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# POPULATION DYNAMICS OF THREE AMPHIBIAN SPECIES ACROSS THE SHEYENNE NATIONAL GRASSLAND OF SOUTHEASTERN NORTH DAKOTA

by

Bartholomew L. Bly Bachelor of Science, University of Wisconsin -- La Crosse, 2001

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota December 2004 This thesis, submitted by Bartholomew L. Bly in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

Chairperson)

This thesis meets the standards for appearance, conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

Dean of Graduate School

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#### ABSTRACT

Concerns over worldwide declines of amphibians, including some to extinction, has increased the urgency for understanding how amphibians interact within local environments and across regional landscapes. Hypotheses for declines include anthropogenic destruction and fragmentation of amphibian habitat, introduction of exotic predators and competitors, increased ultraviolet (UV-B) irradiation, acid precipitation, environmental contamination by pollutants, harsh climatic conditions, over harvesting, and infectious disease. Three different types of models were developed for the northern leopard frog (*Rana pipiens*), gray tiger salamander (*Ambystoma tigrinum diaboli*), and gray tree frog (*Hyla versicolor/chrysoscelis complex*) populations inhabiting the Sheyenne National Grassland (SNG) in southeastern North Dakota. The SNG is a relatively large (283 km<sup>2</sup>) and contiguous piece of tallgrass prairie embedded in a landscape dominated by agriculture, though the predominant land use on the SNG is grazing by domestic <sup>l</sup>ivestock.

Although amphibians often occur in a metapopulation type structure where individual wetlands represent patches, continued fragmentation and isolation of habitat will cause populations to go extinct if colonization is not sufficient to offset local extinction. Because many factors may influence habitat use and occupancy of amphibian populations, best subsets logistic regression was used to develop occupancy and extinction models for these amphibian species using a whole suite of variables related to wetland hydroperiod, wetland isolation, patch quality, and landscape complementation.

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## CHAPTER 1

## AMPHIBIAN ECOLOGY ON THE NORTHERN TALLGRASS PRAIRIE

## "Amphibians were here when the dinosaurs were here, and they survived the age of mammals. They're tough survivors. If they're checking out now, I think it is significant." -- David Wake--

## Introduction

Apparent worldwide declines of amphibian species, some possibly to extinction, gained attention of researchers in the early 1990's (Blaustein and Wake 1990, Pechmann et al. 1991). Since that time much debate has considered whether these declines are real or the result of normal demographic fluctuations. Much research has assumed these declines are real and has focused on determining causative agents for these declines. Hypotheses for these declines include anthropogenic destruction and fragmentation of amphibian habitat, introduction of exotic predators and competitors, increased ultraviolet (UV-B) irradiation, acid precipitation, environmental contamination by pollutants, harsh weather conditions, overharvesting, and infectious disease (Daszak et al. 1999). In recent years the potential role of infectious disease has emerged as a potential mechanism for these declines, because a number of declines and extinctions occurred in relatively pristine landscapes (Pounds and Crump 1994, Laurance et al. 1996). Other research has focused on how the destruction and fragmentation of landscapes impact population dynamics of amphibians inhabiting altered landscapes. Understanding landscape structure (composition and configuration) and its underlying effects on population dynamics (e.g. - migration rates, extinction risk/persistence, and gene flow) is important

for conserving amphibian species in fragmented landscapes (Lehtinen et al. 1999, Pope et al. 2000, Joly et al. 2001, Marsh and Trenham 2001, Guerry and Hunter Jr. 2002).

A metapopulation, or a "population of populations" as described in Levins (1970). can be defined as a group of local populations connected through dispersing individuals (Hanski and Simberloff 1997). Metapopulation theory predicts that smaller more isolated populations are more prone to extinction, and rely on immigration from other populations for persistence (Hanski 1999). Because local populations may go extinct frequently, a regional or landscape level approach is necessary for understanding metapopulation dynamics of populations inhabiting altered landscapes (Hecnar and M'Closkey 1996). Two requirements for long-term persistence of a species are sufficient colonization rates and some degree of asynchrony in local population dynamics (Hanski 1999). Metapopulation models do not focus on single populations, but describe the fraction of all local populations occupied at a given time (Hanski 1994). Although these models are based on metapopulation theory, they have been refined to incorporate more variables and provide more realistic results (Etienne et al. 2004). Amphibian populations fit well into studies exploring metapopulation dynamics, because wetlands form discrete habitat patches that are easily delineated and characterized (Hecnar and M'Closkey 1996, Marsh and Trenham 2001). Metapopulation studies on amphibians began with Gill (1978), and many subsequent studies have evaluated effects of increasing habitat modification on amphibian populations, including possible correlations with global amphibian declines.

Amphibians are important prey and predators in ecosystems, providing important links in food webs that would not exist otherwise (Corn and Peterson 1996). Alteration of amphibian microhabitats has been demonstrated to negatively impact amphibian

populations (Welsh and Ollivier 1999), making amphibians a useful indicator of early ecosystem stress (Corn and Peterson 1996, Welsh and Ollivier 1999). Amphibians are not a diverse group of organisms in the northern Great Plains (Corn and Peterson 1996), but the glacial marshes in the Great Lakes basin and adjacent prairie pothole region provide critical habitat for amphibian reproduction (Lehtinen et al. 1999). Ephemeral pothole ponds are important for amphibian populations, because they are often fishless and persistent enough to allow metamorphosis of amphibian larvae in most years (Corn and Peterson 1996). My study focused on amphibian populations across the Sheyenne National Grassland (SNG) in southeastern North Dakota. Large numbers of wetlands and the landscape heterogeneity (e.g. - tallgrass prairie, grazed pastures, bur oak savannas) associated with the SNG, including the presence of wetlands in the adjacent agricultural land makes the SNG an ideal system for studying impacts of landscape structure on population dynamics, and for identifying the spatial and habitat-related characteristics most important to local amphibian assemblages.

## Study Area

The SNG is located on the western boundary of the tallgrass prairie ecoregion (Jones and Cushman 2004), and is situated within Richland and Ransom Counties in southeastern North Dakota. The SNG, which is managed by the USDA Forest Service, consists of approximately 283 km<sup>2</sup> of tallgrass meadows, fens, sandhills, and bur oak savannas, making it one of the largest remaining portions of contiguous tallgrass prairie in North America (Jones and Cushman 2004). During the Wisconsian glaciation (70,000 to 10,000 years ago), glaciers advanced over much of North Dakota and blocked the major drainages. This blockage caused the formation of glacial Lake Agassiz which

covered the Red River Valley of North Dakota, Minnesota, and Manitoba (Bluemle 1977). A sandy delta was formed where the Sheyenne River entered Lake Agassiz (Bluemle 1977), and because the SNG rests atop of this delta its soil has large quantities of sand. Northern portions of the SNG are characterized by large sand hills that slowly descend into the Sheyenne River Valley, which contains the only contiguous stand of forest in the entire landscape. Central portions of the grassland contain small hummocks and bur oak savannas, which gradually turn into the flat prairie associated with southern portions of the SNG.

Early explorers found that the soil and moisture conditions favoring the growth of big bluestem (a grass synonymous with tallgrass prairie) were also favorable for growing corn, and within a few years of settlement most big bluestem prairies were converted to agriculture (Jones and Cushman 2004). Although the SNG is largely contiguous tallgrass prairie, though not pristine, it is embedded in a landscape dominated by agriculture. Agriculture has been demonstrated to have negative effects on amphibian populations by creating an unsuitable matrix habitat that isolates remnant populations (Joly et al. 2001). Additional wetlands used in this study were found in adjacent tallgrass prairie managed by the Nature Conservancy, and on privately owned land that was usually surrounded by cropland. Other wetlands included several large wetlands managed by the U.S. Fish and Wildlife Service as Waterfowl Production Areas, and although these wetlands were believed to represent suitable amphibian breeding habitat, they were usually embedded within a matrix of row crop agriculture.

Throughout the Pleistocene Epoch, North Dakota has had a continental climate with cold winters and hot summers (Bluemle 1977), and often cycle between periods of

wet and dry years. Increased rainfall in the late 1990's led to abundant and widespread amphibian populations across the SNG (Jones and Cushman 2004), but the spring of 2002 marked the beginning of a dry period that spanned the entire study. Reduced precipitation (snow and rain) in 2002 and 2003 and the sandy soil associated with the SNG caused a great reduction in wetlands. In 2002 the lack of precipitation was severe enough to dry most ephemeral wetlands and few of these contained water in the spring of 2003, making ephemeral wetlands unsuitable for amphibian reproduction in all years. Instead, a majority of wetlands permanent enough to persist over the duration of the study were grazed intensively, contained higher concentrations of predators, and contained lower quality water and vegetation components.

## Impacts on Amphibian Populations

## Habitat Distribution, Surrounding Land Use, and Patch Quality

Many studies (see Chapter 2) have developed statistical models to determine which components of landscape structure and local characteristics are most important in determining amphibian occupancy. As additional grasslands and wetlands are destroyed (e.g. - converted to agriculture) the amount of suitable amphibian habitat may diminish, leaving remnant populations even more isolated. Semlitsch and Bodie (1998) found that the loss of small, isolated wetlands resulted in larger distances between remaining wetlands, making dispersal between wetlands more difficult. Gibbs (1993) found small wetlands to be important because they are often numerically abundant across the landscape, providing unoccupied wetlands with a better chance for recolonization. Many small, ephemeral wetlands containing amphibians may also dilute the impact of predators (such as garter snakes) on permanent wetlands (personal observation). As destruction

and fragmentation of suitable amphibian habitat continues, conservation strategies must determine the extent to which migration occurs between wetlands, and how permeable the intervening matrix habitat is to migration.

Landscape connectivity is dependent upon the interaction of landscape structure and the movement of organisms within that landscape (Merriam 1984). Migration rates are not only affected by the distance between habitat patches, but may also depend on the permeability of the intervening matrix habitat and how well the organism traverses nonsuitable habitat. Connectivity is a process parameter, because the process of organisms moving across the landscape (both suitable and unsuitable) is part of its definition (Merriam 1984). Amphibians are subject to high rates of evaporative water loss because of their semi-permeable skin (Kostinsky et al. 2000), which makes long distance dispersal across an unsuitable matrix (e.g. - row crop agriculture) more difficult. However, adjacent forests and large amounts of woodland in the surrounding landscape have been demonstrated to have positive impacts on amphibian occupancy (Kolozsvary and Swihart 1999, Guerry and Hunter, Jr. 2002). Landscape complementation involves a requirement of more than one critical habitat for completion of a life cycle (Dunning et al. 1992), and has been shown to "mask" metapopulation processes for northern leopard frog (Rana *pipiens*) populations (Pope et al. 2000). Landscape composition and configuration are important for predicting local occupancy and for maintaining regional persistence, but local factors are equally important in determining occupancy patterns.

Local conditions such as water quality (Boyer and Grue 1995), vegetation abundance and diversity (Vos et al. 2000, Hazell et al. 2001), and predator presence (Morin 1983, Knutson 2004) have all been shown to impact amphibian populations

(Bradford et al. 2003, Knutson 2004). Elevated pH, low dissolved oxygen, and high water temperatures may singly or in combination have detrimental effects on amphibian embryos (Boyer and Grue 1995). Presence of emergent vegetation has been shown to positively influence wetland occupancy by amphibians (Wells 1977, Vos et al. 2000, Hazell et al. 2001). Knutson (2004) found amphibian occupancy was negatively affected by the presence of fish in a wetland, and Morin (1983) demonstrated that *Ambystomid* salamander larvae in a pond could extirpate local populations of other amphibians.

## Grazing

Grazing by domestic livestock has been shown to have negative impacts on amphibian populations (Jansen and Healey 2003), and on overall wetland condition because of the tendency for livestock to concentrate around water (Jansen and Robertson 2001). Grazing can impact wetlands by altering water chemistry, degrading aquatic and riparian vegetation, and through repeated disturbances that destroy habitats required by larval amphibians (Knutson et al. 2004). Healey et al. (1997) concluded that differences in adult frog abundance between wetlands were related to disturbance of riparian vegetation by cattle. Although numbers of grazers and grazing duration vary across the SNG, grazing is the predominant land use and cattle are often rotated through nearly all pastures in a given year.

Cattle may potentially be a nuisance to breeding amphibian populations, but other land management practices associated with grazing on the SNG may benefit amphibian populations. In dry years small stock ponds are commonly "dugout" in grazing pastures across the SNG when natural wetlands are scarce. Although these wetlands are heavily impacted by cattle, they are permanent wetlands in which amphibians could potentially

breed. These stock ponds may also increase connectivity of the landscape by lowering interpatch distance during dry years (Baker and Halliday 1999). Baker and Halliday (1999), Hazell et al. (2001), and Knutson et al. (2004) found that constructed ponds often support amphibian populations in agricultural landscapes, and are even critical for some species since they are usually fishless (Baker and Halliday 1999).

## Roads and Vehicular Mortality

Negative effects of road traffic on amphibian populations have been demonstrated by several studies (Fahrig et al. 1995, Vos and Chardon 1998, Linck 2000, and Carr and Fahrig 2001), with greater impacts occurring on more vagile species since they encounter roads more often (Carr and Fahrig 2001). Vehicular mortality is most important when a road with high traffic intensity separates overwintering habitat from breeding habitat (Linck 2000). Because amphibians may routinely use roadside ditches as breeding ponds during wet years (personal observation), vehicular mortality can also be detrimental when metamorphic amphibians emerge from roadside ditches. Although the SNG is largely contiguous, paved roads and gravel roads containing at least moderate traffic intensity are present and may impact amphibian populations across the SNG.

## Amphibians of the Sheyenne National Grassland

At least eight amphibian species have been reported in the SNG (Conant and Collins 1991), but only six were encountered during this study. Species encountered were the northern leopard frog (*Rana pipiens*), the wood frog (*Rana sylvatica*), the gray tree frog (*Hyla versicolor/chrysoscelis* complex), the Canadian toad (*Bufo hemiophrys*), the gray tiger salamander (*Ambystoma tigrinum diaboli*), and the chorus frog (*Pseudacris triseriata*). Species whose range includes the SNG but were not encountered include the

Great Plains toad (*Bufo cognatus*) and Woodhouse's toad (*Bufo woodhousei*). Northern leopard frogs were the most abundant species encountered during all three field seasons, followed in abundance by gray tiger salamanders and gray tree frogs.

## Northern Leopard Frogs

## Natural History

The life history of the northern leopard frog in the upper Midwest has been thoroughly described by Merrell (1977). Northern leopard frogs have the demographic capability to explode under favorable conditions, as was demonstrated in the "Great Frog Uprising" where approximately 175,000,000 metamorphosed leopard frogs invaded Oconto, Wisconsin in 1953. Northern leopard frogs may live 4-5 years in the wild (Leclair and Castanet 1987), and female leopard frogs in Quebec, Canada have attained sexual maturity at two years of age (Gilbert et al. 1994). Fecundity increases with body size in leopard frogs, and females deposit 2600 eggs per mass on average (Gilbert et al. 1994). These demographic capabilities allow northern leopard frog populations to rapidly recover from periods of unfavorable conditions and low numbers.

Although northern leopard frogs require a body of water for hibernation during the winter and for breeding during spring, most of the summer is spent independent of water (Dole 1967) wandering around the surrounding terrestrial habitat (using rain and dew as a water source) foraging on insects, worms, and even other frogs (Conant and Collins 1991; Jones and Cushman 2004). Although northern leopard frogs require a suitable landscape (Pope et al 2000) and may actively select for vegetation structure within the landscape (Beauregard and Leclair Jr. 1988), the largest populations of northern leopard frogs often occur in early successional habitats (Orr et al. 1998).

#### Potential Concerns

Northern leopard frogs were once the most widespread frog species in North America (Jones and Cushman 2004), but have suffered declines in many parts of their range (Orr et al. 1998), including areas in North Dakota (Larson et al. 1998). One problem facing the northern leopard frog is that its status is unknown throughout much of its range (Orr et al. 1998). Northern leopard frog numbers have increased across the SNG recently, most likely because of increased rainfall during the late 1990's (Jones and Cushman 2004). Leopard frogs are vagile, and vehicular mortality has been demonstrated to negatively impact populations near major roads (Linck 2000). Leopard frogs have also been found to utilize rivers for dispersal (DuBois and Stoll 1995, Seburn et al. 1997), but little is known about this dispersal method and how it might benefit populations. The Sheyenne River provides amphibian species inhabiting the SNG with a permanent body of water, but it is unknown if amphibians utilize it during drought years.

Goater (1992) and Goater et al. (1993) demonstrated negative impacts of macroparasites on anurans under experimental conditions, but the extent to which macroparasites affect population dynamics of amphibians in the wild remains unknown. Before exploring impacts of macroparasites on amphibian population dynamics, parasites infecting these populations must be identified and the modes of transmission understood. Time limitations prevented rigorous analysis of parasite-mediated impacts or structuring of amphibian populations, but a survey of helminths infecting leopard frogs across the SNG was conducted in 2003 and is discussed in Chapter 3.

Northern leopard frogs also exhibit dorsal color and pattern polymorphisms, and the mode of inheritance of all three polymorphisms has been demonstrated through multigenerational breeding studies (Hoffman and Blouin 2000). Immigration, and more importantly gene flow, is critical for populations inhabiting fragmented landscapes, because immigrations rates in severely isolated populations may be insufficient to offset the fixation of alleles (Connor and Hartl 2004). Fixation of alleles diminishes the ability of populations to adapt and can lead to localized extinctions (Harrison and Hastings 1996). Time and labor constraints prevented extensive genetic analysis on gene flow and allelic diversity, but visual polymorphisms exhibited by leopard frogs allow for a quick and easy genetic analysis on SNG populations. These results are discussed in Chapter 4.

## Gray Tiger Salamanders

## Natural History

Gray tiger salamanders and various other subspecies are among the most ubiquitous yet seldom encountered amphibian species in the northern prairie (Jones and Cushman 2004). Adult tiger salamanders are rarely encountered because of their fossorial nature, except during spring and late fall rains when large numbers of salamanders often cross roads during migrations (Conant and Collins 1991). During early spring rains, tiger salamanders migrate to breeding ponds and often utilize farm ponds when available (Conant and Collins 1991). Tiger salamanders lack vocalization but instead proceed through elaborate courtship rituals before males deposit a spermatophore that is subsequently picked up in the female's cloaca (Whiteman et al. 1999). Post-breeding migration by ambystomid salamanders has been shown as nonrandom, occurring in a bimodal pattern from the wetland (Kleeberger and Werner 1983).

Carnivorous tiger salamander larvae often prey on aquatic invertebrates and other amphibian tadpoles, including their own larvae if food becomes limited in a wetland

(personal observation). Salamander larvae may also be a determinant in structuring larval amphibian guilds (Morin 1983), and when conditions become ideal within a wetland some salamander species become sexually mature without metamorphosing (paedomorphic) and breed without attaining adult characteristics (Conant and Collins 1991). Paedomorphosis is a polymorphic trait known to occur in several currently recognized subspecies within the *Ambystoma tigrinum* complex (Collins 1981). Paedomorphosis probably arose as a response to local selection (Routman 1993), and is most likely maintained in a population through natural selection, because facultative paedomorphosis is believed to be environmentally induced and occurs in both sexes (Whiteman et al. 1999). *Ambystoma tigrinum diaboli* populations are described in Conant and Collins (1991) as being frequently neotenic, but the extent to which this occurs in North Dakota is unknown.

#### Potential Concerns

Metamorphic tiger salamanders are dependent upon the surrounding terrestrial environment, requiring a burrow that ensures them the proper temperature and humidity level for survival during the cold season (Conant and Collins 1991). Weyrauch and Grubb Jr. (2004) found that landscape associated variables were better at predicting tiger salamander occupancy than wetland associated variables. Ambystomid salamanders are less vagile than anurans, usually exhibiting home ranges of less than 200 meters from natal ponds (Kleeberger and Werner 1983, Dodd 1996). Routman (1993) found little evidence of gene flow between tiger salamander populations separated by 1500 meters in western Nebraska, but Kolozsvary and Swihart (1999) captured an adult tiger salamander in a small forest patch that was more than 1 kilometer away from the nearest potential

breeding site. This suggests that researchers understand little of tiger salamander movement through terrestrial environments, and because tiger salamanders often migrate in large numbers during late fall rains they may be susceptible to impacts by vehicular mortality if a major road intersects migratory routes.

Tiger salamanders have received little attention in terms of global declines, but in summer 1998 a population of gray tiger salamanders in a Waterfowl Production Area in Burleigh County North Dakota experienced a mass die-off of thousands of individuals (Docherty et al. 2003). In the summer of 2000, another large die-off occurred at the Cottonwood Lake Study Area near Jamestown, North Dakota (Jones and Cushman 2004). During amphibian surveys in July 2000, researchers only found a total of 8 salamanders in a wetland where they previously averaged 150 salamanders per trap (Jones and Cushman 2004). Both die-offs were caused by a ranavirus (Green et al. 2002, Docherty et al. 2003), the same type of viral infection associated with global amphibian declines (Daszak et al. 1999). In 1998 a similar ranavirus outbreak occurred in a wood frog population (*Rana sylvatica*) in northeastern North Dakota (Green et al. 2002), but to the best of my knowledge there has not been a documented disease outbreak affecting amphibians across the SNG.

#### Gray Tree Frogs and Cope's Gray Tree Frogs

### Natural History

Gray tree frogs are arboreal outside the breeding period, foraging in small trees and shrubs where they are extremely well camouflaged (Conant and Collins 1991). Breeding occurs later in the summer and can continue into late June on the northern prairie. Because of this phenology and the climate of the northern Great Plains, gray tree

frogs must breed in wetlands that persist until at least mid-August when tree frog tadpoles undergo metamorphosis (personal observation). Gray tree frogs are also known for their ability to tolerate freezing conditions during winter by accumulating high concentrations of sugars within their cells (Schmid 1982).

The range of the gray tree frog (*Hyla versicolor*) and its cryptic relative Cope's gray tree frog (*Hyla chrysoscelis*) is poorly understood in North Dakota (report submitted by Tramontano 2003) because of the inability to differentiate these two species in the field (Conant and Collins 1991). These species are easily differentiated when both are calling, but it is difficult to identify a species without hearing the other. Because they are morphologically identical and may not breed in the same ponds, they have often been misclassified as one species. Their composite range in North Dakota includes the entire eastern edge of the state, and coincides with the edge of the tallgrass prairie (Conant and Collins 1991). However, Tramantano (2003) concluded that both *H. versicolor* and *H. chrysoscelis* inhabit the SNG based on karyotypes of gut epithelium from collected tree frogs. Because "calling" data were not used for analysis, and because there is no other way to differentiate these species in the field, they are hereafter referred to as gray tree frogs (*H. versicolor/chrysoscelis* complex) because they share many life history traits (Conant and Collins 1991).

Jaslow and Vogt (1977) found *H. chrysoscelis* to be a prairie associated species and *H. versicolor* a forest associated species, and both habitat types are represented within the SNG. *Hyla arborea* in the Netherlands have been shown to have an affinity for emergent vegetation in the breeding pond, where presence of emergent vegetation increased a wetlands chance for colonization (Vos et al. 2000). Vos et al. (2000) also

found that tree frogs (*H. arborea*) selectively migrated towards already colonized ponds, coining the term "conspecific attraction" for the phenomena.

#### Potential Concerns

Some regional studies have been conducted on European tree frog (*H. arborea*) metapopulations (Vos and Stumpel 1995, Vos et al. 2000, Carlson and Edenhamn 2000), but I found few published studies on North American tree frogs. However, despite rarely being encountered on the ground (Conant and Collins 1991), metapopulations of *H. arborea* in Europe are thought to remain connected even when migration distances range up to two kilometers (Vos et al. 2000). Since the sandy soil associated with the SNG is very permeable and because gray tree frogs require semi-permanent wetlands related to their late breeding phenology, breeding habitat may become highly fragmented in dry years, increasing the chance for local extinction of smaller more isolated populations.

Gray tree frogs are dormant during daylight hours (Conant and Collins 1991), suggesting that little impact of roads on tree frog populations may exist. Morin (1983) found two competitively inferior species of Hylids (*Pseudacris crucifer and Hyla gratiosa*) to survive better in communities containing increased levels of amphibian predators. This result occurred by salamander larvae actively selecting against the competitively superior anuran larvae, including the Florida leopard frog (*Rana sphenocephala*), which allowed competitively inferior species to exploit habitats where competition was diminished. Wetland conditions similar to these circumstances and with similar species (*Ambystoma tigrinum*, *Rana pipiens*, and *Hyla versicolor/chrysoscelis* complex) are present on the SNG, and similar associations between predation and competition can be explored.

## Additional Species

Breeding populations of wood frogs, chorus frogs, and Canadian toads were less abundant and in fewer patches across the SNG. All three species were heard calling at various sites in the spring of all years, but their low patch occupancy and a potential underestimate of occupancy associated with conducting surveys when they may have already left the pond made modeling these species problematic. The Great Plains toad and Woodhouse's toad were not encountered at all and were eliminated from all analyses.

## Specific Objectives of this Study

- Determine which regional (landscape) and local (wetland) level variables are most important in predicting occupancy of various amphibian species across the SNG and adjacent lands during dry years
- Determine which regional (landscape) and local (wetland) level variables are most responsible for causing local extinctions between years of various amphibian species across the SNG and adjacent lands during dry years
- Use wetland occupancy data and the spatially-explicit incidence function model to estimate extinction and colonization rates for various amphibian species inhabiting the SNG and adjacent lands during dry years
- Survey helminths (including respective prevalence and intensities) infecting northern leopard frogs (*Rana pipiens*) across the SNG during dry years
- Calculate the frequency of color and pattern polymorphisms exhibited by northern leopard frogs (*Rana pipiens*) across the SNG during dry years and use the results to test for deviance between various regions of the SNG

## CHAPTER 2

## MODELING TALLGRASS PRAIRIE AMPHIBIAN POPULATIONS ACROSS THE SHEYENNE NATIONAL GRASSLAND IN SOUTHEASTERN NORTH DAKOTA

### Introduction

Concerns over worldwide declines of amphibians, including some to extinction (Blaustein and Wake 1990, Pechmann et al. 1991), has increased the urgency for understanding how amphibians interact within local environments (Pope et al. 2000, Weyrauch and Grubb 2004) and across regional landscapes (Hecnar and M'Closkey 1996). Hypotheses for declines include anthropogenic destruction and fragmentation of amphibian habitat, introduction of exotic predators and competitors, increased ultraviolet (UV-B) irradiation, acid precipitation, environmental contamination by pollutants, harsh climatic conditions, over harvesting, and infectious disease (Daszak et al. 1999). Although infectious disease has been linked to a number of declines (Green et al. 2002), anthropogenic fragmentation and destruction of habitat required by amphibians has been shown to negatively impact populations (Welsh and Ollivier 1999) and overall species richness (Hecnar and M'Closkey 1996). Because amphibians are important as predators and prey in ecosystems (Corn and Peterson 1996) and because they have been shown to be negatively impacted by habitat alteration (Welsh and Ollivier 1999), amphibians make useful indicators of ecosystem stress (Corn and Peterson 1996, Welsh and Ollivier 1999).

Although amphibians often occur in metapopulation type structures where individual wetlands represent patches (Hecnar and M'Closkey 1996, Marsh and Trenham

2001), continued fragmentation and isolation of habitat will cause populations to go extinct if colonization is not sufficient to offset local extinction (Weyrauch and Grubb 2004). Successful colonization of an unoccupied patch depends on three components, the vagility and behavior of the species, the composition and configuration of habitat across a landscape, and the permeability of matrix habitat separating patches (Laan and Verboom 1990, Weyrauch and Grubb 2004). Many factors likely influence habitat use and occupancy of amphibian populations. The approach taken by many studies is to measure a suite of predictor variables related to local patch quality, matrix quality, and other landscape characteristics including spatial distribution of habitat, and then use logistic regression to analyze relationships between patch occupancy and predictor variables in order to make inferences about the importance of different variables.

Many studies have found associations of amphibian breeding success with habitat variables such as hydroperiod and wetland size (Loman 1988, Snodgrass et al. 2000, Bradford et al. 2003, Knapp et al. 2003, Weyrauch and Grubb 2004), and wetland isolation (Sjogren-Gulve 1994, Kolozsvary and Swihart 1999, Lehtinen et al. 1999, Knapp et al. 2003). Studies have also shown the importance of wetland characteristics in determining amphibian occupancy (Hazell et al. 2001, Bradford et al. 2003, Weyrauch and Grubb 2004, Knutson et al. 2004), including how processes like predation cause local extinctions and increased isolation of remnant populations (Sjogren-Gulve 1994). Water quality variables (e.g. conductivity and pH), vegetation presence and abundance, and predator presence have all been shown to be good indicators of amphibian occupancy, though the direction of associations are not always the same. Amphibians are sensitive to poor water quality conditions (Boyer and Grue 1995), and aquatic vegetation is required

by many amphibian species for deposition of eggs and evasion of predators (Conant and Collins 1991). Predators have differing associations with amphibian diversity, because predators like fish and salamander larvae may extirpate local anuran assemblages, but they may also select for more active tadpoles which allows competitively inferior species to thrive (Morin 1983). Patch characteristics are therefore important for predicting amphibian occupancy and local extinctions, and should therefore be considered when studying amphibian population dynamics.

Manually constructed ponds have also been documented to harbor amphibian populations (Laan and Verboom 1990, Baker and Halliday, Hazell et al. 2001, Knutson et al. 2004), especially when natural wetlands are scarce and unsuitable. The USDA – Forest Service manages a number of stock ponds across the Sheyenne National Grassland (SNG), which are "dugout" with heavy machinery during dry years for use by grazing cattle. The extent to which amphibian populations utilize stock ponds across the SNG is unknown, but the importance of these ponds will be explored, including their occupancy status and potential for increasing landscape connectivity.

The requirement of a suitable breeding pond and a suitable terrestrial habitat for life history completion, or landscape complementation (Dunning et al. 1992), is important for amphibian populations (Pope et al. 2000) and must be considered when studying population dynamics. The primary literature is replete with examples of surrounding landscapes having negative and positive associations with amphibian population dynamics and species diversity (Laan and Verboom 1990, Vos and Stumpel 1995, Hecnar and M'Closkey 1996, Healey et al. 1997, Kolozsvary and Swihart 1999, Pope et al. 2000, Hazell et al. 2001, Joly et al. 2001, Weyrauch and Grubb 2004, Knutson et al. 2004).

Conclusions of these studies were that adjacent or neighboring forest and grassland are significant predictors of amphibian occupancy, because they represent suitable habitat that complements breeding ponds. However, row crop agriculture is less suitable habitat and requires interspersed woodlot refugia or wetlands with long hydroperiods for maintaining viable amphibian populations. Grazing by domestic livestock has also been shown to negatively effect amphibian populations (Jansen and Robertson 2001, Jansen and Healey 2003), though most impacts have been demonstrated through degradation of wetlands (Knutson et al. 2004) and not by impacts on the surrounding terrestrial habitat. Still, heavily grazed pastures may be physiological barriers to dispersing amphibians because the soil may contain less moisture and cause an increased risk of desiccation when vegetative cover is absent or decreased. No study exploring amphibian population dynamics should examine only local (patch) or regional (landscape) variables, but should instead be treated as parts of the whole since both are important for regional persistence.

However, patch occupancy is dynamic and it is then of interest to analyze statetransitions and not simply treat occupancy as being static. State-transition models are similar to models analyzing associations between habitat and occupancy, and can utilize many of the same variables used in occupancy models. However, these models instead determine which factors are most important in occupied patches going extinct (extinction models) and unoccupied patches being colonized (colonization models). Although local populations are expected to undergo stochastic extinctions periodically (Hanski 1999), several variables including wetland isolation and fish presence have been linked to local amphibian extinctions (Sjogren 1991, Sjogren-Gulve 1994). Determining the causative agents responsible for local extinctions, whether purely stochastic or in response to

habitat alteration, is important for conserving amphibian populations with specific habitat requirements. Populations inhabiting fragmented landscapes often go extinct and rely on colonization from neighboring populations, but if colonization is slowed habitat factors, remaining populations will become isolated and local extinction probabilities will be greater. Understanding habitat factors associated with these processes can be used to slow local extinctions and increase colonization probabilities.

Hanski (1994, and see Etienne et al. 2004) introduced a different approach for estimating metapopulation process rates (colonization and extinction) that determine occupancy, where these processes are related to patch size and interpatch distance (which are considered critical to patch occupancy). This method, termed an incidence function model (IFM), is a minimalist model requiring only a single snapshot of occupancy data on which to base estimates of extinction and colonization rates. Incidence function modeling has been conducted on a range species including insects (Moilanen 1999, Wahlberg et al. 2002), mammals (Moilanen 1999), and amphibians (Vos et al. 2000), and has been refined in a number of ways to incorporate more realistic assumptions and include more variables (Etienne et al. 2004). Estimation techniques for IFM parameters have also improved (Moilanen 1999, Etienne et al. 2004), but the IFM is still based on two predictions of metapopulation theory that (1) larger patches will go extinct less frequently because they usually have larger populations and are therefore less impacted by stochastic processes, and (2) more isolated patches are less likely to be colonized and more likely to go extinct because of a reduced chance of rescue through migration from neighboring patches (Hanski 1999).

Much debate concerning the IFM has focused on its primary assumption, which is populations are at an equilibrium between extinction and colonization (Hanski 1994), which is probably not true for populations inhabiting fragmented landscapes. Further scrutiny came from Thomas et al. (2002) when an IFM underestimated minimum viable sizes of a butterfly metapopulation, and Clinchy et al. (2002) found that ecological processes other than extinction and colonization can produce similar occupancy patterns. However, the IFM is a practical and useful tool that provides informative results about population dynamics that may be useful for developing conservation strategies.

#### **Objectives**

My goals in conducting this study on amphibian population dynamics across the SNG were to (1) determine which regional and local level variables are most important in predicting occupancy of three amphibian species across the SNG and its adjacent lands, (2) determine which regional and local level variables are most important in causing local extinction events of three amphibian species across the SNG and its adjacent lands, and (3) using amphibian occupancy data and the spatially-explicit IFM to estimate extinction and colonization rates for three amphibian species across the SNG and its adjacent lands.

## Study Area

The SNG is located on the western boundary of the tallgrass prairie ecoregion (Jones and Cushman 2004), and is situated within Richland and Ransom Counties in southeastern North Dakota (Figure 1). The SNG consists of roughly 283 km<sup>2</sup> of contiguous (though not pristine) tallgrass prairie (Figure 2), making it one of the largest remaining portions of contiguous tallgrass prairie in North America (Jones and Cushman 2004). A sandy delta was formed where the Sheyenne River entered Lake Agassiz



Figure 1. Richland and Ransom counties (in black) located in southeastern North Dakota



Figure 2. The Sheyenne National Grassland (USDA Managed Land is shaded) located in Richland and Ransom counties in southeastern North Dakota, 2002-2004.
during the Wisconsian glaciation, and the SNG rests on this delta and is characterized by having sandy soil (Bluemle 1977). Northern portions of the SNG contain large sand hills that slowly descend into the Sheyenne River Valley, where the only contiguous stand of forest on the SNG is found. Central portions of the grassland are characterized by small hummocks and bur oak savannas, which gradually turn into the flat prairie observed in the southern portions of the SNG. Grazing by cattle is the predominant land use in this area, and the Sheyenne River provides permanent water while running through the SNG.

Although the SNG is composed of contiguous tallgrass prairie, it is embedded in a landscape dominated Ly agriculture, which is the most common land use practice in southeastern North Dakota. Corn and beans are among the most commonly observed crops surrounding the SNG. These fields may represent a more resistant landscape that is less conducive to amphibian dispersal, because they are bare soil (physiological barrier) during amphibian migrations to breeding ponds. In general, the abundance of wetlands and landscape heterogeneity (e.g. - tallgrass prairie, grazed pastures, and bur oak savannas) associated with the SNG, including the presence of wetlands in the adjacent agricultural land makes the SNG an ideal system for studying impacts of landscape structure and local habitat factors on population dynamics.

The northern prairie ecoregion cycles between periods of wet and dry years, and during wet years amphibian populations may "boom" because the abundant prairiepothole ponds associated with this landscape provide critical habitat for reproduction (Lehtinen et al. 1999). However, the spring of 2002 marked the beginning of a dry period that spanned most the study. Reduced precipitation in 2002 and 2003 (Figure 3) combined with the sandy soil of the SNG caused a reduction in the number of wetlands



Figure 3. Total amount of monthly rainfall (cm) recorded by the McLeod, ND weather station (center of the Sheyenne National Grassland) for the active months of amphibians inhabiting the northern prairie, 2001-2004. (figure excludes associated snowmelt)

across the landscape and increased isolation of persistent wetlands. The majority of wetlands that persisted throughout the study were heavily grazed, contained larger numbers of predators, had lower quality water, and the natural vegetation disturbed.

## **Amphibian Species**

Three species of amphibians inhabiting the tallgrass prairie ecoregion were modeled in this study, including the northern leopard frog (Rana pipiens), the gray tiger salamander (Ambystoma tigrinum diaboli), and the gray tree frog complex (Hyla versicolor/chrysoscelis). The life history of the northern leopard frog in the upper Midwest has been thoroughly described by Merrell (1977). Northern leopard frogs are among the most widespread frog species in North America (Jones and Cushman 2004), but suffered declines in many parts of its range (Orr et al. 1998), including areas in North Dakota (Larson et al. 1998). Gray tiger salamanders are one of the most ubiquitous yet seldom encountered amphibians in the northern prairie (Jones and Cushman 2004). Gray tiger salamander larvae are potentially a major determinant in structuring larval amphibian guilds (Morin 1983), and Conant and Collins (1991) describe them as being frequently neotenic (or paedomorphic), but the extent to which this occurs in North Dakota is unknown. In summer 1998 a population of gray tiger salamanders in Burleigh County North Dakota experienced a mass die-off (Docherty et al. 2003), and a similar die-off occurred at the Cottonwood Lake study area near Jamestown, North Dakota in 2000 (Jones and Cushman 2004). Both die-offs were the result of ranavirus outbreaks and were not necessarily linked to habitat alteration, but it is still important to understand tiger salamander population dynamics in order to better manage their populations. Ranges of the gray tree frogs (Hyla versicolor) and its cryptic

relative Cope's gray tree frog (*Hyla chrysoscelis*) are poorly understood in North Dakota (report submitted by Tramontano 2003). Their composite range includes the eastern edge of North Dakota, and Tramantano (2003) found both species inhabiting the SNG. Few studies have explored the population dynamics of North American tree frogs, especially in the northern prairie. Because the status of all three species is poorly understood across the SNG it is important to gain insight into their habitat requirements in order to conserve and properly manage their populations.

#### Methods

#### Site Selection

Most wetlands surveyed in this study were located across the SNG, but additional surveyed wetlands included Waterfowl Production Areas, ponds located on private property, and ponds on Nature Conservancy land. Waterfowl Production Areas and wetlands on private property were usually embedded in agricultural land, but wetlands on Nature Conservancy land were surrounded by forest or grassland. In this study a "wetland" was defined as a body of water not physically connected to another water body (despite drainage ditches flowing into the Sheyenne River) and contained water at the beginning of the amphibian breeding season. The prairie pothole region of North Dakota is characterized by containing numerous small ephemeral wetlands, each capable of harboring an amphibian population in wet years, so wetland proximity was not used in delineating wetlands. A total of 57 wetlands meeting these criteria were surveyed in 2002, 84 wetlands in 2003, and 95 wetlands in 2004 (Table 1, Figure 4). Because a majority of wetlands across the SNG are ephemeral, and because little precipitation was observed during the first two years of this study (Figure 3), many wetlands went dry and

	Tot	al Wetla	ands	Occupied Wetlands		nds
Species	2002	2003	2004	2002	2003	2004
Rana pipiens	57	84	95	25 (43%)	43 (51%)	38 (40%)
Ambystoma tigrinum	57	84	95	9 (16%)	23 (27%)	38 (40%)
Hyla versicolor/chrysoscelis	57	84	95	0 (0%)	12 (14%)	8 (8%)

Table 1. Number of surveyed wetlands included in statistical model creation for all three years, including the number of wetlands occupied (percentage) by each species.

Table 2. Number of surveyed wetlands included in the creation of extinction models, and the number of wetlands where extinction events occurred.

Species	# wetlands used for modeling	# of extinctions
Rana pipiens	39	17
Ambystoma tigrinum	19	8
Hyla versicolor/chrysoscelis	10	7



Figure 4. Distribution of study wetlands across the Sheyenne National Grassland of southeastern North Dakota, and the locations of all paved roads (black), gravel roads (brown), and the Sheyenne River (thick blue line) relative to the surveyed wetlands.

never refilled so additional wetlands were surveyed in subsequent years. Of the 84 wetlands in 2003, only 29 were surveyed in 2002 and 69 of the 95 wetlands in 2004 were surveyed in 2003, but only 24 wetlands persisted all three years.

## Amphibian Surveys

Occupancy surveys were conducted for amphibians in wetlands from 15 July to 25 July during 2002-2004. A wetland was considered occupied if it contained breeding adults or egg masses during respective breeding seasons, or if tadpoles or metamorphs were present during summer months. Occupancy of a wetland was also recorded when amphibian larvae were encountered during other surveys (e.g. - water quality and vegetation surveys). Recruitment was defined as successful reproduction and subsequent metamorphosis into the terrestrial environment. Because few occupied wetlands did not yield recruitment and nearly all ponds with breeding adults produced recruits, differences between occupancy and recruitment were minimal and all analyses used occupancy data. Chorus surveys were not used in determining occupancy because several species were found "calling" in depressions without standing water and was not suitable habitat for reproduction. Inclusion of "calling" data of this manner would overestimate occupancy rates and cause inaccurate results in the statistical models.

Surveys in 2002 and 2003 were conducted by using a dipnet to haphazardly search the littoral zone of the wetland for 10 minutes, and then visually searching the surrounding riparian area for 10 minutes. Stock ponds surveyed in this study were typically deep, had steep banks, and little littoral zone, making visual detection and dipnetting difficult. During occupancy surveys in 2004, the same survey method was used to search shallow and highly vegetated wetlands, but a 5 meter standard minnow

seine (76 mm mesh size, 16 kg capacity) was used to survey stock ponds. Surveys with the seine were effective when wetlands did not contain vegetation or significant debris, and was assumed useful in detecting amphibian larvae of any size, because small macroinvertebrates such as water boatmen (*Corixidae* spp.) and backswimmers (*Notonectidae* spp.) were commonly encountered. A detection rating ranging from one (very poor) to three (very good) was recorded during occupancy surveys in 2003 and 2004, and considered impacts of turbidity, depth, and vegetation on detection ability.

Although populations of wood frogs (*Rana sylvatica*), Canadian toads (*Bufo hemiophrys*), and chorus frogs (*Pseudacris triseriata*) were encountered, these species were not modeled because of a potential underestimate in occupancy resulting from conducting surveys at the end of their breeding phenologies. Because of time and labor constraints associated with additional data collection, occupancy surveys were conducted once, and during a time when these species were encountered as metamorphs (personal observation). Because they were only encountered as metamorphs it was possible that additional wetlands contained these species, but because of breeding plasticity were considered unoccupied because metamorphosis and dispersal may have occurred before the survey (false zero). Including false zeroes creates errors in metapopulation modeling by overestimating migration (MacKenzie et al. 2002, Moilanen 2002). False zeroes were not a problem for northern leopard frogs, gray tree frogs, and tiger salamanders (in 2004) because surveys routinely yielded tadpoles of these species.

Because the seine was more efficient than dipnetting when it could be used, and because the seine gave several wetlands a status of occupied by tiger salamanders that had not previously been occupied. Therefore it was possible that 2002 and 2003 tiger

salamander occupancy data include false zeroes, but 2004 data are believed to be free of this sampling problem. Because tiger salamander larvae are known predators of anuran larvae, salamander presence was used as a predictor variable in other species models. Species of fish encountered during 2004 occupancy surveys include yellow bullhead (*Ameiurus natalis*), largemouth bass (*Micropterus salmoides*), common carp (*Cyprinus carpio*), bigmouth buffalo (*Ictiobus cyprinellus*), white sucker (*Catostomus commersoni*), bluegill (*Lepomis macrochirus*), stickleback (*Culaea inconstans*), fathead minnow (*Pimephales promelas*), and dace (Cyprinidae spp.). Because fish are predators on amphibian populations, presence of fish was used as a predictor variable in 2004 models.

## Spatial Data Variables

Spatial autocorrelation (wetlands having similar characteristics because of close proximity) was not accounted for because the SNG is relatively small (283 km<sup>2</sup>) and is semi-contiguous, so weather conditions and other environmental factors were thought to impact all wetlands similarly. Wetlands were delineated by walking the wetland perimeter with a Trimble GeoExplorer 3 GPS Unit that recorded a waypoint every five seconds in the UTM coordinate system and WGS 84 datum. Wetted perimeter was not used in wetland delineation because water-levels fluctuate greatly within a given year across the SNG, but wetland perimeter remains constant. Wetland polygons were downloaded and differentially corrected using the Clay County Base Station (Moorehead, MN) to reduce systematic error induced by the atmosphere (Kennedy 1996). Polygons were manually edited in ArcMap 8.1 (ESRI 2002) to remove additional errant points. Wetland shapefiles were created for all years and the "calculate area" visual basic script was used to ascertain the surface area of all wetlands for use as a predictor variable.

High resolution aerial photos (www.maptech.com) were used to manually digitize and create shapefiles for all paved roads, gravel roads, and the Sheyenne River using ArcView 3.2 (ESRI 2000). An ArcScript by Jenness (2004) for ArcView 3.2 was used to estimate the distance (m) from the three aforementioned features to all surveyed wetlands for use as predictor variables. Aerial photos were also used to digitize wetlands located on private property, so that the area of these wetlands could be obtained and used in estimating wetland density. Distance from wetlands to the nearest occupied (same species) wetland and the nearest persistent pond were estimated using ArcMap 8.1.

Two different measures of wetland density were estimated for each wetland. Non-surveyed wetlands located on private property were included in wetland density estimates, only if they were known to contain water. All wetlands found during subsequent yearly surveys were included in wetland density estimates for the previous years (assumed to be present based on its subsequent presence). A 1500 meter buffer was constructed around all wetlands using the Buffer Wizard in ArcMap 8.1, and the areas of constructed buffers were calculated with a visual basic script. The first measurement of wetland density was estimated by counting the number of wetlands within the buffer, but the second wetland density measurement estimated the percentage of the buffer composed of wetlands, and was estimated according to the following equation:

(sum of all wetland areas within the buffer / buffer area) \* 100 The minimum number of occupied wetlands (same species) within the buffer was also recorded; but was considered a minimum estimate because not all wetlands within buffer areas were surveyed. This estimate was also not independent of the first wetland density estimate, so correlations of this variable with the wetland density variable were examined

for collinearity before inclusion of either variable in final models. Spatial data were collected for all wetlands in all three years.

## Wetland Habitat and Surrounding Land Use Variables

Wetlands were searched for and identified in mid-April of all years, and a record was kept on whether a pond existed at the beginning of each subsequent year. Electrical conductivity and pH readings were collected between 23-27 June (2003 and 2004) from wetlands using a YSI 63 Water Quality Meter. This time of year corresponds to the larval stage of all modeled amphibians, but is also when water-levels begin to drop during dry years on the SNG (personal observation). Wetlands were also visited in early October (when most ephemeral wetlands were gone) to record which wetlands persisted throughout the year so that wetland hydroperiod could be included in analyses.

Data on wetland vegetation characteristics were collected throughout the month of June in 2003 and 2004. Submergent vegetation data for 2003 were collected using a technique modified from Yin et al. (2000), in which a standard (one-sided) garden rake was used to collect four samples  $(1.4 \times 0.35 \text{ m})$  of submergent vegetation from the littoral zone of each wetland. Voucher specimens were collected and identified to species for all encountered vegetation, and are stored in the University of North Dakota Biology Department. Values of emergent vegetation cover (two perpendicular wetland transects) and the amount of bare soil in the riparian zone (two 50 meter transects running away from the pond) were estimated using a 100 meter measuring tape and averaging the values. Because vegetation sampling was time consuming and labor intensive, vegetation data in 2004 were collected as categorical data, and 2003 vegetation data were converted to categorica! data (absence of vegetation = 0, presence of vegetation = 1). Presence and

absence data of submergent vegetation in 2004 was still collected using the rake method of 2003, but no continuous estimates on abundance and diversity were recorded.

Because all categorical vegetation variables (SUBMERG, EMERG, and SHORE) were highly correlated, they were reduced by Principle Component Analysis (PCA) into two variables that accounted for 92% (2003) and 90% (2004) of the variation between these variables. VEGFACTOR1 eigenvectors for 2003 (SUBMERG = -0.756, EMERG = -0.924, SHORE = -0.760) and 2004 (SUBMERG = -0.729, EMERG = -0.862, SHORE = -0.889) were similar. However, VEGFACTOR2 eigenvectors were different for 2003 (SUBMERG = 0.613, EMERG = -0.003, SHORE = -0.606) and 2004 (SUBMERG = -0.682, EMERG = 0.350, SHORE = 0.220). PCA loadings from the vegetation variables were used as continuous predictor variables in the modeling process.

Three surrounding landscape variables were collected for every wetland in which data were available, and excluded only privately owned grazed land. Data on the timing, duration, and the number of grazers in each pasture was taken from the USDA's Grazing Rotation Schedule (Lisbon, ND Field Office). Number of grazers was not used as a predictor variable because pasture sizes often varied and were unknown. Grazing impacts were tested for by scoring wetlands a "0" if it was first grazed in July or August or not at all, and a "1" if it was first grazed in May or June or throughout the summer. Reasoning behind these scores is that amphibian larvae are exclusively limited to wetlands during May and June, but may be less affected by grazing impacts in July and August when nearing metamorphosis. Effects of forests and croplands were determined by scoring wetlands a "0" if there was not a woodlot/row crop within 25 meters of the wetland, and a "1" if a woodlot/row crop was present within 25 meters of the pond,

despite the composition of the rest of the surrounding landscape. A complete list of all variables used in modeling analyses and their definitions are provided in Table 3.

## Statistical Methods

## Statistical and Extinction Models

Logistic regression and Akaike Information Criterion (AIC) values were used to construct and evaluate statistical occupancy and extinction models for northern leopard frog, gray tree frog, and gray tiger salamander populations across the SNG. The response variable for statistical models was coded according to whether a pond was unoccupied (0) or occupied (1), and these models were constructed in every year for which data allowed. However, the response variable for extinction models was coded according to whether a wetland was occupied in both 2003 and 2004 (0) or the wetland was occupied in 2003 and became unoccupied in 2004 (1). Because at least a portion of all three summers were dry across the SNG (Figure 3), colonization events were rare and were therefore not modeled. Whether a wetland persisted throughout the season was not used as a predictor variable in extinction models because several wetlands went extinct because of not refilling the following year, and therefore received a "0" even though they were incapable of being occupied. It was believed that this lack of differentiation between true temporary ponds and ponds that never existed could hide any importance of temporary wetlands, so these situations were eliminated from final extinction models (Table 2).

Variables were selected for final model inclusion following the variable selection method described in Hosmer and Lemeshow (2000). Variable reduction began with using univariate regression on continuous variables and contingency tables on categorical variables to remove variables with a p-value greater than 0.25. Secondly, any variables

Variable Classification	Variable Name	Description
Wetland Hydroperiod	WETAREA	area of the entire wetland
	WEIPERS	whether wetland persisted the entire season
Wetland Isolation	DISRIVER	distance from wetland to Sheyenne River
	WETDENS1	# of wetlands within 1500m of wetland
	MINOCODENS	% of area within 1500m that is welland # occupied ponds within 1500m of wetland
	DISTOCC	distance (m) to nearest occupied wetland
	DISTPERS	distance (m) to nearest persistent wetland
	PAVEDRD	distance (m) to nearest paved road
	GRAVELRD	distance (m) to nearest gravel road
Local Patch Quality	VEGFACTOR1	PCA of vegetation components of wetland
	VEGFACTOR2	PCA of vegetation components of wetland
	pH	acidity/alkalinity of the wetland
	COND	concentration of ions within wetland
	AMBYSTOMA	presence of salamander larvae in wetland
Landscape Complementation	GRAZING	timing and duration on surrounding land
	WOODS	nearby or adjacent woodlot next to wetland
	CROPLAND	nearby or adjacent cropland next to wetland

Table 3. Hierarchical variable classification, abbreviated name, and variable description.

containing a zero cell in the contingency table were either collapsed to remove the zero or were eliminated, because using these variables with logistic regression causes undesirable numerical outcomes (Hosmer and Lemeshow 2000). All variables meeting these criteria were used as candidates for final model inclusion, but variables eliminated because of a zero cell that were considered important based their direction were noted for later discussion. Scatterplots of candidate variables were analyzed to determine the direction of their associations, and to eliminate variables whose associations were not considered biologically meaningful.

Models were constructed with Statistica 6 software (StatSoft, Inc. 2001), using the "Best Subsets" and "AIC" functions of the LOGIT model in order to construct the three best fit models based on candidate variables. Best Subsets model selection identifies a number of "best" models containing any number of variables up to the single model containing all variables, and evaluates all models compared to the model containing all variables using a likelihood ratio test (Hosmer and Lemeshow 2000). Best Subsets logistic regression is a useful tool for determining best fitted models, but its weaknesses include an inability to handle large numbers of variables and the ability to discriminate against variables containing large standard errors of point estimates (Hosmer and Lemeshow 2000). Models were constructed with remaining variables and respective AIC values were calculated, where smaller AIC values correspond to better explanatory models. However, every variable in the best fit models was further scrutinized by examining the point estimate and standard error for any signs of numerical problems. Any variable with an inflated standard error was removed from final models, but any observed importance of these variables was noted for later discussion. Also, any interactions between candidate variables that improved the explanatory power of models were also scrutinized before final inclusior

After variable reduction and selection was complete, the three best statistical models were compared with each other and four other models that only used variables relating to specific aspects of habitat composition and configuration (see Table 3). Additional models included a wetland hydroperiod model, a wetland isolation model, a 'ocal patch quality model, and a landscape complementation model. Any variable not considered for inclusion in final models because of a zero cell or a non-meaningful association was also excluded from the other models to eliminate any bias in results. A statistical model was not created for tree frogs in 2002 because no tree frogs were encountered. Tiger salamander occupancy was only modeled for 2004 data, because data

from 2002 and 2003 most likely contain false zeroes and differed greatly from the model developed in 2004 (without false zeroes).

## Incidence Function Models

Estimates of all five parameters used in the incidence function model were determined for all three amphibian species using the program SPOMSIM V1.0b (Atte Moilanen 2004, based on Moilanen 1999). Parameters estimated with this program are  $\alpha$ (dispersal parameter), b (connectivity function), y (colonization probability), and u and x (extinction probability). Graphical relationships of how estimated parameters influence the equations predicting colonization and extinction rates were explored using MathCad version 11 (Mathsoft 2002). The dispersal function parameter  $\alpha$  controls the shape of the relationship between dispersal and distance, where larger  $\alpha$  values correspond to lower dispersal rates (Figure 5). Connectivity (b using SPOMSIM or S using Etienne et al. 2004 notation) is a function of the number of patches around a given patch, the distance to each patch, the dispersal function, and the area of each patch which determines the potential contribution of colonists for that patch. The IFM colonization function (C)gives a saturating rate of colonization with increasing connectivity, where the parameter y controls the rate at which colonization increases with increasing connectivity. Meaning if connectivity is constant a larger y means slower colonization saturation (Figure 6). The IFM extinction function (E) is a simple function of wetland area, where parameter xcontrols the rate at which extinction declines with increasing area, and the parameter u(e,using Etienne et al. 2004 notation) is related to the extinction rate in the smallest patch that is capable of supporting a population. Therefore, larger values of x give faster drops in extinction rate with increasing area (Figure 7).



Figure 5. MathCad output showing the relationship of  $\alpha$  to the IFM dispersal function, when  $\alpha = 0.001$  (solid line) and  $\alpha = 0.002$  (dashed line).



Figure 6. MathCad ouput showing the relationship of y to the IFM colonization function, when y = 50 (solid line) and y = 100 (dashed line).



Figure 7. MathCad output showing the relationship of e (u using SPOMSIM notation) and x in the IFM extinction function, when x = 0.1 (solid line) and x = 0.2 (dashed line) and e = 0.5 is constant. If e were 0.6 then the curves would terminate at 0.6 before reaching 1 at smaller areas.

Data for the estimation process were based on occupancy data from 2003 and 2004 (including occupancy data of 2002 ponds that did not exist in 2003, but does not include new ponds in 2004 whose previous occupancy was unknown) for leopard frogs and tree frogs. However, only 2004 occupancy data of tiger salamanders were used in the estimation process because this was the only year considered free of false zeroes. Occupancy data, wetland UTM coordinates, wetland area, and whether the pond was temporary or permanent were formatted for use in SPOMSIM, and each species was loaded as a separate network.

Initially, the only manually adjusted parameter was  $\alpha$ , relating to the dispersal function for each species. Values of  $\alpha$  were roughly based on distances provided in Dodd (1996) for northern leopard frogs (1500 m) and tiger salamanders (160 m), but were slightly overestimated because of the contiguity of the SNG. An estimated  $\alpha$  for the tree frog populations was based on Vos et al. (2000), who did similar modeling of a tree frog metapopulation in Europe, and provided a distance in which the tree frog metapopulation was considered to remain connected. MathCad was used to estimate a value of  $\alpha$  that produced a dispersal curve corresponding to what is known about the dispersal capability of each organism. An initial  $\alpha$  value of 0.001 was used for leopard frogs, 0.005 for tiger salamanders, and 0.0015 for tree frogs. These values have dispersal curves with 20% of leopard frogs dispersing ~2500 m (longest is 5500 m), 20% of tiger salamanders dispersing ~1500 m (longest is 1000 m), and 20% of tree frogs dispersing ~1500 m (longest is 4000 m). These values of  $\alpha$  were defined in the network as an initial estimate, but  $\alpha$  was still estimated with the other parameters based on observed occupancy patterns.

Parameter estimates were first ascertained without regional stochasticity being incorporated, and using the initial estimate of  $\alpha$  in the "Original Incidence Function Model" (OIFM) based on Hanski (1994). All estimates were determined using the OIFM, regardless of whether regional stochasticity was included in the estimation process. Parameters were first estimated with two preliminary runs (no false zeroes, normal initialization range, and a 2x effort level) using the non-linear regression (NLR) estimation method described in Hanski (1994). The two NLR runs are used to better approximate parameter estimates before the final two runs (same settings) with the better suited Monte Carlo (MC) estimation method developed b ' Moilanen (1999). Parameter estimates at this point were considered best approximations but are still estimates because of stochasticity in the estimation process itself, so values presented here may be slightly different if estimated again. Best estimates were then used to simulate population persistence of the observed populations using 100 replications of the first 100 years.

A second set of parameter estimates was calculated using the same method and settings, only two different levels of regional stochasticity were incorporated into the estimation process. Regional stochasticity (e.g. – probability of a regional drought) values were not altered from their original 0.2 level (stochastic event every 5 years, on average), but synchronous regional stochasticity (impacting the entire SNG) and synchronous stochasticity within patch class type (impacting temporary and permanent wetlands differently) were both incorporated. Estimates derived from these conditions may be more reflective of the conditions observed in this study because many temporary ponds went extinct and did not refill for at least two years, which most likely affects population dynamics if these ponds are required for regional persistence. Parameter estimates with regional stochasticity incorporated were also simulated with the same settings to approximate regional population persistence.

Because of an unknown estimation error encountered while estimating tiger salamander parameters, the MC method could not be utilized so estimates and simulations were based on NLR estimation. Although leopard frog and tiger salamander occupancy levels were both 40% in 2004, tree frogs were only found in 8% of wetlands which is below the 20% level recommended by Hanski (1994) for this type of modeling. Despite this shortcoming tree frog population parameters were still estimated and simulated, because all species modeled in this study violate the equilibrium assumption,

where a large number of extinction events were observed and only a few colonization events. Therefore, estimates generated in this analysis may not represent the system during average weather conditions, but are representative of this system during extended dry periods.

#### Results

# Statistical and Extinction Models

Results of the best statistical models for northern leopard frog populations and the best variable-specific models (e.g. – model containing only wetland isolation variables) are presented in Table 4. Variables most often included in leopard frog statistical models were related to wetland isolation and local patch quality, most notably the wetland vegetation characteristics. However, wetland area and landscape composition were both important in 2003 models (Table 4). Data on patch quality were not collected in 2002, and all variables in the final model were related to wetland isolation and proximity of neighboring populations. No landscape variables were included in 2002 leopard frog models, but surrounding cropland was excluded because of a zero cell caused by all five wetlands having adjacent agriculture being occupied. Wetland isolation became less important in 2003, though isolation variables were readily abundant in final models. Wetland area and wetland vegetation characteristics were the most important factors influencing leopard frog occupancy in 2003, but the association of leopard frog occupancy with larger wetlands was the only significant variable. Leopard frogs were more likely to be found in wetlands containing aquatic and riparian vegetation in 2003. Wetland isolation variables included in 2003 were similar to 2002, except for the increased importance of occupancy with distance from the nearest persistent pond.

Rana pipiens Statistical Model - 2002				
Model	AIC			
Statistical Models				
(1) MINOCCDENS (+), WETDENS2 (+), DISRIVER (-)	3	52.73833		
(2) MINOCCDENS (+), WETDENS2 (+)	2	54.10266		
(3) MINOCCDENS (+), WETDENS2 (+), DISRIVER (-),				
DISTPERS (-)	4	54.50694		
Wetland Hydroperiod				
WETAREA (+)	1	79.13782		
Wetland Isolation				
MINOCCDENS (+), WETDENS2 (+), DISRIVER (-)	3	52.73833		
Landscape Complementation				
GRAZING (-)	1	78.61121		
Rana pipiens Statistical Model - 2003				
Model	df	AIC		
Statistical Models				
(1) WETAREA (+), WETDENS2 (+), MINOCCODENS (+),				
DISTPERS (-), WOODS (+), VEGFACTOR1 (-)	6	29.36145		
(2) WETAREA (+), WETDENS2 (+), MINOCCDENS (+)		20.01501		
DISRIVER (-), DISTPERS (-), WOODS (+), VEGFACTORI (-)	/	30.91501		
(3) WETAREA (+), WETDENS2 (+), MINOCCODENS (+),	7	31 00473		
ph (-), DISTPERS (-), WOODS (+), VEGFACTORT (-)		51.00475		
WETADEA (+)	1	83 0904		
Wetland Isolation		05.0704		
DISTOCC (-) WETDENS2 (+) DISRIVER (-)	3	78,4707		
Local Patch Quality				
VEGEACTOR1 (-)	1	63.98518		
Landscape Complementation				
GRAZING (-), WOODS (+), GRAZING*WOODS	2	86.7385		
Rana pipiens Statistical Model - 2004				
Model df		AIC		
Statistical Models				
(1) WETAREA (+), DISTPERS (-), VEGFACTOR1 (-),				
DISTOCC (-), PAVEDRD (+)	5	70.20423		
(2) WETAREA (+), DISTPERS (-), VEGFACTOR1 (-),				
DISTOCC (-), PAVEDRD (+), MINOCCDENS (+)	6	70.85022		
(3) WETAREA (+), DISTPERS (-), VEGFACTOR1 (-),				
DISTOCC (-), PAVEDRD (+), VEGFACTOR2 (+)	6	70.87936		
Wetland Hydroperiod				
WETAREA (+)	1	116.0319		
Wetland Isolation		02 2024		
DISTOCC (-), MINOCCDENS (+), DISTPERS (-), DISRIVER (-)	4	93./0/6		
Local Patch Quality		07 4740		
VEGFACTOR1 (-), VEGFACTOR2 (+), pH (-), AMBYSTOMA (-)	4	87.4762		
Landscape Complementation	2	100 474		
GRAZING (-), WOODS (+), UKUPLAND (+)	13	100.474		

Table 4. Statistical models, variable associations, and AIC values for northern leopard frog populations on the SNG, 2002-2004. (significant variables are in bold)

All the best statistical models constructed in 2003 included a positive influence of nearby forests on leopard frog occupancy. Although similar in composition to 2003 models, the importance of several variables diminished in 2004 while others became more explanatory and significant (Table 4). Presence of wetland vegetation was the most significant predictor of leopard frog occupancy, and was followed in importance and significance by distance to the nearest persistent wetland. Wetland area was less important and was no longer significant, but leopard frog occupancy was still associated with larger ponds and being in closer proximity to occupied wetlands. Occupied wetlands in 2004 were also associated with greater distances from paved roads.

Although no variable included in leopard frog extinction models was significant, wetland vegetation was the most explanatory variable in predicting whether a wetland persisted or went extinct between 2003 and 2004 (Table 5). Ponds having aquatic and riparian vegetation were more likely to have populations that persisted between years, while ponds without vegetation were more likely to go extinct. Wetland area was negatively associated with extinction events, suggesting that smaller wetlands were more likely to go extinct. Leopard frog extinctions were also more likely to occur closer to paved roads, and one of the final extinction models included a variable suggesting ponds with fewer neighboring populations were more likely to go extinct. These were the only variables in final models relating to wetland isolation despite their abundance in the statistical models, suggesting patch quality and wetland size are most important in determining local extinctions of leopard frogs.

Statistical models for gray tiger salamander populations were only generated in 2004 because of a small sample size in 2002 and the likely presence of false zeroes in

Rana pipiens Extinction Models (2003-2004)		
Model	df	AIC
Extinction Models		
(1) VEGFACTOR1 (+), WETAREA (-), PAVEDRD (-)	3	45.90771
(2) WETAREA (-), PAVEDRD (-)	2	46.77678
(3) WETAREA (-), PAVEDRD (-), VEGFACTOR1 (+)		
MINOCCDENS (-)	4	47.03491
Wetland Hydroperiod		
WETAREA (-)	1	59.24363
Wetland Isolation		
DISRIVER (+), WETDENS1 (-), DISTPERS (-), GRAVELRD (+)	4	49.80263
Local Patch Quality		
VEGFACTOR1 (+), VEGFACTOR2 (-), pH (-)	3	48.68065
Landscape Complementation		
WOODS (-)	1	56.26438

Table 5. Extinction models and AIC values of leopard frog populations on the SNG.

Table 6. Statistical models, variable associations, and AIC values of *Amoystoma tigrinum* populations on the SNG, 2004. (significant variables in bold)

Ambystoma tigrinum Statistical Models - 2004		
Model	df	AIC
Statistical Models		
(1) DISRIVER (+), VEGFACTOR1 (+), DISTOCC (-), GRAZING (+)	4	89.0693
(2) DISRIVER (+), VEGFACTOR1 (+), GRAZING (+)	3	90.6691
(3) DISRIVER (+), DISTOCC (-), VEGFACTOR1 (+)	3	90.8228
Wetland Hydroperiod		
WETPERS (+), WETAREA (-)	2	116.1505
Wetland Isolation		
DISRIVER (+), DISTOCC (-), DISTPERS (+)	3	106.475
Local Patch Quality		
VEGFACTOR1 (+), FISH (-), pH (+), CONDUCTIVITY (-)	4	97.9645
Landscape Complementation		
WOODS (-), GRAZING (+), CROPLAND (-)	3	94.6336

Table 7. Extinction models and AIC values of tiger salamander populations on the SNG.

Ambystoma tigrinum Extinction Models (2003-2004	)	
Model	df	AIC
Extinction Models		
(1) VEGFACTOR1 (-)	1	19.1875
(2) WETAREA (-), VEGFACTOR1 (-)	2	19.56221
(3) VEGFACTOR1 (-), GRAZING (-)	2	19.86249
Wetland Hydroperiod		
WETAREA (-)	1	32.22177
Wetland Isolation		
WETDENS2 (+)	1	24.16713
Local Patch Quality		
VEGFACTOR1 (-)	1	19.1875
Landscape Complementation		
GRAZING (-), WOODS (-), GRAZING*WOODS	1	27.98131

2003. Patch quality and wetland isolation were significant predictors of tiger salamander occupancy (Table 6), but in contrast to leopard frog populations the absence of wetland vegetation was significantly associated with occupancy. Tiger salamanders were often found in wetlands containing no vegetation, exploiting stock ponds (27/33 occupied) which were often devoid of vegetation. Provimity to the Sheyenne River was also a significant predictor of tiger salamander occupancy, with wetlands more distant from the river being occupied more often. This result may reflect habitat preference rather than wetland isolation because ponds in close proximity to the river are often surrounded by forest, and ponds further from the river are embedded in prairie and have a greater abundance of stock ponds. Another isolation variable important for tiger salamanders was increased occupancy probability for ponds in closer proximity to other occupied ponds. One landscape feature was also associated with the best models, where ponds first grazed in May of June or all season long were occupied by tiger salamanders more often than ponds first grazed in July or August or not at all. It remains unclear how grazing increases occupancy of tiger salamanders, but its inclusion in final models may be linked to its impact on wetland vegetation.

None of the variables included in the gray tiger salamander extinction models were significant, but patch quality was the most explanatory variable for predicting persistence and extinctions of tiger salamanders (Table 7). Absence of vegetation was associated with persistent salamander populations, and vegetated wetlands were more likely to go extinct. Like leopard frog populations, tiger salamander extinctions were more likely to occur in smaller wetlands. Although stock ponds are not very large (average size is 1300 m<sup>2</sup>), they may harbor large enough populations to offset stochastic

extinctions associated with small population sizes. Wetlands not grazed in May or June were also more likely to go extinct, which may again be linked to wetland vegetation. Tiger salamander extinction models contained no wetland isolation variables, despite half the variables linked to occupancy being associated with wetland isolation.

Wetland isolation and patch quality variables, again, were most important for predicting gray tree frog occupancy, with two different wetland isolation variables being significant and most important in different years (Table 8). Although both variables were present in models in both years, gray tree frogs occupied wetlands with greater distances from persistent pends more often in 2003 and wetlands further from paved roads in 2004. Because ponds further from persistent ponds were more often occupied, one might suggest that gray tree frogs may prefer temporary ponds. The significant effect of paved roads on tree frog occupancy may be related to habitat preference and not roads, because tree frog populations were also associated with wetlands having an adjacent forest and are more abundant near the Shevenne River. Few paved roads go near the river (Figure 4), so resulting distances are large and may not be due to roads necessarily. A whole suite of patch quality variables appeared in final statistical models for both years but none were significant. Among the most important patch quality variables were presence of aquatic vegetation and pH. Ponds having aquatic and riparian vegetation and lower pH values (< 8.25) were more frequently occupied by gray tree frogs. In 2004 models, a negative association was found between tree frog occupancy and wetland persistence, and also included an interaction term with wetland persistence and tiger salamander larvae. This suggests that gray tree frogs may avoid persistent ponds because of increased predation pressure or are eaten out of ponds without vegetation (Table 8). Though not

Hyla versicolor/chrysoscelis Statistical Models - 2003				
Model	df	AIC		
Statistical Models				
(1) DISTPERS (+), VEGFACTOR1 (-), VEGFACTOR2 (+),				
pH (-), WOODS (+)	5	31.78712		
(2) DISTPERS (+), VEGFACTOR1 (-), VEGFACTOR2 (+),				
pH (-), WOODS (+), PAVEDRD (+)	6	32.01763		
(3) <b>DISTPERS (+)</b> , VEGFACTOR1 (-), VEGFACTOR2 (+),				
pH (-), WOODS (+), DISTOCC (-)	6	32.27074		
Wetland Hydroperiod				
WETPERS (-)	1	69.99401		
Wetland Isolation				
DISRIVER (-), WETDENS2 (-), PAVEDRD (+)	3	62.97604		
Local Patch Quality				
VEGFACTOR1 (-), VEGFACTOR2 (+), AMBYSTOMA (-)	2	34.39137		
Landscape Complementation	1			
WOODS (+), GRAZING*WOODS	2	53.15368		
Hyla versicolor/chrysoscelis Statistical Models - 2004				
Model	df	AIC		
Statistical Models				
(1) PAVEDRD (+), DISTPERS (+), VEGFACTOR1 (-), pH (-)	4	41.63704		
(2) PAVEDRD (+), DISTPERS (+), VEGFACTOR1 (-)				
WETPERS (-), WETPERS*AMBYSTOMA (-)	5	41.74296		
(3) PAVEDRD (+), DISTPERS (+), VEGFACTOR1 (-)	3	42.05498		
Wetland Hydroperiod				
WETPERS (-)	1	55.87216		
Wetland Isolation				
DISTOCC (-), MINOCCDENS (-), PAVEDRD (+), DISRIVER (-)	4	39.36125		
Local Patch Quality				
VEGFACTOR1 (-), VEGFACTOR2 (+)	2	48.10468		
Landscape Complementation				
GRAZING (-)	1	58.22279		

Table 8. Statistical models, variable associations, and AIC values for gray tree frog population on the SNG, 2003-2004. (significant terms in bold)

Table 9. Extinction models and AIC values for gray tree frog populations on the SNG.

Hyla versicolor/chrysoscelis Extinction Models (2003-2004)		
Model		AIC
Extinction Models		
(1) VEGFACTOR1 (+)	1	13.32186
(2) VEGFACTOR1 (+), PAVEDRD (-)	2	15.24547
(3) PAVEDRD (-)	1	17.21173
Wetland Hydroperiod		
WETAREA (-)	1	20.19741
Wetland Isolation		
DISTOCC (-), PAVEDRD (-)	2	15.00389
Local Patch Quality		
VEGFACTOR1 (+), AMBYSTOMA, AMBYSTOMA*FISH	1	7.81909
Landscape Complementation		
GRAZING (+)	1	20.29083

significant in 2003 models, presence of a neighboring forest increased occupancy chances for gray tree frogs and was included in all models. However, the neighboring forest variable was excluded from 2004 models because of a zero cell caused by all occupied wetlands having a forest in close proximity. Therefore, this variable is still considered important and meaningful because of its inclusion in 2003 models, the direction of its association with occupancy in 2004, and because trees are foraging and overwintering habitat for gray tree frogs.

Though not significant and based on a small sample size of wetlands (Table 2), the most important variable for explaining tree frog persistence and extinctions was the patch quality variable relating to wetland vegetation (Table 9). Wetlands losing aquatic vegetation between years were more likely to go extinct, but continuously vegetated wetlands were more likely to have persistent tree frog populations between years. Ponds closer to paved roads were also more likely to go extinct, but must be treated with caution because few wetlands containing tree frogs were near paved roads. Although presence of fish and salamander larvae explain nearly half the extinctions and provided a lower AIC score than the best models, inclusion of these variables caused inflated standard errors and unfavorable numerical results and were therefore excluded from final models.

## Incidence Function Models

Final parameter estimates of leopard frog populations without stochasticity using MC estimation were  $\alpha = 0.00007$ , b = 0.0797, y = 74.563,  $\mu = 5.2086$ , and x = 0.2956. Estimated values varied slightly from NLR estimates, but since the MC method provides better estimates some deviance was expected. The modest colonization rate (determined through estimating  $\alpha$ , *b*, and *y*) suggests that during dry periods when colonization events

were observed to be minimal, leopard frogs still move across the SNG and are capable of traversing long distances. Extinction rate is estimated in relation to wetland area, where larger values of x correspond to faster declines in extinction rate with increasing area. The value of x estimated for leopard frog populations without stochasticity is less than the estimate with stochasticity, suggesting that the local extinction probability for leopard frog populations is higher without regional stochasticity incorporated into the estimation process. Simulated results using these estimates on current leopard frog populations (without regional stochasticity) yielded persistence of the metapopulation for the next 100 years on average (Figure 8), but the number of surviving replicates diminishes over time (Figure 9). When regional stochasticity measures were incorporated into the estimation process, estimates of all values changed despite giving similar results to the simulation without stochasticity. When regional stochasticity was incorporated into the model  $\alpha = 0.000024$ , b = 0.000, y = 58.926,  $\mu = 76.802$ , and x = 0.6517. Inclusion of regional stochasticity in parameter estimation increased the colonization rate (smaller y) of leopard frogs and decreased the extinction rate (larger x) across the SNG. Simulation results of leopard frog populations with regional stochasticity incorporated were similar to those not incorporating stochasticity (Figure 8), but stochastic replicates were more likely to survive (Figure 9). Although observed colonization events of leopard frog populations were few during this study, both parameter estimates suggest that leopard frog populations may persist across the SNG during dry years because they are able to occupy larger wetlands that have smaller extinction rates.

Parameter estimates of tiger salamander populations were estimated with the NLR method only, and when regional stochasticity was not incorporated  $\alpha = 0.00004$ ,



Figure 8. Simulations of estimated parameter results for leopard frog populations across the SNG with no regional stochasticity incorporated (left) and when regional stochasticity was incorporated (right). The blue line is the average of all replications.



Figure 9. Proportion of surviving replicates for leopard frog population simulations (with 100 replications) when regional stochasticity not incorporated (left) and when regional stochasticity was incorporated (right).

b = 0.000, y = 224.102,  $\mu = 0.0194$ , and x = 0.000. This value of  $\alpha$  probably led to the modest colonization rate (yet lower than Lopard frogs) for gray tiger salamanders, because they are known to have poor dispersal abilities and exhibit small home ranges (Kleeberger and Werner 1983, Dodd 1996). The low value of x suggests that extinction rates are relatively high, but appear to be offset by colonization because simulated results of with these estimates yielded regional persistence for every replicate (Figure 10). When regional stochasticity was incorporated into the estimation process for tiger salamander populations  $\alpha = 0.000096$ , b = 0.000, y = 138.874,  $\mu = 0.5677$ , and x = 0.43. Inclusion of regional stochasticity increased the colonization rate (smaller y) for gray tiger salamanders across the SNG. Local extinction rates were also lowered (larger x) with the inclusion of regional stochasticity, suggesting that gray tiger salamanders are able to persist through extended dry periods because they are able to colonize and occupy permanent water bodies that are less likely to go extinct. Simulated results of tiger salamander estimates with regional stochasticity included are presented in Figure 10, and are similar to those without stochasticity where all 100 replicates survive all 100 years.

Parameter estimates for gray tree frog populations without regional stochasticity and using MC estimation were  $\alpha = 0.000007$ , b = 0.0301, y = 855.609,  $\mu = 0.2381$ , and x = 0.000. This  $\alpha$  value may reflect the large inter-patch distances observed between occupied ponds, and may also be responsible for the small connectivity estimate. The colonization rate determined with estimated parameters is small and suggests little colonization across the SNG. Extinction rates were high (small *x*) as wetland area increased, and when combined with the low colonization rate caused the regional extinction of gray tree frog populations when simulated with these estimates. Simulation Proportion of occupied patches

Proportion of occupied patches



Figure 10. Simulations of estimated parameter results for tiger salamander populations across the SNG with no regional stochasticity incorporated (left) and when regional stochasticity was incorporated. The blue line is the average of all replications.



Figure 11. Simulations of estimated parameter results for gray tree frog populations across the SNG with no regional stochasticity incorporated (left) and when regional stochasticity was incorporated. The blue line is the average of all replications.

results with these estimates and no regional stochasticity resulted in regional extinction in approximately 10 years for all replicates (Figure 11). When regional stochasticity is incorporated into the estimation process for tree frog populations  $\alpha = 0.004$ , b = 0.152, y = 65.277,  $\mu$  = 1.486, and x = 0.2662. Inclusion of regional stochasticity gave an  $\alpha$  value corresponding to a dispersal curve with less dispersal than was initially estimated based on the organisms known biology. Inclusion of stochasticity increased the colonization rate by increasing connectivity, and the extinction rate was lowered. However, the increased colonization rate was not sufficient to offset extinctions, and simulations using these estimates with stochasticity incorporated also went extinct (Figure 11). Though wetlands were less likely to go extinct with regional stochasticity included, colonization and dispersal rates were insufficient to maintain regional persistence when starting with a small number of occupied ponds that were separated by large distances. Besides the small initial number of occupied ponds, the projected regional extinction of gray tree frog populations seems related to the inability of tree frogs to occupy large wetlands in the absence of smaller ones that disappear during dry years.

## Discussion

Northern leopard frogs were the most abundant and widely distributed amphibian species encountered across the SNG, and their regional persistence seems good (Figure 8) despite the extended dry period experienced during this study (Figure 3). Wetland isolation variables were the most explanatory for predicting leopard frog occupancy when wetlands were abundant, but became less important when ephemeral wetlands became scarce. Ephemeral prairie-pothole ponds are abundant across the SNG in wet years (personal observation), and leopard frogs were observed using them for reproduction in early 2002. When these ponds were present across the SNG, leopard frogs used them and were more likely to occupy them when there were occupied ponds in close proximity. Wetland density and neighboring occupied wetlands were also important in 2003, despite increased isolation of remnant wetlands caused by the removal of ephemeral wetlands. Average wetland percentage in the surrounding landscape for 2002 was 1.75%, declined to 1.47% in 2003, and rebounded to 1.65% in 2004 though wetland density was not important in 2004. However, the rebound experienced in 2004 probably resulted from sampling newly found large ponds instead of numerous small wetlands that contributed to the 2002 estimate. Isolation variables were less important in 2003 and 2004, and were replaced in importance by variables related to wetland hydroperiod and patch quality.

Wetland area was the most significant predictor of leopard frog occupancy in 2003 with larger wetlands being occupied more frequently. This observation agrees with metapopulation theory that predicts smaller patches will go extinct more often and be recolonized less frequently because smaller patches are more prone to extinctions associated with small population sizes (Hanski 1999). Wetland area was also included in 2004 models, though its significance was diminished. In 2003 and 2004 models, ponds closer to persistent ponds were more often occupied by leopard frogs, and this variable was the most important and significant variable in 2004. This suggests that during dry years leopard frogs may occupy breeding ponds based on proximity to overwintering sites, because leopard frogs require a permanent water body for hibernation. Pope et al. (2000) demonstrated the concept of "landscape complementation" for leopard frogs, where leopard frogs selected breeding ponds based on proximity to suitable summer habitat. However, to the best of my knowledge no study has demonstrated an association

between leopard frog occupancy and proximity to overwintering habitat. Although proximity to permanent wetlands increased occupancy probability, permanent wetlands were not always occupied and almost no stock ponds were ever occupied by leopard frogs. However, a visit to wetlands in October of 2003 produced the observation of hundreds of adult leopard frogs in stock ponds, suggesting that leopard frogs were able to successfully overwinter in stock ponds across the SNG.

Patch quality was also important in determining leopard frog occupancy, with the presence of wetland vegetation being among the most explanatory of all variables. Ponds lacking vegetation were less likely to be occupied, since leopard frogs require vegetation for attachment of egg masses. Relationships between amphibian occupancy and diversity with presence of aquatic vegetation have long been acknowledged (Wells 1977, Healey et al. 1997, Hazell et al. 2001). Inclusion of neighboring forest in 2003 models probably resulted from sampling an increased number of wetlands (all occupied) from the Mirror Pool Wildlife Refuge, which is adjacent to the Sheyenne River and is embedded within a contiguous stand of forest. A number of other wetlands in wooded habitat near the Sheyenne River were also occupied in 2003, but a majority of these other wetlands went extinct in 2004. A positive association was also found between leopard frog occupancy and close proximity to agricultural lands, but was more likely related to sampling ponds with longer hydroperiods than agricultural lands aiding with occupancy because negative effects of agriculture have been demonstrated on amphibian occupancy (Joly et al. 2001).

Local extinctions of leopard frogs across the SNG were primarily caused by previously occupied wetlands losing aquatic vegetation components between years, but were also more likely to occur in smaller wetlands. Lack of vegetation may cause local extinctions by removing habitat required for reproduction, or by increased predation resulting from lack of refugia. Metapopulation theory dictates that smaller patches will experience higher extinction rates (Hanski 1999), and appears accurate for leopard frog populations inhabiting the SNG. Paved roads were also important for predicting extinctions of leopard frogs, with populations closer to roads going extinct more often. Negative impacts of roads have been demonstrated on leopard frog populations, especially when roads separate breeding ponds and overwintering sites (Linck 2000).

Results of IFM parameter estimation suggest that leopard frogs are highly vagile and colonize wetlands across the SNG at a rate high enough to offset extinction despite the dry conditions. Extinction rates are relatively high for leopard frog populations inhabiting small wetlands during dry years, but appear to be offset by subsequent colonization. Although wetland isolation was important in explaining occupancy and wetland area was important for explaining local extinctions, patch quality and the surrounding landscape were also important for explaining occupancy and extinctions of leopard frog populations. Therefore, local patch quality measures cannot be ignored for an IFM to be accurate because not all patches are suitable habitat for leopard frogs.

Gray tiger salamanders were also abundant and evenly distributed across the SNG, and their regional persistence also appears good (Figure 10). Variable associations explaining tiger salamander occupancy were surprising, but occupancy was closely linked with wetland isolation and local patch quality variables. Wetlands in close proximity to other occupied wetlands were occupied more often, and wetlands further from the Sheyenne River were also frequently occupied. Gray tiger salamander populations did occupy wetlands near the river, but much less frequently, and their
association with greater distances from the river is probably related to habitat preference. No study has demonstrated negative impacts of neighboring forests on tiger salamander occupancy, but ponds in close proximity of the Sheyenne River were usually embedded in a woodland matrix and were heavily vegetated. Factors associated with wetlands near the Sheyenne River were important because the most important variable influencing gray tiger salamander occupancy was absence of wetland vegetation. Stock ponds have little to no vegetation and were frequently colonized by tiger salamanders, and are more abundant in prairie portions of the SNG and are more distant from the Sheyenne River.

Why salamanders prefer ponds without vegetation and little prey is poorly understood, but salamander larvae inhabiting stock ponds were cannibalistic (personal observation). Occupancy by gray tiger salamanders also increased with early and prolonged grazing, which might be explained either through linkage with the negative effect of grazing on wetland vegetation, or through the creation of a more traversable (shorter grass) matrix for a less vagile species. Inclusion of grazing in the final models may be related to an impact on the surrounding terrestrial habitat because stock ponds usually contain no vegetation from the start. Though not significant, wetlands embedded in an agricultural matrix were occupied less often by salamanders, perhaps resulting from difficulty in traversing unsuitable matrix habitat (Joly et al. 2001). Tiger salamanders also occupied wetlands with extrem *i* y high pH and conductivities. These wetlands were never occupied by other anurans, suggesting that tiger salamanders are very hardy and are more impervious to effects of poor water quality than other amphibians.

Local extinctions of gray tiger salamander populations were best explained by patch quality variables related to wetland vegetation. A possible explanation for

differential persistence of salamanders in ponds varying only in vegetation presence was increased predator evasion in non-vegetated wetlands. Deeper ponds, like stock ponds in this study, have steeper banks and less shallow water zone for predatory birds. Although stock ponds may lack refugia, salamanders occupying deeper ponds may rely on depth and turbidity for predator avoidance, whereas vegetated ponds are often shallow and may be more prone to predation by birds. Salamander populations inhabiting smaller wetlands were more likely to go extinct which is consistent with metapopulation theory (Hanski 1999). Ponds whose pastures were not grazed early in the season were also more likely to go extinct, lending more support to grazing creating a more traversable matrix habitat allowing the less vagile tiger salamander to locate and colonize suitable wetlands.

IFM estimates for tiger salamander populations suggest modest rates of dispersal and colonization for tiger salamander populations across the SNG. Parameter estimates also suggest that extinction rates of local populations are high, but may be offset through recolonization from neighboring populations. This result is given further support by the statistical models results, where ponds in closer proximity to occupied ponds were more likely to be occupied. These results suggest a tiger salamander metapopulation structure characterized by frequent extinction events followed by rapid recolonization. Inclusion of regional stochasticity increased colonization rates of tiger salamanders, but results were similar to those without stochasticity since most observed populations occupied permanent stock ponds and were not impacted by removal of temporary wetlands. Like leopard frog populations, the ability of tiger salamander populations to exploit permanent and temporary wetlands greatly improves the regional persistence probability during dry years. Occupancy of tiger salamanders was associated with wetland isolation variables

and extinctions occurred more often in small wetlands, but patch quality and surrounding landscape variables were again important. Therefore, local conditions affecting tiger salamanders must be better understood, and patch quality variables must be included if using an IFM to estimate salamander extinction and colonization rates across the SNG.

The gray tree frog complex (*Hyla versicolor/chrysoscelis*) was the least abundant and poorly distributed amphibian in this investigation, and their low numbers combined with the observed dry years suggest regional extinction of the metapopulation if more dry years follow (Figure 11). Occupancy of gray tree frogs was closely linked with patch quality, specifically the presence of aquatic vegetation. Wetlands were occupied more frequently if they contained aquatic vegetation, though the importance of riparian vegetation was less critical in 2003. Emergent vegetation provides suitable foraging habitat, while submergent vegetation is important for egg mass attachment. Therefore, absence of aquatic vegetation decreases occupancy probability by eliminating habitat required for reproduction. Gray tree frogs were also the most sensitive species in regard to water quality, because ponds with high pH values (>8.5) were never occupied.

Gray tree frog occupancy was also significantly associated with distance from persistent ponds in 2003 and 2004 with ponds further away from persistent ponds being occupied more often. Additionally, inclusion of the negative association of tree frog occupancy with the wetland persistence/tiger salamander interaction in 2004, suggests that tree frogs may avoid permanent ponds because of the predator communities they harbor. Therefore, it is likely that tree frog populations prefer temporary ponds or permanent ponds embedded in forests which are less frequently occupied by tiger salamanders. Temporary ponds and ponds surrounded by forests are also more likely to

contain aquatic vegetation. Gray tree frogs were found occupying the same wetlands as tiger salamanders, but instances were rare and only occurred in vegetated wetlands. Inclusion of the paved road variable may be associated with the location of suitable tree frog habitat and not related to paved roads necessarily, since tree frog populations were more often found in forested wetlands near the Sheyenne River and not in the habitat near paved roads (Figure 4).

Being near forested area was important in predicting tree frog occupancy in 2003, and although no quantitative measures of woodland extent were incorporated, presence of nearby woodlots was enough to increase occupancy probability. This is informative because it may reflect a similar type of "landscape complementation" exhibited by leopard frog populations across the SNG. Gray tree frogs utilize woods and brush for hibernation during winter and for foraging during summer, so they may select breeding ponds in closer proximity to these other required habitats. However, presence of a neighboring forest was excluded from 2004 models because of a zero cell caused by all wetlands that contained tree frog populations having adjacent woodlots. This finding is biologically meaningful despite its exclusion, and should be considered when creating conservation strategies for tree frog populations across the SNG.

Local extinctions of gray tree frogs were most closely linked with patch quality, specifically wetland vegetation characteristics. Ponds containing populations of tree frogs in 2003 were more often occupied in 2004 if wetland vegetation was also present. When combined with results from the statistical models it appears that gray tree frogs are sensitive to changes in wetland vegetation, and may not be able to occupy wetlands without vegetation. Presence of salamander larvae and fish in wetlands also explained

several extinctions, but were not included in final models because of unfavorable numerical responses. However, it is recommended that conservation plans also consider predation pressures by aquatic predators because gray tree frogs appear to be sensitive.

Results of IFM parameter estimation for gray tree frogs were perhaps less informative than the other species because tree frogs initially occupied few ponds. Unlike leopard frog and tiger salamander extinction models, tree frog extinctions were not associated with wetland area, but instead were related to patch quality. Because wetland area was not a determinant in local extinctions and is how the IFM determines extinction rate, estimated extinction rates may suffer from exclusion of ecologically important factors concerning extinctions. However, parameter estimates for gray tree frogs predict regional extinction in fewer than 20 years, despite the inclusion of regional stochasticity which did extend regional persistence. One factor leading to the regional extinction of gray tree frogs was their inability to occupy permanent wetlands when temporary wetlands become scarce. Leopard frog and tiger salamander populations utilize permanent ponds, but tree frogs may rely on temporary wetlands more than the other species and suffer when they disappear from the landscape. Special care must be taken to conserve gray tree frogs during dry years because their population dynamics appear to be affected in these periods, and may result in regional extinction if dry periods last too long.

Overall, occupancy of amphibians inhabiting the SNG was strongly dictated by wetland isolation and patch quality, whereas local extinctions were linked to wetland size and patch quality. Therefore, variables accounting for wetland hydroperiod, wetland size, wetland isolation, adjoining landscape, and patch quality must all be considered

when modeling amphibian populations. Models exploring occupancy dynamics and local extinction relationships with these variables can be used to develop species-specific conservation strategies, because variables might impact various amphibians differently. IFM parameter estimates can also be used to elucidate factors affecting amphibian metapopulations, but results must be used with caution because patch quality variables have been shown to be important in determining local extinctions of amphibians and are often ignored in parameter estimation. The primary assumption of the IFM concerning equilibrium between extinction and colonization was violated in this study, because the reduced precipitation in 2002 and 2003 caused lots of extinctions and reduced rates of colonization. However, estimates in this study may reflect "true" estimates of these parameters in dry years which is only beneficial for amphibian conservation in dry years, and must not be extended to reflect the dynamics of amphibian populations in wet years.

# CHAPTER 3

# SURVEY OF HELMINTHS INFECTING NORTHERN LEOPARD FROGS (*RANA PIPIENS*) ON THE SHEYENNE NATIONAL GRASSLAND, NORTH DAKOTA

# Introduction

Disappearances of frogs, toads, and salamanders have been reported in areas of North America, Central and South America, Europe, Asia, Africa, and Australia (Blaustein and Wake 1990). Although anthropogenic impacts on habitats required by amphibians have been shown to exhibit negative effects on amphibian populations (Welsh and Ollivier 1999), many population declines have occurred in relatively pristine areas where anthropogenic impacts are minimal (Pounds and Crump 1994; Laurance et al. 1996). Hypotheses for these declines include habitat loss or degradation, introduction of exotic predators and competitors, increased ultraviolet (UV-B) irradiation, acid precipitation, environmental contamination by pollutants, harsh weather conditions, over harvesting, and infectious disease (Daszak et al. 1999).

Microparasitic infections (including Chytridiomycosis, ranaviruses, and even protozoans) have been shown to exhibit profound negative effects on amphibian populations, largely through the extirpation of local populations (Daszak et al 1999; Carey 2000; Green et al. 2002). Macroparasites have been shown to have negative impacts on amphibians by reducing growth and vagility (Goater 1992; Goater et al. 1993), and have even been shown to cause malformities through mechanodisruption of the limb bud during development (Johnson et al. 2002). Negative effects on amphibian populations because of infectious diseases are particularly conspicuous. Although parasitism by helminths has not been related to global amphibian declines, these parasites may still have negative impacts on the population dynamics of their amphibian hosts.

Once the most widespread and common frog in North America, the northern leopard frog (*Rana pipiens*) has suffered declines in parts of its range (Jones and Cushman 2004), including within North Dakota (Larson et al. 1998). Although several studies (McAlpine 1997, Gillilland and Muzzall 1999) have looked at the helminth communities of *Rana pipiens*, few studies (Goldberg et al. 2001) have explored the helminth community of *Rana pipiens* in North Dakota. The Sheyenne National Grassland (SNG) is a relatively contiguous piece of tallgrass prairie located in southeastern North Dakota. The SNG contains a large number of wetlands and good quality terrestrial habitat (grassland) for northern leopard frogs. The objective of this study was to determine which helminths infect northern leopard frogs of various stages across the SNG.

# Methods

Fifty northern leopard frogs were collected with a dipnet from a variety of wetlands and upland habitats across the SNG between April and October 2003. Approximately equal numbers of frogs were collected from early (April, May, and June) and late (July, August, September) months, spanning the entire time that *Rana pipiens* is active during a year in southeastern North Dakota. Sampling in this manner allowed for analysis of temporal trends in parasitic infection rates and species richness. Frogs ranged in stage from emerging metamorph to adult, and before necropsy individual frogs were

weighed and snout-vent lengths were recorded. Frogs were euthanized, following procedures approved by the University Animal Care Committee, before opening the body cavity for dissection. A dissecting microscope was used to examine the mouth cavity, esophagus, lungs, gastrointestinal tract, urinary bladder, kidneys, liver, body cavity, skin, and leg muscle for helminths. Helminths were appropriately heat fixed and preserved in 70% ethanol, and are in storage in the University of North Dakota Biology Department.

Select nematodes were cleared with glycerol through evaporation of an ethanol/glycerol mixture and mounted on slides for identification. Trematodes and cestodes were stained with alum Carmine and mounted in balsam for identification. Helminths found in this study were categorized as either larval (helminth not sexually mature and amphibian is intermediate or paratenic host) or adult (helminth sexually mature and amphibian is definitive host).

DNA was extracted from several helminth taxon using guanidine buffer according to Tkach and Pawlowski (1999). An approximately 1350 bp fragment at the 5' end of the nuclear 28S ribosomal DNA gene, and in some cases ITS region (ITS1+5.8S+ITS2) were amplified by PCR in an Eppendorf Mastercycler machine. For amplification of the 28S fragment, forward primers digl2 and LSU5 and the reverse primer 1500R were used. For amplification of ITS region, forward primers S20T2 or SBr were used in combination with reverse primers 300R or digl2R. PCR products were visualized using horizontal electrophoresis in agarose gel and cleaned-up using Qiagen QiaQuick kit according to manufacturer instructions. Sequencing reactions were prepared using BigDye chemistry and run on an automated capillary sequencer ABI Prism 3100. Sequences were assembled using Sequencher software (GeneCodes Corp., vers.4.2.2), and completed

sequences of larval digeneans were compared with sequences in the NCBI GenBank for identification.

Prevalence, mean intensity, range, and total number (percentage of total community) were calculated for each parasite taxon. In this survey prevalence was defined as the proportion of frogs infected with a given parasite, mean intensity is the mean number of that parasite per infected frog, and range is represented by the lowest infection of a given parasite to the highest observed infection of the same parasite. T-tests were used to test for differences between amphibian sex with total parasite load, and for temporal differences in parasitism, including total parasite load and cumulative species richness with time of year. Because female leopard frogs are often larger than males, and are thought to contain more helminths for this reason (McAlpine 1997), a regression analysis was used to test for an association between frog body size (length) and total parasite load.

## Results

A total of twelve helminth taxa (7 Trematoda, 1 Cestoda, and 4 Nematoda) were found during this survey (Table 10). Nine sequences representing five helminth species were assembled (Appendix A). *Haematoloechus* spp. were both sequenced and identified morphologically, based on the presence of extracecal uterine loops in *H. varioplexus* (Vasyl Tkach, personal communication). *Mesocestoides* sp. did not amplify with the primers used in this survey, but would not have provided any further identification without an adult worm from a definitive host. After searching the NCBI GenBank, the Ochetosomatidae sp. sequenced in this survey matched the sequence of an Ochetosomatid obtained from a garter snake (*Thamnophis sirtalis*) in Nebraska. Although only one

	Life		Mean Intensity		Total Helminths	
	Stage	Prevalence	$\pm 1$ SD	Range	(% community)	
Trematoda						
Haematoloechus medioplexus	Adult	0.2	4.2 <u>+</u> 1.8	0 - 7	42 (0.03)	
Haematoloechus varioplexus	Adult	0.06	$1.3 \pm 0.6$	0 - 2	4 (0.002)	
Gorgodera amplicava	Adult	0.02	1	0 - 1	1 (0.0006)	
Alaria sp. (mesocercariae)	Larval	0.04	10 <u>+</u> 8.5	0 - 16	20 (0.01)	
Ochetosomatidae sp. (metacercariae)	Larval	0.44	$26.6 \pm 40.2$	0 - 138	585 (0.35)	
Fibricola spp. (metacercariae)	Larval	0.54	$26.0 \pm 29.9$	0 - 150	506 (0.31)	
Strigeidae sp. (metacercariae)	Larval	0.12	20.3 <u>+</u> 27.6	0 - 75	122 (0.07)	
Cestoda						
Mesocestoides sp. (tetrathyridia)	Larval	0.14	37.9 <u>+</u> 43.2	0 - 100	265 (0.16)	
Nematoda						
Rhabdias ranae	Adult	0.28	2.6 <u>+</u> 3.4	0 - 14	36 (0.02)	
Cosmocercoides dukae	Adult	0.18	5.4 <u>+</u> 4.1	0 - 12	49 (0.03)	
Spiroxys sp.	Larval	0.02	1	0 - 1	1 (0.0006)	
Spiruridae sp.	Larval	0.22	2.5 <u>+</u> 2.6	0 - 10	28 (0.02)	
Total		0.94	35.3 <u>+</u> 45.1	0 - 165	1659	

Table 10. Prevalence, mean intensity, range, and total number of helminth taxa infecting fifty *Rana pipiens* from the Sheyenne National Grassland during the summer of 2003.

sequence was assembled, based on morphological features of metacercariae I believe there was more than one species of Ochetosomatidae sp. infecting *Rana pipiens* on the SNG. Two sequences which most likely represent two different species of *Fibricola* were obtained, but were considered as one in this survey. Without having an adult worm from a definitive host (bird or mammal), it was difficult to determine whether these specimens are from the genus *Fibricola* or *Neodiplostomum*, because the original phylogeny was based on the definitive host not on characters of the metacercariae (Hong and Shoop 1994). The answer to this question is beyond the scope of this survey, and is treated here by referring to these helminths as *Fibricola* spp.

Of the 1,659 helminths found in this survey, adult helminths made up approximately 8% (132 worms) of the community, whereas the remaining 92% (1,527 worms) were larval helminths using *Rana pipiens* as an intermediate or paratenic host. Forty-seven of the 50 (94%) frogs examined in this survey harbored at least one helminth species, and the average infection intensity was 35.3 parasites per infected frog (Table 10). Of the 47 frogs with helminths, 10 (21%) had only one helminth species, 17 (36%) had two species, 14 (30%) had three species, 4 (9%) had four species, 1 (2%) had five species, and 1 (2%) had six species (Figure 12).

Although total prevalence of helminths in *Rana pipiens* was high and the mean intensity of an infected frog was 35.3 (Table 10), the majority of frogs surveyed had a total between zero and twenty parasites (Figure 13). *Fibricola* spp. and Ochetosomatidae sp. were the most prevalent taxa in the survey; infecting 54% and 44% of the frogs respectively, while cumulatively summing to approximately 65% of the entire parasite community (Table 10). *Mesocestoides* sp. had the highest mean intensity of infection,



Figure 12. Species richness of parasites infecting *Rana pipiens* from the Sheyenne National Grassland during 2003.



Figure 13. Total parasite load of dissected *Rana pipiens* from the Sheyenne National Grassland during 2003.

even though it was less prevalent in the community compared to most other surveyed helminths (Table 10).

Males and females did not differ significantly in parasite load (Mann-Whitney U Test, U = 198.5, N = 45, P = 0.302), but three male frogs (n = 27) were not infected whereas all female frogs (n=18) were infected with helminths. There was no significant difference in total parasite load (Mann-Whitney U Test, U = 229.5, N = 50, P = 0.237) or overall parasite species richness (Mann-Whitney U Test, U = 285, N = 50, P = 0.952) for frogs collected in early months to frogs collected in late months. Moreover, there was no association between total parasite load and snout to vent length (Regression Analysis,  $R^2 = 0.0022$ , P = 0.81), suggesting that body size did not account for any observed variation in parasite load.

# Discussion

Results of this survey were similar to those found in other studies investigating helminth communities of *Rana pipiens*. Both Goldberg et al. (2001) and this study encountered many of the same species, but several species were unique to each study, suggesting there is a larger community of helminths infecting *Rana pipiens* in North Dakota than was previously recognized. *Haematoloechus medioplexus*, *Gorgodera amplicava*, *Mesocestoides* sp., *Cosmocercoides dukae*, and *Spiroxys* sp. were several species encountered in this survey not previously reported to infect northern leopard frogs in North Dakota.

Results of this survey concur with those of McAllister and Conn (1990) and Gillilland and Muzzall (1999) who found low prevalence but high intensity infections of *Mesocestoides* sp. Studies by Goldberg et al. (2001) and McAlpine (1997) found female leopard frogs to be more heavily parasitized than male leopard frogs (though McAlpine's was the only one that demonstrated statistically significant differences), which McAlpine suggested was because female frogs are often larger and therefore provide larger targets for nematode larvae that infect through the skin. Although not statistically significant, female frogs were on average more heavily parasitized than males, which may be due to an age effect; females live longer and accumulate more helminths over time. Because there was no correlation between body size and total parasite load, a case might be made that sampling an equivalent number of females might yield a similar number of non-infected individuals. Alternatively, since four of the five adult helminths encountered in this study require ingestion of infected prey, it is likely that parasitism by adult helminths in this system is dictated by the random chance of ingesting an infected prey.

Juvenile helminths exhibited high prevalence high abundance in this survey. Definitive hosts for the juvenile helminths encountered are birds of prey, snakes, turtles, and small mammals, which are all readily abundant across the SNG. Two common garter snakes (*Thamnophis sirtalis*), one western hognose snake (*Heterodon nasicus*), and one common snapping turtle (*Chelydra serpentina*) were also collected and examined for adult helminths of the juveniles found in *Rana pipiens*. Although Ochetosomatidae sp. adults were not discovered in any snakes, it was still believed that snakes were the definitive host of the juveniles found in *Rana pipiens* (Vasyl Tkach, personal communication).

Two adult *Spiroxys* sp. were detected in the snapping turtle, which also contained an adult female leopard frog in its digestive tract. Although the frog discovery does not necessarily resolve successful transmission of this parasite (uses frog as a paratenic host), it does suggest that it is ecologically plausible for *Spiroxys* sp. to use *Rana pipiens* for completion of its life cycle on the SNG. Unfortunately, the only juvenile *Spiroxys* sp. worm found was cleared in glycerol and mounted on a slide for identification, making it impossible to extract suitable DNA for identification. This worm is usually reported as *Spiroxys contortus*, but it is unclear if this is the encountered species because *S. contortus* was described from Europe, and may be different from those found in North American snapping turtles.

The percentage (8%) of adult helminths found in this survey was lower than Goldberg et al. (2001) reported for the same area of North Dakota. One possible explanation for this observation was a temporal change in the climatic conditions affecting the study area. Goldberg et al. (2001) collected leopard frogs from southeastern North Dakota in 1995 to 1998, a period when leopard frog numbers were high on the SNG because of increased precipitation during the late 1990's (Jones and Cushman 2004). In contrast an extended dry period was observed across the SNG during the summers of 2002 and 2003 when collections for this survey took place.

Dry periods negatively impact amphibian populations because the number of breeding ponds is reduced, and amphibians themselves have an increased risk of desiccation. However, one could perceive the loss of breeding sites being equally hard on the invertebrate intermediate hosts required for successful completion of most helminth life cycles. This lends further support to the hypothesis that parasitism by adult helminths in this system is dictated by the random chance of ingesting infected prey. If there are fewer predators and fewer prey (during dry years) over the same landscape, the

chance of a predator encountering an infected prey may decrease and lead to a smaller percentage of adult helminths.

McAlpine (1997) found that both the host and parasite life histories, and some abiotic features of particular wetlands were the most important factors shaping helminth communities of leopard frogs in New Brunswick, Canada. Conversely, what might become important for amphibian conservation is the role or impact macroparasites have on shaping host communities. Perhaps an important next step is to compare the spatial and temporal dynamics of northern leopard frog populations with observed parasite densities within those populations to explore a potential correlation between extreme macroparasitic infection and local population extinctions. Although malformities have not been linked to global amphibian declines, and Goater (1994) and Goater and Vandenbos (1997) found negligible effects of macroparasites on growth and survival of amphibians, more research is needed to understand ecological circumstances under which macroparasites may have a substantial impact on amphibian population dynamics.

#### CHAPTER 4

# FREQUENCY OF COLOR AND PATTERN POLYMORPHISMS IN *RANA PIPIENS* ACROSS THE SHEYENNE NATIONAL GRASSLAND OF NORTH DAKOTA

#### Introduction

As landscapes become more fragmented, it will become increasingly important to understand the degree to which a species disperses through matrix habitat, and determine what impacts that processes like selection, gene flow, drift, and mutation have on the genetic composition of populations inhabiting these landscapes. Because wetlands form discrete habitat patches required by most amphibians for reproduction, amphibian populations make ideal systems for studying genetic variation amongst and within populations, because amphibians must disperse across potentially unsuitable landscapes to interact with other populations. Migration between populations in a fragmented landscape is important for providing wetlands with additional recruits, while gene flow (resulting from migration) maintains allelic diversity within populations at a fine spatial scale (Newman and Squire 2001). Wetlands may also vary in size, which may determine the size of populations inhabiting these wetlands. This is important because smaller populations often have lower effective population sizes that lose alleles at faster rates than larger populations (Connor and Hartl 2004). Because small populations lose alleles faster than larger populations, rates of gene flow between populations must be high enough to counteract drift, or smaller populations will be hindered in their ability to adapt to local conditions (Harrison and Hastings 1996).

In a review of color and pattern polymorphisms in anurans, Hoffman and Blouin (2000) concluded that northern leopard frogs exhibit three visual polymorphisms (one color polymorphism and two pattern polymorphisms). A polymorphism is the simultaneous occurrence of two or more discrete, allele associated phenotypes in a population, in which the frequency of at least one less common form is higher than can be maintained by recurrent mutation (Connor and Hartl 2004). Visual polymorphisms exhibited by northern leopard frogs include a green/brown color polymorphism, a dorsal spotting polymorphism (spotless phenotype referred to as *Rana pipiens* burnsi), and a mottled polymorphism (mottled phenotype referred to as *Rana pipiens* kandiyohi) (Hoffman and Blouin 2000). Color and pattern polymorphisms appear to follow simple Mendelian inheritance (Hoffman and Blouin 2000), thus providing a simple means of assessing genetic variation within and among populations, but at a small number of loci.

Although inheritance of these polymorphisms may adhere to Mendelian ratios, inheritance may be more complicated and the possibility of both the kandiyohi and burnsi genes being linked cannot be ruled out (Volpe 1956, Volpe 1960). However, inheritance of the kandiyohi and burnsi phenotypes can also be explained by having alleles at two unlinked loci that are both dominant to the common spotted pattern (Volpe 1956, Volpe 1960). Green color has also been shown to be dominant over brown in leopard frogs (Fogleman et al. 1980), which follows the trend of green being dominant to brown in most anuran species exhibiting this color polymorphism (Hoffman and Blouin 2000).

Color and pattern polymorphisms are common in anuran species, but despite their relative abundance little is known on the significance of these polymorphisms (Hoffman and Blouin 2000). Much information on inheritance and genic action is based on

laboratory crosses of single generations (Volpe 1956, Volpe 1960), but adaptation in nature occurs amongst complex ecological processes that can not be replicated in the laboratory (Voss and Shaffer 2000). Although laboratory experiments may be important in understanding phenotypic inheritance within a species, the ecological and evolutionary relevance of genetic and phenotypic variation must be examined with respect to processes such as natural selection, drift, and mutation (Voss and Shaffer 2000). The Shevenne National Grassland (SNG) is a semi-contiguous piece of tallgrass prairie (Figure 14) that provides an ideal location to study phenotypic diversity among northern leopard frog populations across a landscape where selection and other ecological processes are inevitably occurring. Evidence of northern leopard frog migration across a relatively contiguous piece of landscape may be important for developing conservation strategies for leopard frog populations inhabiting highly fragmented landscapes. My goal in studying genetically based polymorphisms is to determine to what extent gene flow and allelic mixing occur across a relatively contiguous landscape, which may later be used as a baseline for developing conservation strategies in highly fragmented landscapes.

#### Methods

In the summer of 2001, frogs were observed during standard terrestrial visual encounter transects (Olson et al. 1997) for visual phenotypic scoring, but frogs observed in 2002 were sampled during occupancy surveys at individual wetlands (Olson et al. 1997). Locations were recorded for every frog, and these locations were subsequently grouped into four study areas based on their geographic proximity (Figure 14). These different study areas vary in topographical and landcover characteristics, and range from rolling hills to flat prairie, and large stands of woodland to no woodland. The four areas

are separated by numerous potential dispersal barriers including cropland, paved roads, railroad tracks, and the Sheyenne River.

Study areas ranged in distance from approximately 5 km (Area 1 - Area 2) to 20 km (Area 1 – Area 4) from one another, but study areas 1 and 2 are essentially detached from the rest of the SNG by the Sheyenne River and small amounts of agricultural land (Figure 14). Phenotypic character states were scored for each frog for all three polymorphisms before subsequent release. Because the inheritance of each of the traits involves dominance, genotype and allele frequencies can only be inferred and not measured directly. Estimation of allele frequencies from phenotype data also requires the questionable assumption of Hardy-Weinberg equilibrium, so phenotype frequencies were calculated and used for analyses rather than estimated allele frequencies. Potential differences in phenotype frequencies between years, different study areas within a year, and the same study area between years were tested for using contingency tables. To improve sample sizes, data on polymorphisms were pooled between areas and years depending on the analysis being performed.

#### Results

Phenotypic occurrences for each study area and year are presented in Table 11. No significant differences were found for color morphs between years ( $\chi^2 = 1.106$ , N = 161, df = 1, P = 0.293), between areas within a year (2001:  $\chi^2 = 1.786$ , N = 76, df = 2, P = 0.41 and 2002:  $\chi^2 = 1.242$ , N = 85, df = 2, P = 0.54), or the same area between years (Area 1:  $\chi^2 = 0.828$ , N = 87, df = 1, P = 0.36 and Area 3:  $\chi^2 = 1.54$ , N = 14, df = 1, P = 0.22). In contrast to the color morphs, significant differences were detected for the mottled polymorphism between years ( $\chi^2 = 8.726$ , N = 16, df = 1, P = 0.003) and for



Figure 14. Property owned and managed by the USDA – Forest Service, and relative locations of the four areas across the Sheyenne National Grassland where phenotypic polymorphisms were observed and compared.

				Mottled					
Year	Location	Spotted		Spotless (burnsi)		(kandiyohi)			
		Green	Brown	Green	Brown	Green	Brown		
2001	Area 1	6	10	0	0	0	2		
	Area 3	5	4	0	0	0	0		
	Area 4	14	32	2	1	0	0		
2002	Area 1	30	30	0	0	1	8		
	Area 2	5	2	0	1	0	3		
	Area 3	1	2	0	0	0	2		
Total		61	80	2	2	1	15		

Table 11. Frequency of visual polymorphisms for all study areas of the Sheyenne National Grassland for the summers of 2001 and 2002.

study area 3 between years ( $\chi^2 = 4.326$ , N = 14, df = 1, P = 0.037). However, tests between study areas within a year (2002:  $\chi^2 = 3.705$ , N = 16, df = 2, P = 0.16) and for study area 1 between years ( $\chi^2 = 0.057$ , N = 11, df = 1, P = 0.811) were not significant. An analysis of the mottled polymorphism between study areas for 2001 was not conducted because of the extremely low number of mottled frogs encountered in 2001 (Table 11). Low frequencies of the spotless phenotype were observed in both 2001 (3.9%) and 2002 (1.2%), and no significant difference was detected between years for this polymorphism ( $\chi^2 = 1.244$ , N = 4, df = 1, P = 0.27). No additional analyses were performed on this polymorphism because of the small number of animals with this phenotype detected during the study.

# Discussion

Amphibian populations occupying small wetlands or isolated pieces of grassland may lose alleles at accelerated rates because of small effective population sizes and drift, but the contiguous grassland matrix of most of the SNG may be sufficient to provide adequate gene flow to small isolated populations and prevent allele fixation. Based on anecdotal capture observations of leopard frogs in Minnesota, Volpe (1956) suggested the mottled and spotless phenotypes occur at an approximate level of 1% in wild populations, which may not be much higher than would be expected to occur through mutation. Lack of a significant difference in green and brown color frequencies between years and areas implies that alleles at this locus are mixed equally across the landscape. Although the green phenotype is dominant to brown (Fogleman et al. 1980), the brown phenotype was more abundant (60%) in this study. Because no significant differences in frequency were found for green and brown frogs, it is possible that this character may be neutral across the SNG. Alternatively, this color polymorphism may be maintained at these frequencies through balancing selection.

Spotless frogs were encountered at a low frequency across the SNG (2.5%), which is in rough agreement with the low frequency (1%) of this allele found in Minnesota populations by Volpe (1956). Such a low allele frequency in a population might be explained simply by normal rates of mutation (Ford 1975). Two spotless frogs were encountered in 2003 in area 1 during collections of leopard frogs for a parasite survey. Combined with the data on spotless observations from 2001 and 2002, these results suggest the spotless allele is found throughout the SNG. However, because of the low frequency of spotless frogs in this survey, I conclude that the spotless allele is not geographically limited in distribution but probably carries no selective advantage.

Mottled (kandiyohi) leopard frogs were encountered at a relatively high level (9.9%) in this study, which is nearly ten-fold higher than the frequency (1%) suggested by Volpe (1956) for wild populations in Minnesota. The majority of mottled individuals (69%) were encountered in study area 1. Mottled frogs were encountered at a frequency of 13% in study area 1, whereas mottled frogs were encountered in the other study areas at a much lower frequency (7%). Encounter rates for the mottled morph were nearly identical for area 1 in 2001 (11%) and 2002 (13%), suggesting that differences in the number of mottled frogs between years was probably related to a sampling deficiency in area 1 during 2001. Likewise, the difference in mottled frequencies in area 3 between 2001 and 2002 was based on extremely low sample sizes (9 and 5 respectively), and all individuals in 2002 were taken from the same pond and may therefore be offspring of the

same cross. Because of the low sample sizes associated with area 3 in both years, these differences must be considered with caution.

One result of interest was the high frequency of mottled frogs in area 1 compared with the rest of the SNG. It is possible that there may be some small selective advantage to being mottled in an area that is more closely associated with woods and brush (e.g. better camouflaged amongst leaf litter). Secondly, the deficiency of mottled frogs in areas 2, 3, and 4 of the grassland may be associated with the transient nature of wetlands in these areas during dry years and subsequent founder events by relatively few mottled frogs. A selective advantage seems less likely since area 3 is almost devoid of woods and still had a similar frequency of mottled frogs when compared to area 1. However, area 4 is composed of flat prairie and area 2 and 3 are associated with small, rolling hummocks which often have shallow wetlands that do not persist in dry years. Area 1 has larger hills which create more permanent wetlands that are capable of persisting through extended dry periods. No leopard frogs and few wetlands were observed in area 4 during surveys in 2002, but large numbers of leopard frogs were encountered during 2001 when rain was plentiful and wetlands more abundant. Therefore, the frequency of mottled frogs in area 1 may reflect a true frequency of this allele in a more permanent wetland landscape, whereas the low frequency in other areas might be related to local extinctions in ephemeral wetlands that are subsequently recolonized by small numbers of frogs carrying this allele. In contrast the high frequency of mottled frogs in area 1 might exist because of reduced gene flow and less allelic mixing between populations across the SNG. This question remains unanswered, but reduced gene flow seems less likely because the other two polymorphisms were spread more or less evenly across the landscape.

Overall, allelic mixing between populations (even when separated by as much as 20 km) separated by relatively contiguous, suitable habitat seems to be occurring at a high enough rate to avoid allele fixation. Some significant differences in mottled frog frequencies were observed, but are likely attributable to sampling biases or to possible founder events of small ephemeral wetlands. One question not addressed in this survey is why most mottled frogs (94%) were brown. Visual polymorphisms are not representative of the entire genome (Connor and Hartl 2004), so more extensive surveys of genetic variation using molecular markers should be conducted on leopard frogs across this landscape to support these data before using these results to develop conservation plans for leopard frog populations inhabiting more highly fragmented landscapes.

#### CHAPTER 5

# DISCUSSION AND AMPHIBIAN CONSERVATION

The primary objective of conservation biology is preventing regional extinctions (Goodman 1987), but declaring recent amphibian declines and extinctions as unusual is difficult because amphibian populations are known to cycle through periods of low and high numbers (Blaustein et al. 1994). Whether recent declines are due to normal demographic fluctuations or represent abnormal declines leading to regional extinctions, it is important to begin developing conservation plans to assist amphibian population preservation. Although many factors, including pathogens (Daszak 1999), have been negatively associated with amphibian population dynamics (including North Dakota populations), anthropogenic destruction and alteration of critical habitat needed by amphibians is the most probable cause for the apparent declines in amphibian populations (Blaustein et al. 1994). Conservation plans for amphibians across the SNG must consider impacts on both the aquatic and terrestrial habitat (Semlitsch 2000), because both habitats are required by all amphibians inhabiting the northern tallgrass prairie (Conant and Collins 1991). Careful management of these habitats at local and landscape levels is important for maintenance of viable populations and regional diversity (Semlitsch 2000). Because amphibian population processes and patch dynamics may vary regionally (Blaustein et al. 1994) and are most likely different between species, ensuring regional persistence of amphibian populations will require species-specific understanding of biotic and abiotic factors influencing patch occupancy (Knapp et al. 2003).

Regional persistence and preservation of amphibian populations is related to three not necessarily independent factors, which include the mean and variance of population growth rate over time (Goodman 1987), rates at which metapopulation processes like colonization and extinction occur across the landscape, and some asynchrony in local population dynamics (Hanski 1999). All three factors must be implemented into conservation plans (Semlitsch 2000), especially for rare species that likely have low populations. Proper management of amphibian populations also requires an understanding of population dynamics when conditions are unfavorable, because comparatively little is gained by studying populations under minimal environmental stress. All these components required for regional persistence of amphibian populations can be negatively impacted by extended dry years. Therefore, understanding the theoretical and observed dynamics of amphibian populations during periods of high environmental (climatic) stress will help in developing conservation plans.

Drought conditions lead to many negative effects on population growth rates of amphibians, including increased risk of desiccation through increased evaporative water loss by reduced substrate moisture, extirpation of local populations breeding in highly ephemeral wetlands, and reduced size at metamorphosis because of earlier pond drying or higher density and therefore high rates of competition in ponds that do persist. Because dry years may cause the population growth rate to become negative, precautions must be taken to either reduce or accommodate this negative growth rate, because a regional population experiencing a negative growth rate for an extended period is likely to go extinct. For regional preservation during extended dry periods, inclusion of "hot spots"

within the study landscape is critical (Goodman 1987). These locations are places where the mean growth rate of the species in question is consistently positive (Goodman 1987), and serve as source populations when conditions become favorable.

Droughts also amplify the impacts of metapopulation processes like extinction and colonization, because droughts often remove small ponds from the landscape which leads to decreased colonization caused by increased isolation of remaining wetlands. Metapopulations encompassing interdependent patches of habitat must be monitored and managed at the landscape scale (Blaustein et al. 1994), because local populations are expected to go extinct periodically and be subsequently recolonized (Hanski 1999). However, metapopulation processes cannot ignore the importance of local dynamics because not all wetlands represent suitable habitat (personal observation), and may have no function in determining extinction and colonization rates across the landscape. Droughts often remove smaller ponds because of short hydroperiods, leaving larger more permanent wetlands that more often contain fish and larger communities of invertebrates that can negatively impact amphibian populations. Highly ephemeral wetlands and permanent water bodies are at the two ends of a spectrum, and Snodgrass et al. (2000) demonstrated that intermediate hydroperiod ponds exhibit the highest species diversity. Also, amphibians found occupying shorter hydroperiod wetlands were often absent from ponds with long hydroperiods (Snodgrass et al. 2000). Matrix habitat between wetlands is also important in judging metapopulation stability, since some land use features like row crop agriculture have been shown to hinder colonization (Joly et al. 2001). Therefore, conservation plans for amphibian populations must include information on

wetland hydroperiod requirements, composition and configuration of suitable habitat (including matrix habitat), and dispersal capabilities of the species in question.

Asynchrony in local patch dynamics is required for regional persistence of populations because simultaneous extinctions of all local populations and eliminates any "rescue" from neighboring populations (Hanski 1999). Droughts may synchronize extinction events across a landscape because all wetlands experience similar conditions. Although longer hydroperiod ponds may be less affected during dry years, ensuring the presence of a range of ponds with different hydroperiods may assist in asynchronizing local dynamics. Local dynamics may also become synchronized through grazing during dry periods because cattle are known to concentrate around water (Jansen and Robertson 2001). When ephemeral wetlands disappear during dry years, cattle congregate around the remaining wetlands and can degrade their condition (Jansen and Robertson 2001). If grazing negatively impacts wetland amphibian assemblages and impacts all wetlands surviving a drought, then potential for synchronized local dynamics exists and should be considered when developing conservation plans for amphibians inhabiting grazed landscapes.

Wetland habitat is required by all amphibian species occupying the northern tallgrass prairie ecoregion for reproduction, and the northern leopard frog even requires a suitable wetland (permanent) for overwintering (Conant and Collins 1991). Loss of wetlands reduces the number and density of breeding sites, which diminishes the capacity of a landscape to maintain local and regional amphibian populations (Semlitsch 2000). Extinctions of local amphibian populations have been associated with increased isolation of wetlands (Sjogren 1991), wetland size, and local patch quality components (see

Chapter 2). Patch quality variables impacting local extinctions may vary among species, conservation plans should be tailored to specific habitat associations. Patch quality variables were also found to be related to amphibian occupancy (see Chapter 2), and these characteristics should also be incorporated in conservation planning. Amphibian Research and Monitoring Initiative (http://edc2.usgs.gov/armi/monitoring.asp#National) or "ARMI" researchers have found that the proportion of occupied area is the only wetland-associated variable that is nationally interpretable and regionally adaptable for predicting amphibian occupancy. Results from this study concur with ARMI, because ponds with a larger number of nearby occupied ponds in the surrounding landscape were occupied more often. A larger proportion of occupied area suggests that persistence of a species is likely, while a smaller proportion of occupied area is linked to an increased risk of regional extinction. The goal of the present study was to visit every possible pond on the Shevenne National Grassland (SNG) instead of creating occupancy estimates based on subsets of ponds across the landscape. Although more labor intensive, ascertaining occupancy for all wetlands across a landscape will provide a better understanding of metapopulation dynamics.

Although the SNG is largely contiguous, it has been grazed extensively and anthropogenic manipulations of wetlands are conducted to allow grazing in pastures where water may otherwise be scarce. These stock ponds are "dugout" with heavy machinery when they become shallow, resulting in ponds with long hydroperiods but limited aquatic and riparian vegetation. Ponds with long hydroperiods (including most SNG stock ponds) often contain salamander larvae and are known to harbor larger invertebrate communities which can negatively impact larval anuran populations

(Snodgrass et al. 2000). Because stock ponds are frequently disturbed by cattle and produced extremely low dissolved oxygen readings (2% oxygen saturation), successful metamorphosis of amphibian populations may not be possible in these ponds. Knutson et al. (2004) demonstrated that man-made ponds in southeastern Minnesota represented suitable breeding habitat for amphibians, while other studies have also shown that newly constructed ponds in agricultural landscapes are often colonized by amphibians (Baker and Halliday 1999). Understanding amphibian population dynamics in these stock ponds across the SNG may be critical for conservation, because stock ponds represent the majority of wetlands present during dry years.

As stated earlier amphibian conservation also relies on proper management of the adjacent terrestrial habitat, as well as aquatic breeding habitat, because a majority of amphibian populations spend large quantities of the active season away from water (Conant and Collins 1991). Amphibian species inhabiting the northern prairie often use the landscape surrounding a wetland for foraging, while others require suitable terrestrial habitat for overwintering (Conant and Collins 1991). Although amounts of adjacent terrestrial habitat required by amphibian species may vary, Semlitsch and Bodie (2003) outlined three zones to consider managing when developing conservation strategies for amphibian populations. Zone 1 is called the aquatic buffer zone and should extend at least 30-60 meters away from the wetland, because this zone catches and removes organic chemicals as well as decreases adjacent terrestrial erosion (Semlitsch and Bodie 2003). Zone 2 is the core habitat required by a given species for completion of its life history, and should extend as far from the pond as the home range of the species in question to ensure that life history requirements are met (Semlitsch and Bodie 2003).

Zone 3 is a buffer around the required terrestrial habitat, but is the most flexible zone in terms of exclusion from amphibian management. Because adjacent landscapes can "mask" metapopulation processes (Pope et al. 2000), and since little is known about terrestrial habitat requirements for many amphibian populations (Marsh and Trenham 2001), sound management of the terrestrial landscape surrounding wetlands will assist in the preservation of regional amphibian populations. An effective management plan for amphibian populations must consider three critical factors, including local population dynamics, ensuring a diversity of wetlands varying in hydroperiod, and the rates of metapopulation processes occurring across the landscape (Semlitsch 2000).

# Northern Leopard Frogs

Northern leopard frogs were once among the most widespread frog species in North America (Jones and Cushman 2004), but have suffered declines in many parts of its range (Orr et al. 1998), including areas in North Dakota (Larson et al. 1998). The status of northern leopard frogs across the SNG appears good, which is supported by them occupying roughly 45% of the surveyed wetlands throughout the study (Table 1). Northern leopard frog success appears to be associated with greater plasticity in life history traits because they were found occupying ephemeral wetlands and permanent lakes, wetlands embedded in agricultural fields and forests, and minimally and extensively grazed wetlands, However, with decreased amounts of precipitation the range of leopard frogs across the SNG became restricted to wetlands in closer proximity to the forest near the Sheyenne River, suggesting habitat associated with the river valley is better for leopard frogs during dry periods. This range restriction may also be related to lack of suitable breeding habitat further from the Sheyenne River, in particular the stock ponds associated with the rest of the SNG. Leopard frogs only successfully reproduced in one stock pond, which was different from most stock ponds because some emergent and submergent vegetation was salvaged during the "digging" process. No other stock ponds yielded successful reproduction by leopard frogs, a result likely linked to the lack of vegetation and abundance of salamanders inhabiting stock ponds. However, stock ponds may have provided leopard frogs with suitable overwintering habitat since hundreds of leopard frogs were observed in stock ponds in early October, suggesting these ponds have an important function in the leopard frog life history across the SNG. However, in their current state stock ponds should not be considered suitable breeding habitat. One local patch quality variable was extremely clear in its impact: leopard frog populations had a hard time reproducing in wetlands without vegetation.

Defining suitable terrestrial habitat for leopard frogs is difficult, because they were observed in a number of wetlands surrounded by different habitat. Though other researchers found effects of surrounding landscape on leopard frog populations (Pope et al. 2000), results from this study suggest that leopard frogs may select breeding ponds closer to overwintering sites during dry years, despite the surrounding landscape. Leopard frogs were often found in natural wetlands impacted by grazing, suggesting that grazing may not significantly impact leopard frogs as long as wetland vegetation is not dramatically altered. Results of the survey of phenotypic polymorphisms (Chapter 4) support leopard frogs selecting preferred habitat because alleles were evenly mixed across the landscape despite large regional distances, suggesting leopard frogs move well across the landscape and could colonize whatever habitat they prefer. Data collected on helminths infecting leopard frogs (Chapter 3) were insufficient to determine negative

impacts of macroparasites of leopard frog populations. However, no *Ribeiroia ondatrae* (parasite associated with amphibian malformations) were encountered, and corresponds with the absence of malformations observed in the study. Understanding the role of parasitic infection in relation to habitat may be important for determining impacts of macroparasites on leopard frog populations, but this was not addressed.

One suggestion for maintaining viable regional populations of leopard frogs across the SNG includes yearly monitoring of wetlands in the Mirror Pool Management Area and Waterfowl Production Areas near Anselm, ND. These ponds were frequently occupied and most likely are "hot spots" for leopard frog populations. Monitoring these areas will help ensure that large populations of leopard frogs are present even during extended dry years, which will help ensure regional persistence of northern leopard frogs across the SNG. Another suggestion is to preserve a portion of the emergent and submergent vegetation (if present) when digging stock ponds. Leopard frog populations require aquatic vegetation for successful breeding and often go extinct in its absence (Chapter 2), so preserving some vegetation will increase the probability of leopard frogs utilizing stock ponds for reproduction instead of just overwintering habitat, which will help forestall extinctions.

# Gray Tiger Salamanders

Gray tiger salamanders are one of the most ubiquitous yet seldom encountered amphibian species in the northern prairie (Jones and Cushman 2004). Gray tiger salamander populations are not in immediate danger of regional extinction across the SNG, and stock ponds appear to be the reason for their observed success. Gray tiger salamanders appear particularly to utilize stock ponds, and exhibited no range restriction

across the SNG during this study. If only stock ponds are considered, the observed metapopulation dynamics of gray tiger salamanders on the SNG are similar to those presented by Gill (1978) for the red-spotted newt. These dynamics are characterized by frequent extinction events followed by immediate recolonization, ultimately resulting in occupancy of nearly all habitat patches. Salamanders occupied nearly 90% of all stock ponds in the summer of 2004, despite the absence of vegetation and low dissolved oxygen readings in these ponds. However, salamander larvae were frequently observed surfacing in these stock ponds during the month of July, most likely a behavioral response to low oxygen levels.

Because these stock ponds were devoid of most prey (including anuran larvae), and since cannibalism was observed amongst captive individuals taken from these ponds, I suggest that cannibalism occurs quite frequently among salamander larvae in SNG stock ponds. When prey items are limited in abundance, salamander populations may possibly regulate their own populations by having high rates of cannibalism and increased metamorphic size for individuals surviving the larval stage. However, it should be noted that several salamander populations inhabiting wetlands maintained by artesian wells were very pale in color and small in size, and most likely will not contribute to future generations despite their ability to breed in ponds with high conductivity. Although gray tiger salamanders are described as being frequently neotenic (Conant and Collins 1991), no paedomorphic individuals were encountered and the status of this polymorphism across the SNG in North Dakota remains unknown.

Though gray tiger salamander populations may exhibit high colonization rates across contiguous grassland, they were less likely to occupy wetlands surrounded by
agriculture. Salamanders are limited in mobility compared to anurans, and agricultural fields may represent a matrix habitat that is resistant to dispersal. Agricultural land may also represent unsuitable overwintering habitat, suggesting that tiger salamanders may also be more likely to use breeding ponds with suitable overwintering habitat nearby. Why salamanders rarely bred in wetlands surrounded by forest was unknown, because Weyrauch et al. (2004) demonstrated that salamanders in Ohio were closely linked to woodland habitat. Therefore, I suggest that gray tiger salamanders inhabiting the SNG prefer prairie habitat and was further supported by the positive association with grazing, a land use practice that rarely occurs in cropland and forests. The relationship between grazing and salamander occupancy is poorly understood, but may be explained by the creation of a less resistant matrix habitat for a species with poor dispersal ability. Regional persistence of gray tiger salamanders across the SNG will likely be continued by increased maintenance of stock ponds, since these ponds almost always harbored salamander populations and most likely contain suitable overwintering habitat.

# Gray Tree Frogs / Cope's Gray Tree Frogs

Conservation of the gray tree frog (*Hyla versicolor*) and its cryptic relative Cope's gray tree frog (*Hyla chrysoscelis*) is complicated because Tramantano (2003) found both *Hyla versicolor* and *Hyla chrysoscelis* inhabiting the SNG. Jaslow and Vogt (1977) found *H. chrysoscelis* to be a prairie associated species and *H. versicolor* a forest associated species, but the extent to which that observation holds true for these species across the SNG remains unknown. These two species were treated as one complex in this study because they are supposed to have similar habitat requirements (Conant and Collins 1991), but if future studies demonstrate them to have different habitat requirements then

results from modeling them as one complex should be treated with caution. Gray tree frog populations were by far the least prevalent of the species modeled, successfully reproducing in only a small fraction of wetlands, which were most often located near the Sheyenne River and its associated forest. Gray tree frog populations exhibited no range reduction during the dry years because they were primarily only found in close proximity to the river.

Populations found in the prairie of the SNG occupied temporary ponds that failed to persist over the summer, while populations of tree frogs were also correlated with greater distances from permanent wetlands. Therefore, SNG tree frog populations may be associated with shorter hydroperiod ponds, which are less likely to contain salamander larvae and fish and are more likely to contain the aquatic vegetation required by tree frogs for successful reproduction and extinction avoidance. Though not included in the final model, half of the observed tree frog extinctions observed between 2003 and 2004 were associated with predator presence. Tree frog populations were more likely to occur in lower pH ponds which were also correlated to temporary ponds and ponds in closer proximity to the river. Ponds typically occupied by tree frogs were old river oxbows which often have low pH values, stands of adjacent forest, no salamander larvae, and the presence of aquatic vegetation. However, because they are in such close proximity to the river, any river flooding introduced fish predators, which were linked to an increased chance for local extinction of tree frog populations. Tree frog populations were also more likely to occupy wetlands with a forest adjacent to the wetland, which is also more common in closer proximity to the river. However, even populations inhabiting prairie wetlands had nearby stands of forest.

One suggestion for gray tree frog complex conservation is improved monitoring and maintenance of wetlands near the Sheyenne River. During wet years tree frog populations may use highly ephemeral wetlands that were abundant across the SNG, but during extended dry periods these ponds were removed from the landscape, and tree frogs must either utilize permanent wetlands or retreat to the Sheyenne River. Results of this study suggest that gray tree frogs are less likely to occupy permanent wetlands, so conservation should be directed towards maintaining the wetlands that tree frogs are known to occupy during dry periods. One example might be to remove fish populations with a seine after spring flooding, because fish can severely impact amphibian population success and abundance. In a wetland near the Sheyenne River where a tree frog and leopard frog population went extinct between 2003 and 2004, a large bullhead was seined from the pond and represents a major potential predator of amphibians.

These suggestions could help preserve these amphibian populations by increasing the quality of habitat across the SNG, largely by providing amphibian populations with more breeding habitat and stepping-stone ponds they can utilize during migrations. Although leopard frog and gray tiger salamander populations appear to be good, careful monitoring should be continued and immediate attention should be given to gray tree frog populations since numbers are extremely low and occupied ponds decreased by almost a half between 2003 and 2004. What is more distressing is that these low numbers occur across a contiguous landscape which should yield better results that those observed in highly fragmented landscapes. It is not too early to begin developing conservation plans that will help ensure the regional persistence of the SNG amphibian populations.

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Appendix A. DNA sequences acquired from the nuclear 28S ribosomal DNA gene and ITS region (ITS1+5.8S+ITS2) for adult and larval helminths collected from *Rana pipiens* across the Sheyenne National Grassland during the summer of 2003.

### Rhabdias ranae

ATAGTTTCTGGATCTTCGGATCTAGCGAACTTCTTAGAGGAATCGGCGGCTTCGATCCGCATG AAATAGAGCAATAACAGGTCTGTGATGCCCTTAGATGTCCGGGGGCTGCACGCGAGTTACTATG GAGAATCCAACGTGTCTACCTTGGCCGAAGGGTCTGGGCAAGCCGTTGAAACGTCTCTTTGAT CGGGATAGGGAATTGTGCAATTATTTCCCTTAAACGAGGAATTCCTAGTAAGAACGAGTCATC AACTCGTTCTGATTTGGTCCCTGCGCTTTGTACACACCGCCCGTCGCTACCCGGGACTGAACTG TTTCGAGAAAACCGGGGATTGACTCACGGGACTTGTTCCTTGTGGTGTTGAGAACCAGTTTAA TCGCGATGGTTTGAACCGGGTAAAAGTCGTAACAAGGTACCGGTAGGTGAACCTGCCGGTGG GTTATAATTGGCGTCTATACGTACTGCTAACGCTCGCGGACCGTCGACGTGTATTGATCAGTA GACTCATTACTACTCCTAGTGGTGGATCACTCGGCTCGCAGGTCGATGAAGAACGCAGCTAGC TGCGATAGTTGGTGCGAATTGCAAACACATTGAGCACTAAACTTTCGAACGCATATTGCGCCG CATGTTGCGTTATTTTGTTTAGCGAGAAGCTCGGTGTGCATGTGTGATATGTGCTAGTGTGTCA CACATGTGTCGTCCTTCTCTGGTTTGTCATTCTCATTGATATAATCATCTGAATCACAAACA CGTGCAAGTTGTGTTGGCTGGTGTTGGTTGATGATATAATATCATTTTGCAATTGCAACCTCAG TGATTCCTTTAGTAACAGCGAGTGAACAAGGAAGAGCCCAGCGCTGAATCTTTCGGTCTATGA CCGCTAAGAATTGTAGCGTATAGGTGTAGCTTTCTACGGCCGATGTATACTCAAAGTCCCTTT GATTGGGGCCACAGTCCTGAGAAGGTGCAAGACCTGTACGAGTTGCATTGTGTTGTAGTCGGT TGCTCCTTGGAGTCGGGTTGCCTGAGAACGCAGCCTGAATTGGGTGGTAAACTCCATCTAAGG CTAAATAGTACTGCGAGTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGCAAAGAACTTT GAAGAGAGAGTTCAAGAGTGCGTGAAACCACTGGGATGGAAACGGATAGAGTTGACGAATTG GGCGATATTCAGCTGTTTTGCGTGAGCAAAGTGGTGTACTTATCGTCTGTGTGCGCTGAGAGT CTTGATTGAACATTCTAAACCGTCGTATTTTGTTGCCCGTCGTCTCACGACGATGGTGTCTTGT GCGTGCGGGTTGGGATGTTTCGGTTAAGTATTTTCGGTGTGAAAGTCGACCACCTATCCGACC CGTCTTGAAACACGGACCAAGGAGTCTAGCGTATGTGCGAGTCATTGGGTGGTAAACCTATTG GCGTAACGAAAGTAAAGGTCGTTTCTTGCGGCTGATATGGGATCCGTGCGGTTTCGATCGTGC GGCGCACCATAGCCCTGTCTCGAAGGCTTGCCTTGAGATGGAGGTAGAGCGCATGCGCTAGG ACCCGAAAGATGGTGAACTATACGTGAGCAGGATGAAGCCGGAGGAAACTCTGGTGGAAGTC CGTAACGGTTCTGACGTGCAAATCGATCGTCTGACTTGCGTATAGGGGGCGAAAGACTAATC

#### Rhabdias ranae

GAACTGTTTCGAGAAAACCGGGGATTGACTCACGGGACTTGTTCCTTGTGGTGTTGAGAACCA GTTTAATCGCGATGGTTTGAACCGGGTAAAAGTCGTAACAAGGTACCGGTAGGTGAACCTGCC **GGTGGATCAATACTGAATATAGACTCAAGTAGTTTTTTATTCGGTGATCAACCCCCTGCCTTTA** AAACGGTTATAATTGGCGTCTATACGTACTGCTAACGCTCGCGGACCGTCGACGTGTATTGAT CAGTATGCATAGCATTCATTAACTCTCACATATATACTTCGTTGTGTACATGTGAGTGTTGATG ACAAAGACTCATTACTACTCCTAGTGGTGGATCACTCGGCTCGCAGGTCGATGAAGAACGCAG CTAGCTGCGATAGTTGGTGCGAATTGCAAACACATTGAGCACTAAACTTTCGAACGCATATTG GCTAGCATGTTGCGTTATTTTGTTTAGCGAGAAGCTCGGTGTGCATGTGTGATATGTGCTAGTG TGTCACACATGTGTCGTCCTTCTCTGGTTTGTCATTCTCATTGATATAATCATCTGAATCAC AAACACGTGCAAGTTGTGTTGGCTGGTGGTTGGTTGATGATATAATATCATTTTGCAATTGCAA CCTCAGATCAGTCCTGATTACCCGCTGAACTTAAGCATATCAGTAAGCGGAGGAAAAGAAAC TAACTATGATTCCTTTAGTAACAGCGAGTGAACAAGGAAGAGCCCAGCGCTGAATCTTTCGGT CTATGACCGCTAAGAATTGTAGCGTATAGGTGTAGCTTTCTACGGCCGATGTATACTCAAAGT CCCTTTGATTGGGGCCACAGTCCTGAGAAGGTGCAAGACCTGTACGAGTTGCATTGTGTTGTA GTCGGTTGCTCCTTGGAGTCGGGTTGCCTGAGAACGCAGCCTGAATTGGGTGGTAAACTCCAT CTAAGGCTAAATAGTACTGCGAGTCCGATAGCAAACAAGTACCGTGAGGGAAAGTTGCAAAG AACTTTGAAGAGAGAGAGTTCAAGAGTGCGTGAAACCACTGGGATGGAAACGGATAGAGTTGAC GAATTGGGCGATATTCAGCTGTTTTGCGTGAGCAAAGTGGTGTACTTATCGTCTGTGTGCGCT GAGAGTCTTGATTGAACATTCTAAACCGTCGTATTTTGTTGCCCGTCGTCTCACGACGATGGTG TCTTGTGCGTGCGGGGTTGGGATGTTTCGGTTAAGTATTTTCGGTGTGAAAGTCGACCACCTATC CGACCCGTCTTGAAACACGGACCAAGGAGTCTAGCGTATGTGCGAGTCATTGGGTGGTAAAC CTATTGGCGTAACGAAAGTAAAGGTCGTTTCTTGCGGCTGATATGGGATCCGTGCGGTTTCGA TCGTGCGGCGCACCATAGCCCTGTCTCGAAGGCTTGCCTTGAGATGGAGGTAGAGCGCATGCG CTAGGACCCGAAAGATGGTGAACTATACGTGAGCAGGATGAAGCCGGAGGAAACTCTGGTGG AAGTCCGTAACGGTTCTGACGTGCAAATCGATCGTCTGACTTGCGTATAGGGGCGAAAGACTA ATC

#### Haematoloechus medioplexus

GGCGAGTGAACAGGGATAAGCCCAGCACCGAAGCCTGTGGCCATTTGGTTACTAGGCAATGT GGTGTTTAGGTCGTTCCGCGGAGGTTCTGCTCCACCCTAAGTCCATCAATGAGTACGGTATTAT GGACATGGCCCACAGAGGGTGAAAGGCCCGTGGGGGGTGGAGATTCGGTAGGCCAGAACTTCC CAAGGCTAAATACTTGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAGTTGAAAAGT ACTTTGAAGAGAGAGAGTAAACAGTGCGTGAAACCGCTCAGAGGTAAACGGGTGGAGTTGAACT GCAAGCTCTGGGAATTCAACTGGTGAGTGTGGGTTTGAGCTTGGTCATATTGGTGGACGTCGGG GTCTGCGTAGAAGCAGGTCTTCGCCTTCGGGTGGGGGATGCGCGATGCACTTATCAAGTGTTGT GCGCCTCGGTTGTTCCCGGCCAACTCGCTAGTGCACTTTCTCAGAGTGGTCACCACGACCGGC ACCGCTGTTTGGCCACTGTGGTTAAACCGAGTTTGCATAGTCCGAGTGGCTTTGTAAGGTCGG GATGGCAGGTAGCTCGCTGACTCTTCTGTGGTTTCGCCATAAGTGTTGTCTTCAAGTCTAATCC GCTGACCGTGGTGGTTCTGTGCAGTGTGTCGGGGGACGGCGGCTCGAGGTGTGTGCATGCGTTG CTGTTGTGCTGACTGGTCCGAGTGTGGTTATTTTGTAGCCTGTTCAATCAGGTCTGTTAGTGGC TCGGATTTGTTCGGCTGACGCTGGCGTGTGTGGCACTGTTCCAAGGGCCAACAGTCTGTGGTG TAGTGGTAGACGATCCACCTGACCCGTCTTGAAACACGGACCAAGGAGAGTAACATGTGCGC GAGTCATTGGGCCCTACGAAACCCAAAGGCGAAGTGAAAGTAAAGGTCTGACTTGTTCAGGC TGAGGTGAGATCCAGTCGTTTCTCACGCGTGGTACTACCAAGCATCGAGCGGCTGGCGCATCA TGTTGAGACCCGAAAGATGGTGAACTATGCTTGCGCAGGTTGAAGCCAGAGGAAACTCTGGT GGAGGACCGCAGCGATTCTGACGTGCAAATCGATCGTCAAACGTGAGTATAGGGGGCGAAAGA CTAATCGAACCATCTA

## Haematoloechus medioplexus

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## Haematoloechus varioplexus

GACTGGTTCGAGTTTGGTTATTTTGTTGCCTGTTCATGCAGGTCTGGTAGTAGCTCGGATTAGT TCGGCTGGCGACTGCGTGTGTGGCATTGTTCCAAGGGCCAACAGTCTGTGGGTGTAGTGGTAGA CTATCCACCTGACCCGTCTTGAAACACGGACCAAGGAGAGTAACATGTGCGCGAGTCATTGG GCTGTACGAAACCCATAGGCGAAGTGAAAGTAAAGGTCTGACTTGTTCAGGCTAAGGTGAGA TCCGGTCGTTTCTCACGCGTGGTACTACCAAGCATCGAGCGGCGGCGCGCATCACCGGCCCGTC CCATGGCAGTTGTTTACCGACAGTTTTCGGTCGGGGCGGAGCATGAGCGTACATGTTGAGACC CGAAAGATGGTGAACTATGCTTGCGCAGGTTGAAGCCAGAGGAAACTCTGGTGGAGGACCGC AGCGATTCTGACGTGCAAATCGATCGTCAAACGTGAGTATAGGGGCGAAAGACTAATCGAAC C

### Ochetosomatidae sp.

GATTCCCCAGTAACGGCGAGTGAACAGGGAAGAGCCCAGCACCGAAGCCTGTGGCCATTTGG TTACTAGGCAATGTGGTGTTTAGGTCGTTCCGCAGAAGTGCTGCTCCACCCTAAGTCCATCAA TGAGTATGGTAGTATGGACATGGCCCATTGAGGGTGAAAGGCCCGTGGGGGTGGAGCGTCGA TTGGTCAGTACTTCTCTGGGTAGACCTTGGAGTCGGGTTGTTTGAGAATGCAGCCCAAAGTGG GTGGTAAACTCCATCCAAGGCTAAATACTAGCACGAGTCCGATAGCAAACAAGTACCGTGAG GGAAAGTTGAAAAGTACTTTGAAGAGAGAGAGAACAGTGCGTGAAACCGCTCAGAGGTAAAC GGGTGGAGTTGAACTGCAAGCTCTGAGAATTCAGCTGATGAGTGTGTTTTGAGCTTGGTCAAT TTGGTGTACTCCGGGGTCTGTGTAGTAGCAGGTCGTCACCTTCGGGTGGTGATGCGCGATGCA CTTATCAAGTGTTGTGCGCTCCGGGTGTTCTTGAACCAACTTGTCAGTGCACTTTCTCAGAGTG GTCACCACGACCGGCGCTGCCGTCTGGCTGCTTTGGTTAAACCGGCTTCGCATAGTCCTTGTG TTTTCGAGTGTAATCAGCTGACCAGAGTGGTTCGGTGCGGTGTCGGAGACGGCGGCTTGTG GTGTGTGCATGCGTTCTGTTCGCTGACTTGTCCGAGTTTGGTTATTATGTTGCCTGTTTAAACA GGCCTGGTGATGGCTCGGATTTGTTCGGTGGGCGGCTGCGTGTGTGGCACTATTCCAAGGGCC AATAGTCTGTGGTGTAGTGGTAGACTTTCCACCCGACCCGTCTTGAAACACGGACCAAGGAGA GTAACATGTGCGCGAGTCATTGGGCGTTACGAAACCCAAAGGCGCAGTGAAAGTGAAGGCTT GACTTGTTCAGGCTGAGGTGAGATCCTGTCGTTTCTCACGCGCGGTACTACCAAGCATCGAGC GGCAGGCGCATCACCGGCCCGTCCCATGGCATTTGTTTTCAAACAGTTTTCAGTCGGGGCGGA GCATGAGCGCACATGTTGAGACCCGAAAGATGGTGAACTATGCTTGCGCAGGTTGAAGCCAG TAGGGGCGAAAGACAATCGAAC

#### Fibricola sp. #1

CCAGCACTGAGTATGGCAACTGGAATGGCCCAAGGAGGGTGAAAGGCCCGTGGGGGGTGGAG AAGCGGGTGGTAAACTCCATCCAAGGCTAAATACTAGCACGAGTCCGATAGCGAACAAGTAC CGTGAGGGAAAGTTGAAAAGTACTTTGAAGAGAGAGAGAACAGTGCGTGAAACCGCTCAGAG GTAAACGGGTGGAGTTGAACTGTAAGCTCCGGGAATTCAGCTGGTGAGTGTGTCATGGGCTTG GTCATTTTCGGCCGGCCTCTAGAGTCCGCTTAGCTGCGGGTCCTTGCTTCTNCGGGAGTAGGG CTTTCTCGGAGTGGTCACCACGACCGGCGTTGTTGTCTTGCTGCTGGTCGAACCGGCTGGGT TGGGTGTAATCAGCTGTCTGCAGCGGTATTGTGCAATGCGTCGGAGACTGCGGCTTTAGATAT TTGCTTTTGTGCCGTTGGCCGGCAGTGTTGAGTTTGACTGGCGTGTTACTCGCTCCGGTGGGTC CGTCGGTAGCTCAGTGCTGTTCGGTTAGCGGTTGCTTGAGTGATATCGTACATGGGCCAATAG TCTGTGGTGTAGCAGCAAACGATCCACCTGACCCGTCTTGAAACACGGACCAAGGAGTTTAAC ATGTGCGCGAGTCATTGGGCGTTACGAAACCCAAAGGCGCAGTGAAAGTAAAGGTTCGGCTT GTCCGAGCTGAGGTGAGATCCTGTAGCTTCTCGTGCGAGGTACCACCAAGCAGTGAGCTATAG GGAGCCTGAGCGCACACGTTGAGACCCGAAAGATGGTGAACTATGCTTGCGCAGGTTGAAGC GTATAGGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT'ITC

### Fibricola sp. #2

TATGGCATCTGGAGTGGCCCATGGAGGGTGAAAGGCCCGTGGGGGATGGAGATCAAGTCGGAC AGTTTTGCCCTGAGTAGACCTTGGAGTCGGGTTGTTTGTGAATGCAGCCCAAAGCGGGTGGTA AACTCCATCCAAGGCTAAATACTAGCACGAGTCCGATAGCGAACAAGTACCGTGAGGGAAAG TTGAAAAGTACTTTGAAGAGAGAGAGTAAACAGTGCGTGAAACCGCTCAGAGGTAAACGGGTGG AGTTGAACTGTAAGCTCCGGGGGATTCAGCTGGTGAGTGTGTCATGGGCTTGGTCATTTCGGC CGGCCTCTGGAGTCCGCTTAGCTGCGGGTCCTTGCTTCTTCGGGAGTAGGGATGTGCGTTGCG GGTCACCACGACCGGCGTTGTTGTCTTGCTGCTGTAGTCGAACCGGTCAGGTTTGGTCCTTGTG GCTGAACTTGATCGGGATGGCAGGTAGCTCATTGGTTGGCTTGTCCAATCTTTGGGTGTAATC AGCTGGCTGCAGTGGTTATGTGCAATACGTCGGAGACGGCGGCTTAAGGTATGTGCTTGTGTG CCGTTGGCTGGCAGTGTCGGGGTTTGACTGGCGTGTTTACCTACTTCGGTAGGTCCGTTGGTAGT TCGATGCTGTTCGGTTAGCGGTTGCATTTGTGATACCGCTCATGGGCCCATAGTCTGTGGTGTA GCAGCAAACGATCCACCTGACCCGTCTTGAAACACGGACCAAGGAGTTTAACATGTGCGCGA GTCATTGGGCGTTACGAAACCCAAAGGCGCAGTGAAAGTGAAGGTTCGGCTTGTCCGAGCTG CCAGCCCGTCTCATGGTGTGGTCATGTAGCCTTGTGTTGCGTGCATCACCGGGGCGGAGCCTG AGCGCACATGTTGAGACCCGAAAGATGGTGAACTATGCTTGCGGAGGTTGAAGCCAGAGGAA GCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGG

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