

THE “FATHEAD MINNOW FACTORY”: EXPLORING HOW A CHANGING CLIMATE
HAS INFLUENCED FISH AND SALAMANDER COMMUNITIES IN THE PRAIRIE
POTHOLE REGION

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Kyle Ian McLean

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Communities in the Prairie Pothole Region

By

Kyle Ian McLean

The Supervisory Committee certifies that this *disquisition* complies with
North Dakota State University’s regulations and meets the accepted
standards for the degree of

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SUPERVISORY COMMITTEE:

Dr. Craig Stockwell
Chair

Dr. David Mushet

Dr. Mark Clark

Dr. Peter Bergholz

Approved:

5/19/2015

Date

Eakalak Khan

Department Chair

ABSTRACT

Global climate change has been linked to changing many ecosystem processes. Early literature on climate change and biological systems predominately focused on individual species responses to temperature gradients. However, altered precipitation patterns can impact the ionic concentrations of aquatic habitats and thus affect the structure of entire communities. Understanding indirect effects of climate change, will be important to predict how whole systems have and will continue to change. Prairie pothole wetlands are well suited to study these processes. Prairie pothole wetlands are typically closed systems with natural hydrological fluctuations that have molded plant and wildlife communities adapted to these changing environments. However, a 20-year wet climate cycle has increased the permanency of many waterbodies facilitating colonization of various fish species, including the fathead minnow (*Pimphales promelas*). Thus, it is important to understand the environmental and biological aspects of prairie pothole wetlands facilitate fish presence under current and projected climate cycles.

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CHAPTER 1. INTRODUCTION

1.1. Prairie Pothole Wetlands

1.1.1. Prairie Pothole Region Characteristics

The Prairie Pothole Region (PPR) covers approximately 777,000 square kilometers spanning from south-central Alberta east to southwest Manitoba and south to northcentral Iowa in the United States (Figure 1.1; Smith et al 1964). This region was formed during the retreat of Pleistocene epoch glaciers 14,000 years ago that left behind an estimated 5-8 million depressional wetlands (Dyke and Prest 1987). These wetlands, commonly referred to as “prairie potholes”, are typically endoheric and have hydrologic regimes ranging from temporary and seasonally flooded to permanently flooded depending on their topographic location along groundwater gradients and climactic conditions (Euliss et al 2004). The water characteristics of prairie potholes vary in dissolved ion concentrations from very fresh to highly saline (Sloan 1972). Prairie potholes also retain surface water runoff, capture sediment deposits, alleviate flooding, and provide highly productive wildlife habitats (Gleason et al 2008). Due to this productivity, the prairie pothole region is often referred to as the “duck factory” of North America and produces an estimated 50-80% of the continental breeding waterfowl (Batt et al 1989). In addition to waterfowl, prairie potholes also host other native fauna such as migrating and breeding waterbirds, aquatic invertebrates and amphibians (Sorenson et al. 1998), as well as non-native freshwater fishes.

1.1.2. Prairie Potholes and Climate Change

The climate of the PPR varies from cold and dry in the northwest to warmer and wetter in the southeast. Annual precipitation across this gradient ranges from 300mm (in the west) to 900mm (in the east). The north to south annual mean temperature gradient ranges from 1°C in the north to 10 °C in the south (Millett et al 2009). The PPR also experiences cyclic dry and wet periods resulting in drought years that can be immediately followed by years of above normal precipitation (Winter and Rosenberry 1998). For example, following a severe drought in the 1990's the PPR of North Dakota entered a record wet period from 1993 to 2012 that resulted in the highest wetland water levels in the last 500 years. The influx of freshwater input from this wet period has been shown to dilute dissolved ion concentrations (Winter and Rosenberry 1998). Responding to the increased precipitation inputs, Devil's Lake has recently experienced historic water-level increases leading to the dilution of dissolved ions (Figure 1.2; see also Covich et al 1997). The freshening of Devils Lake has provided the conditions supporting a large and popular fishery. Many other lakes in the PPR of North Dakota have experienced similar hydrological patterns, but the effects of these changes on fish and amphibian communities is relatively unknown. Climate change models suggest a future climate for the region consisting of both increased temperatures as well as more extreme wet/dry precipitation cycles (Millett et al 2009).



Prairie Pothole Region

Figure 1.1. Map of Prairie Pothole Region. Figure downloaded from: <http://www.ducks.org/conservation/prairie-pothole-region>

1.2. Fish in the Prairie Pothole Region

Fish would not have been native to most prairie pothole wetlands during pre-European settlement times due to frequent drying on most basins, high salt concentrations, and a lack of surface water connections (Peterka 1989, Anteau and Afton 2008). Prior to the last wet period, some larger prairie potholes were found to host populations of small-bodied fishes such as fathead minnows (*Pimephales promelas*) and brook sticklebacks (*Culaea inconstans*). These basins typically lacked larger bodied fishes including gamefish (Peterka 1989, Zimmer et al 2001). However, game fish have been stocked in many wetlands and may have invaded others due to increased connectedness of wetlands because of historically high water levels (Herwig et al 2010). The detrimental effects on macro-invertebrate communities, native amphibian species,

waterfowl, and water quality (Zimmer et al 2001, Hanson and Riggs 1995, Maurer et al 2014).

Thus, understanding how climate change facilitates or limits fish communities in prairie pothole wetlands will be important in identifying how to manage these important habitats under current or future climate scenarios.

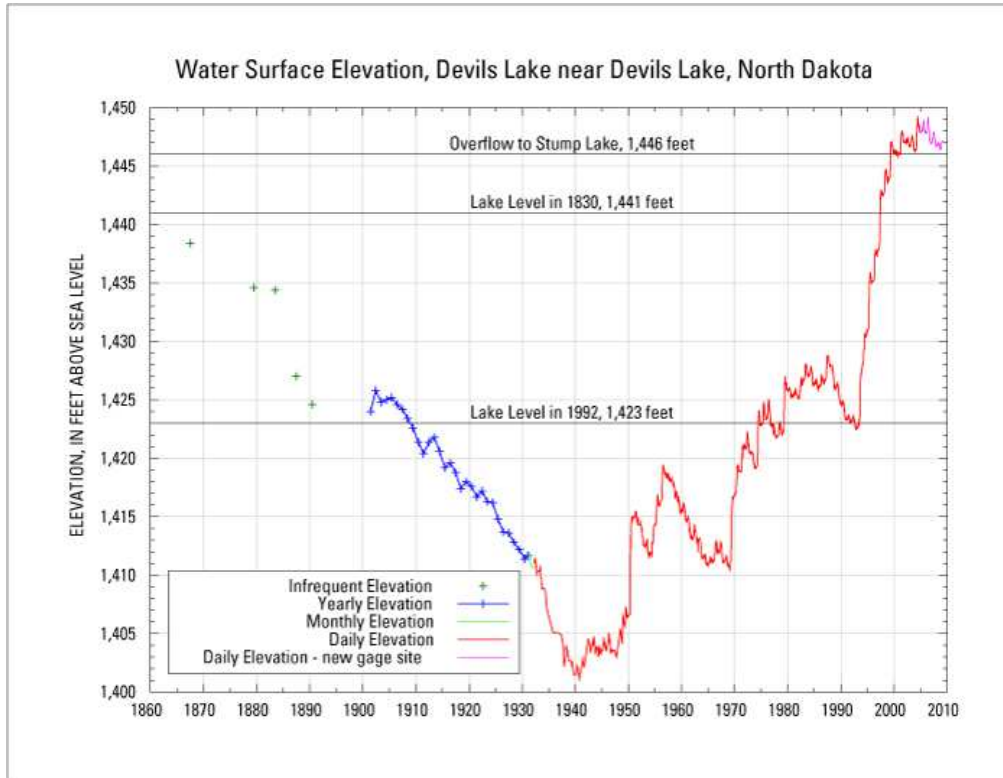


Figure 1.2. Water Level Fluctuations of Devils Lake North Dakota from 1870 to 2010. Figure downloaded from: <http://nd.water.usgs.gov/devilslake/images/DLPOR.gif>

1.3. Organization of Thesis

This thesis consists of three chapters including a general introduction (chapter one) and two chapters that report the results of original research conducted for this thesis. Following the introductory chapter, chapter two of this thesis focuses on fish and salamander communities in the PPR in south central North Dakota. The chapter has two main components. First, fish and

salamander communities are evaluated in relation to gradients in dissolved ion concentrations. The second component uses inferred correlations of species to dissolved ion concentrations to hypothesize about how communities might have changed in response to reduced ionic concentrations as compared to conditions present during an earlier survey of dissolved ion concentrations that existed in the waterbodies in the 1960's-1970's. Chapter three is a descriptive study of cannibalistic morph tiger salamanders found during data collection for chapter two. This chapter gives a brief background of cannibalistic morph tiger salamanders and a detailed account of four populations I discovered in the PPR of North Dakota. The conclusions identify unique properties of the North Dakota populations and a possible explanatory hypothesis that seeks to provide a better understanding of this rare polymorphism.

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CHAPTER 2. INFLUENCE OF CLIMATE CHANGE ON FISH AND SALAMANDER COMMUNITIES IN PRAIRIE POTHOLE LAKES¹

2.1. Abstract

The climate of the Prairie Pothole Region of the United States has historically cycled between wet and dry periods, each period typically lasting five to ten years. However, over the last 22 years (1993-2015), the region has experienced an extended wet period resulting in record water levels in most of the region's lakes and wetlands. We explored effects of this extended wet period on regional fish and salamander communities in 162 wetlands and small lakes previously sampled in the 1960s-70s. Ionic concentrations were considerably lower than previously recorded, largely the result of dilution from increased water volumes. In combination with increased water depths, these chemical changes have allowed fish to populate many previously fishless lakes. Non-metric multidimensional scaling (NMS), principal component analysis (PCA), and bootstrap regressed weighted averages revealed that fathead minnows (*Pimphales promelas*), tiger salamanders (*Ambystoma mavortium*), and brook sticklebacks (*Culaea inconstans*) all occurred across a broad range of the current chemical concentrations. By contrast, yellow perch (*Perca flavescens*) occurred in a much smaller, chemically defined, subset of lakes, and Iowa darters (*Etheostoma exile*) were restricted to lakes with very specific combinations of salinity, pH, and dissolved ions. Fish and tiger salamander abundances were inversely correlated with each other. Thus, it appears that fish restrict salamander abundances in fresher habitats and that salamander's only flourish at sites where higher salinities exclude fish.

¹This material in this chapter was co-authored by Kyle I McLean, David M. Mushet, and Craig A. Stockwell. Kyle McLean had primary responsibility for collecting samples in the field, data analysis, and developer of conclusions. Kyle McLean also drafted and revised all versions of this chapter. David Mushet and Craig Stockwell served as proofreaders and checked the math in the statistical analysis conducted by Kyle McLean.

Yellow perch were present in only 9 waterbodies during the 1960s-1970s sampling period whereas in the 2012-2013 sampling period we identified 54 waterbodies with perch present. The dissolved ion concentrations of the waterbodies recently colonized by yellow perch populations had become significantly fresher compared to the earlier sampling period. A better understanding of relationships between biotic communities and abiotic processes in prairie pothole wetlands and lakes is needed to facilitate informed management responses to changing climate conditions.

2.2. Introduction

While global climate change effects on individual species have received much attention, community-wide effects remain relatively unexplored (Gilman et al. 2010, Walther 2010). However, aquatic community structure is likely to be affected by changes in ionic concentrations driven by global changes in spatial and temporal precipitation patterns (McCarty 2004; Williams 1998; Winter and Rosenberry 1998; Derry et al. 2003). Current knowledge of ionic concentrations effects on aquatic communities has mainly come from cases in which concentrations have increased due to reductions in water volume (Galat et al. 1983; Dickerson and Vinyard 1999). For example, Walker Lake, Nevada experienced continual declines in water volume and concurrent increases in total dissolved solids (TDS) from 2,000 mg/L in 1880 to 13,000 mg/L by 1995 (Dickerson and Vinyard 1999). The TDS increases of Walker Lake resulted in the extirpation of all non-native fish species, two native cladocerans, and cessation of reproduction by native Lahontan Cutthroat Trout (*Oncorhynchus clarki henshawi*; Cooper and Koch 1984, Stockwell 1994, Dickerson and Vinyard 1999). Devils Lake located in east-central North Dakota is another closed-basin lake that has experienced historic changes in ionic

concentrations. In the 1890s, Devils Lake was a large and deep lake with a ferry service and commercial northern pike (*Esox luciosus*) fishery (Young 1924); by 1907 the water level in Devils Lake had receded enough that rising TDS concentrations excluded all fish species except the brook stickleback (*Culeae inconstans*) (Young 1923, 1924). Devil Lake's TDS concentrations increased to 17,500 mg/L by 1919 (Pope 1909, Nerhaus 1920), approaching the upper limit of brook stickleback survival (Scott and Crossman 1973). A continued decline of the lake's water level resulted in TDS concentrations as high as 25,000 mg/L by 1948 (Swensen and Colby 1955). However, beginning in 1967, the lake's water level increased only to decrease again in the early 1990's. This most recent decrease was followed by a long-term increase from 1993 onward to the present. By 2010, Devils Lake reached a historically high level and continued to increase until 2011. Devils Lake has received much attention due to its fishery resources and property damages caused by rising water; however, similar changes occurring in the numerous smaller water bodies occurring throughout the region have gone largely unstudied.

The Prairie Pothole Region (PPR) in North Dakota has experienced a pattern of increased precipitation that in some areas has persisted over the last 22 years. Increased precipitation has resulted in record high water depths and associated diluted dissolved ion concentrations in most of the region's waterbodies. This prolonged wet period has also caused water in many basins to spill into lower-elevation basins (e.g., Leibowitz and Vining 2003), further influencing ion concentrations through overland flows either into or out of a basin. Altered habitat conditions resulting from increased water volumes and associated shifts in ion concentrations are likely to have significant effects on biotic communities.

Historically, lack of overland flows, fluctuating water depths, and high dissolved ion concentrations made most PPR lakes and wetlands unsuitable for fish (Peterka 1989, National

Research Council 1995, Anteau and Afton 1989). The historic lack of fish has contributed to the high invertebrate abundances characteristic of these aquatic habitats. High invertebrate abundances are important because of their value as a food source for breeding and migrating waterfowl; the PPR produces 50-80% of the North American waterfowl population (Batt et al 1989).

To explore if recent changes in the water volumes and ionic composition have impacted vertebrate communities, we resurveyed a set of wetlands and small lakes that were sampled from 1966-1976 by Swanson et al. (1988). This earlier period was characterized by significantly lower precipitation and water-levels. We identified correlations of fish and salamander presence and abundance to contemporary dissolved ion concentrations. Using these data, we compared changes in dissolved ions between the two sampling periods and related differences to observed changes in fish and salamander distributions and abundance. We also examined how dissolve ion concentrations may mediate biotic interactions (competition and predation), further shaping community structure in aquatic habitats of the PPR.

2.3. Study Design and Data Collection

2.3.1. Objectives

- (1) Quantify current presence and abundance of fish species in PPR lakes and wetlands along ionic concentration gradients to identify species-specific thresholds
- (2) Compare historic and contemporary fish distributions in PPR lakes and wetlands and relate observed patterns of occurrence to abiotic thresholds

(3) Identify how changes in fish distribution patterns in PPR wetlands have influenced other biotic communities (e.g., amphibians)

2.3.2. Study Area

We conducted our study in Stutsman County and Kidder County, North Dakota within the PPR of North America (Figure 2.1). These two adjacent counties span a diversity of physiographic and geologic features, all influenced by reoccurring glaciation events. The most recent glaciers receded approximately 14,000 years ago (Bluemle 1972). The retreat patterns varied spatially leading to the physical geography underlying two level IV ecoregions; the Missouri Coteau and the Drift Prairie (Omernik 1987). The Missouri Coteau was formed due to an uneven retreat of glaciers and is characterized by a hummocky knob-and-kettle landscape consisting of thick superglacial drift dotted with numerous depressions formed where buried ice blocks melted and overlying glacial till collapsed into the resulting voids. The other ecoregion is an undulating plain of low-relief ground moraine known as the Drift Prairie. The Drift Prairie was formed where glaciers retreated at a fairly even rate. Across both ecoregions, numerous wetlands and small lakes exist, and differences in the topography, depositions of till, areas of sand and gravel outwash, and thickness of glacial drift contribute to the diverse nature of the landscape and its embedded lakes and wetlands.

The climate of the PPR is highly variable with cold long winters (down to -40 °C) and hot summers (up to 40 °C) and an annual precipitation ranging from 30cm in the western portion to 90cm in the eastern portion. Annual temperature and precipitation vary with the normal wet and dry cycles the region exhibits (Winter and Rosenberry 1995). Climate projections for the SE

prairie pothole region predict an increase of 4 °C and increased precipitation over the next 100 years (IPCC 2007) with an anticipated increase in extreme drought and wet periods (Johnson et al 2004). In fact, average annual precipitation in the region has already increased at a similar rate as predicted global changes (Millet et al 2009).

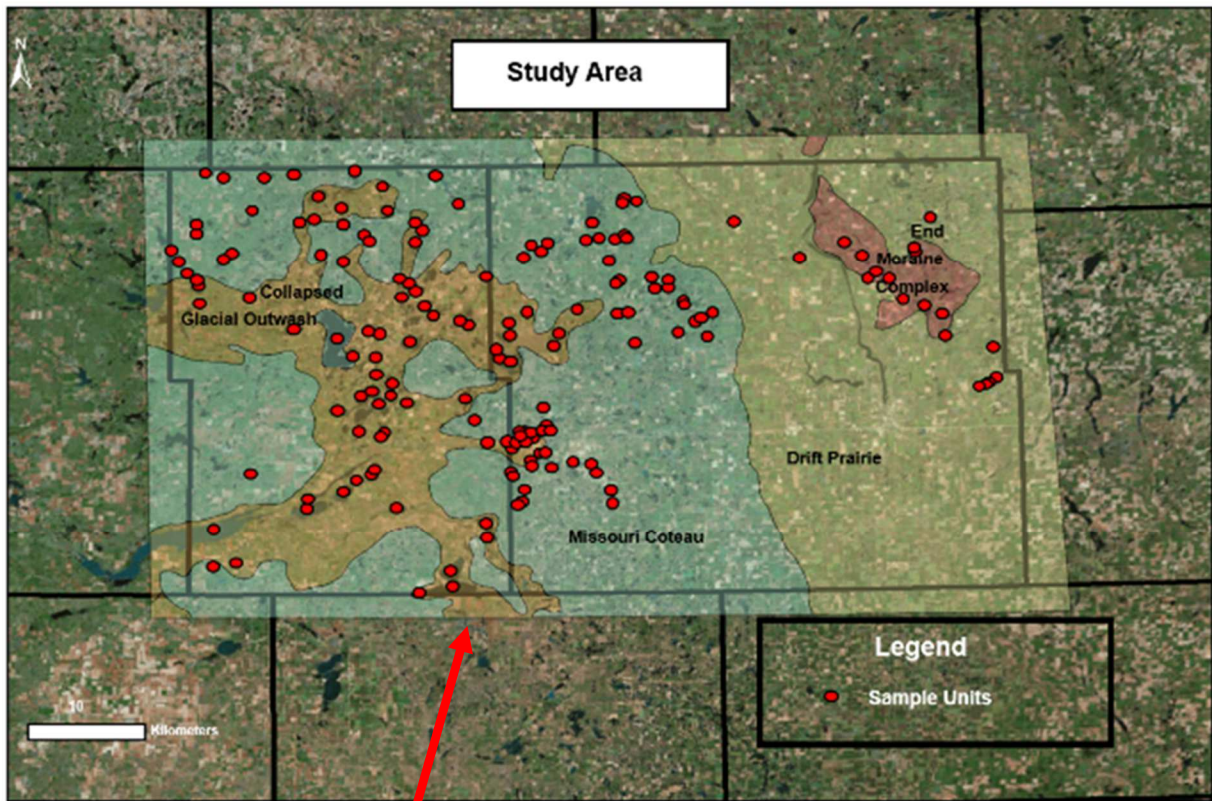


Figure 2.1. Map of 162 waterbodies sampled for dissolved ion concentrations and fish/salamander communities during the summers of 2012-2013 in Stutsman and Kidder County North Dakota. Omernik's level IV ecoregions are highlighted within the study area; Drift Prairie = olive green, Missouri Coteau = blue-green, collapsed glacial outwash = orange.

In 1966-1976, 178 lakes and wetlands in Stutsman and Kidder Counties (Figure 2.1) were sampled to quantify chemical characteristics of prairie lakes and wetlands, and to identify potential influences on fish and other wildlife communities (Swanson et al. 1988). The water bodies selected in this early effort spanned the physiographic and geologic diversity of the Missouri Coteau and the drift plain across the two counties. In 2012 and 2013, we resampled 162 of these water bodies (90%) in an effort to evaluate how altered climactic conditions have influenced water chemical characteristics. The lakes and wetlands that were not sampled were sites for which landowners were unreachable or access permission was denied. Concurrent with the chemical sampling, we quantified fish and salamander communities within each of the revisited lakes and wetlands to evaluate correlations of water chemical characteristics to the aquatic vertebrate communities.

2.3.3. Vertebrate Sampling

We sampled fish and salamander communities in each water body once over the course of two summers (2012 to 2013) during the months of June, July and August. The order in which lakes and wetlands were sampled was adjusted to approximate the seasonal order in which the lakes and wetlands were originally sampled (Swanson et al. 1988). Sampling was conducted at a location on the lake or wetland that had both accessibility to shoreline and landowner permission.

At each site, we placed 7 aquatic vertebrate funnel traps (Mushet et al. 1997) 30 m apart along a transect parallel to the shoreline at a 1-m water-depth contour. The 2-m driftnet and opening of each trap was oriented parallel to the shoreline (Figure 2.2). The traps were set in the

morning and retrieved 24 hours later. Upon retrieval, all individual fish and salamanders captured were identified, enumerated, and released back into the water in a timely manner to reduce stress and harm to individuals. Thus, trap data collected for each lake and wetland consisted of number of captures by species per 24-hour unit of effort. Since larger game fish might not have been as effectively sampled as small fish, we included fish presence information with game-fish stocking and lake survey information from the North Dakota Game and Fish.

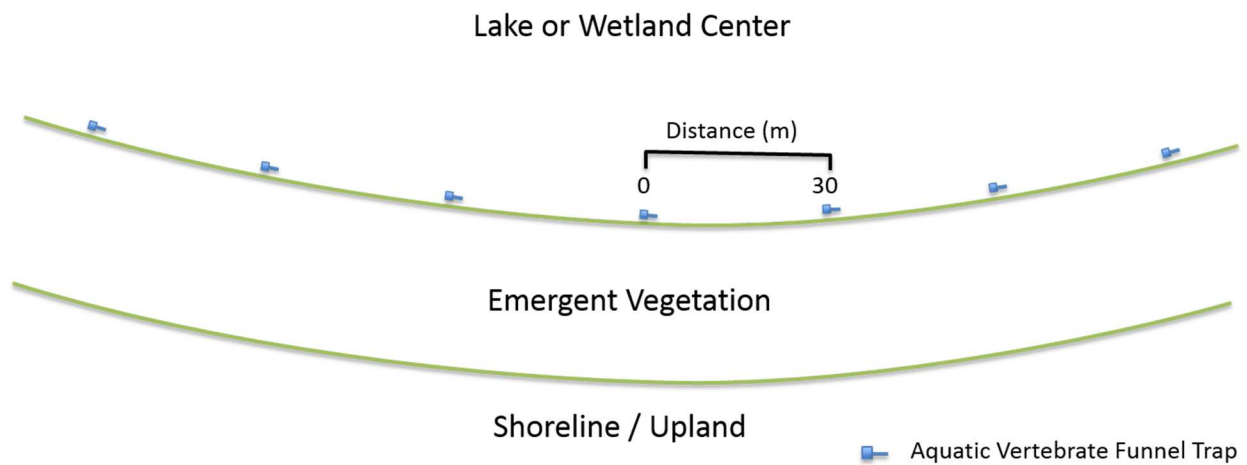


Figure 2.2. Schematic of transect design for aquatic vertebrate funnel trap placement used in each water body sampled (N=162).

2.3.4. Environmental Sampling

We collected a single water sample from the center of each lake and wetland, or outside (i.e., towards the lake or wetland’s center) of the deep-marsh zone as defined by Stewart and Kantrud (1971) at the 1.5 m depth contour, whichever was shallower. A tube-type water sampler (Swanson 1978a) was used to collect water samples so that the water collected was depth integrated (i.e., representative of the overall water column not just a single depth interval). All water samples were stored on ice in the field and then refrigerated until being shipped to the

USGS water analysis lab in Denver, Colorado for analyses of major and minor anions, cations, and alkalinity. At each site, we also measured water pH, specific conductance, temperature, and turbidity. A detailed description of water sample analysis techniques can be found in Mushet et al. (In Review).

2.4. Data Analysis

We used a multivariate analyses approach to explore how aquatic vertebrate communities correlate directly and indirectly with ionic composition of PPR lakes and wetlands. All statistical analysis was conducted using the R statistical programming software (version 2.9.2; R Core Development Team, Vienna, Austria). Most of the analyses were used unconstrained ordination techniques such as Principal Components Analysis (PCA) and Nonmetric Multidimensional Scaling (NMS) to look for correlative patterns. The use of ordinations on the water chemistry and species variable matrices allowed us to visualize sample unit variable composition in reduced dimensionality space (Bruce and McCune 2002). We identified directionality and magnitude of correlations by vector fitting predictor variables to the ordinations (Faith and Norris 1989). Since dissolve ion composition variables were normally distributed, PCA was used as a linear approach for the ordinations. NMS was used with the non-normally distributed fish and salamander community data. For the NMS we removed from analysis two lakes that did not contain any vertebrate species during the sampling giving us a total of 160 lakes for the analysis.

The analyses were completed in an exploratory framework conducted in four steps. The first step was to use NMS and vector fitted linear models to identify correlations of dissolved ion concentrations to NMS ordination of fish and salamander communities. Fish and salamander

species presence in PCA generated dissolved ion space were used to identify additional patterns in species/environment relationships. The second step utilized bootstrap regressed weighted averages and maximum abundances along dissolved ion gradients. This is a way to calculate an estimated niche breadth for species abundances along environmental gradients. The third step used PCA to compare dissolved ion composition in 162 waterbodies from two sampling periods 1966-1976 and 2012-2013 to identify how the ionic chemistry of the lakes have changed over time, thereby allowing us to hypothesize how fish and salamanders communities might have changed as well. The final step used historic and present dissolved ion concentrations and yellow perch (*Perca flavescens*) presence/absence information from the two sampling periods to create PCA ordinations and explore how perch presence responded to ion changes and test if perch presence/absence during the two time periods corresponds with our dissolved ion threshold findings.

2.4.1. Vertebrate Community Patterns

To explore how aquatic vertebrates were correlated with ionic concentrations and community composition, we utilized PCA on the water chemistry variable matrix and NMS on the species composition matrix. We explored species/environment correlations using two similar methods; first we identified how biotic and abiotic characteristics are correlated to species space ordinations. Then we identified where species occurred along ionic composition gradients. Before running the species space ordination, species occurring in <10% of the lakes were removed from analysis. An NMS model was performed on the species and water chemistry matrices and vector fitted with the species and chemistry variables using the function *envfit* in

the *vegan* package. Vector fitted variables were normalized with *Wisconsin Square Root double standardization* which is the default transformation in the *metamds* function in *vegan*. For the dissolved ion concentration PCA plots, individual sample units were scaled and centered and rotated to the direction of the variables contributing most of the variation. Species presence was highlighted in ionic composition PCA plots to visualize where they occur along the gradients. Pearson's correlation coefficients were conducted to look at within group correlations to the distance matrix (Bray's Distance Measure) for the species variables.

2.4.2. Niche Breadth along Ion Concentration Gradients

We identified niche breadth and optimal species abundance using bootstrap regressed weighted averages, abundance maxima, and range of occurrence of fish and salamander abundances along SO_4 and total alkalinity gradients (King et al 2012). SO_4 and total alkalinity were chosen as environmental gradients due to their significant correlation to the NMDS axis and being slightly orthogonal in the PCA components (see results). Additionally, previous work has shown SO_4 and alkalinity being the most toxic components of dissolved ion concentrations to fish in the prairie pothole region (Held and Peterka 1974, Koel and Peterka 1995, McCarraher and Thomas 1968). The weighted average equation for each species in every sample unit was $\text{sum}(y*x)/\text{sum}(y)$ with y being the species abundance and x being the environmental gradient value. Species abundance maxima distinguished the values along the environmental gradients that corresponded to the highest abundances of each species. We used 1,000 bootstraps to establish confidence intervals for the abundance maxima and weighted average (Manly 1997,

Bressler et al 2006). Bootstrapped weighted averages and abundance maxima were computed with the custom functions *wa.boot* and *sppmax.boot* in R 2.9.2 (King et al. 2006).

2.4.3. Ion Concentration Shifts between Sampling Periods

To visualize how dissolved ion concentrations have changed in 162 lakes previously sampled in 1966-1976, we utilized PCA on nine dissolved ion variables for each lake and sampling period using the *prcomp* function from the *stats* package in R (version 2.9.2; R Core Development Team, Vienna, Austria). The groups were highlighted to visualize variation within and between groups and plotted with *ggbiplot* in the *ggplot2* package. We used permutational multivariate analysis of variance using euclidean distance matrices (*Adonis*) from the *vegan* package to test for a significant differences in dissolved ion concentrations between the two sampling periods.

2.4.4. Ionic Composition Shifts and Yellow Perch Presence

Yellow perch was the only fish species quantitatively assessed in the original 1966-1976 sampling. Therefore, we used this species to explore colonization by fish among the historic and current sampling periods. Yellow perch have been shown to have strong correlations to SO_4 concentrations (Koel and Peterka 1995). We used historic and present water chemistry data for two PCA ordinations with four a priori groups highlighted using the *ggbiplot* function in *ggplot2*. The two PCA's followed by permutational multivariate analysis of variance using euclidean distance matrices (*Adonis*) were used to test 1) if ion concentrations of lakes containing perch

statistically differed between time periods and 2) if lakes with perch had statistically different dissolved ion concentrations then lakes where perch were absent.

2.5. Results

2.5.1. Vertebrate Communities Patterns

We captured a total of 12 species of vertebrates (Table 2.1), but the most commonly observed were fathead minnow (*Pimephales promelas*), brook stickleback, yellow perch, Iowa darter (*Etheostoma exile*), and tiger salamander (*Ambystoma mavortium*) (Table 2.1). Species occurring in <10% of the lakes (see species-Table 2.1) were dropped from our analyses. The species space NMS ordination (k=2, stress= .13) identified three inversely related vertebrate communities, each one dominated by a single species; yellow perch dominated; tiger salamander dominated, and fathead minnow dominated communities (Figure 2.3). The dissolved ion concentrations were vector fitted to the NMS ordination; the ions that make up salts were positively correlated (P-value<0.05) with tiger salamander communities and negatively correlated with Iowa darters (Figure 2.3). The species correlations to the distance matrix indicated that fathead minnows, tiger salamanders, and yellow perch all had strong negative correlations to each other (Table 2.2). The PCA using dissolved ion variables revealed that the first two components accounted for 83% of the cumulative variance in the PCA (Figure 2.4a). Thus, we used the first two components in the PCA bi-plot. The first component (PC1) was strongly correlated with concentrations of salts that influence specific conductance while the second (PC2) was strongly correlated with pH and calcium concentrations which represent the

wetland pH buffering system (Figure 2.4a, b). The correlations of dissolved ion variables to the principal components indicated that Na, Cl, and SO₄ were strongly co-correlated. Additionally, pH and calcium concentrations were inversely correlated with each other (Table 2.3b). To obtain a better spread of species presence on the PCA ordinated bi-plot (Figure 2.4b), we removed one waterbody with exceptionally high specific conductance (40,350 µS/cm); well beyond known threshold of local aquatic vertebrates.

Table 2.1. Summary statistics of the vertebrate species captures from 162 waterbodies sampled during the summers of 2012-2013 in Stutsman and Kidder Counties, North Dakota

	Proportion of Lakes Present	Mean Abundance	Total Captured
Fathead Minnow	0.825	2682	364065
Brook Stickleback	0.387	34	2127
Iowa Darter	0.112	16	222
Yellow Perch	0.337	85	4449
Tiger Salamander	0.356	54	3089
Northern Pike	0.031	1.2	6
Walleye	0.074	3.8	38
Smallmouth Bass	0.006	0.7	5
Bluegill	0.006	0.3	2
Common Carp	0.018	0.6	13
Black Bullhead	0.018	0.4	9

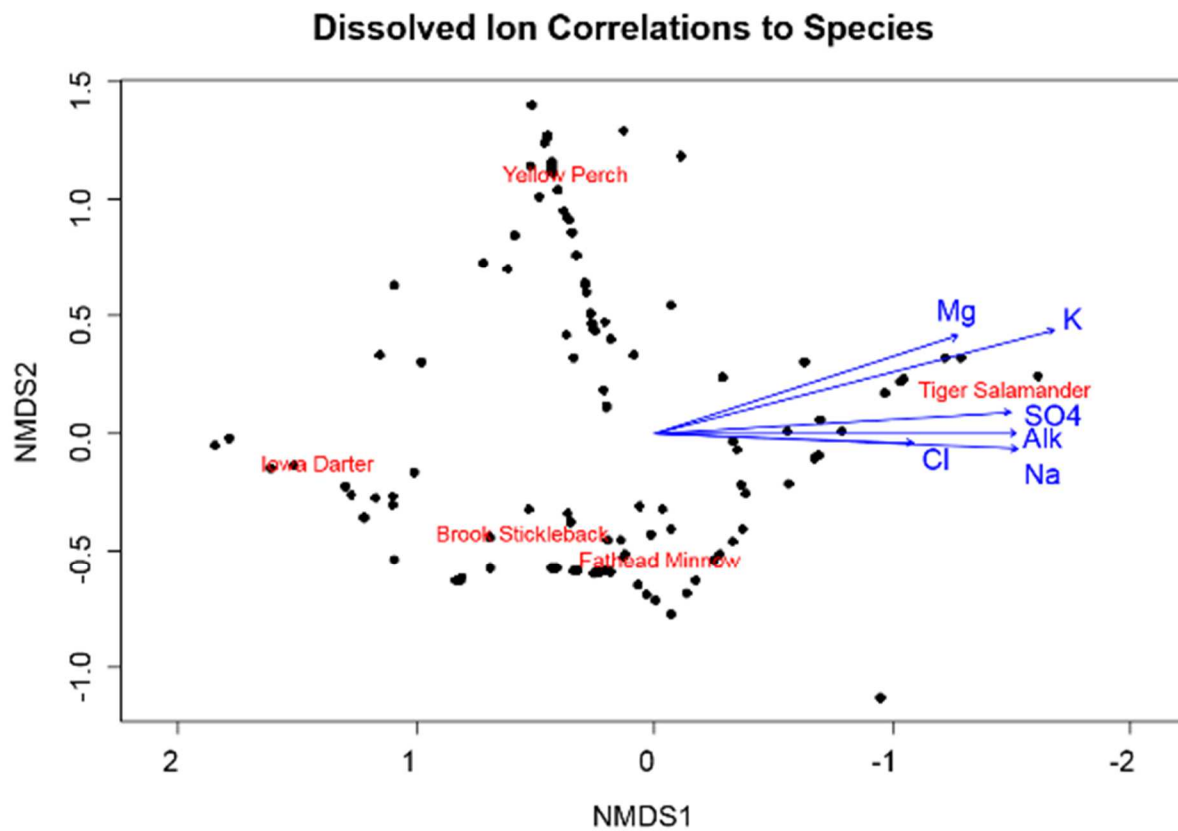


Figure 2.3. NMS ordination (k=2, stress=0.13) from fish and salamanders species abundance matrix of 160 waterbodies in Stutsman and Kidder Counties sampled in 2012 and 2013. Species names represent the species centroids. Blue lines and arrows are vector fitted linear models from the dissolved ion concentration matrix. Only significantly correlated (p-value<0.05) variables were plotted.

Table 2.2. Pearson’s correlations of fish and salamander species to distance matrix (bray) of species abundances in 160 waterbodies in Stutsman and Kidder counties, North Dakota, sampled in 2012-2013

Pearson’s Correlation	Fathead Minnow	Brook Stickleback	Iowa Darter	Yellow Perch	Tiger Salamander
Fathead Minnow	1				
Brook Stickleback	0.054	1			
Iowa Darter	-0.170	-0.005	1		
Yellow Perch	-0.336	-0.249	-0.143	1	
Tiger Salamander	-0.404	-0.247	-0.154	-0.297	1

Tiger salamanders occurred throughout the salinity gradient (after removal of the most saline lake; PC1) with most located on the more saline side of the axis (Figure 2.5a). Fathead minnows and brook sticklebacks also occurred throughout the salinity gradient and were the most prevalent species (Figure 2.5b, Figure 2.55c). Yellow perch occurrence was restricted along the PC1 axis (Figure 2.5d), and Iowa darters showed a tight grouping in the “fresher” lakes (Figure 2.5e).

Table 2.3. (a) Importance of principal components from current dissolved ion concentrations PCA for 2012-2013. (b) Importance of principal components from current dissolved ion concentrations PCA for 2012-2013

(a)	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Stand. Dev.	2.145	1.405	0.719	0.673	0.475	0.336	0.267	0.038
Proportion of Variance	0.580	0.246	0.064	0.056	0.028	0.014	0.008	0.000
Cumulative Proportion	0.580	0.827	0.891	0.948	0.976	0.990	0.999	1

(b)	PC1	PC2
Element		
pH	-0.04	0.57
Alk	-0.27	0.46
SO ₄	-0.45	-0.12
Cl	-0.43	-0.02
Mg	-0.37	-0.27
Ca	-0.02	-0.60
Na	-0.45	0.08
K	-0.45	-0.01

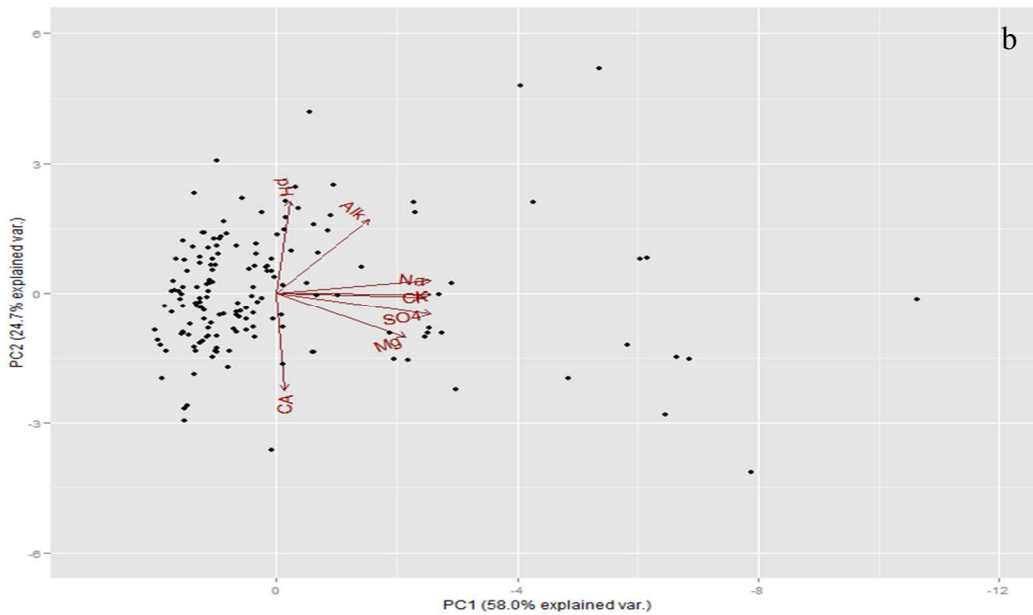
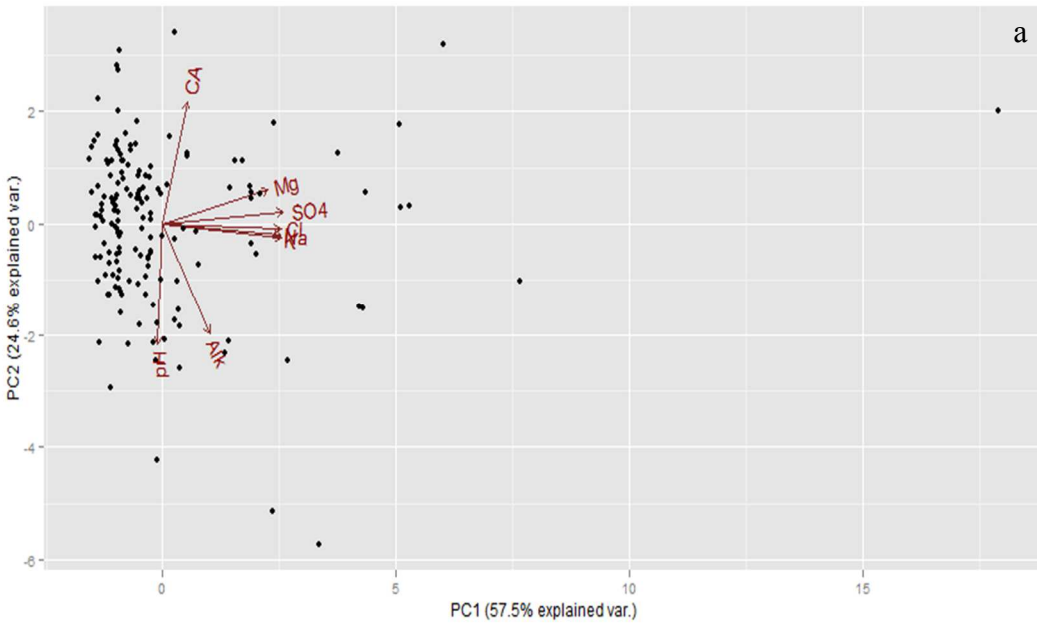


Figure 2.4. (a) PCA bi-plot representing PC1 (x-axis, 58% variance explained) and PC2 (y-axis, 25% variance explained) of dissolved ion concentrations from waterbodies sampled in 2012-2013 in Stutsman and Kidder County, North Dakota. (b) bi-plot including Lake #144 and (b) bi-plot excluding lake #144.

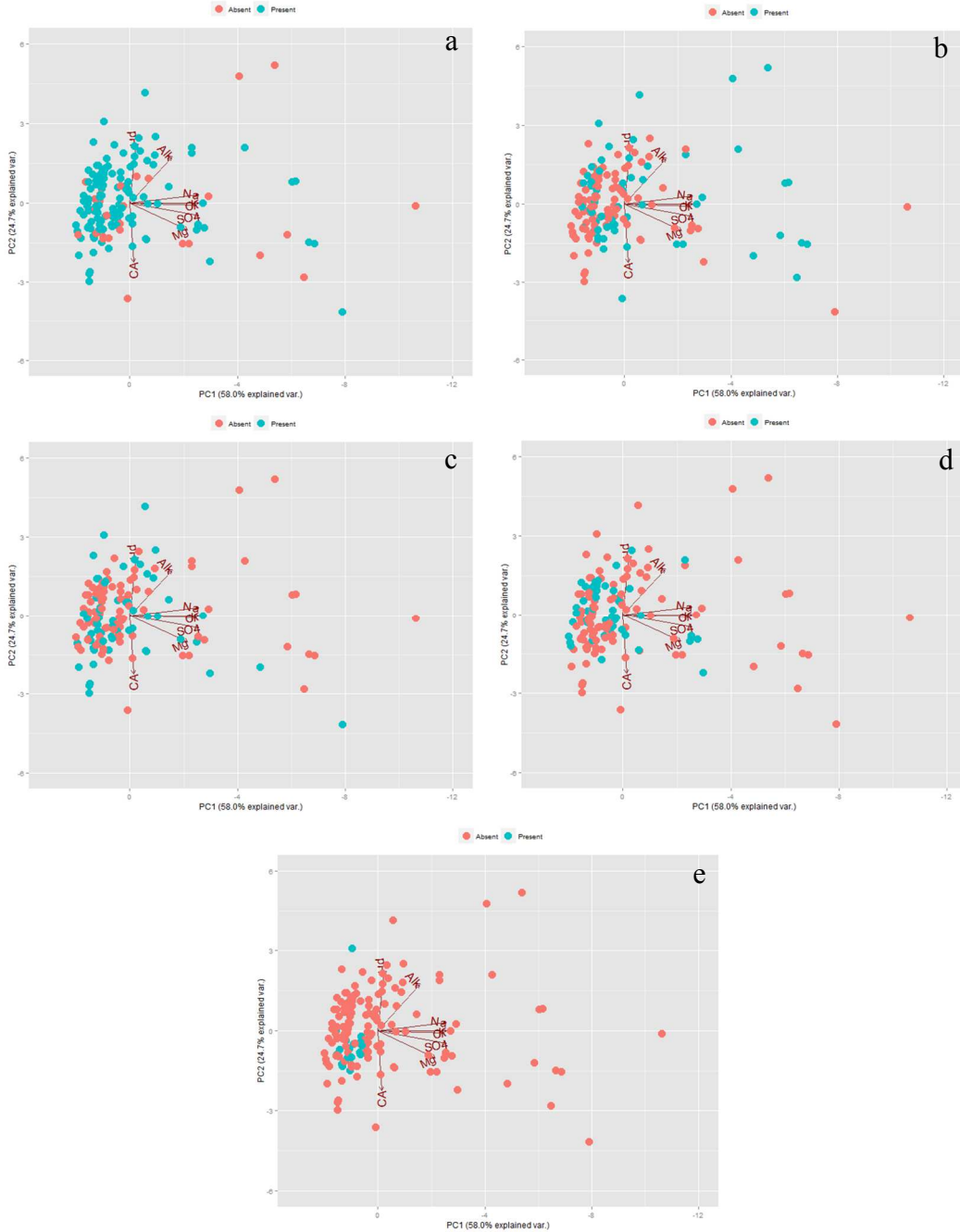


Figure 2.5. PCA bi-plot representing PC1 (x-axis, 58% variance explained) and PC2 (y-axis, 25% variance explained) of dissolved ion concentrations from 161 waterbodies sampled in 2012-2013 in Stutsman and Kidder County, North Dakota. To spread out the axis waterbody #144 was excluded due to its extreme salinity. Each bi-plot highlights a species presence with a blue circle and absence with a red circle, (a) fathead minnow (*Pimephales promelas*), (b) tiger salamander (*Ambystoma mavortium*), (c) brook stickleback (*Culaea inconstans*), (d) yellow perch (*Perca flavescens*), and e) Iowa darter (*Etheostoma exile*).

2.5.2. Niche Breadth along Ionic Gradients

Among all vertebrate species sampled, fathead minnows and brook sticklebacks had the largest range relative to SO_4 concentrations; 33 to 8590 mg/L. Regressed weighted averages and abundance maxima's for both species indicate an optimal range of occurrence in the fresher waters that are less than then 3,000mg/L SO_4 (Figure 2.6a, Table 2.4a). However, the optima in fresher waters may be an artifact due to the majority of waterbodies being fresh.

Tiger salamanders had a similar range of occurrence but were optimally located higher on the gradient than the other vertebrate species. Yellow perch and Iowa darters were restricted in range of occurrence but had a similar optimal range to brook sticklebacks and fathead minnows (Figure 2.6a, Table 2.4a). Species presence on the alkalinity (carbonate/bicarbonate) gradient showed all fish species disappearing above 1200 mg/L while tiger salamanders occurred in wetlands with alkalinity as high as 2,600 mg/L (Figure 2.6b, Figure 2.4b). All fish species had an optimal alkalinity defined niche between 200-900 mg/L and the tiger salamanders optimal niche was 500-2,370 mg/L (Figure 2.6b, Table 2.4b).

Table 2.4. (a) Fish and salamander ranges of occurrence, weighted average (5% CI), and abundance maxima (95% CI) along SO₄ (mg/L) gradient from 161 waterbodies sampled in 2012-2013. (b) Fish and salamander ranges of occurrence, weighted average (5% CI), and abundance maxima (95% CI) along alkalinity (mg/L) gradient from 162 waterbodies sampled in 2012-2013

(a)			
Species	Weighted Average	Abundance Maxima	Range of Occurrence
Tiger Salamander	1119-3123	448-5390	10.0-7314
Fathead Minnow	759-1561	358-964	33-8560
Brook Stickleback	579-1708	248-2760	59-8560
Yellow Perch	592-1189	308-1230	18-2917
Iowa Darter	389-879	137-726	137-964
(b)			
Species	Weighted Average	Abundance Maxima	Range of Occurrence
Tiger Salamander	628-1071	538-2370	262-2590
Fathead Minnow	521-664	338-945	223-1272
Brook Stickleback	440-667	262-568	229-1236
Yellow Perch	482-645	353-657	223-1050
Iowa Darter	350-542	319-644	305-644

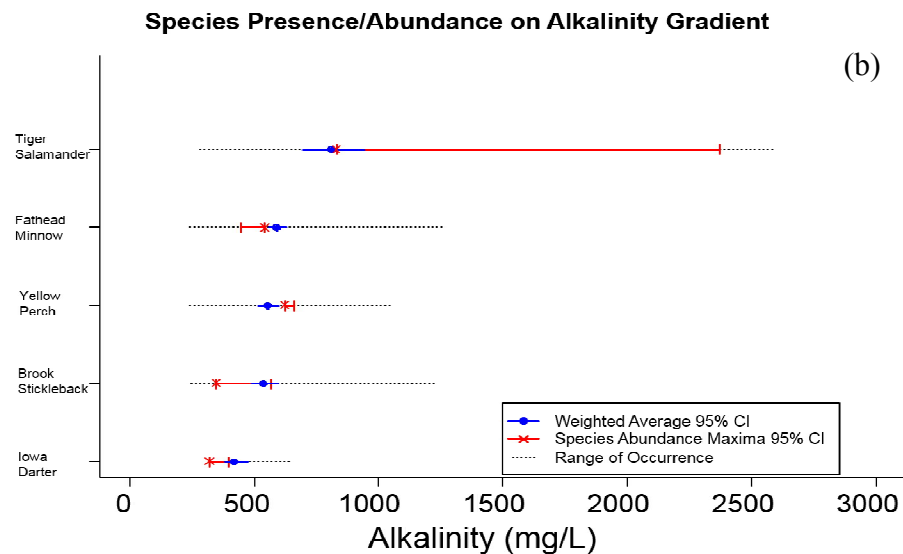
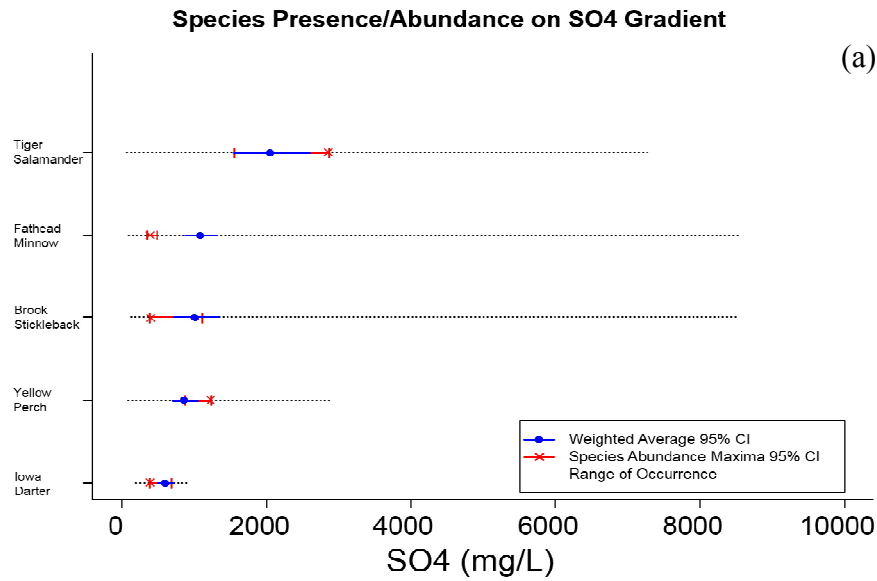


Figure 2.6. Distribution of fish species abundances with increasing SO₄ (a) and alkalinity (b). Blue horizontal lines are empirical confidence intervals (CIs; 5th–95th percentile) of weighted-average abundance among 1000 bootstraps whereas red lines span the 5th–95th bootstrap percentiles of the maximum abundance observed among sample units. The black dashed line represents the total range of values over which a species was found in. Taxa are plotted in rank order of the upper CI (95th percentile) of maximum abundance. Only taxa that were collected in over 10% of waterbodies are shown.

2.5.3. Ionic Composition Shifts between Sampling Periods

The PCA model using dissolved ion concentrations from two sampling periods (1966-1976 and 2012-2013; 162 wetlands sampled during each sample period) identified two principle components (PC1 and PC2) that explained 73% of the variation in our data (Table 2.5a). Inclusion of PC3 only added an additional 11% of variation explained and did not contribute to data interpretations. Similar to the previous PCA, PC1 represented the variation in dissolve ions associated with salinity and PC2 was associated with pH and calcium (Figure 2.7 Table 2.5b). The ordination plot showed greater dissimilarities of dissolved ion concentrations and greater numbers of waterbodies on the saltier side of the gradient for the earlier sampling period when compared to the more current sampling (Figure 2.7). The permutational multivariate analysis of variance using distance matrices model indicated that dissolved ion concentrations were significantly higher for the latter period ($P < 0.05$; Table 2.6).

2.5.4. Ionic Composition Shifts and Yellow Perch Presence

Nine lakes had yellow perch in the 1966-1976 sampling period, but increased by 600% to 54 lakes containing perch in our 2012-2013 sampling. PCA bi-plots indicated that lakes where perch historically occurred had very similar dissolved ion concentrations to the lakes where we captured perch in 2012-2013 (Figure 2.8). An ordination using historic dissolved ion concentrations for lakes recently colonized by perch indicate a majority of these lakes have become fresher (Figure 2.9). The dissolved ion correlations to the first two components was similar for both models (Tables 2.7 and 2.9). The follow up permutational multivariate analysis

of variance using distance matrices did not find any significant difference in dissolved ion concentrations of historic and current lakes containing perch (Table 2.8). Further, the lakes recently colonized by perch had become significantly fresher from what they were in the 1960's-1970 when they did not contain perch (Table 2.10).

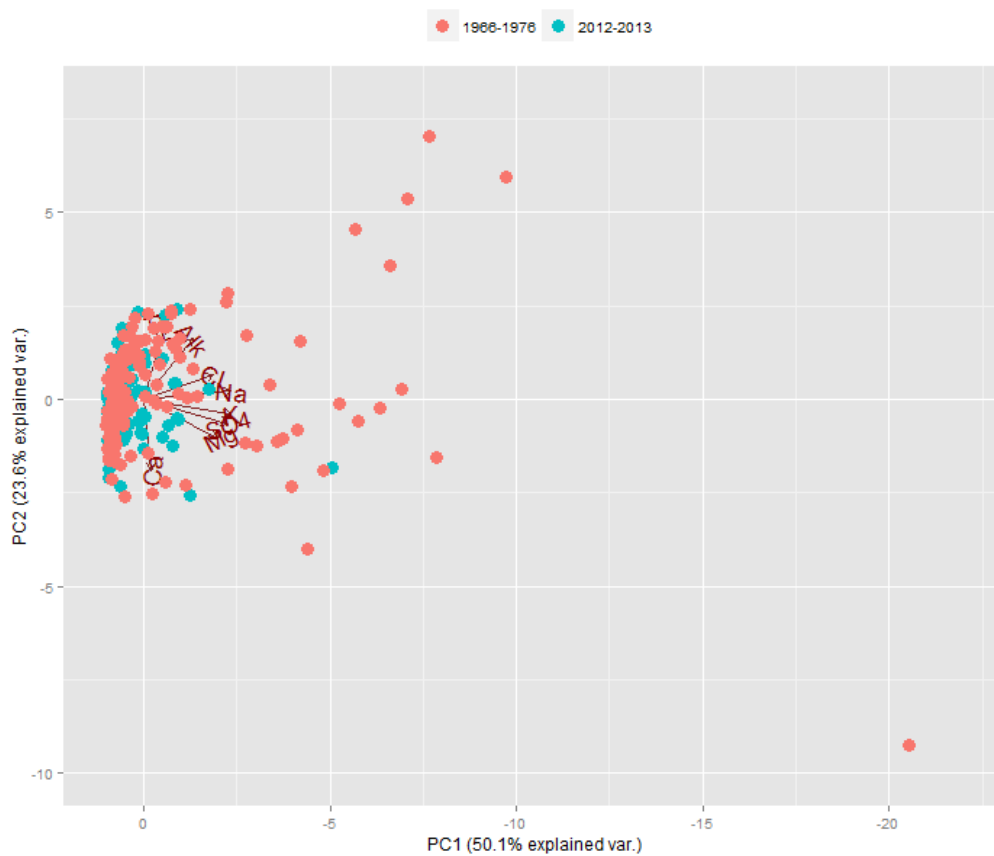


Figure 2.7. PCA bi-plot representing PC1 (x-axis, 58% variance explained) and PC2 (y-axis, 24% variance explained) of dissolved ion concentrations of 162 waterbodies sampled in Stutsman and Kidder Counties, North Dakota, from 1960's-1970's sampling period (red) and 2012-2013 sample period (blue).

Table 2.5. (a) Importance of principal components from current dissolved ion concentrations PCA from 1966-1976 and 2012-2013. (b) Dissolved ion correlations to rotated PCA bi-plot from 1966-1976 and 2012-2013 Sampling Period

(a)	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Stand. Dev.	2.001	1.373	0.953	0.719	0.588	0.383	0.370	0.226
Proportion of Variance	0.500	0.235	0.113	0.064	0.043	0.018	0.017	0.006
Cumulative Proportion	0.500	0.736	0.849	0.914	0.958	0.976	0.993	1
(b)	PC1	PC2						
pH	-0.1	0.55						
Alk	-0.26	0.46						
SO ₄	-0.45	-0.18						
Cl	-0.37	0.18						
Mg	-0.39	-0.3						
Ca	-0.03	-0.55						
Na	-0.46	0.06						
K	-0.46	-0.11						

Table 2.6. Differences between 1966-1976 and 2012-2013 sampling period dissolved ion concentrations in multivariate distance

<i>Adonis Model</i>	Df	Sums Squares	Mean Squares	F. Model	R2	Pr(>F)
Sampling Period	1	8.56E+10	856489901	14.015	0.0417	1e-04***
Residuals	322	1.97E+10	61113067		0.95829	
Total	323	2.05E+10			1	

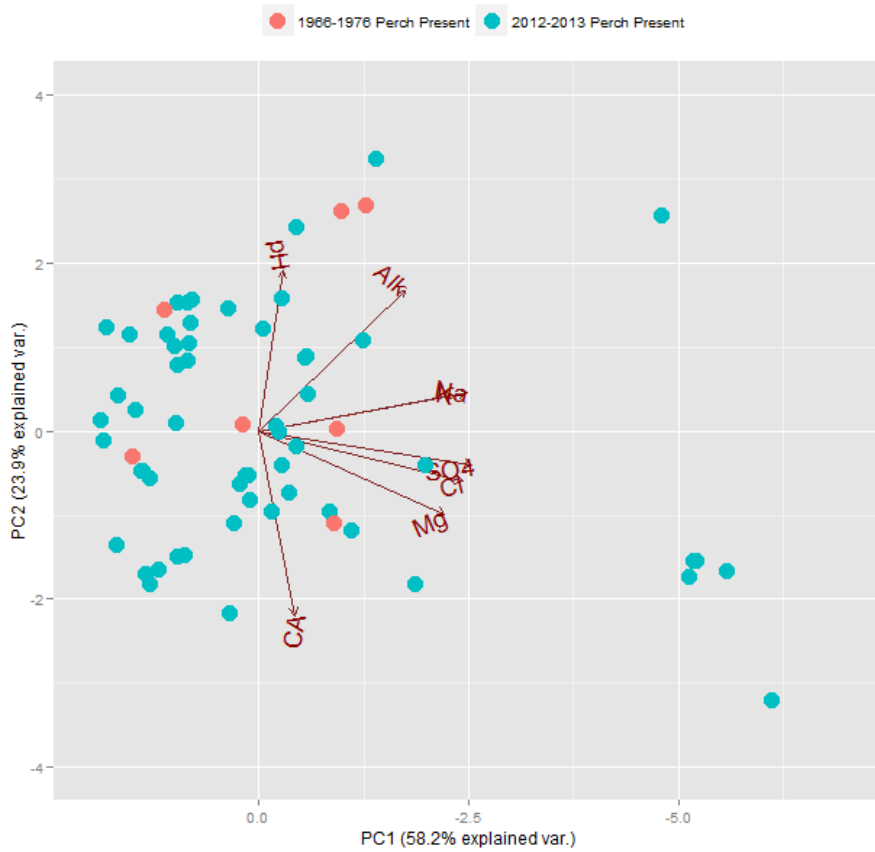


Figure 2.8. PCA bi-plot representing PC1 (x-axis, 54% variance explained) and PC2 (y-axis, 22% variance explained) of dissolved ion concentrations of waterbodies containing yellow perch (*Perca flavescens*) in Stutsman and Kidder Counties from the 1960's-1970's sampling period (red) and the 2012-2013 sample period (blue).

Table 2.7. Dissolved ion correlations to PC1 and PC2 (82% variance explained) for lakes with perch present in the 1966-1976 and 2012-2013 sampling periods in multivariate distance

	PC1	PC2
pH	-0.05126	0.526151
Alk	-0.30802	0.462604
SO ₄	-0.44822	-0.1114
Cl	-0.4295	-0.16579
Mg	-0.39067	-0.27187
CA	-0.07459	-0.60481
Na	-0.43743	0.12539
K	-0.40938	0.11762

Table 2.8. Differences in dissolved ion concentrations of lakes with yellow perch (*Perca flavescens*) present in the 1966-1976 and 2012-2013 sampling periods in multivariate distance

<i>Adonis Model</i>	Df	Sums Squares	Mean Squares	F. Model	R2	Pr(>F)
Sampling Period	1	4532822156	354683	0.459	0.006	0.527
Residuals	322	9266122863	771417		0.994	
Total	323	20535000000			1	

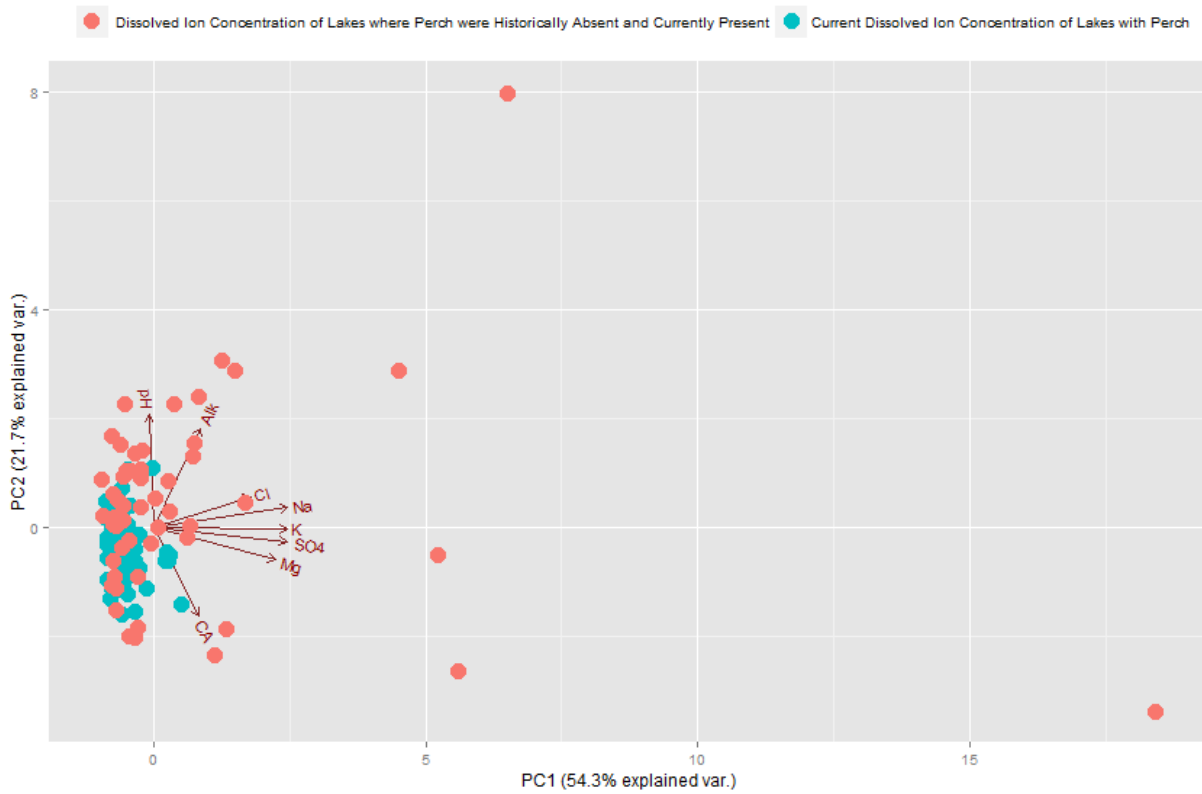


Figure 2.9. PCA bi-plot representing PC1 (x-axis, 54% variance explained) and PC2 (y-axis, 22% variance explained) of dissolved ion concentrations of waterbodies containing yellow perch (*Perca flavescens*) in Stutsman and Kidder Counties from the 1960's-1970's sampling period (red) and the 2012-2013 sample period (blue).

Table 2.9. Dissolved ion correlations to PC1 and PC2 (74% variance explained) for lakes with yellow perch (*Perca flavescens*) absent in the 1966-1976 and present in 2012-2013 sampling periods in multivariate distance

	PC1	PC2
pH	-0.10317	0.552468
Alk	-0.2606	0.465819
SO ₄	-0.44743	-0.18544
Cl	-0.36806	0.184204
Mg	-0.39429	-0.30063
Ca	-0.03856	-0.54962
Na	-0.46334	0.06323
K	-0.46276	-0.11407

Table 2.10. Differences in dissolved ion concentrations of lakes with yellow perch (*Perca flavescens*) absent in the 1966-1976 and present in 2012-2013 sampling periods in multivariate distance

<i>Adonis Model</i>	Df	Sums Squares	Mean Squares	F. Model	R2	Pr(>F)
Sampling Period	1	4532822156	4532822156	5.6813	0.04669	1e-04***
Residuals	116	9266122863	79880370		0.95331	
Total	117	9719945019			1	

2.6. Discussion

Our results show that 84% of the sampled water bodies hosted fish populations in 2012 and 2013, indicating a major change to this formerly fishless region. Increased water levels in prairie pothole lakes and wetlands have resulted in decreased dissolved ion concentrations and increased water permanency, reducing the effects to these two limiting factors on fish occurrence. The 1966-1976 sampling identified 33 lakes having a specific conductance of 14,500 $\mu\text{S}/\text{cm}$ or greater (Swanson et al 1988); but by 2012-2013, only two lakes continued to be that saline. Fish colonization may have been facilitated by newly formed surface connections among wetlands due to increased water levels. In addition to increased connectivity, fish colonization has likely been facilitated through human translocations. In fact, game fish are often stocked in suitable lakes (<http://www.gf.nd.gov/>). In North Dakota, the number of lakes in which game fish are managed has risen by 259% since 1988 (<http://www.gf.nd.gov/>). Additionally, the commercial baitfish industry (Litvak and Mandrak 1993) has led to the transport of baitfish (i.e., fathead minnow) to numerous water bodies throughout the upper Great Plains (Hanson and Riggs 1995), this transportation of minnows has likely also included the incidental introduction of other fishes such as brook sticklebacks and Iowa darters.

Fathead minnow and yellow perch are typically restricted to SO_4 concentrations less than 4,500 and 8,000 mg/L, respectively (Held and Peterka 1974, Rawson and Moore 1944, Koel and Peterka 1995). While information on brook stickleback tolerance to SO_4 , is not available, this species is limited by total dissolved solids concentrations of 17,000-25,000 mg/L (Scott and Crossman 1973). Our findings are consistent with these defined tolerance limits, with one exception; a population of fathead minnows occurring in a lake with an SO_4 concentration of

8,560 mg/L, however, this population was apparently extirpated within the next two years (personal observation). Iowa darters were found to be the fish species most restricted by SO_4 concentrations; this species did not occur in water bodies with SO_4 concentrations exceeding 2,500 mg/L.

While fish were prevalent throughout our study area, they did not occur in some lakes with apparently suitable ion concentrations. The lack of fish in these lakes was likely the result of other factors that limit their presence. Three factors that seem most likely preventing presence in these “favorable” habitats are 1) the lakes are topographically situated such that surface connections do not form even during high water periods, 2) movements of fish to these sites were not assisted by humans (e.g., game fish or bait fish stockings), and 3) shallow depths making them susceptible to winterkill.

The increased presence of fish in the region has likely changed the aquatic communities of many PPR water bodies. Barred tiger salamanders are native to prairie pothole lakes and wetlands and were historically the top predator within the wetlands. Their biphasic life histories allow them to leave wetlands when conditions become unfavorable. Fish and tiger salamanders typically do not co-occur in high abundances (Zimmer et al 2000 and 2001). This could be an important consideration for tiger salamander populations with the freshening of water bodies in the area making them more favorable to fish. We found that tiger salamanders were most abundant near the 6,000 mg/L SO_4 threshold where fathead minnows typically did not occur. However, tiger salamanders can occur in much fresher waters. We suspect the pattern we observed resulted from salamanders being excluded from the fresher wetlands by the presence of fish. Tiger salamanders also were able to occur at alkalinity levels greater than 2,000 mg/L where fathead minnows no longer occurred, which is consistent with previous studies looking at

the tolerance of fathead minnows to alkalinity in Nebraska bicarbonate wetlands (McCarragher and Thomas 1968).

Previous research has shown that fathead minnows can also alter invertebrate communities and aquatic vegetation, which indirectly change physical properties such as turbidity (Zimmer et al 2001). Therefore, the increase of fish presence on the PPR landscape could have implications for a wide range of wildlife dependent on these aquatic habitats. As an example, breeding waterfowl are dependent upon aquatic invertebrate communities as forage. Therefore, shifting aquatic invertebrate communities resulting from increased occurrence of fish in the prairie pothole region will likely influence waterfowl reproduction in this region considered to be the “duck factory of North America” (Batt et al 1989). Additionally, a future drought cycle that leads to widespread extirpations of these recently established fish populations could have major implications on the aquatic communities. Since evapotranspiration is much higher in smaller water bodies, we could lose many smaller fishless wetlands leaving the larger more permanent “fish filled” wetlands on the landscape which could reduce suitable habitat for tiger salamanders, foraging waterfowl, and other native vertebrates inhabiting this portion of the North American continent. Similarly if high water conditions persist, the PPR may ultimately become known as the “fathead minnow factory” of North America rather than its “duck factory.”

2.7. Acknowledgments

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CHAPTER 3. CANNIBALISTIC-MORPH TIGER SALAMANDERS IN UNEXPECTED ECOLOGICAL CONTEXTS¹

3.1. Abstract

Barred tiger salamanders (*Ambystoma mavortium* (Baird 1850)) exhibit two trophic morphologies; a typical and a cannibalistic morph. Cannibalistic morphs, distinguished by enlarged vomerine teeth, wide heads, slender bodies, and cannibalistic tendencies, are often found where conspecifics occur at high density. During 2012 and 2013, 162 North Dakota wetlands and lakes were sampled for salamanders. Fifty-one contained *A. mavortium* populations; four of these contained cannibalistic morph individuals. Two populations with cannibalistic morphs occurred at sites with high abundances of conspecifics. However, the other two populations occurred at sites with unexpectedly low conspecific but high fathead minnow minnows (*Pimephales promelas* (Rafinesque, 1820)) abundances. Further, no typical morphs were observed in either of these later two populations, contrasting with earlier research suggesting cannibalistic morphs only occur at low frequencies in salamander populations. Another anomaly of all four populations was the occurrence of cannibalistic morphs in permanent water sites, suggesting their presence was due to factors other than faster growth allowing them to occupy ephemeral habitats. Therefore, our findings suggest environmental factors inducing the cannibalistic morphism may be more complex than previously thought.

¹This material in this chapter was co-authored by Kyle I McLean, David M. Mushet, and Craig A. Stockwell (*American Midland Naturalist*). Kyle McLean had primary responsibility for collecting samples in the field, data analysis, and developer of conclusions. Kyle McLean also drafted and revised all versions of this chapter. David Mushet and Craig Stockwell served as proofreaders and checked the math in the statistical analysis conducted by Kyle McLean.

3.2. Introduction

The barred tiger salamander (*Ambystoma mavortium* (Baird, 1850)) is a polytypic species with multiple distinct morphologies, making it popular for studies of phenotypic plasticity and evolution. Among *A. mavortium* polymorphisms are two distinct trophic phenotypes; a typical morph and a cannibalistic morph (Powers, 1907). Cannibalistic morph tiger salamanders often occur where there is a relatively high prevalence of conspecific larvae (Powers, 1907; Rose and Armentrout, 1976; Lannoo and Bachman, 1984). Cannibalistic morph individuals differ from typical morphs by their enlarged vomerine teeth, wider heads, long slender bodies, and tendency to consume conspecifics (Powers, 1907; Rose and Armentrout, 1976; Lannoo and Bachman, 1984). These characteristics allow cannibalistic morphs to effectively feed on conspecifics. The elongated and recurved vomerine teeth increase an individual's ability to capture and hold conspecific prey (Reilly *et al.*, 1992), while their wide head facilitates ingestion of conspecifics and other large prey items that would otherwise be unavailable due to gape size limitations (Zaret, 1980).

Competition appears to be an important environmental trigger inducing the cannibalistic morph. In fact experimental work shows cannibalistic morph characteristics can be induced by increasing conspecific density, varying population size structure, reducing prey density, or combining both high intraspecific competition and moderate interspecific competition (Hoffman and Pfennig, 1999; Collins and Cheek, 1983; Ghioca and Smith, 2008a; Loeb *et al.*, 1998; Whiteman *et al.*, 2002). It is noteworthy, cannibalistic morphs have been found primarily in ephemeral habitats with high densities of conspecifics (Collins and Cheek, 1983; Lannoo and Bachman, 1984) where rapid growth should be favored. The altered diet of cannibalistic morphs

may reduce competition with typical morphs by allowing them to feed on larger prey items (including conspecifics) unavailable to the typical morph (Ghioca and Smith, 2008b). This feeding niche separation, in combination with direct reduction of conspecific competition through cannibalism, allows cannibalistic morph individuals to grow and metamorphose quicker (but *see* Rose and Armentrout, 1973), thereby reducing desiccation risk in rapidly drying habitats (Lannoo *et al.*, 1988; Ghioca and Smith, 2008a).

Although the presence of cannibalistic morph salamanders is linked to intra-specific competition in ephemeral habitats, it seems likely enhanced interspecific competition may also facilitate the development of cannibalistic morphs. Here, we report on the presence of cannibalistic morph individuals in four populations of the tiger salamander (*A. mavortium*). Only two of these populations harbored high densities of conspecifics, while the other two occurred in habitats with low salamander densities but high densities of fish competitors.

3.3. Materials and Methods

During the summers (June-August) of 2012 and 2013, we sampled amphibian communities of 162 wetlands and small lakes in Stutsman and Kidder counties, North Dakota, U.S.A (Figure 3.1). We sampled each location with seven funnel-type amphibian traps (Mushet *et al.*, 1997) placed thirty meters apart along the 1 m depth contour parallel to the shoreline. Each trap had a 2m attached drift fence that was also situated parallel to the shoreline. Traps were left in place for 24 h before being checked for captures.

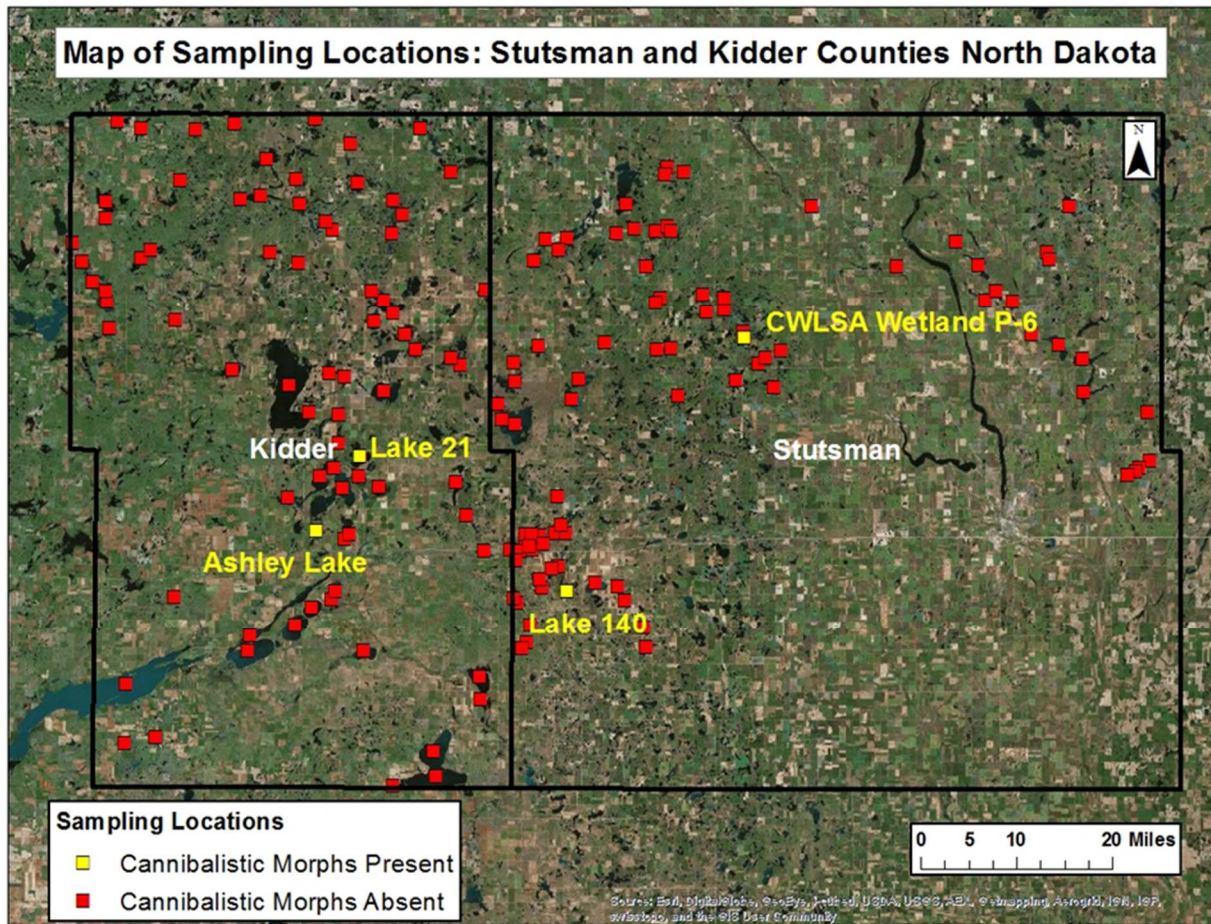


Figure 3.1. Location of 162 sites sampled for amphibians during 2012 and 2013 in Stutsman County and Kidder County, North Dakota. Location information for all sites are provided in Swanson *et al.* (1988). Four sites where cannibalistic morphs occurred are identified as Lake 21, Lake 140, Ashley Lake, and CLSA Wetland P-6

All captured individuals were identified, measured, enumerated, and classified as being either typical or cannibalistic morphs. Cannibalistic morph determinations were initially made in the field based on visual observations of the presence of hypertrophied vomerine teeth; typical morph individuals had much smaller, peg-like, teeth (Pederson, 1991, Fig. 3.2). Suspected cannibalistic morphs were collected and euthanized with MS-222. In addition 34 typical morph individuals were collected from one of the lakes containing cannibalistic morphs (*i.e.*, Ashley Lake). All other captured individuals were immediately released back into the wetland or lake.

Following collection specimens were transported to the aquatics laboratory at the USGS Northern Prairie Wildlife Research Center in Jamestown, North Dakota where a digital caliper was used to measure snout-vent length (SVL) and greatest skull width (GSW of all collected specimens); GSW is the skull measurement shown to differ greatest between morphs (Pedersen, 1993).

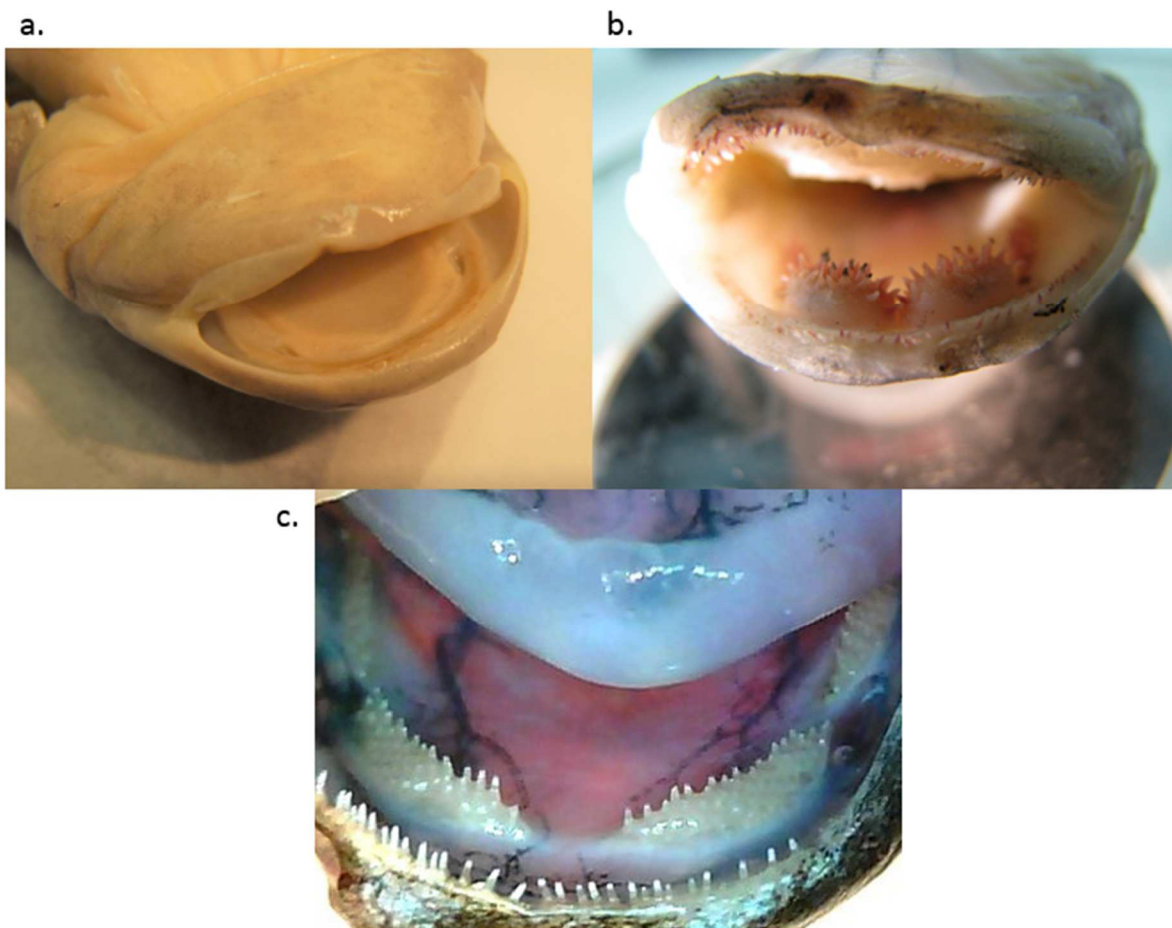


Figure 3.2. Vomerine teeth of (a) typical-morph *Ambystoma mavortium* from Lake Ashley, (b) cannibalistic morph *A. mavortium* from Lake Ashley, and (c) cannibalistic morph *A. mavortium* from Lake 21.

We used analysis of covariance (ANCOVA) to test the hypothesis cannibalistic morphs from high conspecific populations, cannibalistic morphs from a population with high fathead

minnow densities, and typic morphs differed in growth patterns and GSW. For this analysis we used the GSW as the dependent variable, SVL as the predictor variable, and morph diagnosis (*i.e.*, typic, cannibalistic) as the grouping variable (GSW~SVL+MORPH). Both GSW and SVL were log transformed before analyses were performed. Using SVL as a covariate allowed for the interaction of body length and skull size to be corrected for when testing for skull size differences among groups. For the ANCOVA, we used both an analysis of variance (ANOVA) table and a linear model to identify differences in slope and intercept. We utilized Tukey's HSD multiple comparisons test on the ANOVA for pairwise comparisons. In the case of all groups having similar slopes, we conducted an ANOVA using the Arcsine square root transformed ratio of GSW/SVL followed by a Tukey's post-hoc pairwise comparison. These analyses were conducted to: (1) identify if the slopes were different, (2) test significance in the y-intercept, and (3) test differences in the ratio of GSW/SVL. We used R statistical programming software (R Developmental Core Team, 2008) for all analyses.

3.4. Results

We captured *A. mavortium* individuals in 51 of the 162 sites sampled. In 2012 we captured two larvae with hypertrophied vomerine teeth in Unnamed Lake 21 (hereafter Lake 21; Swanson, 1988). Following this initial discovery, we sampled Lake 21 on two additional occasions in July 2012 to gain more information on this population. In total 52 *A. mavortium* larvae were captured from Lake 21; all had enhanced vomerine teeth. In 2013 we discovered cannibalistic morph *A. mavortium* individuals at three additional sites, Unnamed Lake 140 (hereafter Lake 140), Ashley Lake, and Cottonwood Lake Study Area (CLSA) Wetland P6. A

total of 13 cannibalistic morph individuals were collected from these additional sites, one from Lake 140, nine from Ashley Lake, and three from CLSA Wetland P6.

From the capture data from the initial standardized seven trap 24 h sampling period, the four sites with cannibalistic morphs, Ashley Lake and CLSA Wetland P6, had a high relative abundance of conspecific typic-morph individuals and a corresponding low frequency of cannibalistic-morph individuals (frequency = < 8%). The other two sites (Lake 21 and Lake 140) had low abundances of conspecifics, all of which were cannibalistic morphs (*i.e.*, frequency of cannibalistic morphs was 100%). While these two sites had low conspecific abundance, they had high abundances of fathead minnows (*Pimephales promelas* (Rafinesque, 1820); Fig. 3.3). It should be noted that at one of the two sites with high minnow abundance (Lake 140) only a single salamander larvae, a cannibalistic morph, was captured.

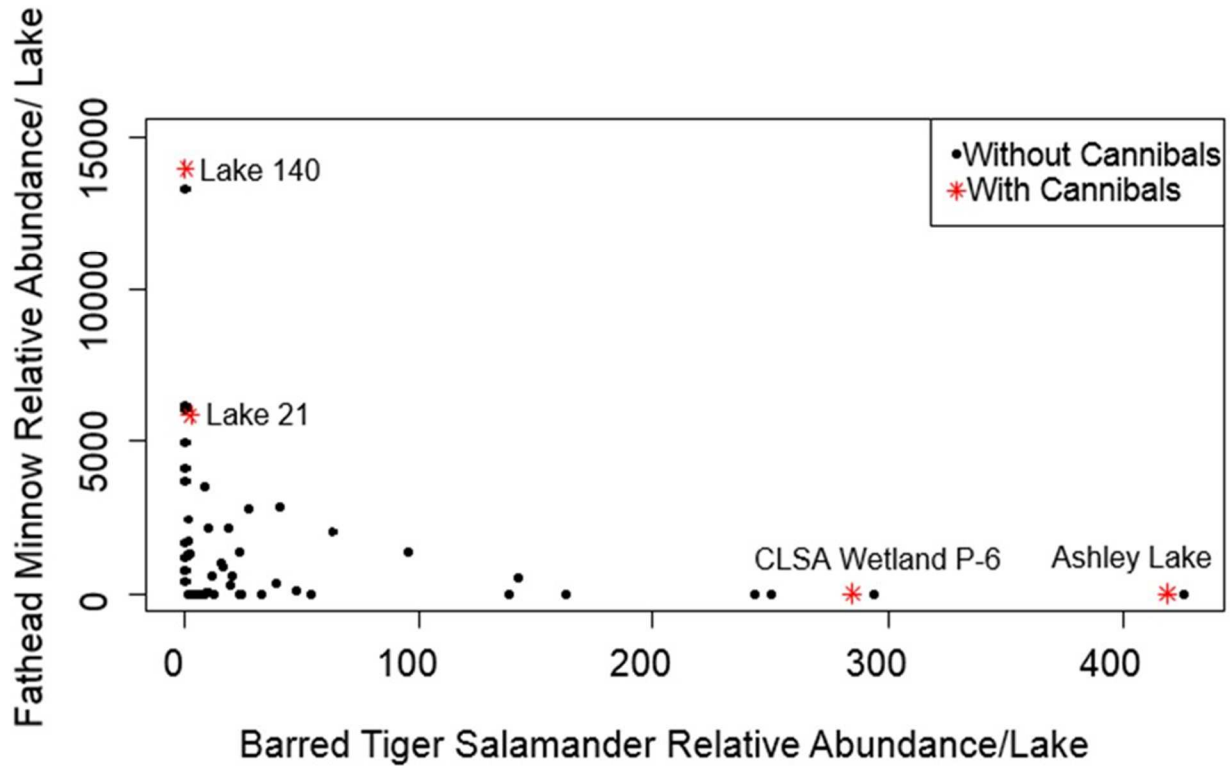


Figure 3.3. Relative abundance of tiger salamander (*Ambystoma mavortium*) larvae and fathead minnows (*Pimephales promelas*). Sites labeled Lake 21, Lake 140, Ashley Lake, and CLSA Wetland P6 refer to sites at which cannibalistic morphs occurred.

There was a noticeable difference in the vomerine teeth elongation of cannibalistic morphs from the high conspecific abundance lakes (Ashley Lake and CLSA Wetland P6) compared to the high minnow abundance lakes (Lake 21 and Lake 140). Cannibalistic morph individuals in lakes with high minnow abundances had smaller vomerine teeth than similar morphs in the other two lakes (Figure 3.2).

The full ANCOVA was conducted to test skull growth differences among three groups; cannibalistic morphs from a high fathead minnow density population (Lake 21), cannibalistic morphs from high conspecific populations (Lake Ashley and CLSA Wetland P6), and typical morphs (Lake Ashley). The first ANCOVA model, $GSW \sim SVL * MORPH$, showed the group factor (MORPH) had a significant effect on SVL, but the interaction was not significant (P-

Value=0.181), indicating that the three groups have similar regression slopes for logSVL and logGSW.

The second, more parsimonious, model without the interaction (GSW~SVL+Pop) fitted to test for differences in slope intercept showed a significant MORPH effect, indicating the skull growth rates were different among groups (Table 3.1). The Tukey’s HSD pairwise comparison indicated the intercepts of logGSW for each group were significantly different (Table 3.2). The linear model was logGSW~logSVL for all groups with the common slope (0.96) showed Lake Ashley/CWLSA Wetland P-6 cannibalistic morphs had the largest logGSW (Intercept -0.36), followed by Lake 21 cannibalistic morphs (Intercept = -0.42), and lastly Lake Ashley typical morphs (Intercept = -0.56; Fig. 3.4). Lake 21 morph skulls are significantly wider than the typical morphs verifying their classification as cannibalistic morphs.

Table 3.1. Anova model LogGSW~LogSVL+MORPH for typic tiger salamander (*Ambystoma mavortium*) larvae captured in 2012 and 2013 as compared to Kidder and Stutsman counties, North Dakota, cannibalistic morphs from populations with high conspecific abundance, and populations with low conspecific abundance but high fathead minnow (*Pimephales promelas*) abundance

	Deg. Freedom	Sum Squares	Mean Squares	F-Value	Pr(>F)
LogSVL	1	0.9775	0.9775	723.8	< 0.0001
MORPH	2	0.3130	0.1565	115.9	< 0.0001
Residuals	94	0.1269	0.0014		

Table 3.2 Tukey's HSD pairwise post hoc comparisons of intercept from ANOVA model $\text{LogGSW} \sim \text{LogSVL} + \text{MORPH}$ comparing larval growth patterns of tiger salamander (*Ambystoma mavortium*) larvae morphotypes.

Pairs	Estimate	Std.	Std. Error	T-Value	P-Value
INTRA-INTER	0.0604	0.0c	4.974	< 0.0001	
TYP-INTER	-0.1047	0.0087	-11.835	< 0.0001	
TYP-INTRA	-0.1644	0.0123	-13.324	< 0.0001	

Note: INTRA, cannibalistic morphs populations with high conspecific abundance. INTER, cannibalistic morphs population with high abundances of fathead minnows (*Pimephales promelas*). TYP, typic morph larvae

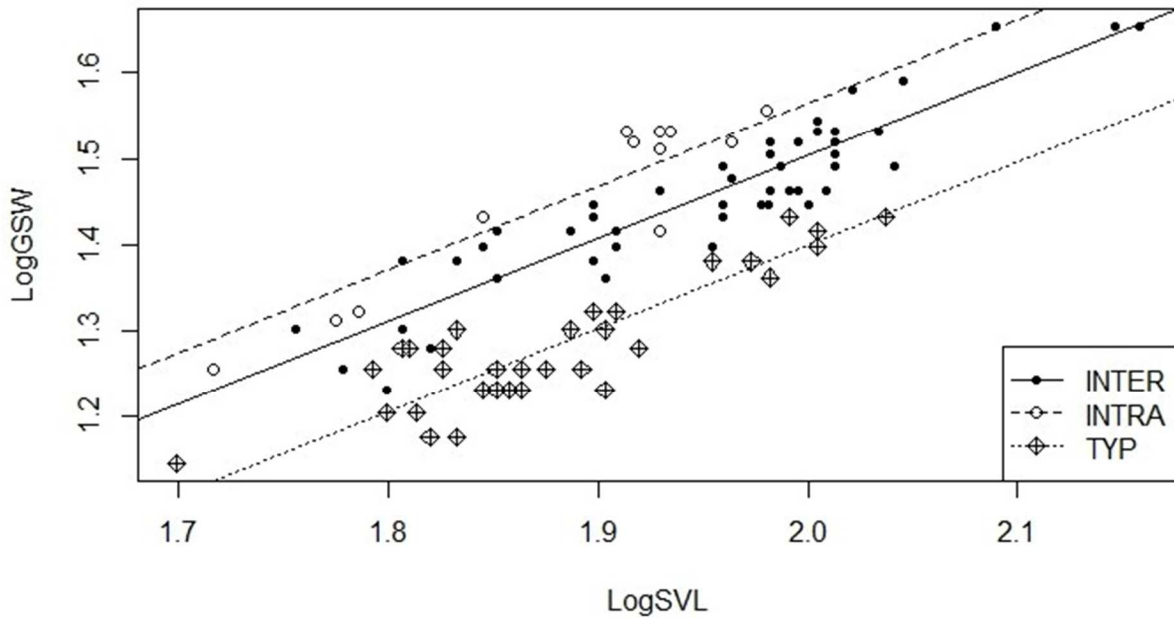


Figure 3.4. Greatest skull width (GSW) and snout vent length (SVL) of typic morph (TYP), high conspecific population cannibalistic morph (INTRA), and high interspecific population cannibalistic morph (INTER) tiger salamanders (*Ambystoma mavortium*) larvae collected in Stutsman County and Kidder County, North Dakota.

We used ANOVA to evaluate skull width differences between morphs by comparing the Arcsine square root transformed ratio of GSW/SVL among groups. The ANOVA model $GSW/SVL \sim MORPH$ indicated a significant difference among groups (Table 3.3). The follow up Tukey's HSD showed a significant difference between all paired morphs with high conspecific population cannibals having the GSW/SVL ratio followed by high interspecific population cannibal morphs, and lastly the typic morphs (Table 3.4, Figure 3.5).

Table 3.3 Anova model arcsine square root transformed $GSW/SVL \sim MORPH$ for typic tiger salamander (*Ambystoma mavortium*) larvae captured in 2012 and 2013 as compared to Kidder and Stutsman counties, North Dakota, cannibalistic morphs from populations with high conspecific abundance, and populations with low conspecific abundance but high fathead minnow (*Pimephales promelas*) abundance from populations in Kidder and Stutsman counties, North Dakota

	Deg Freedom	Sum Squares	Mean Squares	F-Value	Pr(>F)
MORPH	2	0.0183	0.0915	117.4	< 0.0001
Residuals	95	0.0741	0.0007		

Table 3.4 Tukey's HSD pairwise post hoc comparisons of intercept from ANOVA model arcsine square root transformed GSW/SVL~MORPH comparing growth patterns of comparing larval growth patterns of tiger salamander (*Ambystoma mavortium*) larvae morphotypes

Pairs	Estimate	Std. Error	T-Value	P-Value
INTRA-INTER	0.052	0.008	5.879	< 0.0001
TYP-INTER	-0.073	0.006	-12.015	< 0.0001
TYP-INTRA	-0.126	0.009	-13.499	< 0.0001

Note: INTRA, cannibalistic morphs populations with high conspecific abundance. INTER, cannibalistic morphs population with high abundances of fathead minnows (*Pimephales promelas*). TYP, typic morph larvae

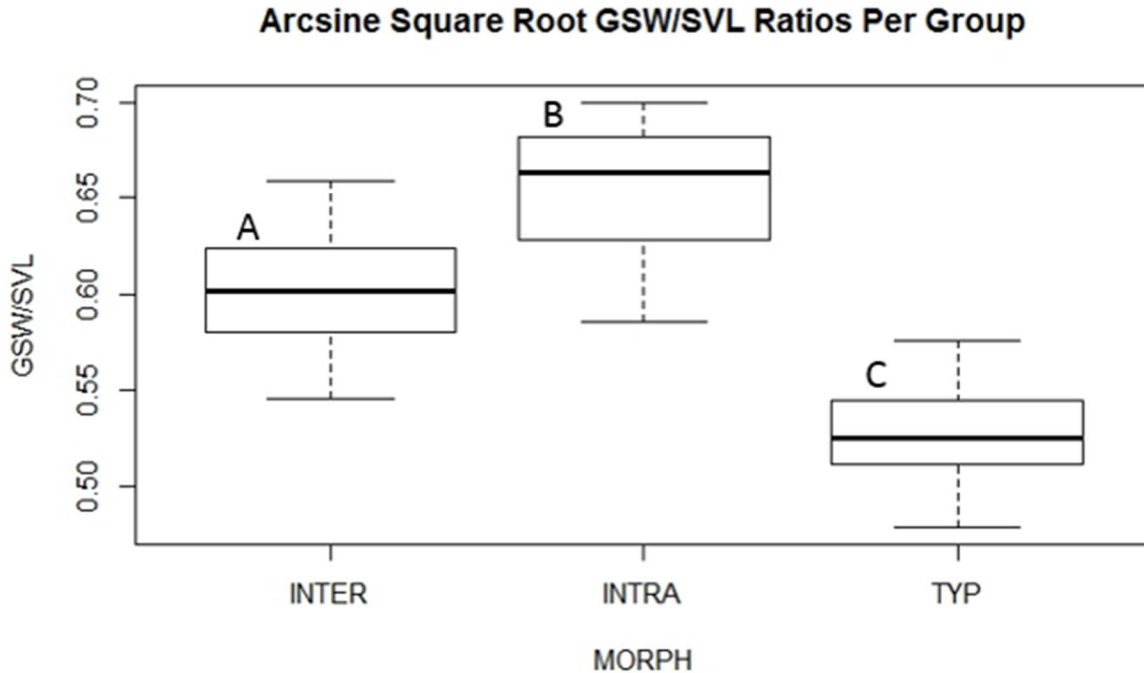


Figure 3.5. Box Plot of Arcsine Square Root GSW/SVL ratio for typical morph (TYP), high conspecific population cannibalistic morph (INTRA), and high interspecific population cannibalistic morph (INTER) tiger salamanders (*Ambystoma mavortium*) larvae collected in Stutsman County and Kidder County, North Dakota, USA. Groups sharing letters were not significantly different.

3.5. Discussion

In addition to being the northernmost records of cannibalistic morphs and first record in North Dakota as well as for the *Ambystoma mavortium diaboli* (Dunn, 1940) subspecies, our reports are noteworthy because cannibalistic morphs were observed in an unexpected ecological context, namely low abundance of conspecifics, but high abundance of fathead minnows. The degree of vomerine tooth hypertrophy varied among populations. One notable observation was that among the four populations, cannibalistic morphs from sites with high conspecific abundances and sites with low conspecific abundances differed. In general the vomerine teeth

and relative head size were much reduced in cannibalistic morphs from sites with low conspecific abundances but high abundance of minnows. This is consistent with the hypothesis presented by Powers (1907) and supported by Reilly (1983) that cannibalistic morphology can be influenced by prey characteristics. Additionally, In Lake 21, six individuals were identified as cannibalistic morph pedomorphic adult by their large size (>120mm) and presence of enlarged cloaca's, representing the second record of cannibalistic morph morphology occurring as a pedomorphic individual. The first record of cannibalistic morph pedomorphs occurred in South Dakota in a wetland where individuals also co-occur with fathead minnows (Larsen *et al.*, 1999).

It has been suggested cannibalistic morphs are adapted to arid climates and associated traits allow them to metamorphose earlier and potentially avoid habitat desiccation (Ghioca and Smith, 2008). In shallow water habitats, competition for resources is high, and early metamorphosis might be advantageous. However, all of our populations occurred in lakes or wetlands that rarely dry, a finding contrary to the hypothesis that cannibalistic morphs occur primarily in habitats where rapid drying presents great desiccation risks. Additionally, our finding of two populations in which all individuals were cannibalistic morphs is contrary to previously held beliefs that the cannibalistic morph always occurs at low frequencies in a population.

Our research identifies a scenario where an invasive competitor/prey species presence might have induced the cannibal morph phenotype. Like most habitats where tiger salamanders occur as the top predator (Collins and Holomuzki, 1984), prairie pothole wetlands were historically fishless, but fathead minnows have been stocked as baitfish in these closed basin prairie potholes (Zimmer *et al.*, 2000). Earlier workers have shown fathead minnows to have dietary overlap with tiger salamanders (Held and Peterka, 1974; Deutschman and Peterka, 1988;

Benoy, 2008). Therefore, dense populations of invasive fathead minnows are likely to deplete zooplankton communities and competitively exclude salamanders from occupying such wetlands (Zimmer *et al.*, 2002). Because both tiger salamanders and fathead minnows are gape limited (Zaret, 1980; Held and Peterka, 1974), increased head/gape sizes should allow cannibals to exploit larger prey items. Larger gape sizes may also allow cannibalistic morphs to prey on fathead minnows. In fact larval cannibalistic morphs from Lake 21 at SVL as small as 70mm had fathead minnows in their stomach (K. McLean, pers. Obs.). When viewed as a whole, our findings suggest other factors such as inter-specific competition may favor the presence of cannibalistic morphs, suggesting the presence of this polymorphism may be context specific. Our findings also suggest the degree of vomerine elongation and skull size differences are influenced by different environmental factors. Clearly, more work is needed to improve our understanding of the environmental and genetic factors that maintain this highly variable polymorphism and the role these factors will play in rapidly changing prairie pothole environments.

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**APPENDIX. FUNNEL TRAP CAPTURE DATA OF ALL AQUATIC VERTEBRATES
CAUGHT IN 162 WATERBODIES SAMPLED IN 2012-2013 IN STUTSMAN AND
KIDDER COUNTIES NORTH DAKOTA**

Lake Number	Fathead Minnows	Brook Stickleback	Iowa Darter	Yellow Perch	Northern Pike	Walleye	Smallmouth Bass	Bluegill	Common Carp	Black Bullhead	Tiger Salamander	Northern Leopard Frog
144	0	0	0	0	0	0	0	0	0	0	0	0
145	842	87	0	0	0	0	0	0	0	0	0	2
146	0	0	0	0	0	0	0	0	0	0	243	0
147	0	0	0	0	0	0	0	0	0	0	163	0
148	3962	15	0	0	0	0	0	0	0	0	0	0
150	101	0	0	7	0	0	0	0	0	0	0	0
151	200	67	0	2	0	0	0	0	0	0	0	0
152	857	2	0	77	0	0	0	0	0	0	0	1
153	41	19	0	8	0	0	0	0	0	0	0	0
154	422	0	0	10	0	1	0	0	0	0	0	0
155	14	1	0	231	0	0	0	0	0	0	0	0
156	297	0	0	15	0	0	0	0	0	0	0	0
157	137	0	0	91	0	0	0	0	0	0	0	0
159	8064	0	0	0	0	0	0	0	0	0	0	0
160	9131	4	0	0	0	0	0	0	0	0	0	0
161	11	0	0	54	0	0	0	0	0	0	0	0
162	10	1	0	12	0	0	0	2	0	0	0	0
164	237	2	8	0	0	0	0	0	0	0	0	0
165	13339	9	11	0	0	0	0	0	0	0	1	0
166	48	0	0	49	0	0	0	0	0	0	0	0
167	106	0	0	0	0	0	0	0	0	0	48	0
168	31	0	3	0	0	20	0	0	0	0	0	0
169	0	0	0	0	0	0	0	0	0	0	419	0
170	737	2	0	56	0	0	0	0	0	0	1	0
171	0	0	0	0	0	0	0	0	0	0	294	0
172	1	0	0	0	2	0	0	0	0	0	0	0

Lake Number	Fathead Minnows	Brook Stickleback	Iowa Darter	Yellow Perch	Northern Pike	Walleye	Smallmouth Bass	Bluegill	Common Carp	Black Bullhead	Tiger Salamander	Northern Leopard Frog
173	0	0	0	0	0	0	0	0	0	0	3	0
174	0	0	0	0	0	0	0	0	0	0	9	0
175	0	0	0	0	0	0	0	0	0	0	7	0
176	0	0	0	0	0	0	0	0	0	0	285	0
177	568	0	0	0	0	0	0	0	0	0	0	798
178	7879	0	0	0	0	0	0	0	0	0	0	5
180	4975	35	6	0	0	0	0	0	0	0	1	0
181	0	0	0	0	0	0	0	0	0	0	5	0
