2008), and the northern leopard frog (*Lithobates pipiens*; Corn and Fogleman 1984, Leonard et al. 1999).

Detecting declines for widely distributed amphibian species is challenging due to their natural temporal variance in population size (Caldwell et al. 1991, Pechman and Wilbur 1994, Marsh 2001). However, genetic tools may provide insights to population demographics of species with great temporal variation (Whitlock 1992). Furthermore, genetic surveys can provide important baseline information for future monitoring and conservation assessments (Demarias et al. 1993, Oostermeijer et al. 2003, Lesbarrères et al. 2014).

Here, we use molecular markers to evaluate the status of the northern leopard frog in North Dakota. This widespread species has drawn recent attention due to range-wide declines, particularly in western populations of this species (Corn and Fogleman 1984, Leonard et al. 1999). The conservation status of western *L. pipiens* populations has been evaluated for 17 of 18 U.S. states, the exception being North Dakota (NatureServe 2014)(Figure 2.1a). Populations east of North Dakota have been considered secure, but populations to the west are considered at risk. This suggests that landscape factors affect *L. pipiens* population dynamics changes across the state.

Notably, the degree of aridity changes spatially across North Dakota, with western portions of the state receiving about half as much precipitation (34cm) as eastern portions (62cm; 50-year average annual precipitation; PRISM Climate Group 2014)(Figure 2.1b). Further, North Dakota is bisected by the 100th meridian, a historically observed breakpoint that roughly delineates the arid western half of the United States and the wet eastern half (Powell 1879, Sabo et al. 2010). Across North Dakota, anthropogenic disturbance also varies spatially with relatively higher levels of disturbance in the east. Wetland densities also vary spatially across the North Dakota with higher densities of wetlands in the eastern half, owing to the geomorphology of the Prairie Pothole region (Mushet et al. 2014), and fewer wetlands in the western half, particularly so in the southwest (Euliss and Mushet 2004). These three factors, precipitation, disturbance, and wetland density, may play an important role influencing the viability of *L. pipiens* populations in North Dakota. Here, we evaluated *L. pipiens* genetic diversity across the state. We used a model selection approach to further elucidate the role of precipitation, anthropogenic disturbance and wetland density on genetic diversity.

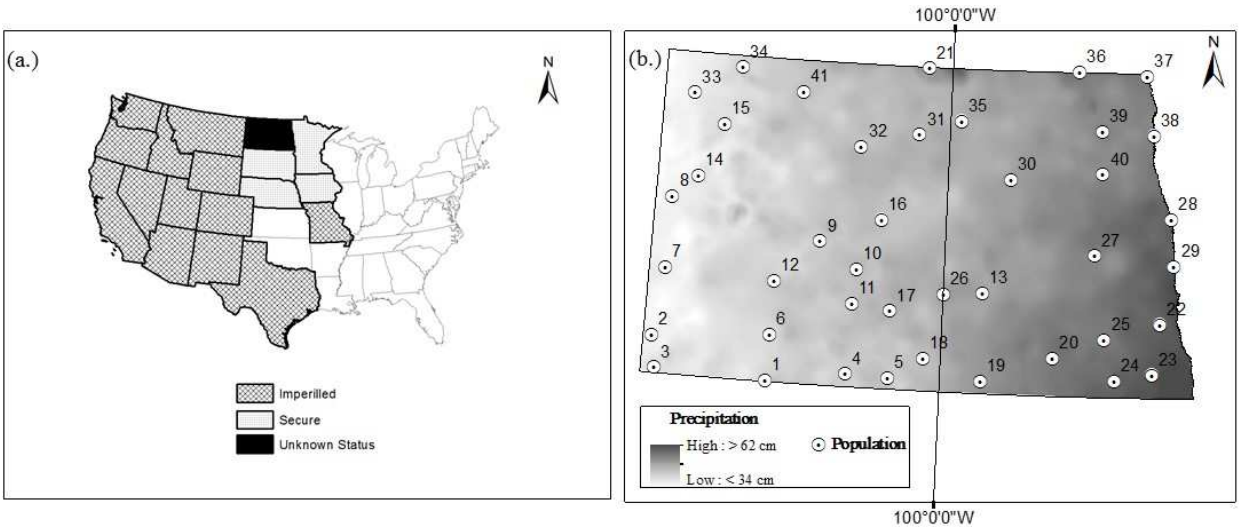

Figure 2.1. (a.) NatureServe conservation status of western population of *L. pipiens* found in the United States and (b.) sampling sites of *L. pipiens* throughout North Dakota and precipitation pattern found throughout state.

2.3. Materials and Methods

We sampled 41 populations of *L. pipiens* throughout North Dakota (Figure 2.1b). All sampling sites were at least 30km distant from each other and occurred in wetlands classified as being either a permanent or semi-permanent wetland according the U.S. Fish & Wildlife Service National Wetland Inventory (http://www.fws.gov/wetlands). At each collection site, *L. pipiens* toe clippings were collected from 30 individuals following NDSU IACUC protocol #A10047. Toe clippings were stored in individually marked vials containing 95% ethanol alcohol.

Total genomic DNA was extracted and purified using DNeasy® Blood and Tissue kits (Qiagen® Corporation). We amplified seven microsatellite loci using primers developed by Hoffman et al. (2003) for *L. pipiens* (Rpi 100, Rpi 101, Rpi 103, Rpi 104, Rpi 106, Rpi 107, Rpi 108), two microsatellite loci using primers developed by Hoffman and Blouin (2004) for the Oregon spotted frog (*Rana pretiosa*; RP197 and RP415) and two microsatellite loci using primers developed by McKay et al. (2011) for the Southern leopard frog (*Lithobates sphenocephala*; Rasp09 and Rasp20). Amplifications were conducted using polymerase chain

reactions (PCR) on Eppendorf Mastercyclers following PCR mixtures published with each respective locus. Lengths of PCR products (i.e. microsatellite fragments) were visualized using an Applied Biosystems 3130 automated sequencer.

MICRO-CHECKER (version 2.2.3; van Oosterhout et al. 2004) was used to evaluate our data set for genotyping errors and null alleles. GENEPOP'007 (Rousset 2008) was used to test for deviations from Hardy-Weinberg Equilibrium (*HWE*) and Linkage Disequilibrium (*LD*). Summary statistics for population diversity estimates were calculated using the Microsoft Excel add-in GenAlEx 6.0 (Peakall and Smouse 2006) including observed (H_O), expected (H_E) and, and mean number of alleles per locus (N_A).

To test for possible spatial pattern in genetic diversity we used linear regression to determine if mean number of alleles per locus and expected heterozygosity could be predicted from latitude and longitude. Further, we examined spatial variation by examining diversity between and within the eastern and western parts of the state; east and west of 100th meridian.

We obtained 2011 National Land Cover Data (NLCD) and the National Wetland Inventory (NWI) data from the North Dakota GIS Hub (www.nd.gov/gis) and used ArcGIS 9.3 ® to extract landcover types in a 15km buffer radius surrounding each population sample location. We chose the 15km radius size as a probabilistic area for potential individual movements under ideal conditions of wet seasons since the last drought period.

Once clipped, we reclassified the NLCD to combine all disturbed areas which included cultivated fields, roadways, and towns. We used the NWI data to calculate wetland density, i.e., number of permanent and semi-permanent wetlands per km^2, around each sample location. Lastly, we obtained point estimates of 50-year average annual precipitation from each sample location from the PRISM Climate Group dataset (http://prism.oregonstate.edu; PRISM 2014).

We used multiple regression analysis to evaluate correlations of landscape attributes with genetic metrics: H_E and N_A. We developed seven *a priori* landscape level models and evaluated each using a model selection approach. Model parameters included: 1) disturbance area, 2) wetland density and 3) average annual precipitation. Models were ranked using Akaike's Information Criterion which was corrected for small sample size (AICc). Models were subsequently evaluated using relative weights with the resulting AICc weight. Variable significance was further evaluated using model averaging estimates using unconditional confidence intervals (Burnham and Anderson 2002). Additionally, we reported correlation coefficients and associated p-values with significance adjusted with a sequential Bonferroni correction (Rice 1989). All analysis was conducted in Program R using the packages "foreign", "MASS", and "AICcmodavg".

2.4. Results

Examination of all 11 loci revealed no deviations from Hardy Weinberg Equilibrium and no null alleles were identified. Additionally, we found no signification deviations from linkage-disequilibrium within our data set. Heterozygosity varied widely (H_O: 0.684 - 0.839; H_E: 0.695 - 0.853), as did the average number of alleles (N_A: 5.364 – 11.182)(Table 2.1).

Table 2.1. Genetic diversity metrics of *L. pipiens* populations sample throughout North Dakota; Pop=sampled location numerical designation, H_O = observed heterozygosity, H_E = expected heterozygosity, and N_A = average number of alleles.

Pop	Latitude	Longitude	H_O	H_E	N_A
1	45.9459	-102.3523	0.699	0.710	5.545
2	46.2917	-103.9303	0.705	0.717	5.818
3	45.9886	-103.8614	0.694	0.706	5.364
4	46.0603	-101.2729	0.718	0.730	6.818
5	46.0444	-100.7024	0.687	0.699	5.364
6	46.3817	-102.3276	0.684	0.696	5.636
7	46.9448	-103.8166	0.729	0.742	7.182
8	47.6199	-103.809	0.763	0.776	7.636
9	47.2982	-101.7223	0.745	0.757	7.455
10	47.0524	-101.206	0.754	0.767	7.727
11	46.7295	-101.2365	0.740	0.752	8.000
12	46.8975	-102.32	0.757	0.769	7.818
13	46.8855	-99.465	0.789	0.803	8.273
14	47.8343	-103.4848	0.765	0.778	7.455
15	48.3387	-103.172	0.783	0.796	8.182
16	47.536	-100.8975	0.809	0.823	10.273
17	46.6797	-100.7111	0.791	0.804	8.636
18	46.2469	-100.2325	0.780	0.793	8.091
19	46.05	-99.4417	0.811	0.825	9.455
20	46.2902	-98.4735	0.803	0.816	9.091
21	48.9853	-100.3384	0.705	0.717	5.909
22	46.6303	-97.0144	0.819	0.833	10.364
23	46.1521	-97.1195	0.835	0.849	11.182
24	46.0874	-97.6337	0.839	0.853	10.636
25	46.4796	-97.7752	0.805	0.819	8.909
26	46.8596	-99.9971	0.797	0.810	9.545
27	47.27	-97.9363	0.817	0.831	9.000
28	47.6232	-96.8768	0.804	0.817	10.000
29	47.1793	-96.8362	0.802	0.815	9.000
30	47.9666	-99.1254	0.809	0.823	9.000
31	48.354	-100.4244	0.784	0.798	8.727
32	48.214	-101.2344	0.792	0.805	8.545
33	48.6072	-103.6239	0.749	0.761	7.455
34	48.8831	-102.9811	0.766	0.779	8.091
35	48.501	-99.847	0.797	0.811	9.091
36	48.9965	-98.1966	0.808	0.822	10.182
37	48.9705	-97.2387	0.812	0.826	10.000
38	48.413	-97.1386	0.811	0.825	10.364
39	48.4417	-97.8601	0.815	0.828	10.182
40	48.0383	-97.8393	0.804	0.818	10.091
41	48.69105	-102.09507	0.775	0.788	8.000

Expected heterozygosity (HE) was negatively correlated with longitude; relatively lower heterozygosity for western populations compared to eastern populations (R^2= 0.522, p < 0.001) (Figure 2.2a). Average number of alleles (NA) was also negatively correlated with longitude (R^2 = 0.572, p < 0.001)(Figure 2.2b). We failed to detect any correlation between latitude and average number of alleles (R^2 = 0.065, p = 0.108) or between latitude and expected heterozygosity (R^2 = 0.063, p = 0.112), respectively.

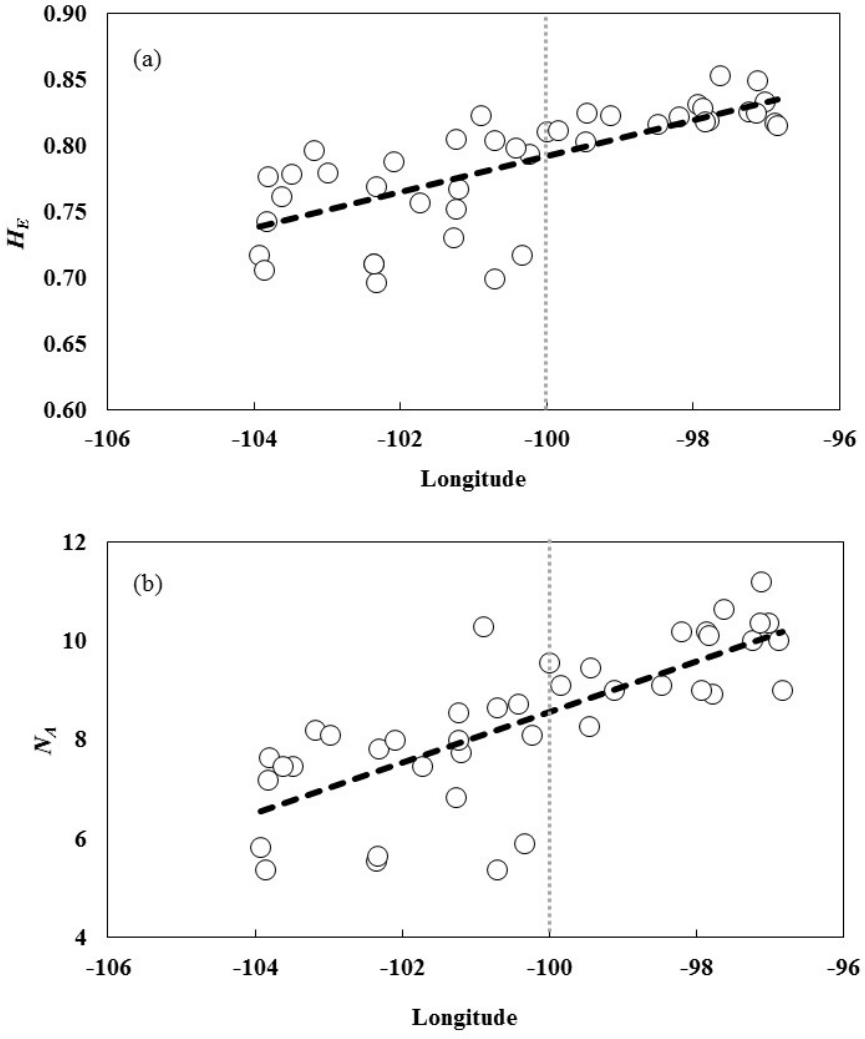

Figure 2.2. (a.) Expected heterozygosity (H_E) versus longitude of sampled *L. pipiens* populations across North Dakota and (b.) average number of alleles (N_A) versus longitude of sampled *L. pipiens* populations across North Dakota.

We further explored geographic patterns in diversity by comparing genetic diversity between populations east versus west of the 100th Meridian. Expected heterozygosity was significantly lower for western populations (n =23; 0.76 ± 0.008; mean ± standard error of the mean) compared to eastern populations (n = 18; 0.82 ± 0.003); paired-sample t-test showed significant differences in scores (t = 7.122, $p < 0.001$). Similarly, average number of alleles was significantly lower for western populations (7.38 ± 0.265) compared to eastern populations (9.69 ± 0.178); paired-sample t-test showed significant differences in scores (t = 7.754, $p < 0.001$). Expected heterozygosity was negatively correlated with longitude in the eastern half (R^2= 0.240, p = 0.039) but failed to show correlation in western half (R^2= 0.049, p = 0.324)(Figure 2.3a). Average number of alleles was negatively correlated with longitude in the eastern half (R^2= 0.2960, p = 0.019) but not correlated with longitude in the western half of the state (R^2= 0.036, p = 0.198)(Figure 2.3b).

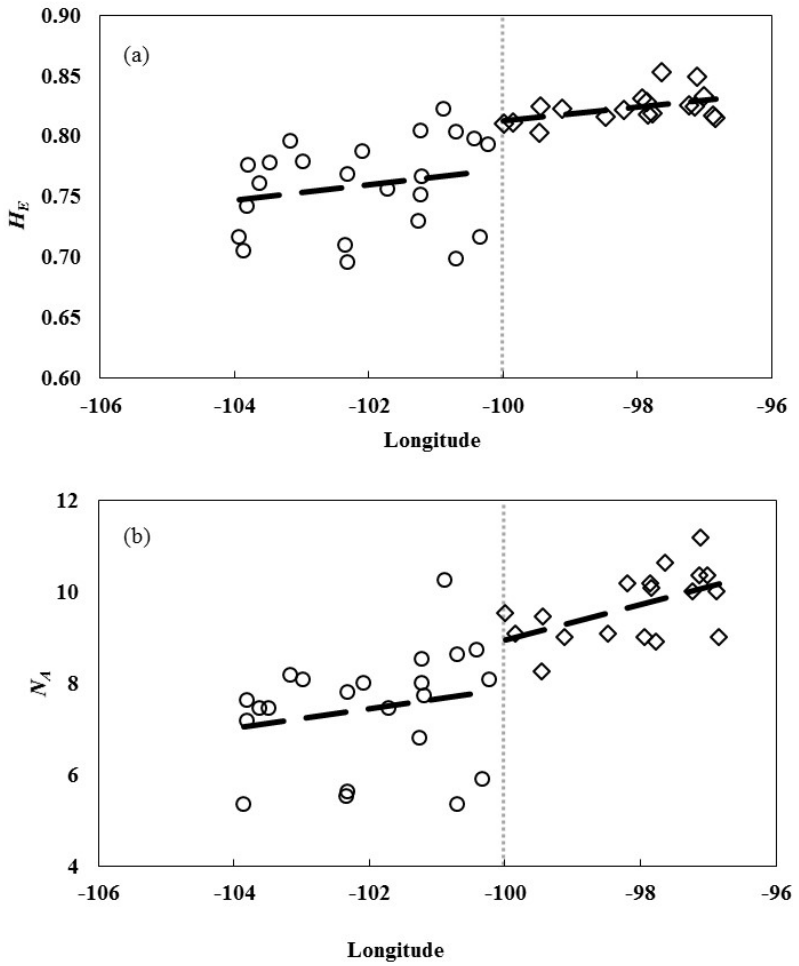

Figure 2.3. (a.) Expected heterozygosity (H_E) and (b.) average number of alleles (N_A) versus longitude of sampled *L. pipiens* populations sampled across North Dakota with designated east and west populations demarcated by 100th meridian; western sampled locations are marked as open circles while eastern sampled locations are open diamonds.

All seven multiple regression analysis models predicting expected heterozygosity (H_E) with various combinations of disturbance area, wetland densities, and average precipitation were significant with R^2 values ranging from 0.09 to 0.58 (Table 2.2). The two models out performing others were the global model (Wetland Density + Disturbance Area + Precipitation; Δ AIC = 0.00) and the model including only Wetland Density and Disturbance Area (Δ AIC = 0.66). Model averaging indicated that the only significant variable was Wetland Density (Table 2.3).

Table 2.2. Model selection results of associated landscape attributes to heterozygosity; bolded *p*-values indicate significance following sequential Bonferroni correction.

Variables	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log Likelihood	Adjusted *R* Squared	*P*-Value
Wetland Densities + Disturbance Area + Precipitation	-162.86	0.00	0.57	0.57	87.31	0.58	**0.0000**
Wetland Densities + Disturbance Area	-162.20	0.66	0.41	0.98	85.67	0.55	**0.0000**
Disturbance Area + Precipitation	-156.59	6.27	0.02	1.00	82.86	0.49	**0.0000**
Disturbance Area	-151.91	10.95	0.00	1.00	79.29	0.40	**0.0000**
Wetland Densities	-148.43	14.43	0.00	1.00	77.55	0.35	**0.0000**
Wetland Densities + Precipitation	-147.16	15.70	0.00	1.00	78.15	0.35	**0.0001**
Precipitation	-134.98	27.88	0.00	1.00	70.82	0.09	**0.0371**
Intercept Only Model	-132.69	30.17	0.00	1.00	68.51	NA	NA

Table 2.3. Variable model averaging results of landscape attributes in heterozygosity models; significant variables (i.e., variables that do not overlap zero) are bolded.

Variable	Overall Variable Weight	Model Averaging
Wetland Densities	0.98	***0.02 (95% CI 0.01, 0.02) Significant***
Disturbance Areas	1.00	0 (95% CI 0, 0) Not Significant
Precipitation	0.59	0 (95% CI 0, 0) Not Significant

Predicted average number of alleles (N_A) from the seven model combinations produced all significant models with R^2 values ranging from 0.08 to 0.55 (Table 2.4). Of these, the two models that performed better than the others were the global model (Wetland Densities + Disturbance Area + Precipitation; Δ AIC = 0.00) and the Wetland Densities + Disturbance Area model (Δ AIC = 0.98; Table 2.4). Variable model averaging results indicated that only the significant variable was Wetland Densities (Table 2.5).

Table 2.4. Model selection results of associated landscape attributes to average number of alleles; bolded *p*-values indicate significance following sequential Bonferroni correction.

Variables	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log Likelihood	Adjusted *R* Squared	*P*-Value
Wetland Densities + Disturbance Area + Precipitation	126.15	0.00	0.51	0.51	-57.19	0.55	**0.0000**
Wetland Densities + Disturbance Area	127.12	0.98	0.31	0.82	-58.99	0.52	**0.0000**
Disturbance Area + Precipitation	128.46	2.32	0.16	0.98	-59.66	0.51	**0.0000**
Disturbance Area	132.84	6.69	0.02	1.00	-63.09	0.43	**0.0000**
Wetland Densities	142.69	16.55	0.00	1.00	-68.01	0.27	**0.0003**
Wetland Densities + Precipitation	143.93	17.78	0.00	1.00	-67.39	0.27	**0.0010**
Precipitation	152.05	25.91	0.00	1.00	-72.69	0.08	**0.0453**
Intercept Only Model	153.98	27.84	0.00	1.00	-74.83	NA	NA

Table 2.5. Variable model averaging results of landscape attributes in average number of alleles models; significant variables (i.e., variables that do not overlap zero) are bolded.

Variable	Overall Variable Weight	Model Averaging
Wetland Densities	0.82	***0.42 (95% CI 0.07, 0.78) Significant***
Disturbance Areas	1.00	0 (95% CI 0, 001) Not Significant
Precipitation	0.67	0 (95% CI 0, 0.01) Not Significant

Finally, we tested the same models for predicting genetic variation for western populations as well as for western populations. Model selection results of eastern populations failed to produce any significant models (Table 2.6 and 2.7). Analyses of western populations to predict expected heterozygosity (H_E) and average number of alleles (N_A) from the seven models produced significant models but variable model averaging failed to identify any variable of importance (Tables 2.8 and 2.9).

Table 2.6. Model selection results of associated landscape attributes to heterozygosity east of the 100th meridian.

Variables	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log Likelihood	Adjusted *R* Squared	*P*-Value
Intercept Only Model	-102.69	0.00	0.33	0.33	53.74	NA	NA
Wetland Densities + Disturbance Area	-102.08	0.61	0.24	0.57	56.58	0.17	0.094
Disturbance Area	-101.13	1.56	0.15	0.72	54.42	0.01	0.280
Wetland Densities	-100.71	1.98	0.12	0.84	54.21	-0.01	0.369
Precipitation	-99.83	2.85	0.08	0.92	53.77	-0.06	0.820
Wetland Densities + Disturbance Area + Precipitation	-98.28	4.41	0.04	0.96	56.64	0.12	0.199
Disturbance Area + Precipitation	-97.77	4.92	0.03	0.99	54.42	-0.05	0.569
Wetland Densities + Precipitation	-97.42	5.27	0.02	1.00	54.25	-0.07	0.656

Table2.7. Model selection results of associated landscape attributes to average number of alleles east of the 100th meridian.

Variables	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log Likelihood	Adjusted *R* Squared	*P*-Value
Disturbance Area	43.89	0.00	0.40	0.40	-18.09	0.14	0.071
Intercept Only Model	44.76	0.88	0.26	0.66	-19.98	NA	NA
Disturbance Area + Precipitation	46.87	2.98	0.09	0.75	-17.90	0.10	0.176
Wetland Densities + Disturbance Area	46.99	3.10	0.09	0.84	-17.95	0.95	0.185
Wetland Densities	47.28	3.40	0.07	0.91	-19.78	-0.04	0.559
Precipitation	47.67	3.79	0.06	0.97	-19.98	-0.06	0.941
Wetland Densities + Disturbance Area + Precipitation	50.42	6.53	0.02	0.99	-17.71	0.06	0.301
Wetland Densities + Precipitation	50.64	6.75	0.01	1.00	-19.78	-0.11	0.845

Table 2.8. Model selection results of associated landscape attributes to heterozygosity west of the 100th meridian.

Variables	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log Likelihood	Adjusted *R* Squared	*P*-Value
Disturbance Area	-80.97	0.00	0.34	0.34	44.15	0.21	0.0182
Wetland Densities	-79.88	1.09	0.20	0.54	43.61	0.17	0.0318
Wetland Densities + Disturbance Area	-79.42	1.35	0.18	0.72	44.99	0.23	0.0321
Disturbance Area + Precipitation	-78.98	1.99	0.13	0.85	44.67	0.21	0.0423
Intercept Only Model	-77.38	3.59	0.06	0.91	41.01	NA	NA
Wetland Densities + Precipitation	-77.02	3.95	0.05	0.96	43.69	0.13	0.0987
Wetland Densities + Disturbance Area + Precipitation	-76.47	4.50	0.04	1.00	45.11	0.20	0.0756
Precipitation	-74.70	6.27	0.01	1.00	41.01	-0.05	0.9050

Table 2.9. Model selection results of associated landscape attributes to average number of alleles west of the 100th meridian.

Variables	AICc	Delta AICc	AICc Weight	Cumulative Weight	Log Likelihood	Adjusted *R* Squared	*P*-Value
Disturbance Area	74.26	0.00	0.32	0.32	-33.47	0.19	0.0241
Wetland Densities	74.95	0.68	0.23	0.55	-33.81	0.16	0.0343
Wetland Densities + Disturbance Area	75.58	1.31	0.17	0.72	-32.61	0.21	0.0402
Disturbance Area + Precipitation	76.77	2.51	0.09	0.81	-33.21	0.17	0.0673
Intercept Only Model	77.30	3.03	0.07	0.88	-36.33	NA	NA
Wetland Densities + Precipitation	77.56	3.29	0.06	0.94	-33.60	0.14	0.0945
Wetland Densities + Disturbance Area + Precipitation	78.96	4.69	0.03	0.97	-32.60	0.17	0.0994
Precipitation	79.99	5.73	0.02	1.00	-36.33	-0.05	0.9395

2.5. Discussion

The high level of genetic diversity we observed is consistent with earlier published work on *L. pipiens* (Hoffman et al. 2004; O'Donnell and Mock 2012; Mushet et al. 2013). Further, Phillipsen et al. (2011) showed that genetic diversity was negatively correlated with longitude when measured across the entire range of *L. pipiens*. They hypothesized that this pattern could reflect recent divergence and range expansion of northwestern populations or that the reduced diversity of northwestern populations was due to habitat specific factors that depress effective population size in this region. Our findings suggest a third hypothesis that spatial variation in diversity is broadly associated with precipitation, and more locally by wetland density.

We observed significant spatial variation in genetic diversity that was correlated with longitude, but not latitude. The lack of a latitudinal effect is not surprising due to the fact that our latitudinal sampling breadth was less than half of the longitudinal sampling breadth (3°, 44'; 7°, 60', respectively). Further, we sampled across the 100th meridian, well known for delineating the arid west from the wet east (Powell 1879, Sabo et al. 2010), as well as the common transition region from eastern tallgrass prairie to western shortgrass prairie (Frey 1992).

Although many eastern amphibians reach their western limits near the 100th meridian (Bock and Smith, 1982), the range of *L. pipiens* spans regions east and west of the 100th meridian. Nonetheless, our results shows that genetic diversity changes beyond the 100th meridian. The change was reflected in a statistically significant reduction in genetic diversity for western populations compared to eastern populations. Further, our model selection results show different patterns between west and east, however, these findings are limited by relatively small sample sizes.

Interestingly, wetland density appears to be the most important factor influencing genetic diversity. Wetland density was positively correlated with both heterozygosity and the average number of alleles. These findings are consistent with *L. pipiens* habitat requirements that include a mosaic of wetlands to survive and reproduce especially in more northern latitudes where winter *L. pipiens* will overwinter by settling at the bottom of wetlands, and only survive if the ponds do not completely freeze or experience winterkill conditions due to deep snow (Cory 1952, Dole 1965, Merrell and Rodell 1968). In North Dakota, winter conditions can produce ice thickness of up to a meter deep so it is important for *L. pipiens* to overwinter in deeper wetlands (Barica 1979, Mushet et al. 2013). Thus, the spatial and temporal distribution of winter refugia are very important for the persistence of northern leopard frogs.

We also examined whether land-use patterns affected genetic diversity as it is widely assumed that agricultural activities negatively influence amphibian populations (Semlitsch 2000). In North Dakota, we expected that areas of intense agricultural practices, particularly the eastern half, would show signs of reduced genetic diversity owing to restricted gene flow among local populations. While agricultural disturbance was a variable commonly found within our top models, it was not a significant variable based on model averaging. However, our analyses are

inherently limited due to the fact that spatial patterns of agriculture are confounded with the precipitation gradient. Agricultural crop production in the wetter east has recently been dominated by corn and soybeans, while the drier western area is heavily used for haying as well as small grain crop production such as spring, winter, and durum wheat varieties (USDA 2014).

Within the mosaic of agriculture, there are important grassland habitats of ecological importance for *L. pipiens*. The historic protection of these areas has been afforded through the U.S. Department of Agriculture (USDA) Conservation Reserve Program (CRP). The CRP program is voluntary with monetary incentives to establish and maintain perennial cover on upland areas that were previously used for agricultural production (Mushet et al. 2014). However, CRP protection can be lost when commodity prices increase thereby reducing the incentives for participation (Rashford 2011). In fact, during the last decade a high percentage of CRP lands have been converted back to agricultural production (Fargione 2009, Wright and Wimberly 2013, Mushet et al. 2014). Such trends could reduce migratory corridors that allow gene flow and recolonization of amphibians including *L. pipiens*. Thus, additional genetic monitoring would be advisable to evaluate the effect of reduced CRP lands.

Our findings help provide insights on the conservation status of northern leopard frogs in North Dakota. The high genetic diversity we found in eastern populations suggests large population sizes suggesting that these populations may be considered relatively secure. By contrast, western populations in the state had significantly lower levels of genetic diversity and thus are correspondingly less secure. These findings are consistent with large-scale assessments of *L. pipiens*, as populations east of North Dakota are considered secure while populations in states to the west of North Dakota are considered at risk (NatureServe). Our findings further illustrate the value of genetic markers as tools for assessing population status and supports

proposals to include genetic markers in assessing species conservation statuses (Rivers et al. 2014).

Lastly, the product of our research can serve as baseline information for future studies, especially in light of changing climatic conditions. Multiple reports and models predict changing temperatures and precipitation patterns that will influence the regional wetland hydrology and future vulnerability of wetland desiccation (Larson 1995, Poiani et al. 1996, Sorenson et al. 1998, Conly and van der Kamp 2001, Johnson et al. 2005, Johnson et al. 2010, Niemuth et al. 2010, Wright 2010). Thus, the spatial variation in diversity may provide a glimpse of the potential temporal changes in genetic diversity. Specifically, climate change induced reductions in precipitation and/or wetland densities likely would have negative impacts on *L. pipiens* genetic diversity, which in turn may compromise evolutionary responses to climate change.

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CHAPTER 3. NORTHERN LEOPARD FROG GENETIC STRUCTURE AND CONSERVATION UNITS IN NORTH DAKOTA[1]

3.1. Abstract

One critical component of biodiversity conservation is the recognition and protection of intra-specific conservation units. Most previous work has focused on defining evolutionarily significant units as a way to protect important intra-specific variation within rare species; however, defining conservation units may prove useful due to the global decline of many common species. We evaluated the northern leopard frog (*Lithobates pipiens*) in light of recent petition for federal protection. Our work focused on populations in North Dakota to fill an important information gap on the conservation status of this species. Microsatellite marker analyses included traditional and Bayesian analyses programs, which all produced concordant patterns of genetic spatial structure. These analyses all identified structure that occurred at two spatial scales. Strong population structuring was defined by the Missouri River, which we defined as the *Western Badlands* and *Western Prairie* conservation units. Finer scale structuring of *L. pipiens* occurs within these two defined conservation units, with four units and six units in the *Western Badlands* and *Western Prairie* conservation units, respectively. These fine scale units are roughly aligned with local watersheds. The most unique population was the Turtle Mountain population that was nested within the *Western Prairie* conservation unit. We used approximate Bayesian analyses to evaluate coalescence times among the 10 defined units. The *Western Prairie and Western Badlands unit* shared common ancestry 13,600 to 18,100

[1] The material in this chapter was co-authored by Justin D. L. Fisher, Kevin M. Purcell David M. Mushet, and Craig A. Stockwell. Justin D. L. Fisher had the primary responsibility for collecting samples in the field as well as processing samples in the laboratory. Justin D. L. Fisher was the primary developer of the conclusions described here within. Justin D. L. Fisher also drafted and revised all previous versions of this chapter. Kevin M. Purcell, David M. Mushet, and Craig A. Stockwell served as a proofreaders and supplied constructive comments for an improved chapter.

generations ago. The coalescence times of the 6 populations within the *Western Prairie* unit varied from as recently as 588 generations to 10,900 generations, while populations within the *Western Badlands* unit varied as recently as 2,890 generations to 5,220 generations. These patterns suggest structure of *L. pipiens* reflects that spatial structure was established during and after the Wisconsin glaciation, and that finer scale structure is associated with watersheds. Future management efforts should be directed toward conserving intraspecific diversity of *L. pipiens* at multiple scales in order to conserve the historic evolutionary legacy of this species.

3.2. Introduction

Understanding and protecting intra-specific variation is an important means of conserving biodiversity (Waples 1991; Frankham et al. 2002). In fact, the Endangered Species Act (ESA) provides for the conservation of biodiversity below the species level through the protection of subspecies as well as *distinct population segments* (Pennock and Dimmick 1997; Haig et al. 2006). However, the recognition of subspecies varies widely among taxonomic groups (Haig et al. 2006) creating a need for alternative conceptual frameworks for recognizing and protecting diversity below the species level (Ryder 1986; Waples 1991; Moritz 1994; Crandall et al. 2000; Fraser and Bernatchez 2001).

The most prominent conservation unit concept has been the Evolutionarily Significant Unit (ESU) which Ryder (1986) defined as a set of conspecific populations with a distinct and long-term evolutionary history, mostly separated from other such units. First explicitly used by Waples (1991), ESUs had to meet two criteria which included 1) reproductive isolation and 2) "evolutionary legacy" or adaptive distinctiveness. This definition was used under ESA to protect designated ESUs of various Pacific salmon species, such as the Upper Columbia River spring-run of Chinook salmon (*Oncorhynchus tshawytscha)*(Good et al. 2005; Miller et al. 2011).

Aside from ESUs, intraspecific variation is protected as *Distinct Population Segments* under the Endangered Species Act (see Pennock and Dimmick 1997; Waples 1998).

One major challenge with the ESU concept was its vague operational definition, leading Moritz (1994) to define ESUs as reciprocally monophyletic for mitochondrial DNA haplotypes and significant differences for allele frequencies at nuclear loci. Moritz (1994) also provided an avenue for protecting biodiversity at finer spatial scales as *management units* (MU) that should be managed to ensure the viability of the larger ESU. He defined MUs as conspecific populations that are demographically autonomous and have a significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles (Avise, 1995; Moritz, 1999).

Several other definitions of ESUs have been introduced, but a consensus definition seems to be as difficult and controversial as debates concerning various species concepts (Moritz 1994a,b; 1999; Moritz et al., 1995; Taylor and Dizon, 1996; Pennock and Dimick, 1997; Waples, 1998). However, Fraser and Bernatchez (2001) suggested that all ESU concepts can be used, depending on the taxa, evolutionary forces and temporal scales under consideration. For instance, Moritz (1999) recognized the conservation value of genetic structural diversity by pointing out that unique geographical lineages are irreplaceable. Therefore, molecular assessments of spatial genetic population structure remain a powerful tool for recognizing conservation units.

While conservation units for have been recognized for a large number of rare species (Crandall et al. 2000; de Guia and Saitoh, 2007), few studies have considered the pre-emptive benefit of designating conservation units for widespread and relatively common species (Moraes-Barros et al. 2007). However, such studies of intraspecific diversity can identify

significant biogeographic regions. Further, defining conservation units may prove useful since many taxa have experienced widespread declines in abundance as has been the case with North American chiropterans (Blehert et al. 2009) as well as numerous amphibian species (Dirzo et al. 2014).

The northern leopard frog (*Lithobates pipiens*) is a widespread species which provides a nice case study for evaluating conservation units at different spatial scales. Both Hoffman et al. (2004) and O'Donnell and Mock (2012) recognized two distinct conservation units for *L. pipiens* as the *Eastern* and *Western* populations which are segregated by the Mississippi River. Furthermore, a third distinct conservation unit located in the far western region was recently recognized by the *Committee on the Status of Endangered Wildlife in Canada* (COSEWIC 2009), using data provide by Wilson et al. (2008). These units are probably best considered as *distinct population segments* for this species. In fact, the USFWS implicitly recognized these units when only the *Western population of L. pipiens* for proposed for listing under the ESA (USFWS 2009). Beyond ESA listing, it is useful to further evaluate fine scale structure of this species to determine whether localized management units should be recognized and independently managed.

Here, we apply molecular markers to evaluate the genetic structure of *L. pipiens* across the state of North Dakota, as this is the only state in which the status of the *western L. pipiens population* has not been evaluated. Northern leopard frog are considered secure in states to the east of North Dakota while they have declined in states west of North Dakota (NatureServe 2014))Figure 3.1). Such a change in species security suggests that landscape factors change across North Dakota. Thus, understanding the genetic structure within and across North Dakota will provide insights to identifying and understanding local conservation units. For instance, like

the Mississippi, the Missouri River is a likely barrier to gene flow. Further, the only non-glaciated area during the Pleistocene in North Dakota lies to the southwest of the Missouri River (Bluemle 1972). Thus, it seems likely that the Missouri River may structure the genetic variation of *L. pipiens* in North Dakota.

We evaluated spatial genetic structure with standard and emerging Bayesian-based structuring methods. Further, we evaluated temporal patterns of divergence using approximate Bayesian computational methods (Cornuet et al. 2008) to evaluate time of coalescence among *L. pipiens* populations in North Dakota. These temporal and spatial analyses should provide insights into the biogeographic history of *L. pipiens* populations in North Dakota, and thus fill an important information gap concerning the spatial conservation status of this widespread species.

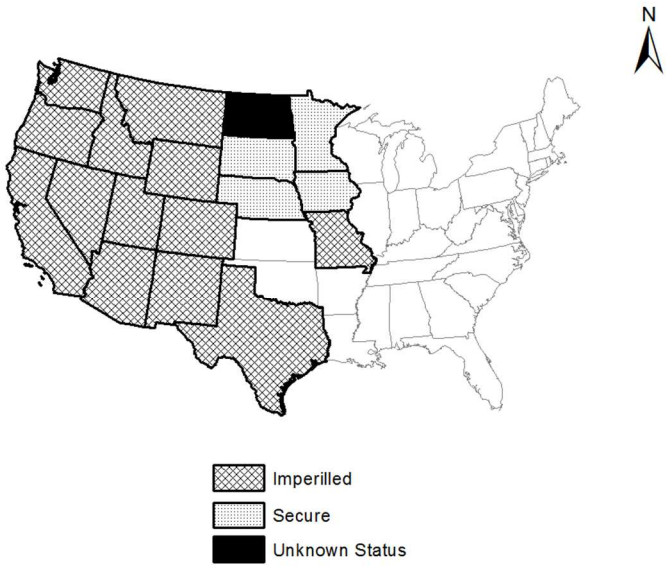

Figure 3.1. NatureServe conservation status of *L. pipiens* among western population.

3.3. Materials and Methods

We sampled 41 populations of *L. pipiens* throughout North Dakota (Figure 3.2). Potential sampling sites were selected *a priori* as permanent or semi-permanent wetlands as classified by the U.S. Fish & Wildlife Service National Wetland Inventory (USFWS 2012). The minimum distance between sampling sites was at least 30km and no greater than 85km. At each site, we actively searched the wetland perimeter for specimens and, as captures were made, focused on spreading out distances between sampled specimens to reduce likelihood of sampling related individuals. At each collection site, *L. pipiens* toe clippings were collected from 30 individuals following NDSU IACUC protocol #A10047. Toe clippings were stored in individually marked vials containing 95% ethanol alcohol.

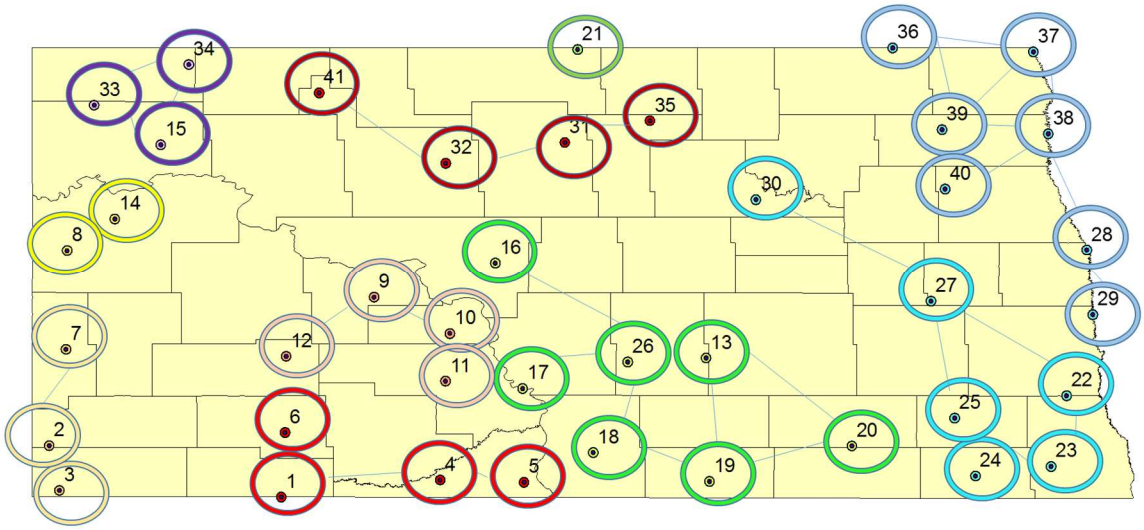

Figure 3.2. Sampling locations of *L. pipiens* populations throughout North Dakota; color coded circles with connecting lines indicate population structuring association.

Total genomic DNA was extracted and purified in our laboratory at North Dakota State University using DNeasy® Blood and Tissue kits (Qiagen® Corporation). We amplified seven

microsatellite loci primers developed by Hoffman et al. (2003) for *L. pipiens* (Rpi 100, Rpi 101, Rpi 103, Rpi 104, Rpi 106, Rpi 107, Rpi 108), two microsatellite loci primers developed by Hoffman and Blouin (2004) for the Oregon Spotted Frog (*Rana pretiosa*; RP197 and RP415) and two primers developed by McKay et al. (2011) for the southern leopard frog (*Rana sphenocephala*; Rasp09 and Rasp20). Amplification was conducted using polymerase chain reactions (PCR) on Eppendorf Mastercyclers following PCR mixtures published for each locus. PCR products were shipped to Ohio State University where lengths of PCR products (i.e. microsatellite fragments) were visualized using an Applied Biosystems 3130 automated sequencer. We scored the resulting electropherograms and cross checked with 2% of samples randomly re-run to ensure accuracy of scores.

We assessed the integrity our data set and derived population level metrics of genetic diversity. MICRO-CHECKER (version 2.2.3; van Oosterhout et al. 2004) was used to evaluate our data set for genotyping errors and null alleles. GENEPOP'007 (Rousset 2008) was used to test for deviations from Hardy-Weinberg Equilibrium (*HWE*) and Linkage Disequilibrium (*LD*). ARLEQUIN 3.5 was used to examine pairwise F_{ST} and significant testing of differences in F_{ST} values with a permutation test using 1000 iterations (Excoffier et al. 2005). We used Mantel tests in ARLEQUIN 3.5 to determine the relationship between genetic and geographic distance; to test for isolation by distance (*IBD*) (see Escoffier and Lischer 2010)). To further explore genetic structure, we calculated Cavalli-Sforza chord distance in the program TREEFIT (Kalinowski 2009) and visualized the chord distance as a UPGMA tree produced in TREEVIEW (Page 1996).

We also analyzed genetic structure using the Bayesian clustering programs STRUCTURE 2.3 (Pritchard et al. 2000), BAPS 3.2 (Corander et al. 2008), and GENELAND

4.0.4 (Guillot et al. 2005; Guillot and Santos 2009). These three programs model genetic population structure by describing the genetic variation in each sub-population using a separate joint probability distribution over all the observed loci. BAPS and GENELAND both allow for evaluation of population structure using geographic location as a model parameter.

Our STRUCTURE analyses consisted of an admixture model with correlated allele frequencies for each potential number of clusters (*K*) and prior information about the source populations was not used (i.e. LOCPRIOR model not used). Each analysis consisted of 200,000 simulations after an initial burn-in of 20,000 simulations which was significant for convergence. Our analysis was run for *K* values ranging from 1 to 41 inferred clusters with 10 independent runs each. We assessed the best *K* value supported by the data using the ΔK method described by Evanno et al. (2005) via STRUCTURE Harvester 0.6.94 web application (Earl and von Holdt 2012*)*. This application uses the rate of change in successive posterior probabilities over the range of *K* values to identify the best *K*. If inconsistent results in *K* values were found compared to BAPS and GENELAND, additional STRUCTURE analyses was performed individually for each *K* group (Breton et al. 2008; Pereira-Lorenzo et al. 2010). These additional STRUCTURE analyses allow for identification of potential sub-structuring which may have been missed.

Our BAPS analyses were initially performed by both "clustering of individuals" and "spatially clustering of individuals" models within the population mixture analysis. We performed these analyses using K_{max} ranging from 1 to 41 and ran 10,000 iterations to estimate the admixture coefficients of each sample. We ran ten replicates for both models with consistent results across all models results.

In GENELAND, we ran analysis of 10 runs to infer *K* using 200,000 Markov chain Monte Carlo (MCMC) iterations, a maximum rate of Poisson process fixed to 100, *K* ranging

from 1 to 41, and uncertainty attached to the spatial coordinates fixed at 5m. We used the Dirichlet allelic frequency distribution model as recommended by Guilliot et al. (2005). We inferred the *K* value for our analysis from the modal *K* value from the initial 10 runs. Using the inferred *K* value we ran a subsequent analysis consisting of 10 replicates using the same MCMC iterations and Poisson process as described above.

Finally, we used DIYABC 2.0.4 (Cornuet et al. 2008), an Approximate Bayesian Computation (ABC) method (Beaumont et al. 2008; Bertorelle et al. 2010; Csillery et al. 2010), to estimate coalescence times among the population clusters identified in the UPGMA tree. We also used the UPGMA tree to set up the coalescence scenario (Figure 3.2). We simulated 1,000,000 multilocus datasets based on two different sets of summary statistics. Following Beaumont (2008) our summary statistics for the first model included the one sample and two sample summary statistics of *mean number of alleles*, *heterozygosity*, and *allele size variance*. Our second model included one sample summary statistics of *mean number of alleles*, *heterozygosity*, and *Garza-Williamson's* M, as well as two sample statistics including F_{ST} and *individual likelihood assignments* (from population *i* but assigned to population *j*; L*i-j*). We used the default stepwise mutation model with a mutation rate of $10^{-3} – 10^{-4}$ (Guillemaud et al. 2010) and set uniform priors for population size (1-20,000) and time of coalescence (1-20,000 generations).

3.4. Results

We failed to detect any significant deviations from Hardy Weinberg equilibrium for all 11 loci and no null alleles were identified. Additionally, we found no signification deviations from linkage-disequilibrium. We found clear evidence of population structuring. First, we observed a strong signal of isolation by distance ($R^2 = 0.293$; $p < 0.01$) (Figure 3.3). Further, all

4 different analyses of spatial structure found largely concordant patterns. The chord distance UPGMA tree (Figure 3.4) provided clear evidence of two primary branches that corresponded to populations northeast and southwest of the Missouri River, suggesting it as a primary barrier to gene flow. The tree also showed 4 major branches in the southwest and 6 major branches in the northeast. The four southwestern branches roughly corresponded with the following watersheds: 1) Little Missouri, 2) Lower Yellowstone, 3) Cannonball and 4) Heart-Knife (Figure 3.5). The six northeastern branches correspond with the following watershed/land features: 1) Sakakawea), 2) Souris River, 3) Turtle Mountains), 4) Lake Oahe (here after referred to as Potholes), 5) Devils Lake – Sheyenne (hereafter referred to as Sheyenne), and 6) Lower Red River (here after referred to as Red River) (Figure 3.5).

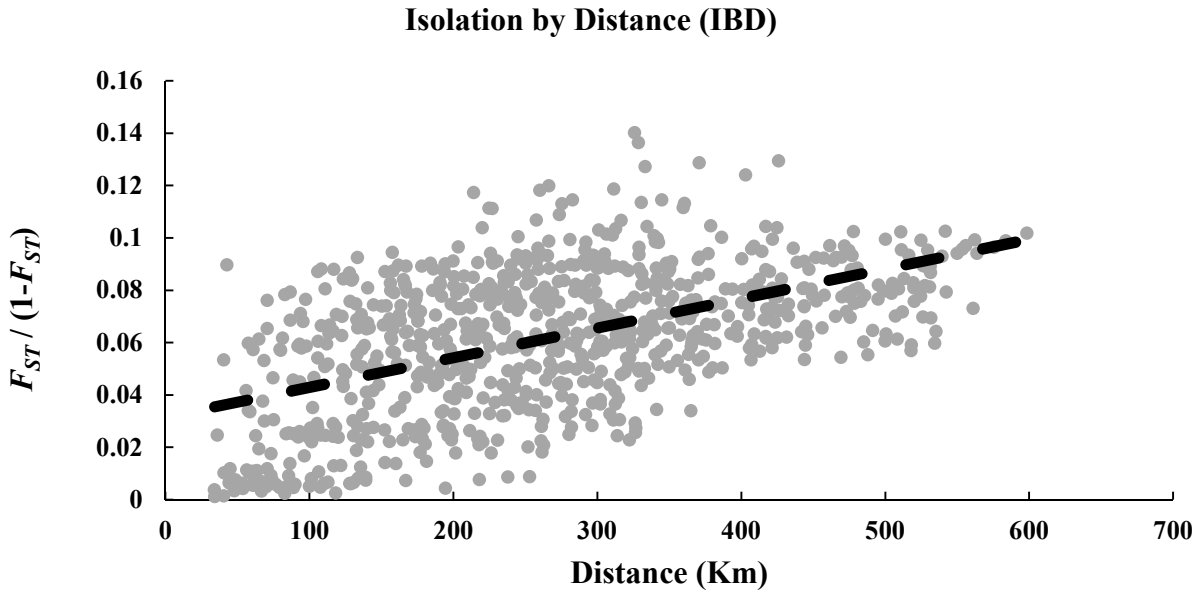

Figure 3.3. Isolation by distance.

STRUCTURE results also revealed an initial *K* of 2 paralleling the two major branches of the chord distance tree; northeast and southwest of the Missouri River. Similarly, further

STRUCTURE hierarchical analysis within each of these two groups, produced a total *K* of 10; with a *K* of 4 (southwest) and a *K* of 6 (northeast), which also corresponded with the major watershed-associated branches in the UPGMA tree (Figure 3.5).

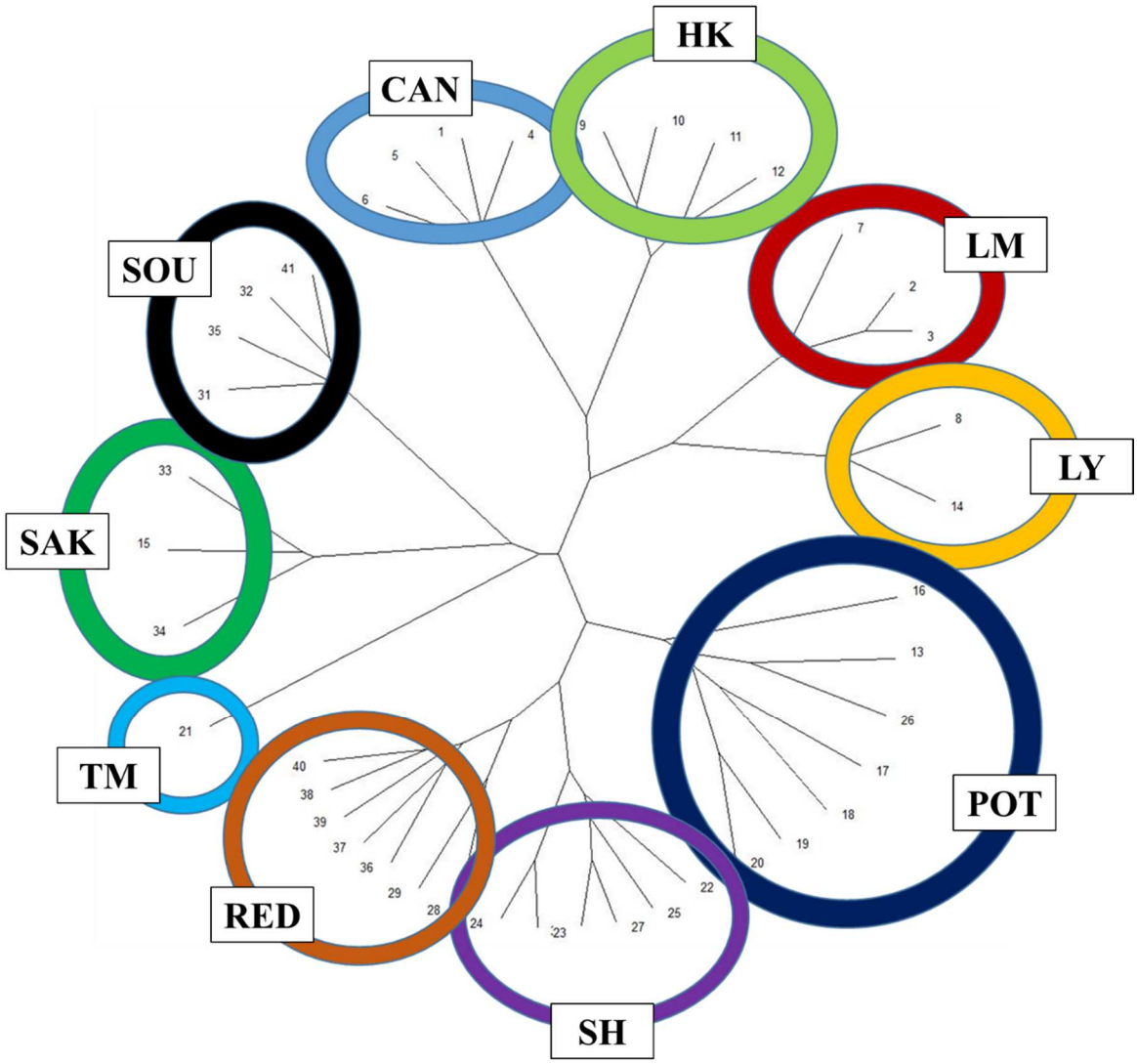

Figure 3.4. UPGMA tree using Cavalli-Sforza chord distance; circled populations indicate clusters identified via population structuring programs (SAK = Sakakawea; SOU = Souris; TM = Turtle Mountains; RED = Red River; SH = Sheyenne; POT = Pothole; CAN = Cannonball; HK = Heart-Knife; LM = Little Missouri; and LY = Lower Yellowstone).

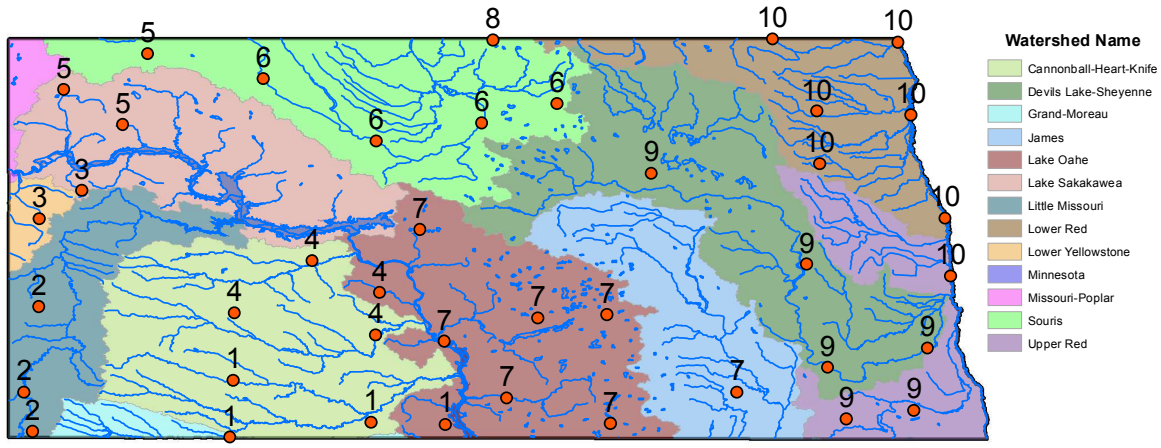

Figure 3.5. Population structuring overlaid on watersheds; grouping numbers coincide to DIYABC model clusters as 1 = Cannonball, 2 = Little Missouri, 3 = Lower Yellowstone, 4 = Heart – Knife, 5 = Sakakawea, 6 = Souris, 7 = Potholes, 8 = Turtle Mountains, 9 = Sheyenne, and 10 = Red River.

STRUCTURE results also revealed an initial *K* of 2 paralleling the two major branches of the chord distance tree; northeast and southwest of the Missouri River. Similarly, further STRUCTURE hierarchical analysis within each of these two groups, produced a total *K* of 10; with a *K* of 4 (southwest) and a *K* of 6 (northeast), which also corresponded with the major watershed-associated branches in the UPGMA tree (Figure 3.5).

BAPS and GENELAND results were both consistent with STRUCTURE and produced *K* values equal to 10. Visual inspection of the population groupings produced by these three population structuring programs produced the same assignments of sampled locations (Figure 3.5). Again, southwestern clusters were geographically smaller than most of the northeastern clusters. One population that clustered alone in all analyses was the Turtle Mountain region of upper north central state boarding Canada. The Turtle Mountain population is the most closely related to the Souris Cluster (Figure 3.5).

DIYABC indicated that median coalescence times among all 10 clusters varied from 638 to 18,100 generations for the Beaumont model (Figure 3.6a; Table 3.1) and 588 to 13,600

generations for the Cornuet-Miller model (Figure 3.6b; Table 3.1). The median coalescence times for the southwestern and northeastern clusters separated by the Missouri River was 13,600 generation and 18,100 generations for the Cornuet-Miller and Beaumont models, respectively (Figure 3.6; Table 3.1).

Focusing only on populations north and east of the Missouri (i.e. eastern populations), median coalescence times for the Beaumont model varied from 638 to 9,970 generations; whereas median coalescence times for the Cornuet-Miller model varied from 588 to 10,900 generations. Sequential coalescence for the Beaumont model produced 638 generations for median coalescence time between the Red River and the Sheyenne watersheds. Subsequent coalescence between the Sheyenne and the Southcentral Potholes watersheds occurred in 3,230 generations, Sakakawea and the Souris watersheds in 7,140 generations, Souris and the Turtle Mountains watersheds in 6,830 generations, and finally the Souris and Red River watersheds in 9,970 generations. Similar sequential divergence times by generations were approximated using the Cornuet-Miller model.

Median coalescence time for the southwestern population clusters varied from 5,220 to 8,550 generations for the Beaumont model while median coalescence times for the Cornuet-Miller model varied from 2,980 to 6,430 generations (Figure 3.6a; Table 3.1). Sequential coalescence times for the Beaumont model was 5,220 generations between the Little Missouri and the Lower Yellowstone watersheds. Subsequent coalescence between the Cannonball and Heart-Knife watersheds occurred in 7,770 generations and the Lower Yellowstone and Heart – Knife watersheds reached coalescence in 8,550 generations. Similar sequential divergence times were approximated using the Cornuet – Miller model (Figure 3.6b; Table 3.1).

Table 3.1. Time of divergence estimates for both Beaumont and Cornuet – Miller models; Missouri River location indicates if divergence was on eastern or western side of river; divergent location is designated as either WP = Western Prairie conservation unit or WB = Western Badlands conservation unit.

Time	Converged Units	Beaumont Model	Cornuet - Miller Model
		Median (± Confidence Interval)	**Median (± Confidence Interval)**
1	Red River – Sheyenne (*WP*)	638 (129, 5,770)	588 (128, 4,630)
2	Little Missouri – Lower Yellowstone (*WB*)	5220 (1,470, 13,400)	2890 (733, 8,850)
3	Heart-Knife – Cannonball (*WB*)	7,770 (2,320, 14,200)	7,880 (2,390, 13,700)
4	Red River – Potholes (*WP*)	3,230 (685, 14,000)	3,190 (783, 13,200)
5	Sakakawea – Souris (*WP*)	7,140 (2,220, 14,600)	5,490 (1,650, 12,600)
6	Lower Yellowstone – Heart-Knife (*WB*)	8,550 (3,470, 16,400)	6,430 (2,620, 14,200)
7	Sakakawea – Turtle Mountains (*WP*)	6,830 (2,000, 14,400)	8,600 (3,200, 15,700)
8	Red River – Sakakawea (*WP*)	9,970 (3,620, 19,000)	10,900 (4,290, 19,100)
9	*Western Prairie* and *Western Badlands* (*WP + WB*)	18,100 (13,100, 19,900)	13,600 (6,390, 19,600)

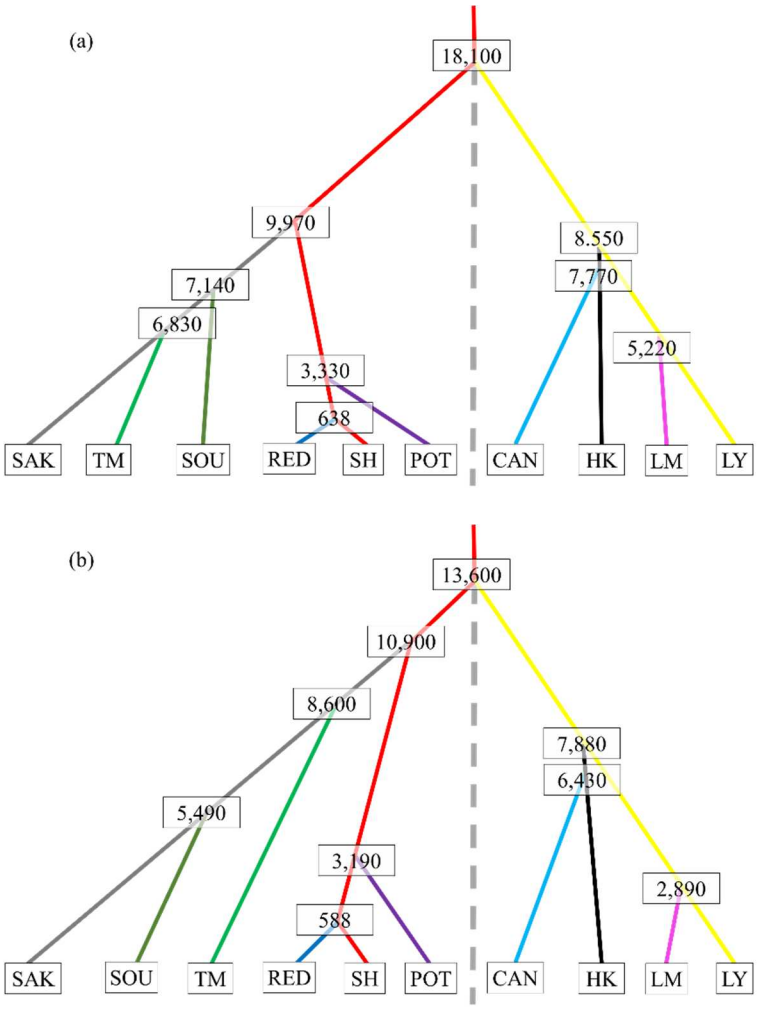

Figure 3.6. (a) DIYABC model with time of divergence values based off of Beaumont model parameters, grey dashed line separates populations east and west of Missouri River; (b) DIYABC model with time of divergence values based off of Cornuet – Miller model parameters, grey dashed separates populations east and west of Missouri River (SAK = Sakakawea; SOU = Souris; TM = Turtle Mountains; RED = Red River; SH = Sheyenne; POT = Pothole; CAN = Cannonball; HK = Heart-Knife; LM = Little Missouri; and LY = Lower Yellowstone).

3.5. Discussion

The ability to delineate conservation units is dependent up on detecting genetic structure (Crandall et al. 2000; Moritz 2002) and our results show nested genetic structure for *L. pipiens* across North Dakota. At the macro-scale we see two clear conservation units of *L. pipiens* in

North Dakota which are separated by the Missouri River. This break was clear from both the UPGMA tree as well as the initial STRUCTURE analysis, and suggests that the Missouri River is a barrier to gene flow. This finding is consistent with the observation that another larger river, the Mississippi, delineates the border between the *western* and *eastern* populations for *L. pipiens* (Hoffman et al. 2004, O'Donnell and Mock 20012).

The Missouri River also is an important biogeographical marker in North Dakota, as it originally formed as a product of water-melt along the edge of Pleistocene glaciation (Bluemle 1972). The area to the southwest of the Missouri was the only non-glaciated portion of North Dakota (Bluemle 1972) and this area was thus possibly occupied by *L. pipiens* population during the Wisconsin glacial period. Therefore, the genetic divergence of these two populations approximately aligns with the Pleistocene glacial spatial patterns. Further, these two populations show median coalescence times of 13,600 to 18,100 generations, further reflecting the long-term evolutionary independence of these two populations. Based on these findings, we recommend managing *L. pipiens* in North Dakota as the *Western Badlands* (southwestern ND) and *Western Prairie* conservation units.

Finer scale genetic differentiation is also apparent, as our analyses identified 4 and 6 genetic clusters within the *Western Badlands* and *Western Prairie* conservation units, respectively. These 10 clusters were consistently identified by both traditional and Bayesian based analyses programs. It is striking that these ten population clusters are roughly associated with major watersheds. This would suggest high gene flow along major riparian areas. This watershed association also appears to produce finer scale structure for the western population clusters which are associated with smaller watersheds relative to the larger watersheds of the north (Souris) and east (Red River and Potholes).

The differentiation of populations may also reflect the boom-bust long-term population contractions and expansions that corresponded with well-known inter-annual variability in air temperature and recurring drought to wet climatic cycles that have historically occurred every 10 to 20 years (Karl and Riebsame 1984; Mushet et al. 2013). Mushet (2010) showed that populations likely persist through droughts by overwintering in the few remaining deep wetlands that do not freeze solid and still maintain enough oxygen in the water for winter survival (Cory 1952, Emery et al. 1972, Canjak 1986, Wagner 1997, Ultsch et al. 2004). Mushet (2010) reported evidence that the spatial contraction and expansion of leopard frog populations was correlated with drought and wet periods, respectively. Thus, we hypothesize that this same process may contribute to genetic structuring of *L. pipiens* populations in North Dakota.

The divergence among the population clusters also shows some interesting temporal patterns. Coalescence times of populations within the *Western Prairie* conservation unit were as recent as 588 to 638 generations ago. By contrast, populations within the *Western Badland* unit coalesced much earlier (2,890 to 5,220 generations). These differences may suggest higher gene flow permeability among *L. pipiens* populations in the *Western Prairie* unit compared to the *Western Badlands* conservation unit, a hypothesis that is consistent with the higher density of permanent wetlands in the *Western Prairie* conservation unit.

Another striking finding was the distinctiveness of the Turtle Mountains population which forms its own cluster and was isolated from all other populations for 6,830 (Beaumont model) to 8,600 (Cornuet-Miller model) generations. The Turtle Mountains is notably one of the most unique areas in the North Dakota as it is an oval-shaped area of glaciated hills marked with depressional wetlands (Bailey 1926, Potter and Moir 1961). This hill structure at one point was vegetated by spruce, fir, and hemlock species but has been succeeded by deciduous forest

species, with much of the dominant species being aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) (Potter and Moir 1961). Additional work will be needed to better understand the history of this unique population.

Future conservation efforts of *L. pipiens* populations in North Dakota might be best directed towards the use of macro-scale population grouping when evaluating regional declines, particularly in the southwestern portion of North Dakota. The *Western Badlands unit,* is geographically unique and this unglaciated landscape is quite different compared to the rest of North Dakota. Specifically, river structures with dramatic elevation contours shape this region and more permanent wetlands are relatively rare in this region compared the rest of state. We recommend targeting conservation efforts towards rivers and natural permanent waters within watersheds is imperative.

Management of the *Western Prairie* unit, poses a more unique situation as this unit lies within the Prairie Pothole region (PPR). The PPR is commonly described as a region with high densities of depressional wetlands with intensive agricultural practices. Since the late 1800's much of this region has been converted from natural grasslands to crop production (Euliss and Mushet 1999). Dahl (1990) estimates that nearly half of the wetland area originally present in the PPR has been filled or drained to produced more tillable area for agriculture. Additionally, many of the remaining wetlands are degraded owing to chemical drift (Grue et al. 1989) and altered hydrological cycles (Euliss and Mushet 1996 and Euliss and Mushet 1999). Thus, for the *Western Prairie* unit, conservation efforts should be directed towards enrolling at risk wetlands in wetland conservation programs, such as the U.S. Department of Agriculture's former Wetland Reserve Program. Additionally, managers should aim to reduce and possibly eliminate chemical

drift and siltation of wetlands efforts and also maintain vegetative buffers between wetlands and agricultural activities.

In general, our results suggest that colonization patterns during and after the Wisconsin glaciation has sculpted the genetic structure of *L. pipiens* populations in North Dakota. Further, finer scale structure has been created over many millenia. Thus, protecting conservation units at multiple scales will protect these historic legacies. Further, our findings show that evaluating the spatial structure of can provide guidance on how to protect the rich spatial and temporal diversity within widespread species.

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CHAPTER 4. POTENTIAL FOR PARASITE-INDUCED BIASES IN AQUATIC INVERTEBRATE POPULATION STUDIES[1]

4.1. Abstract

Recent studies highlight the need to include estimates of detection/capture probability in population studies. This need is particularly important in studies where detection and/or capture probability is influenced by parasite-induced behavioral alterations. We assessed potential biases associated with sampling a population of the amphipod *Gammarus lacustris* in the presence of *Polymorphus* spp. acanthocephalan parasites shown to increase positive phototaxis in their amphipod hosts. We trapped *G. lacustris* at two water depths (benthic and surface) and compared number of captures and number of parasitized individuals at each depth. While we captured the greatest number of *G. lacustris* individuals in benthic traps, parasitized individuals were captured most often in surface traps. These results reflect the phototaxic movement of infected individuals from benthic locations to sunlit surface waters. We then explored the influence of varying infection rates on a simulated population held at a constant level of abundance. Simulations resulted in increasingly biased abundance estimates as infection rates increased. Our results highlight the need to consider parasite-induced biases when quantifying detection and/or capture probability in studies of aquatic invertebrate populations

[1] The material in this chapter was co-authored by Justin D. L. Fisher David M. Mushet, and Craig A. Stockwell. Justin D. L. Fisher had the primary responsibility for collecting samples in the field as well as processing samples in the laboratory. Justin D. L. Fisher was the primary developer of the conclusions described here within. Justin D. L. Fisher also drafted and revised all previous versions of this chapter. David M. Mushet and Craig A. Stockwell served as a proofreader and supplied constructive comments for an improved chapter. This chapter has been published in *Hydrobiologia* 722:199-204, http://dx.doi.org/ 10.1007/s10750-013-1700-9.

4.2. Introduction

Abundance estimates of aquatic organisms are critical to many ecological studies, yet these estimates can be influenced by variations in detection and/or capture probabilities (MacKenzie et al. 2006). Additionally, biotic factors such as parasitism can influence behavior and, thereby, detection/capture probability. Some parasites alter their host's activity patterns (Hindsbo 1972, Holmes & Bethel 1973, Bakker et al. 1997), presumably to increase the probability of the infected host being consumed by a definitive host (Bethel & Holmes 1977, Moore 1984). For instance, Lafferty and Morris (1996) reported that killifish (*Fundulus parvipinnis*) infected with a trematode parasite (*Euhaplorchis californiensis*) displayed conspicuous behaviors and were more vulnerable to predation by bird species. Dezfuli et al. (2003) determined that amphipods (*Echinogammarus stammeri*) infected with the acanthocephalan parasite *Pomphorhynchus laevis* showed overall increased activity and lack of avoidance in the presence of a fish predator (*Leuciscus cephalus* L.). Likewise, amphipods infected with various acanthocephalan parasites, *Polymorphus* spp., have been shown to display increased positive phototaxis making them more vulnerable to definitive host waterfowl (Cézilly et al. 2000, Bauer et al. 2005).

Despite support for the *parasite-manipulation hypothesis* (Holmes & Bethel 1972, Kennedy et al. 1978, Bakker et al. 1997, Bauer et al. 2000, Cézilly et al. 2000, Poulin 2000), scant attention has been paid to the potential for altered behavior to bias abundance estimates. In one pioneering report, Rothschild (1962) highlighted a case of trematode infected snails being larger and less likely to conceal themselves from definitive host predators. Rothschild (1962) noted that these parasite-induced changes should lead to sampling bias towards collection of infected compared to uninfected individuals. However, the hypothesis that parasite-altered

behavior may affect population estimates (Rothschild 1962) has not been rigorously tested. We explored the effects of parasite-induced behavioral changes on capture rates and population estimates of the amphipod *Gammarus lacustris* in the presence of a behavior altering parasite, *Polymorphus* spp.

4.3. Material and Methods

4.3.1. Study Area

Our study was conducted in a 4.9 ha, fishless, closed-basin, semi-permanent wetland (wetland P1) within the Cottonwood Lakes Study Area, Stutsman County, North Dakota, USA (47° 05' 54" N, -99° 05' 56" W). The Cottonwood Lake Study Area has been the focus of long-term ecological research efforts since 1967 (Swanson et al. 2003). Wetland P1 is representative of semi-permanent wetlands located in the prairie pothole region of North America (Swanson et al. 2003). At the time of our sampling, wetland P1 was approximately 4.9 ha in size and in the open-water phase (Stewart & Kantrud 1971) lacking significant areas of emergent vegetation. Wetland P1 has historically supported a population of amphipods (Swanson et al. 2003), a group of invertebrates that typically inhabits semi-permanent wetlands in the region (Kantrud et al. 1989). Wetland P1 is fishless, and thus the acanthocephalan parasites in this site were most likely limited to species that utilize waterfowl as their definitive hosts (e.g. *Polymorphous paradoxus*; Bethel & Holmes 1973).

4.3.2. Sampling Methods

We estimated baseline infection rates in wetland P1 by seining along three randomly selected transects with a 1-m tall by 3-m long seine having 5-mm diameter meshing. For each transect, we seined the entire water column from the shoreline until reaching 1-m water depth and then seined in the opposite direction while returning to shore. *G. lacustris* captured in our

seine hauls were preserved in 80% ethyl alcohol and subsequently counted and individually inspected under a dissecting microscope for the presence or absence of cystacanths. The brightly colored cystacanth of *Polymorphus* spp. is easily visible through the cuticle of an infected *G. lacustris* (Hyman 1951, Bethel & Holmes 1973, Marriott et al. 1989).

On five consecutive days from 17-21 July 2006, we set activity traps (Swanson 1978) at a 1-m-depth along each of 30 transects (1 trap per transect for a total of 20 traps per event and 100 traps total) that we established along randomly selected compass bearings (1^{o} to 360^{o}) radiating from the study wetland's center. Distance of each trap from the shore varied due to uneven wetland morphology. Each day, ten of the 20 total traps were vertically suspended near the surface so that the trap openings were oriented towards the wetland bottom. Ten additional traps were also vertically oriented but placed 10-cm from the benthos (Figure 4.1). Trap placement (surface or benthic) was randomly assigned for each transect. Traps remained in place for 24 hours before samples were collected and preserved in 80% ethyl alcohol. Subsequently, all captured amphipods were counted and examined for cystacanths as described above.

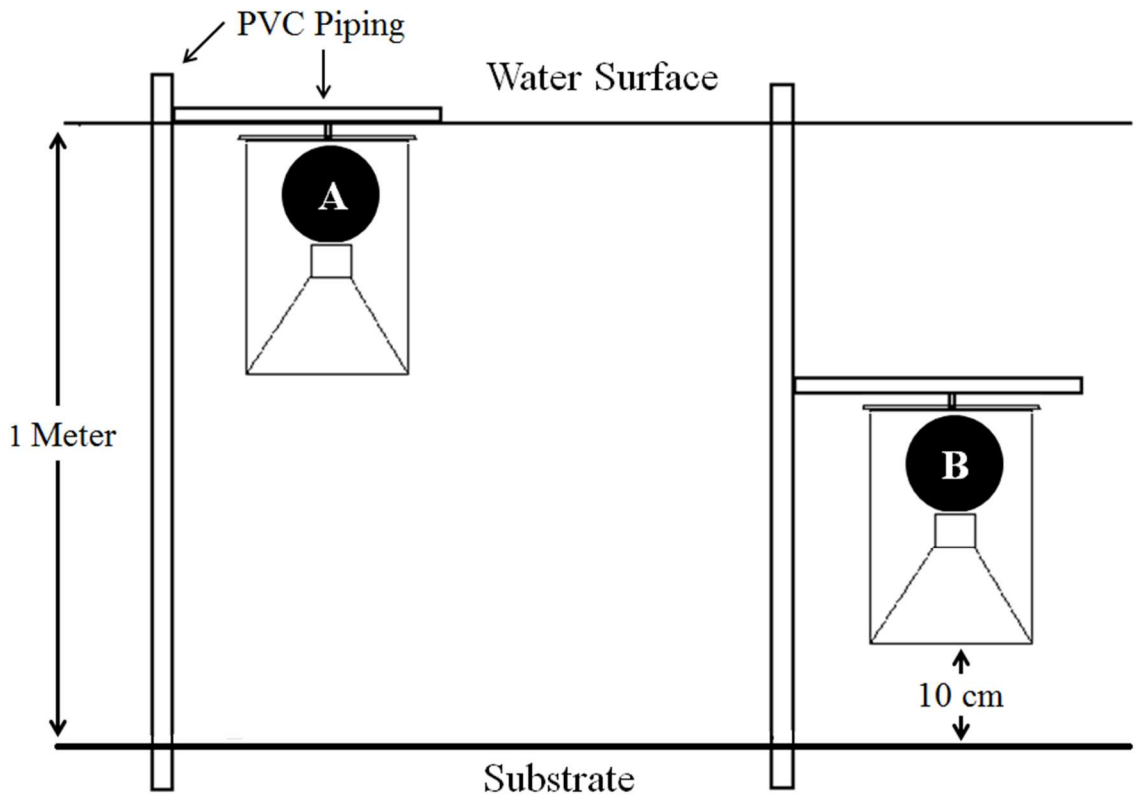

Figure 4.1. Illustration of activity trap placement vertically at the wetland surface (A) and vertically at the benthos (B).

4.3.3. Data Analyses

We estimated parasite prevalence (Margolis et al. 1982) as the proportion of *G. lacustris* infected with at least one cystacanth relative to the total number of *G. lacustris* captured in a trap. Total *G. lacustris* captures departed from Kolmogorov-Smirnov normality tests ($p < 0.05$) and thus were square-root transformed to meet normality assumptions. Repeated measures (sampling occasion) analysis of variance (ANOVA) was used to estimate the main and interactive effects of trapping location and trap date on total captures and infection rates. There was no effect of trapping session day on either total capture or infection rates. Thus, data from all five trapping sessions were combined for subsequent analyses (Table 3.1). Pairwise post-hoc comparisons were conducted by performing Tukey-Kramer tests and experimental error rates

were maintained at 0.05 using a sequential Bonferroni correction (Rice 1989). All statistical analyses were conducted using SAS® software version 9.2.

Table 4.1. Repeated-measures ANOVA results for captures and infection rates.

Dependent Variable	Model	*DF*	*F*-value	*P*-value
Captures	Depth	1	855.92	< 0.001
	Day	4	0.31	0.8701
	Day x Depth	4	0.22	0.9294
Infection Rate	Depth	1	343.62	< 0.001
	Day	4	0.40	0.8069
	Day x Depth	4	0.36	0.8370

4.3.4. Simulations

We simulated a single amphipod population with a consistent abundance of 10,000 individuals and varied treatments by using infection rates of 0%, 5%, 10%, and 20%. These infection rates are reflective of the range of infection rates reported by Spencer (1974) for a Colorado population of *G. lacustris*. In our baseline simulation (i.e., 0% infection rate), we set the vertical distribution of amphipods at benthic and surface depths to match the vertical distribution (*Dist*) of un-infected amphipods captured in our activity trap sampling of wetland P1. Those values were obtained for each respective depth as follows:

$$Dist = \frac{(Ni - Ii)}{\sum Ni} \; x \; 100$$

where *Ni* was average number of individuals captured, *Ii* was the average number of infected individuals. Thus, the proportion of un-infected individuals occurring in benthic and surface depths were 77%, and 23%, respectively. In subsequent simulations of infection rates, we used the depth-specific infection rates estimated from our activity trap data to distribute various percentages of infected individuals across the three water column depths. Those depth-specific infection rates were obtained as follows:

$$\Delta\, Dist = \left[\frac{(Ni - Ii)}{\sum Ni}\; x\; IR\right] + Dist$$

where *IR* was infection rate. We then calculated changes in abundance as the difference between the number of individuals at a specific depth under an infection scenario and the number of individuals at that same depth under the 0% infection rate scenario. Using these simulations, we explored how changes in infection rates could influence *G. lacustris* abundance estimates even when actual population size is constant.

4.4. Results

G. lacustris total captures differed significantly among trapping depths ($F_{(1, 99)} = 855.92$, $p < 0.001$; Figure 4.2a). Benthic activity traps captured significantly more amphipods (19.72 ± 0.59; mean ± 95% confidence interval) than surface traps (7.50 ± 0.53).

The *G. lacustris* parasite infection rate in the wetland P1 as estimated from our seine haul data was 4.99% ± 0.84% (mean ± 95% confidence interval). The infection rate estimate from activity traps with data from all trap locations combined was very similar (5.90% ± 0.59%) to our baseline estimate. However, infection rates estimated from activity trap captures differed significantly between trapping locations ($F_{(1, 99)} = 343.62$, $p < 0.001$). Estimated infection rates of benthic samples (0.14% ± 0.07%) were significantly lower compared to the infection rate of surface samples (21.05% ± 2.15%) (Figure 4.2b).

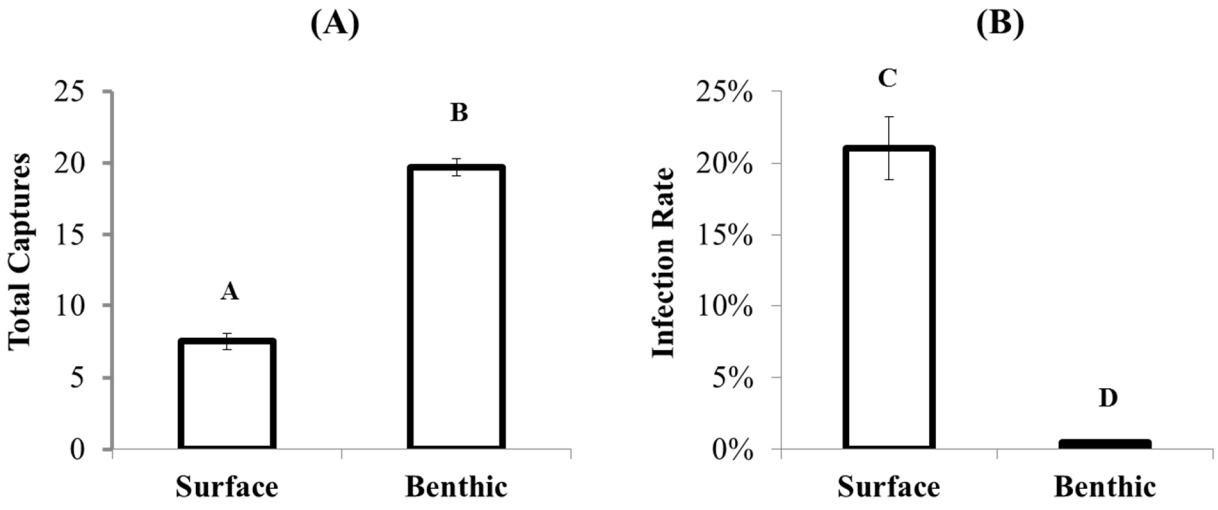

Figure 4.2. Average (±95% confidence limits) number of *Gammarus lacustris* captures (A) and percentage of individuals infected with *Polymorphus* spp. (B). Data are from activity trap sampling at two locations (surface and benthic) within a 1-m water depth wetland in Stutsman County, North Dakota. Treatment means sharing a common letter were not significantly different.

Our simulations showed that studies using capture data from traps at surface locations had the potential to overestimate changes in amphipod abundance by 21% to 85% at infection rates ranging from 5% and 20%. By contrast, estimates utilizing data from benthic located traps and the same 5% and 20% infection range underestimated changes in abundance by 6% to 26% (Figure 4.3).

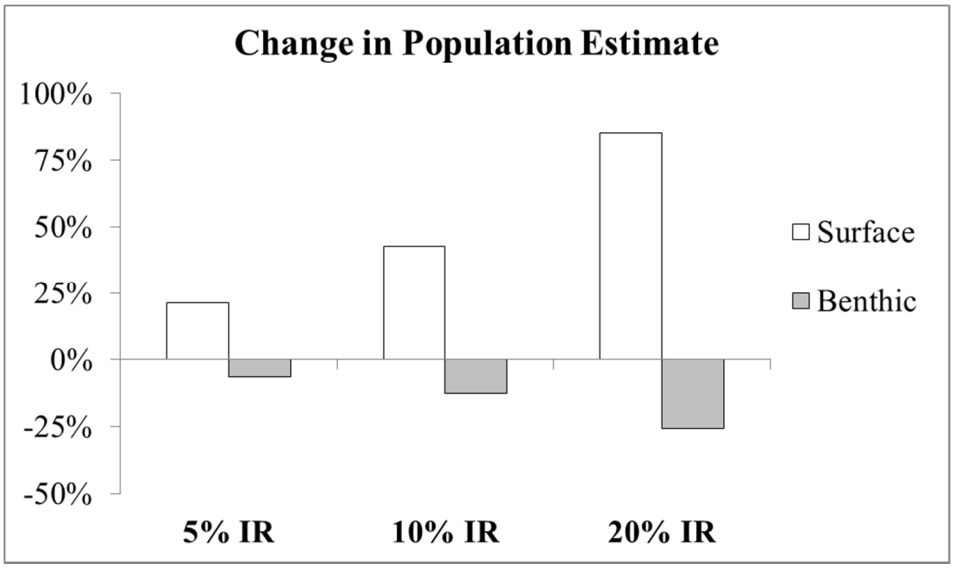

Figure 4.3. Simulated change (percent) in abundance estimates for a population of 10,000 *Gammarus lacustris* individuals with varying levels of *Polymorphus spp.* infection rates (IR). In simulations, individuals preferentially moved to mid- and surface water depths as a result of parasite-induced behavioral alterations.

4.5. Discussion

Our results showed that infection status had a strong effect on amphipod activity patterns. Uninfected amphipods were most abundant in benthic traps, while infected amphipods were most abundant in surface traps. This finding is consistent with previous work showing that un-infected amphipods commonly utilize the benthos where they feed on epiphytic growth on aquatic plants, dead animal and plant material, and filamentous green algae (Bethel & Holmes 1973, Thorp & Covich 2001). Conversely, amphipods infected with waterfowl associated acanthocephalans often display positive phototaxis and are attracted to sunlit surface waters where they are more vulnerable to predation (Bakker et al. 1997, Bauer et al. 2000, Cézilly et al. 2000). Such findings have been interpreted as evidence for the parasite manipulation hypothesis (Holmes & Bethel 1972, Kennedy et al. 1978, Bakker et al. 1997, Bauer et al. 2000, Cézilly et al.

2000, Poulin 2000), whereby altered behavior of the intermediate host increases risk of predation by the parasite's definitive host.

Although parasite altered behavioral patterns have been well documented (Kennedy et al. 1978, Bakker et al. 1997, Bauer et al. 2000, Cézilly et al. 2000), very limited work has considered how altered behavior can affect the accuracy of population sampling. Population sampling will be most affected in situations where sampling is focused in certain habitats. For instance, invertebrate abundance has been regularly evaluated using surface-deployed activity traps (e.g., Cieminski & Flake 1995, Hanson et al. 2000, Gernes & Helgen 2002, Miller et al. 2008, Hanson et al. 2010). However, for some taxa, captures in these traps may more closely track changes in parasite infection rates rather than changes in population numbers. Other sampling devices (e.g., D-frame and other nets, core samplers, grab samplers; Merritt et al. 2008) that sample specific locations in a wetland will likely be vulnerable to similar biases.

The reliability of invertebrate abundance estimates will depend on both sample location and parasite prevalence. Our study revealed an overall infection rate of 5% in the wetland we sampled, but other studies have reported seasonal infection rates approaching 20% (Spencer 1974). Our simulations show that such differences in parasite prevalence can result in nearly an 85% overestimation of amphipod abundance if only data from surface traps are used. Likewise, underestimations of abundance changes of 26% can result from relying solely on data from benthic traps. In addition to spatial variation, parasite infection rates fluctuate over time (Bates et al. 2010). Thus, perceived temporal shifts in population estimates may be more reflective of temporal shifts in parasite prevalence rather than actual changes in population size. Therefore, the potential for parasites to bias sampling data must be considered when evaluating both spatial and temporal abundance trends in aquatic invertebrate studies. Furthermore, additional research

is warranted to evaluate the generality of the patterns we report here and to further evaluate spatial and temporal bias across regions.

In addition to being applicable to a broad range of invertebrate sampling methodologies, our results are broadly applicable to other systems where parasites alter the spatial behavior of their hosts. For instance, many aquatic species such as fishes (Crowden & Broom 1980, Radabaugh 1980, Lafferty & Morris 1996, Lafferty 1999), mollusks (Bourne 1993, Lowenberger & Rau 1994), mosquitoes (Webber et al. 1987), and chaetognaths (Pearre 1979) have been reported to move to the top of water column when infected by various behavior modifying parasites. The possible effect of behavior altering parasites on population estimates adds another level of complexity that must be considered when designing population sampling studies and interpreting results.

4.6. Acknowledgements

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CHAPTER 5. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

5.1. Overview of Need

In light of the global decline of amphibian species, conservation practitioners need to be cognizant of the various factors that have been associated to these declines. However, addressing current species of concern may not be enough as we continue down the path of defaunation in the Anthropocene (Dirzo et al. 2014). To this extent, surveying and monitoring common species may aid in gaining baseline information for future conservation needs. Thus, the work presented in this dissertation fills important information gaps of a widespread amphibian species, the northern leopard frog. The goal was to aid local state and federal managers gather a genetic baseline and utilize this information to determine an appropriate regional conservation status. Furthermore, the data gathered will act as a baseline for potential future genetic sampling efforts.

5.2. Overview of Results

In chapters two and three I utilized microsatellite markers to describe the genetic diversity and population structure of sampled populations of northern leopard frogs throughout North Dakota. Genetic diversity was found to be negatively correlated with longitude, with reduced genetic diversity in the western side of the state. In fact, I described a distinct reduction in genetic diversity near the 100th meridian, a historical boundary Powell (1890) described as the approximate longitude where the arid west meets the wet east. Further analyses of the genetic diversity data revealed a significant correlation to deep wetland densities. This finding is consistent with *L. pipiens* lifecycle requirements for winter refugia.

The recent petition for listing the northern leopard frog under Endangered Species Act protection was spurred by genetic discontinuities found along the Mississippi River. Therefore,

it would seem plausible that the Missouri River in North Dakota would also act as a barrier to gene flow. In fact, I describe two major conservation units, the *western prairie* and *western badlands* that are bounded by the Missouri River. I also described finer scale population genetic structure with 6 and 4 population clusters within the *western badlands* and *western prairie* conservation units, respectively. These 10 population clusters were roughly correlated with watersheds. These findings suggest that conservation of northern leopard frogs in North Dakota should focus on permanent waters including rivers as well as wetlands.

Future research efforts could work off of the current results looking to answer questions regarding gene flow and wetland and river importance at a finer resolution. Ideally, the use of genetic markers on populations sampled within 10 to 15km from each site would allow for addressing key issues relating to landscape permeability and understanding how wetland densities and river basins are correlated to genetic diversity. These types of questions could help managers identify specific wetlands and/or river segments that need protective actions for short and long-term persistence of northern leopard frog populations.

The findings in chapter four also provide insights into understanding the aquatic communities within wetlands and the importance of sampling aquatic invertebrates, particularly with uncertainties associated to parasites behavioral modifications that may bias sampling results. These results, and follow-on simulated results, demonstrate the importance for understanding the potential bias associated to sampling methodologies. Future research and study designs targeting both vertebrate and invertebrate species should consider the behavioral effects of parasites, especially in aquatic systems.

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APPENDIX. AMPHIBIAN ACTIVE SAMPLING ECOUNTER DATA

While sampling northern leopard frog populations, I also recorded all amphibian and reptile species encountered and associated GPS coordinates. In total, I encountered a total of 6 amphibian and 6 reptile species including northern leopard frog (*Lithobates pipiens*), wood frog (*Lithobates sylvatica*), western chorus frog (*Pseudacris triseriata*), gray tree frog (*Hyla versicolor*), Great Plains toad (*Bufo cognatus*), tiger salamander (*Ambystoma tigrinum*), western pained turtle (*Chrysemys picta belli*), bullsnake (*Pituophis caternifer*), common garter snake (*Thamnophis sirtalis*), plains garter snake (*Thamnophis radix*), prairie rattlesnake (*Crotalus viridis*), and smooth green snake (*Opheodrys veranalis*) (Table A.1).

Table A.1. Amphibian encounter data: northern leopard frog = NLF, wood frog = WF, western chorus frog = CF, gray tree frog = GTF, Great Plains toad = GPT, tiger salamander = TS, western pained turtle = WPT, bullsnake = BS, common garter snake = CGS, prairie rattlesnake = PRS, and smooth green snake = SGS. All grid coordinates are given in WGS 1984 format.

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Adams	45.9459	-102.352	X	-	-	-	-	-	-	-	-	-	-	-
Adams	46.287	-102.968	-	-	-	-	-	-	-	-	-	-	-	-
Adams	45.9965	-102.641	-	-	-	-	-	-	-	-	-	-	-	-
Adams	46.0292	-102.352	-	-	-	-	-	-	-	-	-	-	-	-
Adams	46.0112	-102.16	-	-	-	-	-	-	-	-	-	-	-	-
Barnes	46.9076	-98.209	X	-	-	-	-	-	-	-	-	-	-	-
Barnes	46.9359	-98.2006	-	-	-	-	-	-	-	-	-	-	-	-
Barnes	47.1583	-98.0059	-	-	-	-	-	-	-	-	-	-	-	-
Benson	47.9666	-99.1254	X	-	-	-	-	-	-	-	-	-	-	-
Benson	48.3391	-99.8037	-	-	-	-	-	-	-	-	-	-	-	-
Benson	47.979	-99.2104	-	-	-	-	-	-	-	-	X	-	-	-
Billings	46.888	-103.273	-	-	-	-	-	-	-	-	-	-	-	-
Billings	46.8757	-103.349	-	-	-	-	-	-	-	-	-	-	-	-
Billings	46.8945	-103.541	-	-	-	-	-	-	-	-	-	-	-	-
Bottineau	48.7777	-101.394	-	-	-	-	-	X	-	-	-	-	-	-
Bottineau	48.8117	-101.489	-	-	-	-	X	-	-	-	-	-	-	-
Bottineau	48.792	-101.147	-	-	-	-	X	-	-	-	-	-	-	-
Bottineau	48.8139	-101.146	-	-	-	-	X	-	-	-	-	-	-	-
Bottineau	48.6237	-100.3	-	-	-	-	X	-	-	-	-	-	-	-
Bottineau	48.9853	-100.338	X	X	X	-	-	-	-	-	-	-	-	-
Bottineau	48.9528	-100.347	-	-	-	-	-	-	-	-	-	X	-	-
Bowman	46.0457	-103.902	-	-	-	-	-	X	-	-	-	-	-	-
Bowman	46.0494	-103.884	X	-	X	-	-	-	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Bowman	46.2137	-103.944	-	-	X	-	-	-	-	-	-	-	-	-
Bowman	46.1485	-103.953	-	-	X	-	-	-	-	-	-	-	-	-
Bowman	45.9886	-103.861	X	-	-	-	-	-	-	-	-	-	-	-
Bowman	46.2211	-103.74	-	-	-	-	-	-	-	-	-	-	X	-
Bowman	45.997	-103.281	-	-	-	-	-	-	-	-	-	-	X	-
Bowman	46.1329	-103.093	-	-	-	-	-	-	-	-	-	-	-	-
Burke	48.5507	-102.637	-	-	X	-	-	-	-	-	-	-	-	-
Burke	48.5507	-102.669	X	-	X	-	-	-	-	-	-	-	-	-
Burke	48.5729	-102.683	-	-	-	-	-	-	-	-	-	-	-	-
Burke	48.7975	-102.251	-	-	-	-	-	-	-	-	-	-	-	-
Burleigh	47.1407	-100.324	X	-	X	-	-	X	-	-	-	-	-	-
Burleigh	46.8375	-100.593	-	-	-	-	-	X	-	-	-	-	-	-
Burleigh	47.1582	-100.444	X	-	-	-	-	-	-	-	-	-	-	-
Burleigh	47.1634	-100.349	X	-	-	-	-	-	-	-	-	-	-	-
Burleigh	47.1669	-100.29	X	-	-	-	-	-	-	-	-	-	-	-
Burleigh	46.8665	-100.871	X	-	-	-	-	-	-	-	-	-	-	-
Burleigh	46.6797	-100.711	X	-	-	-	-	-	-	-	-	X	-	-
Burleigh	46.8255	-100.639	-	-	-	-	-	-	-	-	X	-	-	-
Burleigh	46.8233	-100.435	-	-	-	-	-	-	-	-	-	-	-	-
Burleigh	46.8032	-100.277	-	-	-	-	-	-	-	-	-	-	-	-
Cass	47.1793	-96.8362	X	X	X	-	-	-	-	-	-	-	-	-
Cass	47.1941	-96.8471	X	-	-	-	-	-	-	-	-	-	-	-
Cass	46.6303	-97.0144	X	-	-	-	-	-	-	-	-	-	-	-
Cavalier	48.9965	-98.1966	X	X	X	-	-	-	-	-	X	-	-	-
Cavalier	48.9286	-98.3347	-	-	-	-	-	-	-	-	-	-	-	-
Cavalier	48.8914	-98.4964	-	-	-	-	-	-	-	-	X	X	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Cavalier	48.9025	-98.2344	-	-	-	-	-	-	-	-	-	-	-	-
Dickey	46.0045	-98.6708	X	X	-	-	-	-	-	-	-	-	-	-
Divide	48.9158	-103.775	-	-	-	-	-	X	-	-	-	-	-	-
Divide	48.7208	-102.984	-	-	-	-	-	X	-	-	X	-	-	-
Divide	48.8831	-102.981	X	-	X	-	-	-	-	-	-	-	-	-
Divide	48.9522	-103.883	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.9448	-103.887	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.9678	-103.897	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.946	-104.016	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.892	-104.042	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.8677	-103.963	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.8404	-103.962	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.6893	-103.921	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.7009	-102.948	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.7156	-102.997	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.8028	-103.006	-	-	-	-	-	-	-	-	-	-	-	-
Divide	48.8936	-103.174	-	-	-	-	-	-	-	-	-	-	-	-
Dunn	47.2852	-102.608	X	-	-	-	X	-	-	-	-	X	-	-
Dunn	47.3505	-102.66	X	-	-	-	-	-	-	-	X	-	-	-
Dunn	46.9895	-102.79	-	-	-	-	-	-	-	-	-	-	-	-
Eddy	47.832	-99.1041	X	X	-	-	-	-	-	-	-	-	-	-
Eddy	47.7605	-99.1917	X	-	-	-	-	-	-	-	-	-	-	-
Eddy	47.7312	-99.0902	X	-	-	-	-	-	-	-	-	-	-	-
Eddy	47.839	-99.1253	X	-	-	-	-	-	-	-	-	-	-	-
Emmons	46.5423	-100.118	X	-	-	-	-	-	-	-	-	-	-	-
Emmons	46.2469	-100.233	X	-	-	-	-	-	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Emmons	46.2474	-100.216	-	-	-	-	-	-	-	-	-	-	-	-
Foster	47.4567	-98.5787	-	-	-	-	X	-	-	-	X	-	-	-
Foster	47.4574	-98.7994	X	-	-	-	-	-	-	-	-	-	-	-
Foster	47.5323	-98.7545	X	-	-	-	-	-	-	-	-	-	-	-
Foster	47.4569	-98.6539	-	-	-	-	X	-	-	-	-	-	-	-
Foster	47.4574	-99.2572	-	-	-	-	-	-	-	-	-	-	-	-
Golden Valley	46.631	-103.96	X	-	-	-	-	X	-	-	-	-	-	-
Golden Valley	46.9448	-103.817	X	-	-	-	X	-	-	X	-	-	-	-
Golden Valley	46.9194	-103.987	-	-	-	-	-	-	-	-	-	-	-	-
Golden Valley	46.6471	-103.986	-	-	-	-	-	-	-	-	-	-	-	-
Golden Valley	47.0389	-104.012	-	-	-	-	-	-	-	-	-	-	-	-
Grand Forks	48.0383	-97.8393	X	-	X	-	-	-	-	-	-	-	-	-
Grand Forks	47.7448	-97.7218	-	-	-	-	-	-	-	-	-	-	-	-
Grand Forks	47.9352	-97.5946	-	-	-	-	-	-	-	-	-	-	-	-
Grand Forks	47.9366	-97.4934	-	-	-	-	-	-	-	-	X	-	-	-
Grand Forks	47.9751	-97.284	-	-	-	-	-	-	-	-	-	-	-	-
Grant	46.4183	-101.483	X	-	-	-	-	-	-	-	-	-	-	-
Griggs	47.6483	-98.3312	X	-	-	-	-	-	-	-	-	-	-	-
Hettinger	46.3817	-102.328	X	-	-	-	-	-	-	-	-	-	-	-
Hettinger	46.4292	-102.516	-	-	-	-	-	-	-	-	-	-	-	-
Kidder	46.8855	-99.465	X	-	-	-	-	-	-	-	-	-	-	-
Kidder	46.8596	-99.9971	X	-	-	-	-	-	-	-	-	-	-	-
Kidder	46.6381	-99.7655	-	-	-	-	-	-	-	-	-	-	-	-
Kidder	46.676	-99.7654	-	-	-	-	-	-	-	-	-	-	-	-
Kidder	46.8122	-99.7622	-	-	-	-	-	-	-	-	-	-	-	-
Kidder	46.8506	-100.029	-	-	-	-	-	-	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
LaMoure	46.2902	-98.4735	X	-	X	-	X	-	-	-	-	-	-	-
LaMoure	46.3002	-98.2838	-	-	-	-	-	-	-	-	-	-	-	-
Logan	46.5086	-99.8149	X	X	X	-	-	-	-	-	-	-	-	-
Logan	46.5097	-99.8088	X	X	-	-	-	-	-	-	-	-	-	-
Logan	46.5074	-99.8093	X	-	-	-	-	-	-	-	-	-	-	-
McHenry	48.0577	-100.939	X	-	-	-	-	-	-	-	-	-	-	-
McHenry	48.354	-100.424	X	-	-	-	-	-	-	-	-	-	-	-
McHenry	47.9218	-100.376	X	-	-	-	-	-	-	-	-	-	-	-
McIntosh	46.086	-99.4816	-	-	-	-	-	X	-	-	-	-	-	-
McIntosh	46.05	-99.4417	X	X	X	-	-	-	-	-	-	-	-	-
McIntosh	46.0818	-99.4376	-	-	-	-	-	-	-	-	-	-	-	-
McKenzie	47.6199	-103.809	X	-	X	-	X	X	-	-	-	-	-	-
McKenzie	47.8041	-103.049	-	-	X	-	-	-	-	-	-	-	-	-
McKenzie	47.5495	-103.779	-	-	X	-	-	-	-	-	-	-	-	-
McKenzie	47.8343	-103.485	X	-	-	-	-	-	-	-	-	-	-	X
McKenzie	48.0597	-103.82	X	-	-	-	-	-	-	-	-	-	-	-
McKenzie	47.9924	-103.17	X	-	-	-	-	-	-	-	-	-	-	-
McKenzie	47.6768	-103.877	X	-	-	-	-	-	-	-	-	-	-	-
McKenzie	47.6726	-103.809	X	-	-	-	-	-	-	-	-	-	-	-
McKenzie	48.08	-103.684	-	-	-	-	-	-	-	-	-	-	-	-
McKenzie	48.1073	-103.71	-	-	-	-	-	-	-	-	-	-	-	-
McKenzie	47.873	-103.818	-	-	-	-	-	-	-	-	X	-	-	-
McKenzie	47.9765	-103.083	-	-	-	-	-	-	-	-	-	-	-	-
McLean	47.6476	-101.383	-	-	-	-	-	X	-	-	-	-	-	-
McLean	47.536	-100.898	X	-	X	-	-	-	-	-	-	-	-	-
Mercer	47.3206	-101.679	-	-	-	-	-	X	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Mercer	47.2982	-101.722	X	-	-	-	-	-	-	-	-	-	-	-
Morton	46.7285	-101.866	X	-	-	-	-	-	-	-	-	-	-	-
Morton	46.7295	-101.237	X	-	-	-	-	-	-	-	-	-	-	-
Morton	46.466	-101.189	-	-	-	-	-	-	-	-	-	-	-	-
Mountrail	48.3248	-101.948	-	-	-	-	-	X	-	-	-	-	-	-
Mountrail	48.3179	-102.752	-	-	-	-	-	X	-	-	X	-	-	-
Mountrail	48.1633	-102.394	X	-	-	-	-	-	-	-	-	-	-	-
Mountrail	48.209	-102.614	X	-	-	-	-	-	-	-	-	-	-	-
Mountrail	48.2113	-102.624	X	-	-	-	-	-	-	-	-	-	-	-
Mountrail	48.3241	-102.254	-	-	-	-	-	-	-	-	-	X	-	-
Mountrail	48.1246	-102.483	-	-	-	-	-	-	-	-	-	-	-	-
Nelson	47.7442	-97.9511	-	-	X	-	-	-	-	-	-	-	-	-
Nelson	47.7892	-98.2359	X	-	X	-	-	-	-	-	-	-	-	-
Nelson	47.76	-98.1657	-	-	-	-	-	-	-	-	-	-	-	-
Oliver	47.0524	-101.206	X	-	X	-	-	-	-	-	-	-	-	-
Pembina	48.5729	-97.1492	-	-	-	-	-	X	-	-	-	-	-	-
Pembina	48.9705	-97.2387	X	X	-	-	-	-	-	-	-	-	-	-
Pembina	48.9628	-97.436	-	-	-	-	-	-	-	-	-	-	-	-
Pierce	48.4789	-100.197	-	-	-	-	X	-	-	-	-	-	-	-
Pierce	48.501	-99.847	X	-	-	-	-	-	-	-	-	-	-	-
Pierce	48.3579	-99.976	-	-	-	-	X	-	-	-	-	-	-	-
Ramsey	48.05	-98.5485	X	-	X	-	-	-	-	-	-	-	-	-
Ramsey	48.3079	-99.1982	-	-	-	-	-	-	-	-	-	-	-	-
Ransom	46.4796	-97.7752	X	X	-	-	-	-	-	-	-	-	-	-
Renville	48.722	-101.846	-	-	-	-	-	X	-	-	-	-	-	-
Renville	48.9227	-101.628	-	-	-	-	-	-	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Renville	48.794	-101.795	X	X	-	-	-	-	-	-	-	-	-	-
Renville	48.8256	-101.954	-	-	-	-	-	-	-	-	-	-	-	-
Renville	48.7672	-101.975	-	-	-	-	-	-	-	-	-	-	-	-
Renville	48.7648	-101.981	-	-	-	-	-	-	-	-	-	-	-	-
Renville	48.9992	-101.96	-	-	-	-	-	-	-	-	-	-	-	-
Renville	48.9987	-101.79	-	-	-	-	-	-	-	-	-	-	-	-
Renville	48.7637	-101.581	-	-	-	-	-	-	-	-	-	-	-	-
Renville	48.7983	-101.497	-	-	-	-	-	-	-	-	-	-	-	-
Richland	46.1521	-97.1195	X	-	-	-	-	X	-	-	-	-	-	-
Richland	46.1678	-97.0115	-	-	-	-	-	-	-	-	-	-	-	-
Rolette	48.9505	-99.6207	X	-	-	-	-	-	-	-	-	-	-	-
Rolette	48.9095	-99.6115	-	-	-	-	-	-	-	-	-	-	-	-
Sargent	46.0874	-97.6337	X	-	-	-	-	-	-	-	-	-	-	-
Sheridan	47.5163	-100.463	-	-	-	-	-	-	-	-	-	-	-	-
Sheridan	47.466	-100.616	-	-	-	-	-	-	-	-	-	-	-	-
Sioux	46.0444	-100.702	X	-	X	-	-	X	-	-	X	-	-	-
Sioux	46.0603	-101.273	X	-	-	-	-	-	X	-	X	-	-	-
Sioux	46.2115	-100.648	-	-	-	-	-	-	-	-	-	-	-	-
Sioux	46.2123	-100.649	-	-	-	-	-	-	-	-	-	X	-	-
Sioux	46.269	-100.635	-	-	-	-	-	-	-	-	-	-	-	-
Slope	46.4766	-103.196	X	-	-	-	-	-	-	-	X	-	-	-
Slope	46.2917	-103.93	X	-	-	-	-	-	-	-	-	X	-	-
Slope	45.958	-102.604	X	-	-	-	-	-	-	-	X	-	-	-
Stark	46.8975	-102.32	X	-	-	-	-	-	-	-	-	-	-	-
Stark	46.8658	-102.849	-	-	-	-	-	-	-	-	X	-	-	-
Steele	47.27	-97.9363	X	-	-	-	X	-	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Stutsman	47.2362	-99.272	X	-	-	-	-	-	-	-	-	-	-	-
Stutsman	47.2691	-99.0582	X	-	-	-	-	-	-	-	-	-	-	-
Stutsman	47.1538	-99.1401	-	-	-	-	-	-	-	-	-	-	-	-
Towner	48.7936	-99.25	X	-	-	-	-	-	-	-	X	-	-	-
Towner	48.6318	-99.2637	X	-	-	-	-	-	-	-	-	-	-	-
Towner	48.9447	-99.3917	-	-	-	-	-	-	-	-	-	-	-	-
Towner	48.9498	-99.3475	-	-	-	-	-	-	-	-	-	-	-	-
Towner	48.8835	-99.4189	-	-	-	-	-	-	-	-	-	-	-	-
Towner	48.8634	-99.4968	-	-	-	-	-	-	-	-	-	-	-	-
Towner	48.7904	-99.1947	-	-	-	-	-	-	-	-	-	-	-	-
Traill	47.4988	-97.3415	-	-	-	-	-	X	-	-	-	-	-	-
Traill	47.6232	-96.8768	X	-	-	-	-	-	-	-	-	-	-	-
Walsh	48.4058	-97.798	X	-	-	-	X	-	-	-	-	-	-	-
Walsh	48.4417	-97.8601	X	X	X	X	-	-	-	-	-	-	-	-
Walsh	48.413	-97.1386	X	X	-	-	-	-	-	-	-	-	-	-
Ward	48.214	-101.234	X	X	X	-	-	-	-	-	-	-	-	-
Ward	48.6924	-102.095	X	-	-	-	-	-	-	-	-	-	-	-
Ward	48.5586	-101.962	-	-	-	-	-	-	-	-	-	-	-	-
Wells	47.4791	-99.3991	-	-	-	-	-	X	-	-	-	-	-	-
Wells	47.7622	-99.9232	-	-	-	-	-	-	-	-	-	-	-	-
Wells	47.6708	-99.5116	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.5868	-103.444	-	-	-	-	-	X	-	-	-	-	-	-
Williams	48.5709	-103.668	X	X	-	-	-	-	-	-	-	-	-	-
Williams	48.6072	-103.624	X	-	-	-	-	-	-	-	-	-	-	-
Williams	48.3387	-103.172	X	-	-	-	-	-	-	-	-	-	-	-
Williams	48.6131	-104.017	-	-	-	-	-	-	-	-	-	-	-	-

County	Latitude	Longitude	NLF	WF	CF	GTF	GPT	TS	WPT	BS	CGS	PGS	PRS	SGS
Williams	48.6238	-104.025	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.5787	-103.591	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.4707	-103.69	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.4338	-103.735	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.5784	-103.129	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.5954	-102.913	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.5843	-102.935	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.1656	-103.14	-	-	-	-	-	-	-	-	-	-	-	-
Williams	48.2566	-103.428	-	-	-	-	-	-	-	-	-	-	-	-