AN EXAMINATION OF THE EFFECTS OF ANTHROPOGENIC HABITAT MODIFICATION AND CONTAMINANTS ON MISSOURI RIVER VALLEY FAUNA

By

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B.S., Lehigh University, 2007

A Thesis Submitted in Partial Fulfillment of
The Requirements for the Degree of
Master of Science

Department of Biology
In the Graduate School
The University of South Dakota
May 2012
The members of the Committee appointed to examine the dissertation of Kirsten Wert find it satisfactory and recommend that it be accepted.

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Acknowledgements

I would like to thank the following agencies and people for their generous assistance.

My field research on the Niobrara Delta was made possible by funding from the South Dakota Game Fish and Parks Wildlife Action Plan Competitive Grant program. The National Park Service provided funding for Songmeter digital wildlife sound recorders and Songscope sound analysis software through their small grants program.

Professor Dave Swanson spent many early hours on the Niobrara Delta assisting with bird surveys, and also offered helpful suggestions on thesis revisions as a member of my advisory committee.

Professor Tim Cowman graciously agreed to serve as the third member of my committee during my last semester at the University of South Dakota.

Aaron Gregor conducted trapping for turtles on the Niobrara Delta, and Emy Monroe demonstrated how to perform tactile searches for freshwater mussels.

The following people were instrumental in assisting in various ways, including gathering field data, analysis of samples for Bd or ATV, analyzing audio recordings and/or conducting lab experiments: Jenn Brown, Ben Mabee, Dylan Lehrbaum, Travis Snyders, Erica Geerdes, Katie Ferguson, Matt Kerby and Danielle Quist.

My advisor, Jacob Kerby not only helped me gain knowledge and practical skills in my field but also helped me improve on interpersonal and communication skills that are key in any profession. His warmth and sense of humor have been very encouraging throughout my entire graduate career.

Thank you to my parents, June and Randy Wert, along with my boyfriend, Andy Luxon, for consistently encouraging me and believing in me.

Last but not least I want to thank the entire USD Biology Department for creating an encouraging and supportive academic, professional, and social environment for all graduate students.
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Introduction

Habitat modification is one of the largest threats wildlife species currently face worldwide. In North America, wetland and riparian habitats in particular have been decimated since European settlement. Over three quarters of riparian woodland in the western US has been destroyed, and over half of all wetlands in the lower forty-eight States have been drained or filled (Partners in Flight/USFWS, 1999). The damming and channelization of rivers has been a major contributor to the loss of riparian and floodplain wetland habitat in particular. Natural hydrological conditions such as flood disturbance regimes and channel migration that formerly created diverse habitats for wildlife have been altered, allowing the conversion of floodplain habitat to agriculture and causing loss of sandbar habitat. Over 95% of riparian forests have been lost in western North America due to conversion to agriculture and alteration of hydrology (Brawn et al., 2001).

Prior to the construction of dams and channelization features on the Missouri River south of Sioux City, Iowa, constant erosion and deposition took place on the river. Outer edges of river bends were eroded, while sediment was deposited on the inner edges and onto mid-channel bars, creating bare substrate where pioneer vegetation could take hold, creating important diversity in riparian vegetation, recruitment of cottonwood (Populus deltoides) and willow (Salix spp.), and also creating sidewaters, sloughs and backwaters beneficial to various wildlife species. Dam construction, along with channelization starting in the mid 1940s replaced dynamic riparian habitats with a fixed navigation channel and stable floodplain shorelines. This significantly reduced habitat formerly provided by pre-regulation channel migration (CMRRASMI: NRC, 2010).
Habitat modification is one of the top threats to amphibian species today (Cushman, 2006). Habitat loss and fragmentation and play an important role in amphibian declines and extinctions (Knutson et al. 1999). Anthropogenic wetland habitat modification and loss in the upper Midwestern US has become widespread, with over 75% of wetlands lost over large portions of the region through conversion to agricultural, industrial, or urban land uses (Lehtinen et al. 1999). These land use changes have been associated with declines of amphibian populations (Mushet et al. 2006). The most common land use type in the Midwest is intensive row-crop agriculture. Modern farming practices employed to grow these crops often lead to habitat loss and modification (Knutson et al. 1999). Reversing declines in amphibian populations will require identifying and retaining high-quality habitat on the landscape (Lehtinen et al. 1999).

Habitat modification, especially through intensive agriculture, also has negative consequences for wetland bird populations in the Great Plains (Igl and Johnson, 1997; Brawn et al., 2001; Partners in Flight/USFWS, 1999). Wetland and riparian areas provide critical habitat for endangered and declining bird species. Large unregulated floodplain systems in the Midwest historically underwent seasonal flood pulses that created backwaters and open sandbar habitats, and allowed establishment of plant communities comprising successional habitat gradients, including marshes and willow/cottonwood forests. These habitats once supported diverse bird communities, but as these habitats and the disturbance regimes responsible for their formation have been lost to dams and agriculture, wildlife has declined. Loss of sandbars resulting from flood control has been implicated in declines of the endangered Interior Least Tern (*Sterna antillarum*) and Piping Plover (*Charadrius melodus*) (Brawn et al., 2001). In addition, there have been
large reductions in populations of marsh bird species such as King Rail (*Rallus elegans*), Virginia rail (*Rallus limicola*) and Sora (*Porzana carolina*) in the last 30 years (Partners in Flight/USFWS, 1999).

Where possible, wildlife habitat use should be monitored before habitat modification takes place. This is especially true when resulting data could be used to prevent or lessen future impacts to wildlife. The Niobrara Delta is one example of this. Over the past several decades, sediment has been settling out and accumulating in the upper reaches of Lewis and Clark Lake, beginning at the mouth of the Niobrara River, and over time, extending farther and farther downstream, forming what is now known as the Niobrara Delta (CMRRASMI; NRC, 2010, Army Corps of Engineers (USACE), 2004). This process has created both marsh habitat and various problems that must eventually be dealt with. The goal of my research was to determine what wildlife species might be impacted by proposed future sediment management that would alter the habitat.

Gavins Point Dam was constructed in 1955 and by 1957, Lewis and Clark Lake was filled. This reservoir is officially designated for multiple uses, including fish and wildlife habitat alongside regulation of flows for navigation, hydropower, flood control, recreation, water supply, water quality and irrigation, (Boyd *et al.*, 2010, Committee on Missouri River Recovery and Associated Sediment Management Issues; National Research Council, aka CMRRASMI: NRC, 2010). When the dam was first completed, engineers were aware of future sedimentation issues and predicted similar volumes of sedimentation buildup to what have been observed so far (USACE, 2004). Roughly 6 million tons of sediment enter Lewis and Clark Lake every year (Coker *et al.*, 2009), while sediment discharge measured at Yankton, just downstream of Gavins Point dam is
measured at only 0.25 million tons per year (Jacobson et al., 2009). This means that since
the completion of reservoir filling, roughly 316 million tons of sediment have gradually
settled out into Lewis and Clark Lake. Over half of incoming sediment originates on the
Niobrara River, which joins the Missouri roughly 31 miles upstream of Gavins Point
dam. This river drains mainly the Sand Hills region of Nebraska, and thus carries a
relatively large sediment load. The successive buildup of the Niobrara Delta over time
can be seen in aerial photography through the 1980s, 1990s and 2000s (Figures 1.1-1.3).
The downstream edge of the delta migrates approximately 500 to 600 feet per year, and
the total length of Lewis and Clark Lake has been reduced over time from 25 miles to 17
miles (Boyd et al., 2010).

The effects of this deposition include reduction in lake capacity, damage to
boating ramps, changes in channel morphology resulting in difficult boating, higher water
surface and groundwater elevation resulting in more frequent flooding and reduction of
farmable land, loss of cottonwood trees and changes in fish habitat (USACE, 2004).
Water storage has decreased by over 20% so far, and is being lost annually at a rate of
0.42%, and the expected lifespan of Lewis and Clark Lake is the shortest of all the main
stem reservoirs at 190 years (CMRRASMI; NRC, 2010). If no action is taken to mitigate
sedimentation, in roughly 140 years the reservoir will have completely filled with
sediment and the river channel location will change, finding the path of least resistance
(USACE, 2004).

Despite the various problems created by sedimentation, the Niobrara Delta also
contains potentially valuable wildlife habitat. Much of the Niobrara Delta, particularly
the leading downstream edge, is made up of side channel and sandbar habitats now
lacking throughout much of the rest of the Missouri River. These habitats vary in stages of stabilization and successional vegetation growth, ranging from bare sandbars to marshy areas with cattails, rushes and sedges, to areas with willow and cottonwood saplings, and potentially benefit a wide variety of wildlife, including several species of high conservation concern in South Dakota. Species of conservation concern include King Rail, False Map, Smooth Softshell, and Spiny Softshell turtles and freshwater mussels. King Rail has only three South Dakota breeding records from 1952, 1974 and 1977. The most recent status assessment for this species in the Midwest estimated a breeding population of zero to five pairs in South Dakota (Cooper, 2008). The Niobrara Delta is considered one of the only potential habitats in the state where this species could breed. False Map, Spiny Softshell and Smooth Softshell Turtles are monitored by the South Dakota Natural Heritage Program due to their rarity in the state. In addition, False Map Turtles are listed as a state threatened species by the South Dakota Game Fish and Parks Commission. All three species tend to use riverine habitats, preferring backwaters, lakes and floodplains to typical reservoir habitat (Kiesow, 2006). Freshwater mussels are also an important taxon to monitor, since twenty-five out of the thirty-four mussel species of South Dakota are considered vulnerable, imperiled or critically imperiled by the South Dakota Game Fish and Parks.

Nesting endangered Least Terns and Piping Plovers, as well as some turtle species, prefer bare sandbar habitat for laying eggs, whereas marsh bird species prefer shallow inundated areas with dense vegetation. Slower water velocity and areas of sediment accretion also provide habitat for various amphibian species that cannot live in deep reservoir or river channel habitats. Natural aquatic habitats, such as emergent
wetlands, streams and lakes may be important in maintaining anuran populations in largely agricultural landscapes (Knutson et al. 1999). While most natural lakes have shallow wetland complexes associated with them, reservoirs as a rule do not (Knutson et al. 1999). Lewis and Clark Lake is an exception due to sedimentation processes that create potentially productive shallow-water amphibian habitat.

There are several proposed solutions to sedimentation problems that may affect this habitat. The Army Corps of Engineers has proposed flushing as a solution for moving sediment past the dam (Boyd, USACE, 2011). Flushing is already performed in two reservoirs within the Missouri River basin: Guernsey Reservoir on the North Platte River, and Spencer Dam on the Niobrara River. This process is achieved by opening low-level outlets in the dam, draining the reservoir, and allowing river-like high velocity flows to move the sediment deposits through the dam and downstream. The U.S. Army Corps of Engineers has recently been using computer models to determine the engineering practicality of moving sediment through Lewis and Clark Lake to the river downstream of Gavins Point Dam (USACE, 2007). Only smaller particles such as silts and clays would be transported past the dam while most of the sand would re-deposit within the reservoir. USACE expects that continued flushes would increase efficiency but this had not been modeled at the time of the presentation of data. Silts and clays would not be useful for providing emergent sandbar habitat necessary for nesting terns and plovers. Discharges would also redistribute sediments below Gavins Point Dam. High discharges used for sediment flushing could also cause mild to major flooding in certain locations (Boyd, USACE, 2011).
Sediment management plans that would move large amounts of sediment would significantly alter existing delta habitat but the effects of such activities on wildlife species in the Niobrara Delta area are unknown. The only previous faunal surveys conducted on the delta involved fish and mussel species. South Dakota Game Fish and Parks annually conducts a general fisheries survey of Lewis and Clark Lake and the USACE annually reports on the pallid sturgeon population and associated fish communities in the Missouri River, including Segment 9, which comprises most of the Niobrara Delta (Steffenson, 2009). Survey sites included in a 2005 mussel survey included an area near the mouth of the Niobrara River but no mussels were found there (Shearer et al., 2005). No previous survey work had been undertaken for amphibians, reptiles or birds.

I also planned to examine the potential for trematode infection in amphibian, snail, and bird hosts. In nearby Minnesota, amphibian deformities are known to be caused by a trematode parasite called *Ribeiroia ondatrae* (Johnson and Sutherland, 2003; Johnson et al., 2010). I intentionally searched for snails of the family Planorbidae that transmit the parasite to amphibians in order to attempt to determine the prevalence of the disease on the delta.

**Methods**

This project involved three monthly surveys in the summers of 2010 and one survey in May 2011. These surveys examined the presence and abundance of birds, amphibians, turtles, and freshwater mussels. I also conducted similar surveys in Clay County, South Dakota during the summer of 2011 for comparison of species habitat use. Species of conservation concern in South Dakota were especially targeted by my surveys,
including king rail (*Rallus elegans*), false map (*Graptemys pseudogeographica*) and softshell turtles (*Apalone mutica* and *spinifera*), and several species of freshwater mussels (unionid species). Surveys were conducted at 15 randomly selected sites on the delta. GIS was used to create a polygon map of the entire delta area. This was then divided into five sections of roughly equal area. Three sites were chosen at random within each of the five sections. This ensured that my surveys were representative of the entire delta, but still randomly placed. During the first week of sampling, I attempted to get as close to the official coordinates as possible. Some of the planned survey sites proved inaccessible. In these cases, I chose an accessible site as close to the original coordinates as possible for my survey site (Figures 1.4 and 1.5). In 2010, sites were sampled three times over the summer; in May, June, and July, except for turtle trapping, which was done during August. At every site, standard survey techniques were used to determine the presence of particular taxa. Water quality was also measured using a YSI meter. Severe flooding in June and July 2011 prevented access to all of the delta sites (Figure 1.6). I decided to survey accessible off-river wetland areas in order to compare the bird and amphibian species present between the two habitat types. Fifteen sites were surveyed in Clay County, northeast of the town of Vermillion. The distances between these sites and the Missouri River ranged from 5.6 to 8.4 kilometers. The same survey techniques employed on the delta were used at these sites except for mussel and turtle surveying, since those species were not expected to be present at off-river sites.

*Survey Types*
Crews used survey techniques focusing on four main types of organisms: Marsh birds, amphibians, turtles, and freshwater invertebrates. While targeting these specific organisms, any fauna encountered during surveys were recorded and included in analysis. 

Marsh Birds: The North American Marsh Bird Survey Protocol (Conway, 2009) uses several methods to target secretive marsh bird species, including playback of bird calls and visual scanning from broadcast points. I began surveys as soon as it was light enough to navigate on the delta, soon after sunrise. Small portable speakers were aimed into the marsh and an .mp3 file of calls of each focal species was played. The audio file contained exactly 30 seconds of calls of each of the focal marsh bird species that I suspected may have been breeding in the area, interspersed with 30 seconds of silence between each species. Focal species included King Rail, Virginia Rail, Sora, Black Rail (Laterallus jamaicensis), Yellow Rail (Coturnicops noveboracensis), American Bittern (Botaurus lentiginosus), Least Bittern (Ixobrychus exilis), Pied-billed Grebe (Podilymbus podiceps), American Coot (Fulica americana) and Common Gallinule (Gallinula galeata). I placed considerable emphasis on the King Rail given its status as an accidental species in South Dakota. I also recorded the presence of any other bird species I could detect and identify at each site.

Amphibians: I employed Visual Encounter Surveys (Corn and Bury, 1990) along three 50 meter transects at each site. I also used three one-meter swipes with a dip net at each site to search for tadpoles and egg masses. All captured amphibians were also swabbed for chytrid fungus and inspected for parasite induced deformities.

Turtles: Aaron Gregor sampled for turtles at each site on the delta with baited hoop traps (Anderson et al., 2009) from 18-20 August 2010. False Map and Softshell turtles were
targeted. Turtles were also weighed, measured, sexed, and marked using Cagle’s notching scheme (Cagle, 1939) for all species except softshell turtles, which were marked exteriorly. (Figure 1.5 shows trap locations.) Lack of river access due to severe flooding prevented summer sampling of turtles in 2011.

*Freshwater Invertebrates:* I used visual and tactile searches in transects 1 meter wide by 12 meters long to search for mussels and snails. Dr. Emy Monroe (USD) assisted me with mussel sampling techniques and identification. I also intentionally searched for planorbid snails that could carry *Ribeiroia ondatrae.*

*SongMeter II Recorders:* I collaborated with the National Park Service, borrowing two Song Meter SM2 digital field recorders to record nighttime amphibian sounds in 2010. An additional grant in 2011 allowed me to use three additional recorders. SongMeter recorders were attached to stakes and placed at different survey sites each day of sampling during afternoon amphibian surveys, and picked up during early morning bird surveys. They were programmed to begin recording at 19:00 and stop recording at 03:00. Data from these recorders were analyzed with Song Scope Bioacoustics software.

*Data Analyses:* During data interpretation, delta survey sites were categorized into age classes by apparent successional age (Table 1.1). As the delta formed over time, it spread from the mouth of the Niobrara towards the reservoir, and from the banks towards the middle of the channel. This can be seen in aerial photos taken in successive years after the dam was closed. GIS aerial photography layers from different years were compared to each other to determine age classes. As more map layers became available I was able to improve the estimation of site ages and refine my analyses. My data were subsequently arranged according to site age class.
Results

Detection Data

Standardized North American Marsh Bird Monitoring Results:

I was able to detect five of my target marsh bird species with call playback surveys on the Niobrara Delta, and the same species, except for Least Bittern (Ixobrychus exilis), and one additional target species, Common Gallinule (Gallinula galeata), at off-river wetland sites (Table 1.2, Figure 1.7). While average numbers of Sora and Pied-billed Grebe (Podilymbus podiceps) were comparable between the Niobrara Delta and the off-river wetland areas, American Coots (Fulica americana) were detected in significantly greater averages at off-river wetland sites (Figure 1.8). Common gallinule was not detected on the delta, but one individual was detected at an off-river wetland site. This species is listed by Tallman et al. (2002) as a casual migrant in South Dakota, with less than 20 summer records, so this is a noteworthy record.

Visual Detection of Other Bird Species:

Although I did not find King Rail (Rallus elegans), I found 11 other bird species considered uncommon in the state of South Dakota, including Swamp Sparrow (Melospiza georgiana) (Table 1.2) (Tallman et al., 2002). These species were found at a variety of different age class sites (Figure 1.9). There were fewer bird species detected overall at the off-river wetland sites than on the delta, and Green Herons (Butorides virescens) were the only uncommon species found at the off-river wetland sites near Vermillion (Table 1.2). Many of the species found at both the Niobrara delta and off-river sites were detected at relatively similar numbers, or numbers too low to be statistically compared between different habitat types. However Killdeer (Charadrius
vociferus), Common Yellowthroat (Geothlypis trichas), Yellow-headed Blackbird (Xanthocephalus xanthocephalus) and Red-winged Blackbird (Agelaius phoeniceus) numbers differed significantly between the delta and off-river sites. Common Yellowthroats were found in greater abundance on the delta, while Killdeer and Yellow-headed Blackbirds were found in greater numbers at off-river wetland sites (Figure 1.10).

**Amphibian Visual Encounter Survey Results:**

The amphibian species I detected at the delta included bullfrogs (Lithobates catesbeiana), northern leopard frogs (Lithobates pipiens), Woodhouse’s toads (Anaxyrus woodhousii), boreal chorus frogs (Pseudacris maculata), and northern cricket frogs (Acris crepitans) (Table 1.3). Combined 2010 and 2011 amphibian detection data from the delta were again organized by apparent survey site age (Figure 1.11). Combined 2011 daytime and nocturnal amphibian detection data, as well as 2011 daytime amphibian detections alone were arranged by site age class as well (Figures 1.12 and 1.13). Species found on the Niobrara Delta in May 2011 were also compared to those found at off-river wetland areas in June of the same year. Tiger salamander (Ambystoma tigrinum) larvae were found only at off-river sites. Boreal chorus frogs and Northern Cricket Frogs were detected only on the delta. In 2011, numbers of leopard frogs were comparable between the delta and off-river wetland areas while overall detection for bullfrogs was low (Figure 1.14).

**Turtle Trapping Results**

Turtle species detected at the delta include False Map (Graptemys pseudogeographica), Spiny Softshell (Apalone spinifera), Snapping (Chelydra serpentine), and Painted turtles (Chrysemys picta) (Table 1.4). Detection data were again
arranged according to the percentage of sites of different age class with detections.
Species detection seems relatively evenly spread out over sites of different age classes (Figure 1.15).

*Freshwater Invertebrate Detections*

In 2010, one live freshwater mussel, a white heelsplitter (*Lasmigona complanata*) was found, at site 104, although empty shells were found at other sites. Planorbid snails were not present on the delta.

*Songmeter Recordings*

Amphibians recorded at night included Northern and Plains Leopard Frogs, Boreal Chorus Frogs, Northern Cricket Frogs, Bullfrogs and Woodhouse’s Toads (Figure 1.12). In 2010, only two songmeter digital recorders were available, which prevented analysis of which species might have been using habitat of differing successional age class. Plains leopard frogs were not detected in recordings from 2010. In 2011, I was able to combine nocturnal presence/absence data with daytime detections and arrange the data by site age class, as for daytime detection data (Figure 1.13).

**Discussion**

In 2011, I was able to conduct surveys at off-river wetland sites with similar water depths and vegetation to that found on much of the Niobrara Delta, and compare species found off-river to those found on the Delta. I found interesting differences in species occurrence between the delta and off-river wetland sites.

Overall wetland bird diversity on the Niobrara Delta was low, particularly for rail species, which need relatively stable water depths in order to successfully brood, hatch and raise young. Results of my call playback surveys suggest that sites older than 50
years may be the most important to secretive marsh bird species, since these were the sites at which the greatest numbers of target species were detected. Sites classified as successional older may be less susceptible to water level fluctuations affecting the rest of the delta.

The single Least Bittern detected on the delta was found in an area with very tall vegetation and deeper water habitat, which fulfills expectations according to their preferred habitat (Poole et al., 2009). The site where the single Virginia Rail was found during 2010 delta surveys was successional older than would have been expected for this species. Flooding of younger aged sites could have forced the individual to nest in nearby sub-optimal habitat. 2011 flooding mainly impacted areas along the Missouri River, and water levels at off-river sites did not experience the same level of fluctuations, which could explain the higher numbers of Virginia Rails off-river for 2011. Soras also nest above shallow water (18-22 cm deep) but differences in foraging behavior and diet may allow Soras to forage in a wider range of water depths than Virginia Rails, which seem to prefer shallower water (Melvin and Gibbs, 1996). This could help explain why there were far more Soras found overall on the delta than Virginia Rails.

There were more bird species detected on the delta overall than at off-river wetland sites, and only one uncommon species was found at the off-river wetland sites near Vermillion while ten were found on the delta (Table 1.2). One reason for this is the timing of surveys. May is peak migration time for many bird species, and most of the shorebirds detected on the delta do not breed along the Missouri River but were simply using it as stopover habitat on the way to breeding grounds. Common Yellowthroats were most likely found in larger numbers at the delta because they tend to forage in low trees
(Guzy and Ritchison, 1999), and there were far more of these on the delta than at off-river sites. Killdeers are better able to make use of human-disturbed habitats of the kind adjacent to the off-river wetlands I surveyed, which would explain their higher numbers there. Yellow-headed Blackbirds nest colonially and tend to displace Red-winged Blackbirds when both species attempt to use the same habitat for breeding. However, Yellow-headed Blackbirds are more specialized than Red-winged Blackbirds and after breeding this species forages in cropland and grassland (Miller, 1968; Twedt and Crawford, 1995). This could account for why I found more Yellow-headed Blackbirds at the off-river wetland sites, which were surrounded by cropland and grassland, while there were more Red-winged Blackbirds at the delta area, where adjoining habitat is composed of more forested areas.

Amphibian species detected on the delta are typically found in backwaters, side-channels, islands, sandbars and river banks. These habitat types have been diminished by damming and channelization and are rare elsewhere on the river, so the presence of these amphibians indicates that the delta could be serving as important habitat. Boreal chorus frogs and Northern cricket frogs were detected only the delta, however this was likely because these species have moved away from breeding areas by June. Tiger salamander larvae were likely found at off-river sites due to a lack of fish predators, which are abundant on the delta.

Turtle trapping in 2010 resulted in captures of all species expected except for smooth softshell (Apalone mutica). The apparent absence of softshell turtles was surprising, since they prefer large riverine habitats with large sandbars very similar to
habitat on the delta (MNDNR). It is possible that this species is present on the delta and has so far gone undetected.

The habitat at most of the sites was not ideal for mussels. Mussels prefer habitats with rocky, pebbly, or coarse sandy substrates, and many of the survey sites had very fine silt, and water with a high sediment load (Perkins and Backlund, 2000). However rocky shoreline areas do exist in the reservoir. The planorbid snails that carry amphibian parasites were not found on the delta during surveys, and none of the amphibians I found were visibly deformed or infected.

Our call recorders detected a number of nocturnally calling amphibian species. Amphibian species call mainly at night rather than during the day. Combining daytime with nocturnal species detections enhanced my ability to detect which species were occurring at which sites. Technical difficulties prevented Songmeter recorders from recording at sites ND110, ND113 and ND115, which may have prevented amphibian species detections at two thirds of the 25-50 year age class sites, and at one out of six of the oldest age class sites (Table 1.1). However comparing daytime detections alone (Figure 1.12) to daytime detections combined with nocturnal audio detections (Figure 1.13) clearly demonstrates that deploying Songmeter recorders at night can substantially increase amphibian species detection capability. Every amphibian species except for bullfrogs was detected at substantially higher numbers of survey sites when night-time data was included (Figures 1.12 and 1.13).

Future Work

Whether the Army Corps of Engineers uses sediment flushing, dredging or any other method of sediment management, wildlife on the Delta should be continually
surveyed in the future to determine both short-term and long-term effects. I will be conducting a third summer of surveys this year. The severe floods of 2011 moved large amounts of sediment into the upper reach of Lewis and Clark Lake, and also rearranged sections of the Delta’s channel and sandbar morphology. Surveying both before and after this flood event will give me the opportunity to determine the effects of scouring, erosion, deposition and channel migration processes on river delta habitat use one year later.

Summary

Several bird species found on the Niobrara Delta were not detected at off-river wetland sites. However, this may have been largely due to differences in survey location areas. Some migrant species detected on the delta are associated with the Missouri River in general and may not depend on the delta as a unique habitat. Also, all 15 of the off-river survey sites were located with 5.4 kilometers of each other, while sites on the delta were spread as far apart as 25.8 kilometers. Surveys over larger areas tend to find larger numbers of species, and this may have confounded my results.

The wide range of successional ages of habitats on the delta could be beneficial for biodiversity. The delta appears to provide good migration stopover habitat, but is not ideal for many breeding marsh bird species due to abrupt rises in water level in May and June. Artificial flow regimes are likely to reduce the potential for the delta to provide breeding habitat for bird and amphibian species. Selectively dredging certain areas of the delta may have less of an impact on species using the delta than the manipulation of flows on the Missouri River that are currently an integral part of USACE Missouri River management strategies.
Different bird species appear to be able to use each habitat age class, so dredging different areas of the delta may have consequences for different species. These patterns were not as clear with amphibians and turtles. The extent of habitat use by freshwater mussels appears minimal, most likely because of the silty substrate in most of the delta area. However the results make it clear that this habitat may be valuable to a range of amphibian, reptile and bird species.

There is currently a lack of information on how sediment flushing may affect delta habitat in reservoirs. Two other Midwest dam/reservoir units have been or are subject to periodic flushing by resource management agencies: Guernsey Reservoir on the North Platte River in Wyoming and Spencer Dam on the Niobrara River in Nebraska. Guernsey Reservoir was only flushed for four years in the 1950s after which time construction of an upstream dam reduced incoming sediment enough to make flushing unnecessary (White, 2001). The reservoir behind Spencer Dam on the Niobrara River (which is much smaller than Lewis and Clark Lake) is currently flushed at least twice every year. Downstream effects of flushing on water quality and fish have proven very negative (Hesse and Newcomb, 1982).

Since the sediment management study conducted by USACE has so far not looked at environmental impacts, it is as yet unclear how much of the habitat would be altered. Presumably, at the least, sedimentation processes in the reservoir would cease if flushing was conducted annually. If the USACE eventually decides to design a Programmatic Sediment Management Plan, an Environmental Impact Statement will most likely be necessary. Since wildlife is one of the designated purposes of Lewis and Clark Lake, the USACE has an obligation to avoid heavy impacts to wildlife in future
sediment management plans (Boyd, 2011). A variety of birds, amphibians and reptiles currently use Niobrara Delta habitat. It is of great importance to consider effects on wildlife habitat when designing future sediment management plans.
Chapter 2: Effects of the herbicide acetochlor and pathogenic Bd fungus on two species of amphibians, the cricket frog (*Acris crepitans*) and the leopard frog (*Lithobates pipiens*).

**Introduction**

Amphibian species worldwide are currently facing an extinction crisis. Over 120 species have likely gone extinct since 1980 (Whitfield *et al.* 2007) and more than 70% of the world’s remaining amphibian species are in decline (Hayes *et al.*, 2010). Complex interactions of several anthropogenic factors are most likely playing a part in causing these declines, including emerging diseases and contaminants (Collins and Storfer, 2003). A better understanding of these basic interactive effects is crucial to conservation efforts.

One of the major contributing factors underlying worldwide amphibian declines is a disease called chytridiomycosis caused by the chytrid fungus *Batrachochytrium dendrobatidis* (or Bd for short). Bd has been implicated in massive population declines and even extirpations throughout the globe (Retallick *et al.*, 2004, Rachowicz *et al.* 2006, Venesky *et al.*, 2010). It has already caused severe declines or extinctions in over 200 amphibian species worldwide and has been called the “greatest threat to biodiversity of any known disease” (Wake and Vredenburg, 2008, pg. 11466). It affects not only adult amphibians but tadpoles as well, infecting their mouthparts and decreasing foraging efficiency (Venesky *et al.*, 2010).

Contaminants are another important factor contributing to amphibian population declines (Bridges and Semlitch, 2000). In particular, pesticides and herbicides applied ubiquitously on agricultural lands are issues of major concern for amphibian populations in the US. Several studies suggest that pesticides can have significant impacts on
amphibian populations (Hayes et al. 2002; Relyea et al. 2005; Hayes et al. 2006; Forson and Storfer 2006; Oka et al. 2008; Kerby and Storfer, 2009). These contaminants can impact the immune system, increasing susceptibility to disease, even at low, ecologically relevant levels (Forson and Storfer, 2006; Kerby and Storfer, 2998; Hayes et al., 2010).

The effects of emerging infectious diseases and environmental contaminants are commonly studied separately, but in nature, animals are often exposed to multiple stressors simultaneously. Several recent studies have examined the combined effects of contaminants, disease and/or non-anthropogenic stressors, such as predator cues, on amphibians (Kerby and Storfer, 2009; Kerby et al. 2011; Sih et al., 2004; Brown, 2011).

Several studies have argued that the prominent impact of Bd fungus on amphibian populations is due to its being a novel pathogen, to which naive populations have no evolved resistance. However, pathogens such as ranavirus have co-evolved with native populations and potentially cause epizootic events due to exacerbation by contaminants, particularly pesticides and herbicides. In fact, for some contaminants, concentrations that appear relatively safe when tested as a single stress factor can become deadly for amphibians when combined with disease or predator stress (Relyea and Mills, 2001; Sih et al., 2004; Kerby et al., 2011). Anthropogenic and natural stressors can also have sub-lethal impacts on mass, body size, development rates, behavior and activity levels (Kerby et al., 2011). Stress resulting from detection of predator chemical cues can significantly alter prey behavior, including reduction of foraging effort (Luttbeg and Kerby, 2005).

While stress interaction studies have been conducted on a number of well known pesticides and herbicides including carbaryl, chlorpyrifos and atrazine, acetochlor has not yet been investigated in combination with other stressors. Acetochlor is an herbicide
commonly used on Midwest corn crops, and is the third most common herbicide detected in rivers and lakes (Foley et al., 2008). A recent study examining the presence of contaminants in habitats used by amphibians in southeast South Dakota found acetochlor levels of .07-.09 parts per billion at five out of twenty pond and wetland survey sites (Brown, 2011). Acetochlor can affect thyroid hormone receptor gene expression, which may lead to reductions in metamorphosis rate (Turque et al. 2005; Crump et al. 2002; Cheek et al. 1999) and alterations of brain function (Helbing et al., 2006). Therefore time to metamorphosis and tadpole behavior could be important variables to monitor during exposure to environmentally relevant levels of acetochlor.

The purpose of my experiments was to examine the effects of acetochlor, Bd fungus and predator stress, both alone and in combination with each other, on tadpoles of amphibian species in southeast South Dakota. I expected increased mortality, a change in growth rate, and changes in behavior and feeding in experimental groups, with interacting stressors having greater effects than stressors acting alone.

**Methods**

*Experiment 1: Effects of Acetochlor, Bd Fungus and Predator cue on Lithobates pipiens tadpoles*

*Experimental Setup*

For the first experiment, 240 individual Lithobates pipiens tadpoles were hatched from four egg masses found on the Niobrara Delta (Bon Homme County, SD). Three egg masses were collected at UTM Coordinates 591781 4743681 on May 17, 2011, and a fourth was collected at UTM Coordinates 585692 4737592 on May 18, 2011. Individual
tadpoles from each egg mass were randomly assigned to eight treatment groups in order to avoid confounding genetic effects. A 2 x 2 x 2 factorial design was used, with 30 replicates in each treatment group. Tadpoles were kept in 100 milliliters of treatment water in 150 ml, 7 cm diameter, 8 cm deep, non-reactive plastic urine-sample cups for the duration of the experiment. Treatments were assigned random positions on shelving units to avoid any potential confounding effects of the testing environment. Containers were positioned on three shelves of each side of every shelving unit, so tadpoles on each side could be scanned easily and with little disturbance for mortality, position, behavior, and feeding within one minute. Tadpoles were fed fish flakes at the beginning of the 8 day experiment. They were checked twice daily, at 0900 and 2100 from 13-20 July, 2011.

Experimental Groups

Dragonfly larvae were captured at wetland sites roughly 2-4 miles northeast of Vermillion, South Dakota. (UTM coordinates 672402 4740069, 674206 4740109, and 673489 4740496.) Larvae were individually housed and fed *L. pipiens* tadpoles every 2-3 days until the beginning of the experiment. Predator cue was produced by moving the twelve individually housed dragonfly larvae into a single garbage container of 45 liters of reverse osmosis water reconstituted with RO Rite 48 hours prior to the experiment. Dragonfly cups were covered with mesh sides that allowed movement of water into and out of cups within the garbage container. This water was then subsequently used to fill experimental cups in predator cue treatments at the experiment’s beginning.

Bd fungus was grown for two weeks on 120 petri dishes of sterile agar, and then rinsed from each petri dish into a communal beaker with 3 ml of reverse osmosis water.
A haemocytometer and microscope were then used to estimate the concentration of zoospores, which was 11.2 million zoospores per milliliter. One milliliter of zoospore laden water was added to each Bd treatment. Controls for Bd were created by swirling 3 ml of reverse osmosis water in petri dishes of plain sterile agar in the same manner, combining the swirled water in one beaker and adding one milliliter to each treatment cup.

For acetochlor treatment groups, a stock solution of 1 mg/L of technical grade acetochlor in a 10% DMSO solvent was created and verified via HPLC. One mL of this solution was added to 100 ml of water in each acetochlor experimental treatment cup, creating a final concentration of 100 µg/L. One milliliter of 10% DMSO solution was added to control treatment cups to serve as a solvent control.

*Mortality, Illness, Mass and Length*

At the beginning of the experiment, photos of each tadpole were taken using a Canon Digital DS126071 Rebel XT/EOS SLR camera with EFS 18-55 mm lens. These photos were later measured using the software program ImageJ to determine snout vent length (SVL) and total length. Each tadpole was placed next to a centimeter ruler before being photographed, allowing the scale of each photo to be standardized. As tadpoles were being checked, mortality was recorded and dead tadpoles were immediately photographed again for later measurement. Dead tadpoles were preserved in 2 ml centrifuge tubes of ethanol labeled with treatment group and date. Any tadpoles positioned upside down or with curled tails were listed as “Ill” and monitored closely for
death. At the end of the experiment all survivors were photographed once more for
measurement, mass was recorded, and tadpoles were labeled and preserved.

Behavioral observations and stress assays

Each side of every shelving unit was scanned for one timed minute. Tadpole
position was recorded in three categories: Top (touching the surface of the water),
Bottom (on the bottom surface of the cup), or Middle (anywhere in between the two).
Movement was rated on a scale of 1-3 with 0 being no movement, 1 being any detectable
slight movement, 2 being moderate movement and 3 being very erratic movement.
Feeding activity was also recorded when individuals made characteristic pecking motions
along the bottom of the cup. Mid-way through the experiment, half of every treatment
group was euthanized to acquire brain tissue for assays to determine corticosterone
levels, which are indicative of stress. These methods and results are presented in a
separate undergraduate honors thesis (Snyders, 2012).

Experiment 2: Effects of acetochlor and delayed Bd fungus exposure on Acris crepitans
tadpoles.

Experimental Setup

My second experiment used only acetochlor and Bd as stressors but reversed the
order of exposure to Bd. Acris crepitans tadpoles were collected at the tadpole stage from
a single pond (UTM coordinates 663803  4729525) and assigned to one of four treatment
groups using a 2 acetochlor x 2 Bd factorial design. The control group had 18 replicates
and each of the experimental groups had 17 replicates. Pesticide preparation was the
same as experiment one with the exception that 2 mL of stock solution was added to larger 200 mL containers. (Round Berry Plastics treatment cups were roughly 13 cm diameter and 7 cm deep.) Larger containers were used due to larger tadpole size.

Experimental Groups

The same protocols from experiment one were followed to make Bd exposures but only 40 petri dishes were required to grow Bd this time due to a reduced number of treatment cups. Acetochlor was added on the morning of 5 August 2011 and Bd was added the afternoon of 8 August 2011, three days later. This allowed me to measure the residual effects of contaminant exposure on disease susceptibility.

Mass, Length, Mortality, Illness and Metamorphosis

Tadpoles were photographed for measurement as in the previous experiment, and weighed both at the beginning and end of the experiment. Tadpoles were checked at the same times, 5-15 August 2011 not only for mortality and apparent illness but also for front leg emergence. Tadpoles were removed from the experiment when front legs appeared, signaling complete metamorphosis, to avoid mortality related to drowning rather than acetochlor or Bd exposure. Metamorphosed or dead individuals were photographed for measurement and weighed before being preserved in the same manner used in experiment one, as were all survivors at the end of the experiment. No movement or feeding behavior was recorded.
**Statistical analyses**

Since death and illness were binomial response variables, I used a multi-way ANOVA to determine the sum of squares, F values and p values and determine whether I had significantly higher mortality or illness in any of the experimental groups. Final mass and final body length were entered into General Linear Models along with predictor variables.

I used a Kruskal-Wallis test to examine the response variables: average movement scale, proportion of checks during which tadpoles were moving, and proportion of checks during which tadpoles were feeding. These tests were done only for data involving surviving tadpoles because tadpoles that died during the experiment were measured for a shorter amount of time (some only with only a few observations) thereby altering the interpretation of behavioral results.

General linear models were also used to examine data from the second experiment. Bd exposure and acetochlor exposure were entered as predictor variables and final body mass and final body lengths were entered as response variables. Mortality and illness were also compared between experimental groups with percentage tables and a two-sample proportions test.

**Results**

**Experiment 1: Effects of Acetochlor, Bd Fungus and Predator cue on *Lithobates pipiens* tadpoles**

Our first experiment examined three predictor variables; Bd exposure, acetochlor exposure, and predator cue exposure, and seven response variables; death, illness, final body length, final body mass, average movement scale, proportion of checks where tadpoles were moving, and proportion of checks where tadpoles were feeding. No
relationships were found for death, final body length, or final body mass (Tables 2.1-2.2). Significant negative relationships were found between Bd exposure and average movement scale, between Bd and proportion of checks during which tadpoles were moving, between Bd and proportion of checks during which tadpoles were feeding, between predator cue and average movement scale, and between predator cue and proportion of checks during which tadpoles were moving (Table 2.4; Figures 2.1-2.5). The negative relationship between predator cue and proportion of checks during which feeding was detected was not statistically significant, and pesticide did not have a significant relationship with any movement or feeding measurement (Table 2.4).

**Experiment 2: Effects of acetochlor and delayed Bd fungus exposure on prometamorphic *Acris crepitans* tadpoles**

General linear models indicated that neither final body length nor final body mass were significantly different for any particular experimental group (Tables 2.5 and 2.6). Five tadpoles appeared to be ill and three died during the second experiment. P-values resulting from two-sample proportions tests were not significant, indicating that exposure to Bd, acetochlor, or both Bd and acetochlor was not correlated with higher or lower tadpole mortality or illness (Tables 2.7-2.9).

**Discussion**

During the first experiment high levels of mortalities were observed across all treatments. This prevented my being able to detect any differences in death due to treatment effects. The cause of mortality was unknown but occurred suddenly across all treatments. Despite this, I was able to detect significant differences in several sub-lethal variables.
Mean movement scale was significantly reduced in the Bd experimental group compared to the control group (Figure 2.1). This indicates that Bd exposure was likely to have exerted a mild suppressive effect on the average speed of tadpole movement. Bd exposure also lowered the total amount of time tadpoles spent moving (Figure 2.2). These two variables are clearly related though and the low values of the movement scales infer that the effect was based more on reducing movement at all rather than slowing down very active tadpoles. The mean proportion of time spent feeding also decreased with Bd exposure (Table 2.1; Figure 2.3). Mean movement scale and mean proportion of checks during which tadpoles were detected moving were also lower for predator experimental groups versus control groups (Figures 2.4 and 2.5). These decreases in time spent moving and feeding are presumably directly due to the exposure to disease or predator cue, or due to tadpoles attempting to shunt energy into faster development rather than foraging (Warne et al., 2010). Despite these reductions in foraging, I did not detect differences in final body length between the respective experimental groups. This is perhaps due to the short timeframe of the experiment (five days) or the high degree of mortality immediately preceding the experiment.

Interestingly, the results of the second experiment showed no significant relationships between Bd or acetochlor and mortality, illness, final body mass or final body length (Tables 2.7-2.11). The lack of any effects from Bd exposure during experiment two was somewhat surprising. It is typically thought that leopard frogs are somewhat resilient to Bd exposure, so I expected to see greater impacts in the cricket frog. However, body size in the second experiment was significantly larger, with organisms that were farther along in larval development. The first experiment found
results in activity and feeding levels, but these behaviors were not measured in cricket frog tadpoles, so whether sub-lethal effects are present will require further study.

I used 100 micrograms per liter as the experimental acetochlor concentration. Previous studies found median acetochlor concentrations of roughly 0.05 - 1.19 micrograms per liter in surface water near acetochlor application sites (Kolpin et al. 1996 qtd. in Cheeck et al. 1999). These levels are higher still than those found by Brown (2011) of .07-.09 ppb. Therefore my testing levels were well above the range I would have expected in most natural tadpole habitats. However, the suggested application rate for acetochlor is 23 grams per liter (Miller, 2010). Pesticide drift can potentially directly expose amphibians to concentrations close to application rates and has been implicated in amphibian declines in California (Davidson et al., 2002). In order to obtain high enough numbers of replicates to generate adequate statistical power, I used only one level of acetochlor. Ideally, with a higher number of tadpoles available, could have examined multiple levels of acetochlor, including a low level at ecologically relevant levels, a high level at application concentrations, and a medium level between the two values. Using multiple experimental concentrations of acetochlor could also have allowed me to detect possible hormetic effects. Hormesis occurs when the deleterious effects of a contaminant are stronger at lower levels than at higher levels. This phenomenon can obscure negative effects if the only concentration being tested is a higher concentration that produces little or no response.

No previous studies have examined acetochlor’s effect specifically on Northern cricket frog tadpoles. This species may have a greater resistance to acetochlor than other amphibian species that have previously been studied. Another possible reason for the lack
of effects is the late developmental stage at which the second experiment was begun. Studies of acetochlor’s effects on other amphibian species have found that the contaminant is a thyroid targeting endocrine disrupter that specifically affects the process of metamorphosis (Crump et al. 2002). Tadpoles used for experiment two were already in the prometamorphic stage, with hind limbs present, and forelimbs close to completion of development. It is possible that development was too far along for tadpoles to be severely affected by exposure.

Although acetochlor is known to have a possible effect on metamorphosis timing, my experiments were unable to examine this aspect as a response variable. The first experiment used tadpoles raised from egg masses in the lab, but ended during prematamorphosis. Tadpoles used in the second experiment were collected from a pond as free-swimming individuals, not hatched from egg masses in the lab, and therefore had undetermined hatch dates. Future studies of effects on metamorphosis timing would ideally follow protocols used in previous experiments, in which tadpoles were raised from egg masses in the lab, treated with exogenous thyroid hormone in order to induce metamorphosis (Wright et al. 1994), re-dosed every 48 hours (Cheek et al. 1999) and monitored until metamorphosis.
Chapter 3: Effects of Projected Agricultural Runoff and Cattle Presence on Amphibian Abundance and Disease in Natural and Man-made Ponds of Northeast Nebraska and Southeast South Dakota.

Introduction

Habitat loss and degradation are major contributors to biodiversity loss, particularly for amphibian species. A number of studies suggest that contamination from agricultural runoff poses a threat to amphibian populations (Kiesecker, 2002; Hayes, et al., 2006; Rohr, 2010). Herbicide contaminants are commonly found in aquatic habitats in agricultural landscapes. A large number of studies have examined effects of these pesticides and herbicides on amphibians in laboratory studies. Some have direct effects, such as glyphosate, which is used ubiquitously for US crop production, and has detrimental effects on larval and adult amphibian mortality (Relyea 2005; Relyea 2005). Other pesticides have more indirect effects through lowered immune responses and increases in susceptibility to disease. For instance, four of the most commonly used agricultural pesticides and herbicides markedly increase trematode parasite load in Green Frog tadpoles (Rohr J. R. 2008) and carbaryl reduces skin peptides important for defense against chytrid fungus (Davidson, 2007).

Ambystoma tigrinum virus (ATV) is another disease that interacts with contaminants, and can play a role in increased mortality of Abystoma tigrinum (Tiger Salamanders) (Kerby and Storfer, 2009). This disease was originally endemic, with local populations having developed resistance to local strains of the virus. However, in recent years the fishing bait trade has moved large numbers of Tiger Salamander larvae, some of which are infected, from Midwestern states such as Nebraska to Southwest states such as New Mexico and Arizona. This has spread genetically distinct strains of the virus to new
areas, where it is taking a toll on populations of endangered Tiger Salamander subspecies (Jancovich et al., 2005; Storfer et al., 2007; Picco A. M and J. P. Collins, 2008). Determining whether herbicide–mediated increases in susceptibility of Tiger Salamander larvae to ATV observed in the lab have similar effects in natural habitats could be an important factor in understanding the recent spread of novel ATV strains to new areas. ATV prevalence is as yet unknown in South Dakota, although surveys have been conducted in North Dakota and Nebraska (Kerby, personal communication, 2011).

Although many experimental studies have determined relationships between contaminants and increased susceptibility to disease in controlled lab or mesocosm settings, it is important to determine if these relationships occur in natural amphibian habitat as well. Complex interactions between a wide array of biotic and abiotic factors can exist. Some of these interactions can be recreated in lab or mesocosm settings, which have been instrumental in determining basic mechanisms of effects and in demonstrating that these interactions can dramatically increase the severity of anthropogenic effects. However it is not realistic to account for every possible interactive factor that may change how amphibian populations react to anthropogenic habitat modification or contamination. Previous studies on interactive effects of herbicide, disease and predator stress found that the source habitat of amphibian larvae was the most important factor related to survival (Brown, 2011). More studies of altered versus natural habitats are needed in order to determine general effects on populations in nature.

This study focused on comparing estimated amphibian abundance and species diversity at pond sites in northeastern Nebraska and southeast South Dakota. This landscape provides an ideal area to examine the impacts of agricultural inputs. Several
sites are situated in the middle of agricultural fields and have high levels of pesticide inputs, while others are somewhat protected and contain very little detectable contaminants. In addition, several ponds served as watering areas for cattle and thus afforded the opportunity to incorporate possible effects of cattle on amphibian habitat use. Wetland habitat loss and modification over the past century, particularly for cropland, has forced amphibians in many areas of the Midwest to use any remaining habitat in the largely agricultural landscape (Knutson et al., 1999, Knutson et al., 2004). This includes cattle ponds, which are considered sub-optimal habitat for many anurans. However these ponds can also be important for a few species such as the Eastern Tiger Salamander (A. t. tigrinum). These sites allow for the examination not only of Tiger Salamander larval abundance but also ATV prevalence.

I predicted that I would find lower numbers of individuals at sites with higher projected agricultural runoff impacts and at sites with cattle present. I did not expect other factors such as pH to differ sufficiently between sites to produce any statistical relationships.

Methods

Twenty local ponds in South Dakota and northeastern Nebraska were chosen for surveying. GIS map layers were used to find sites expected to have no or low herbicide input from agricultural surface runoff as well as sites expected to have high herbicide input from agricultural surface runoff. Partially transparent aspect and slope map layers were overlain on aerial photography layers to find 10 ponds that were topographically isolated from cropland and 10 ponds which appeared to be downslope from adjacent
cropland in the same sub-watersheds. After amphibian surveys were completed the circumference of each pond was measured using ArcGIS.

Ponds were surveyed in late June and early July 2011 by teams of two to three surveyors. Two surveyors walked 100 meters each in opposite directions along the pond shoreline, using the visual encounter technique (Corn, 1990) to record any amphibians visually or aurally detected. GPS and rangefinders were used to keep track of distance walked along the shoreline. Detected individuals were caught, measured, weighed and swabbed for chytrid fungus whenever possible. A third surveyor used a dipnet to make five one-meter sweeps for tadpoles. Up to 50 tadpoles at each site were identified. Where *Ambystoma tigrinum* (Tiger Salamander) larvae appeared to be present, two surveyors used a seine net one meter tall and 2 meters wide to make three one-meter sweeps. Larvae from each seine sweep were measured, recorded, and kept in plastic bags until all were counted to prevent re-capture. Small, non-lethal tail samples were taken from of up to 10 individual *Ambystoma tigrinum* larvae at each site for future *Ambystoma tigrinum* virus (ATV) detection, before releasing all larvae back to the pond. Scissors used to clip tail samples were sterilized with bleach and rinsed between each individual to prevent cross contamination of samples. Samples were immediately placed on ice and stored in a -20° C freezer at the University of South Dakota before being analyzed for the presence of ATV.

Many of the pond sites were found to be man-made dug-outs used to water cows on grazing land, and were impacted to varying degrees by cattle use. Presence or absence of cattle at each site was recorded. Evidence of cattle use included droppings, hoof prints, cattle trails or heavy compaction of soil around pond edges.
A water quality multimeter (YSI Probe) was used to collect measurements of water temperature, conductivity, pH, chlorophyll and dissolved oxygen levels. Air temperature was also recorded.

**Statistical Analyses**

Relationships between numerical response variables and categorical predictor variable groups were analyzed with general linear models using a Gaussian distribution and identity link function setting in the R commander program in R 2.11.1 for Windows. Projected agricultural runoff impact (high or low) and cattle presence were the categorical predictor variables tested, while numerical predictor variables included pH and pond circumference. Numerical response variables included total detections for adult Northern Cricket Frogs and Boreal Chorus Frogs, and detections of Gray Tree Frog tadpoles, Leopard Frog tadpoles and Tiger Salamander larvae. Water pH and pond circumference were also treated as response variables in order to rule out co-variation with runoff impact and cattle presence/absence. Generalized linear models with Gaussian distributions and identity link functions were also used to test separate and interactive affects of pH and cattle presence for Gray Tree Frog tadpole detections because mean pH was significantly higher where cattle were present.

Projected runoff impact and Tiger Salamander larvae body length data were entered into a general linear model using a Gaussian distribution and identity link function, while ATV detection was treated as a categorical variable, using a glm with a binomial distribution and logit link function. Because only four sites contained Tiger Salamander larvae, all of which happened to be cattle ponds, and the pH dataset would
only contain four values, presence of cattle and pH were not used as predictor variables with ATV detection or body length.

**Results:**

A statistically significant positive relationship was found between pH and cattle presence, with mean pH at sites with cattle present being 8.3 while pH at sites without cattle was 7.6 (Figure 3.1). Gray Tree Frog tadpoles were detected at three out of twenty survey sites. Detections were negatively correlated with pH and were also lower on average with cattle present (Table 3.1, Figures 3.2 and 3.3). Treating pH as a covariate with cattle presence in a general linear model with Gray Tree Frog tadpoles as the response variable showed that neither relationship was due only to interaction effects between the predictor variables (Table 3.1). Leopard Frog tadpoles were detected at six out of twenty survey sites. Detections were negatively correlated only with pH and were not lower on average with cattle presence (Table 3.1, Figure 3.4).

Adult Boreal Chorus Frog abundances also displayed a significant negative correlation with pH (Table 3.1, Figure 3.5), but were not higher or lower at sites without cattle (Table 3.1). This species was only present at projected low runoff impact sites, however difference in detection was not found to be statistically significant (Table 3.1). This species was detected at very low levels at only two of the 20 sites surveyed.

There was a strong trend of a positive relationship of Leopard Frog tadpoles at low projected runoff impact sites (Table 3.1 Figure 3.6). Total adult cricket frogs and cattle absence was another strong trend (Table 3.1, Figure 3.7).
Three *Ambystoma tigrinum* larvae tested positive for *Ambystoma tigrinum* virus. Although these were from three different ponds with no apparent trend, this marks the first known finding of ATV in extreme northeast Nebraska. ATV did not show any significant trends in regards to runoff impacts (Table 3.2), however Tiger Salamander larvae had significantly larger snout-vent length at high impact sites (Table 3.2, Figure 3.8).

Water temperature, conductivity, chlorophyll, dissolved oxygen levels, and air temperature were also entered in general linear models with predicted runoff impact and cattle presence (Table 3.3). Although pond circumference co-varied with projected runoff impact level, and conductivity co-varied with cattle presence, there were no relationships found between these abiotic factors and species abundances (Table 3.4).

**Discussion**

Some interesting relationships became apparent when examining sites with and without cattle present and with regard to pH levels. Cattle pond use and pH were linked to abundance estimates of several amphibian species found at the sites.

Tree Frog tadpoles, Leopard Frog tadpoles and adult Boreal Chorus Frog detections were significantly negatively correlated to higher pH (Figures 3.2, 3.4 and 3.5). Gray Tree Frog tadpole detections were also lower on average with cattle present (Figure 3.3). Although pH and cattle presence were positively correlated (Figure 3.1), this did not produce a false relationship with Gray Tree Frog tadpoles and either pH or cattle presence. Both relationships were statistically significant when they were used as predictor variables in a general linear model with Gray Tree Frog detections as the
response variable. The increase in pH at sites with cattle use may be due to addition of ammonia to the ponds in cattle waste. pH was not significantly different between projected high and low runoff impacts in this study. However, pH, along with other water quality parameters can be dramatically affected by fertilizer runoff (Hart, et al., 2004; Mcleod and Hegg, 1983). Presence of cattle is known to have negative effects on amphibian presence, abundance and diversity. The main reasons for this include the overgrazing and trampling of edge and emergent vegetation that serves as cover for avoiding predators and feeding sites for amphibian larvae, as well as negative effects on water quality, including increased nitrogenous waste inputs and increased turbidity (Schmutzer et al., 2008 and Burton et al, 2009). Cattle may even sometimes directly trample egg masses (Schmutzer 2008).

Adult Boreal Chorus Frog and Leopard Frog tadpole detections were also negatively correlated with pH, but not cattle presence. Due to the low number of non-cattle sites that I sampled, it is unclear whether the relationship between pH and cattle did not apply for these species, or was simply not detectable. I observed fewer Leopard Frog tadpoles overall (22) and adult Boreal Chorus Frog adults (7) as compared to Gray Tree Frog tadpoles (42).

pH above 8.0 in aquatic habitats has been shown to be detrimental to amphibians. One reason for this is that ammonium/ammonia equilibrium shifts toward higher toxic ammonia concentrations as pH increases (Poole, 2012). Data demonstrate that many survey sites did display pH levels above 8.0, with ponds with cattle present having significantly higher mean pH than those without cattle. pH averaged 8.3 at ponds with cattle present versus 7.6 on average at ponds without cattle (Table 3.1). Although
amphibians are not as sensitive to ammonia as fish are, many amphibian species hibernate or reproduce on the bottom of ponds, where ammonia can be released from sediments (Jofre and Karasov, 1999). While cattle ponds are not generally ideal habitat for amphibians, these ponds may actually be ideal for Tiger Salamanders in terms of water depth. Surveys found larval Tiger Salamanders at four out of 20 total sites, all of which were man-made ponds for use in watering cattle. This species requires deeper pond habitats for reproduction. Although there was no relationship found between Tiger Salamander larvae and pH, low sample size may have obscured the effect pH may have on this species. This relationship is potentially important, particularly if cattle ponds make up a high proportion of suitable egg-laying habitats that are locally available.

Although possible relationships between ATV prevalence and runoff or cattle impacts could not be determined, average body length of Tiger Salamander larvae was significantly larger at projected high impact sites (Figure 3.8). However SVL data was collected from only four sites. Differences among these sites may have been due to any number of confounding factors, such as small differences in breeding timing or population densities. Other water quality parameters that were measured and entered in general linear models to determine possible relationships with predicted runoff impacts and cattle presence included dissolved oxygen, conductivity, chlorophyll and water temperature. Air temperature was measured as well (Table 3.3). No relationships were found between these parameters that also affected species abundance (Table 3.4).

Leopard Frog tadpoles were found at higher numbers at low projected runoff impact sites and this relationship was nearly statistically significant (Figure 3.6). However low sample size for most amphibian species may have created high variation
that obscured some statistical relationships. Low sample size clearly affected significance of adult Boreal Chorus Frog detection differences. This species was only detected at low projected runoff impact sites, but overall was only detected at two out of 20 sites, with overall detection numbers being 5 at one site and 2 at another site.

Other factors may have been at work in the relationships I detected. Beyond its association previously noted, pH might also be correlated with general habitat degradation or factors such as edge vegetation or surrounding land use which one might expect to have effects on abundances. In future studies, it would be best to more closely account for these other potential contributing factors by measuring and estimating the extent of their presence at each site.

*Future Directions*

Water quality samples were collected in one-liter amber glass bottles and frozen to allow possible future analysis for herbicides and pesticides present at survey sites. This will allow future verification of which sites were actually high and low impact sites in regards to pesticide and herbicide contamination.

While numerous studies have been conducted concerning herbicide runoff and how agricultural contaminants arising from crop production affect amphibians, relatively few studies have focused on impacts of cattle, and none have focused on how cattle presence may impact amphibian disease. Grazed landscapes containing cattle ponds make up a very large proportion of land in the Midwestern United States. In some areas, natural wetland habitat has declined so severely that agricultural ponds may be very important as some of the only viable amphibian habitat left (Knutson *et al.* 2004). Use of ponds for
watering cattle can dramatically alter vegetative habitat and water quality, markedly
decrease numbers of postmetamorphic individuals of certain species and decrease overall
amphibian reproductive success (Schmutzer et al. 2008, Burton et al. 2009 and Knutson
et al. 2004). These effects are pervasive, and future studies focusing on the effects of
cattle would contribute important information to the conservation biology of amphibians
in largely agricultural landscapes.
Figure 1.1. Aerial photo of Niobrara Delta showing downstream edge in 1982 (Tworek, 2001).
**Figure 1.2.** Aerial photo of Niobrara Delta showing downstream edge of delta in early 1991 (Tworek, 2001).
Figure 1.3. The Niobrara Delta in 2005.
Figure 1.4. Map of Survey Points on the Delta.
Figure 1.5. Map of 2010 Turtle Trapping Sites.
Traps were set in suitable habitat as close as possible to the same survey points used to
survey for other species. Site ND101 is at the Northeast end of the Delta and does not
show on the map because the data layer is missing. Site 107 was inaccessible during
turtle trapping due to flooding, however a trap was set as close as possible to the original
point.
Figure 1.6. Severe flooding on the Niobrara Delta in 2011.
Figure 1.7. Proportion of Niobrara Delta sites at which marsh bird species were detected during 2010-2011. Data is divided by estimated site age class.
Figure 1.8. Comparing between marsh bird call playback survey results at Niobrara Delta Sites in May 2011 compared to off-river wetland sites near Vermillion in June of 2011. (This data is from 2011 only. Off-river sites were not surveyed in 2010.)
Figure 1.9. Proportion of Niobrara Delta sites at which uncommon bird species were detected during 2010-2011. Data is divided by estimated site age class.
Figure 1.10. Detections of bird species at Niobrara Delta Sites in May 2011 compared to off-river wetland sites near Vermillion in June of 2011. These were species with significant differences in detected individuals between delta and off-river sites.
Figure 1.11. Proportion of Niobrara Delta sites at which amphibian species were detected during the day in 2010 and 2011. Data is divided by estimated site age class.
Figure 1.12. 2011 daytime Niobrara Delta amphibian species detection data.
Figure 1.13. 2011 combined nocturnal and daytime Niobrara Delta amphibian species detection data.
Figure 1.14. Amphibian detection data at Niobrara Delta Sites in late May 2011 compared to off-river wetland sites in early June 2011.
Figure 1.15. Proportion of Niobrara Delta sites at which turtle species were detected during 2011. Data is divided by estimated site age class.
Figure 2.1. Plot of mean movement scale of surviving tadpoles in Bd experimental groups compared to those in the Bd control groups. Includes standard error bars. Tadpoles exposed to Bd moved significantly slower on average.
Figure 2.2. Plot of mean proportion of checks during which surviving tadpoles were detected moving compared between Bd experimental and control groups. Includes error bars. Data indicates that Bd exposed tadpoles were moving less of the time than those not exposed to Bd.
Figure 2.3. Plot of mean proportions of checks where surviving tadpoles were detected feeding compared between Bd treatment and control groups. Standard error bars included. Tadpoles exposed to Bd were observed feeding significantly less often when checked.
Figure 2.4. Mean movement scale of tadpoles exposed to predator cue compared to unexposed tadpoles. Tadpoles exposed to predator cue were moving significantly more slowly on average.
Figure 2.5. Plot of mean proportion of checks during which tadpoles were seen moving for predator cue experimental groups compared to control groups. Proportion of spent moving was significantly lower for tadpoles exposed to predator cue than for those who were not.
Figure 3.1. Mean pH at sites with cattle present compared to sites without cattle.

General Linear Model resulted in a t value = 2.494, p < 0.05*. 

* indicates significance at the 0.05 level.
Figure 3.2. Gray Tree Frog tadpole detections plotted against pH for sites with and without cattle. Gray Tree Frog tadpole detections were negatively correlated with increasing pH values. This species was detected at three out of twenty survey sites.
Figure 3.3. Leopard Frog tadpole and adult Boreal Chorus Frog detections plotted against pH. Detections of Leopard Frog tadpoles and adult Boreal Chorus Frogs were negatively correlated with increasing pH values. Leopard Frog tadpoles were detected at six out of twenty survey sites while adult Boreal Chorus Frogs were detected at two out of twenty survey sites.
Figure 3.4. Mean Leopard Frog tadpole detections at sites predicted to have high runoff impacts compared to sites predicted to have low runoff impacts.
Figure 3.5. Mean adult Northern Cricket Frogs detected at sites with cattle present compared to sites without cattle.
Figure 3.6. Mean Tiger Salamander larvae snout-vent length at projected high impact sites compared to projected low impact sites.
Table 1.1. Site Age Classes.

<table>
<thead>
<tr>
<th>Estimated Age Classes</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 yrs</td>
<td>108, 109</td>
</tr>
<tr>
<td>10-25 yrs</td>
<td>101, 102, 103, 106</td>
</tr>
<tr>
<td>25-50 yrs</td>
<td>104, 110, 115</td>
</tr>
<tr>
<td>&gt;50 yrs</td>
<td>105, 107, 111, 112, 113, 114</td>
</tr>
</tbody>
</table>

Sites were divided by apparent successional age for analyses.
Table 1.2. Complete List of bird species detected on the Niobrara Delta and at off-river wetland sites in 2010 and 2011.

<table>
<thead>
<tr>
<th>Detected ONLY on Niobrara Delta</th>
<th>Detected on Niobrara Delta AND Off-river Sites</th>
<th>Detected ONLY at Off-river Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wading Birds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Least Bittern*</td>
<td>Wading Birds</td>
<td></td>
</tr>
<tr>
<td>Great Blue Heron</td>
<td>Green Heron*</td>
<td></td>
</tr>
<tr>
<td><strong>Geese and Ducks</strong></td>
<td>Geese Ducks and Grebes</td>
<td></td>
</tr>
<tr>
<td>Canada Goose</td>
<td>Wood Duck</td>
<td></td>
</tr>
<tr>
<td>Gadwall</td>
<td>Mallard</td>
<td></td>
</tr>
<tr>
<td><strong>Gulls and Terns</strong></td>
<td>Blue-winged Teal</td>
<td></td>
</tr>
<tr>
<td>Ring-billed Gull</td>
<td>Northern Shoveler</td>
<td></td>
</tr>
<tr>
<td>Forster’s Tern*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Least Tern*</td>
<td>Pied-billed Grebe</td>
<td></td>
</tr>
<tr>
<td><strong>Raptors</strong></td>
<td>Virginia Rail</td>
<td></td>
</tr>
<tr>
<td>Bald Eagle</td>
<td>Sora</td>
<td></td>
</tr>
<tr>
<td><strong>Plovers, Sandpipers, etc.</strong></td>
<td>American Coot</td>
<td></td>
</tr>
<tr>
<td>American Avocet</td>
<td>Killdeer</td>
<td></td>
</tr>
<tr>
<td>Piping Plover*</td>
<td>Yellow Warbler</td>
<td></td>
</tr>
<tr>
<td>Lesser Yellowlegs</td>
<td>Song Sparrow</td>
<td></td>
</tr>
<tr>
<td>Semi-palomed Sandpiper</td>
<td>Red-winged Blackbird</td>
<td></td>
</tr>
<tr>
<td>Least Sandpiper</td>
<td>Yellow-headed Blackbird</td>
<td></td>
</tr>
<tr>
<td>Pectoral Sandpiper</td>
<td>Common Grackle</td>
<td></td>
</tr>
<tr>
<td>Wilson’s Phalarope</td>
<td>Eastern Kingbird</td>
<td></td>
</tr>
<tr>
<td><strong>Passerines</strong></td>
<td>Tree Swallow</td>
<td></td>
</tr>
<tr>
<td>Eastern Wood-peewee</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Willow Flycatcher*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsh Wren</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray Catbird</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bell’s Vireo*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common Yellowthroat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rose-breasted Grosbeak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chipping Sparrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swamp Sparrow*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Meadowlark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Goldfinch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown-headed Cowbird</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orchard Oriole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baltimore Oriole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bank Swallow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cliff Swallow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barn Swallow</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Pelican</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belted Kingfisher*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mourning Dove</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow-billed Cuckoo*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Flicker</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Bold** denotes species targeted by call playback surveys

*Species considered uncommon in South Dakota*
Table 1.3. Amphibian species totals.

<table>
<thead>
<tr>
<th>Species</th>
<th>2010 Niobrara Delta Totals</th>
<th>May 2011 Niobrara Delta Totals</th>
<th>June 2011 Off-river Wetlands Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullfrog</td>
<td>Too numerous</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Leopard Frog</td>
<td>9</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Northern Cricket Frog</td>
<td>11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Boreal Chorus Frog</td>
<td>&gt;14</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Woodhouse Toad</td>
<td>5</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Tiger Salamander</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total amphibians</strong></td>
<td><strong>&gt;&gt; 58</strong></td>
<td><strong>29</strong></td>
<td><strong>38</strong></td>
</tr>
</tbody>
</table>

Table 1.4. Turtle species totals.

<table>
<thead>
<tr>
<th>Species</th>
<th># Individuals Caught</th>
</tr>
</thead>
<tbody>
<tr>
<td>False Map</td>
<td>19</td>
</tr>
<tr>
<td>Spiny Softshell</td>
<td>6</td>
</tr>
<tr>
<td>Snapping</td>
<td>25</td>
</tr>
<tr>
<td>Painted</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 2.1. ANOVA Table (Type II tests) for response variable death in Experiment 1.

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>Sum of Squares</th>
<th>F Value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd</td>
<td>0.038</td>
<td>0.1596</td>
<td>0.6899</td>
</tr>
<tr>
<td>Pest</td>
<td>0.033</td>
<td>0.1419</td>
<td>0.7067</td>
</tr>
<tr>
<td>Pred</td>
<td>0.133</td>
<td>0.5676</td>
<td>0.452</td>
</tr>
<tr>
<td>Bd:Pest</td>
<td>0.008</td>
<td>0.0355</td>
<td>0.8508</td>
</tr>
<tr>
<td>Bd:Pred</td>
<td>0.075</td>
<td>0.3193</td>
<td>0.5726</td>
</tr>
<tr>
<td>Pest:Pred</td>
<td>0.033</td>
<td>0.1419</td>
<td>0.7067</td>
</tr>
<tr>
<td>Bd:Pest:Pred</td>
<td>0.075</td>
<td>0.3193</td>
<td>0.5726</td>
</tr>
</tbody>
</table>

Table 2.2. ANOVA Table (Type II tests) for response variable illness in Experiment 1.

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>Sum of Squares</th>
<th>F Value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pest</td>
<td>0.208</td>
<td>0.8547</td>
<td>0.35619</td>
</tr>
<tr>
<td>Pred</td>
<td>0.675</td>
<td>2.7692</td>
<td>0.09744</td>
</tr>
<tr>
<td>Bd:Pest</td>
<td>0.3</td>
<td>1.2308</td>
<td>0.26841</td>
</tr>
<tr>
<td>Bd:Pred</td>
<td>0.033</td>
<td>0.1368</td>
<td>0.71187</td>
</tr>
<tr>
<td>Pest:Pred</td>
<td>0.3</td>
<td>1.2308</td>
<td>0.26841</td>
</tr>
<tr>
<td>Bd:Pest:Pred</td>
<td>0.075</td>
<td>0.3077</td>
<td>0.57963</td>
</tr>
</tbody>
</table>
Table 2.3. Non-significant results for general linear models using Experiment 1 final Leopard Frog tadpole body mass and body length data.

<table>
<thead>
<tr>
<th>General Linear Model</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Mass ~ Pest</td>
<td>0.01853</td>
<td>0.01400</td>
<td>1.323</td>
<td>0.18800</td>
</tr>
<tr>
<td>Final Mass ~ Bd</td>
<td>0.00095</td>
<td>0.01137</td>
<td>0.08300</td>
<td>0.934</td>
</tr>
<tr>
<td>Final Mass ~ Pred</td>
<td>-0.01166</td>
<td>0.01438</td>
<td>-0.81000</td>
<td>0.41900</td>
</tr>
<tr>
<td>Final Mass ~ Bd * Pest</td>
<td>-0.016981</td>
<td>0.0192367</td>
<td>-0.883</td>
<td>0.379</td>
</tr>
<tr>
<td>Final Mass ~ Bd * Pred</td>
<td>0.0098048</td>
<td>0.0202177</td>
<td>0.485</td>
<td>0.628</td>
</tr>
<tr>
<td>Final Mass ~ Pred * Pest</td>
<td>-0.0026895</td>
<td>0.0200099</td>
<td>-0.134</td>
<td>0.893</td>
</tr>
<tr>
<td>Final Mass ~ Pred * Pest * Bd</td>
<td>0.007183</td>
<td>0.0279999</td>
<td>0.257</td>
<td>0.798</td>
</tr>
<tr>
<td>Final Body Length ~ Pest</td>
<td>0.01329</td>
<td>0.04546</td>
<td>0.29200</td>
<td>0.77000</td>
</tr>
<tr>
<td>Final Body Length ~ Bd</td>
<td>0.00935</td>
<td>0.03590</td>
<td>0.26000</td>
<td>0.79500</td>
</tr>
<tr>
<td>Final Body Length ~ Pred</td>
<td>-0.02615</td>
<td>0.04388</td>
<td>-0.59600</td>
<td>0.55200</td>
</tr>
<tr>
<td>Final Body length ~ Bd * Pest</td>
<td>-0.013527</td>
<td>0.063581</td>
<td>-0.213</td>
<td>0.832</td>
</tr>
<tr>
<td>Final Body length ~ Bd * Pred</td>
<td>0.008154</td>
<td>0.06192</td>
<td>0.132</td>
<td>0.895</td>
</tr>
<tr>
<td>Final Body length ~ Pest * Pred</td>
<td>-0.007791</td>
<td>0.063052</td>
<td>-0.124</td>
<td>0.902</td>
</tr>
<tr>
<td>Final Body length ~ Pred * Pest * Bd</td>
<td>0.028159</td>
<td>0.088847</td>
<td>0.317</td>
<td>0.752</td>
</tr>
</tbody>
</table>

Table 2.4. Kruskal-Wallis test results for surviving Leopard Frog tadpoles in Experiment 1

Significance codes are the same for all data shown:
p << 0.001 = ***, p < 0.001 = **, p < 0.01 = *, p < .1 = .

<table>
<thead>
<tr>
<th>Relationships</th>
<th>Kruskal-Wallis chi-squared</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Movement Scale ~ Bd</td>
<td>13.76720</td>
<td>0.0002069***</td>
</tr>
<tr>
<td>Proportion Checks Moving ~ Bd</td>
<td>16.49640</td>
<td>0.00004874***</td>
</tr>
<tr>
<td>Proportion Checks Feeding ~ Bd</td>
<td>10.34330</td>
<td>0.001299**</td>
</tr>
<tr>
<td>Average Movement Scale ~ Pred</td>
<td>6.8693</td>
<td>0.008769**</td>
</tr>
<tr>
<td>Proportion Checks Moving ~ Pred</td>
<td>4.44870</td>
<td>0.03493*</td>
</tr>
<tr>
<td>Proportion Checks Feeding ~ Pred</td>
<td>1.88610</td>
<td>0.16960</td>
</tr>
<tr>
<td>Average Movement Scale ~ Pest</td>
<td>0.934</td>
<td>0.33380</td>
</tr>
<tr>
<td>Proportion Checks Moving ~ Pest</td>
<td>0.70260</td>
<td>0.40190</td>
</tr>
<tr>
<td>Proportion Checks Feeding ~ Pest</td>
<td>0.08800</td>
<td>0.76680</td>
</tr>
</tbody>
</table>
**Table 2.5.** Main effects of acetochlor and Bd exposure on final body length and final body mass of Northern Cricket Frog tadpoles in Experiment 2.

<table>
<thead>
<tr>
<th>General Linear Model Tested</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Body Length ~ Pest</td>
<td>0.004182</td>
<td>0.041602</td>
<td>0.101</td>
<td>0.92</td>
</tr>
<tr>
<td>Final Body Length ~ Bd</td>
<td>0.001996</td>
<td>0.041563</td>
<td>0.048</td>
<td>0.962</td>
</tr>
<tr>
<td>Final Body Mass ~ Pest</td>
<td>-0.02324</td>
<td>0.02032</td>
<td>-1.143</td>
<td>0.257</td>
</tr>
<tr>
<td>Final Body Mass ~ Bd</td>
<td>-0.01116</td>
<td>0.02048</td>
<td>-0.545</td>
<td>0.588</td>
</tr>
</tbody>
</table>

**Table 2.6.** Interactive effects of acetochlor and Bd exposure on final body length and final body mass of Northern Cricket Frog tadpoles in Experiment 2.

<table>
<thead>
<tr>
<th>General Linear Model Tested</th>
<th>Variable</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Body Mass ~ Pest * Bd</td>
<td>Bd</td>
<td>0.0165</td>
<td>0.028485</td>
<td>0.579</td>
<td>0.564</td>
</tr>
<tr>
<td></td>
<td>Pest</td>
<td>0.005735</td>
<td>0.028877</td>
<td>0.199</td>
<td>0.843</td>
</tr>
<tr>
<td></td>
<td>Bd * Pest</td>
<td>-0.05824</td>
<td>0.040562</td>
<td>-1.436</td>
<td>0.156</td>
</tr>
<tr>
<td>Final Body Length ~ Pest * Bd</td>
<td>Bd</td>
<td>0.04367</td>
<td>0.0581</td>
<td>0.752</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>Pest</td>
<td>0.04804</td>
<td>0.05973</td>
<td>0.804</td>
<td>0.424</td>
</tr>
<tr>
<td></td>
<td>Bd * Pest</td>
<td>-0.08661</td>
<td>0.08411</td>
<td>-1.03</td>
<td>0.307</td>
</tr>
</tbody>
</table>

**Table 2.7.** Percentage table comparing proportions of surviving Northern Cricket Frog tadpoles between treatments in Experiment 2.

<table>
<thead>
<tr>
<th>Pest</th>
<th>N</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd</td>
<td>58.3</td>
<td>41.7</td>
</tr>
<tr>
<td>Y</td>
<td>35.7</td>
<td>64.3</td>
</tr>
</tbody>
</table>
Table 2.8. Percentage table comparing proportions of Northern Cricket Frog tadpoles that did not fall ill between different treatments in Experiment 2.

<table>
<thead>
<tr>
<th>Pest</th>
<th>N</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>51.6</td>
<td>48.4</td>
</tr>
</tbody>
</table>

Table 2.9. Results of two-sample proportions test to determine whether exposure to Bd, acetochlor, or both had effects on Northern Cricket Frog tadpole mortality or illness in Experiment 2.

<table>
<thead>
<tr>
<th>Response variables compared between experimental groups</th>
<th>X-squared</th>
<th>p-value</th>
<th>95 Percent Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion Surviving</td>
<td>1.3302</td>
<td>0.2488</td>
<td>-0.1490495 0.6014304</td>
</tr>
<tr>
<td>Proportion Not Ill</td>
<td>0.0164</td>
<td>0.8981</td>
<td>-0.2630271 0.2307691</td>
</tr>
</tbody>
</table>
**Table 3.1.** General linear model z-value and t-value results using pH, cattle presence, amphibian detection, Tiger Salamander larvae snout-vent length and ATV detection data.

<table>
<thead>
<tr>
<th></th>
<th>Projected Runoff Impact</th>
<th>Cattle Presence</th>
<th>pH</th>
<th>Cattle Presence and pH Interactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray Tree Frog Tadpoles</td>
<td>0.234</td>
<td>-4.351***</td>
<td>-4.466***</td>
<td>4.146***</td>
</tr>
<tr>
<td>Leopard Frog Tadpoles</td>
<td>1.964</td>
<td>-1.587</td>
<td>-3.728**</td>
<td>1.554</td>
</tr>
<tr>
<td>Adult Boreal Chorus Frogs</td>
<td>1.353</td>
<td>-0.106</td>
<td>-2.346*</td>
<td>0.248</td>
</tr>
<tr>
<td>Adult Northern Cricket Frogs</td>
<td>-0.529</td>
<td>-2.057.</td>
<td>-0.360</td>
<td>-1.752 .</td>
</tr>
<tr>
<td>Tiger Salamander larvae</td>
<td>0.031</td>
<td>1.067</td>
<td>0.751</td>
<td>0.097</td>
</tr>
</tbody>
</table>

**Table 3.2.** General linear model results for Tiger Salamander larvae body length and ATV detection

<table>
<thead>
<tr>
<th></th>
<th>Projected Runoff Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATV Detections</td>
<td>-0.131</td>
</tr>
<tr>
<td>Tiger Salamander SVL</td>
<td>-2.046*</td>
</tr>
</tbody>
</table>

**Table 3.3.** P values for general linear model results between water quality parameters, pond circumference, air temperature and projected impact and cattle presence.

<table>
<thead>
<tr>
<th></th>
<th>Projected Runoff Impact</th>
<th>Cattle Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond Circumference</td>
<td>0.0132 *</td>
<td>0.116</td>
</tr>
<tr>
<td>pH</td>
<td>0.153</td>
<td>0.0226 *</td>
</tr>
<tr>
<td>DO</td>
<td>0.73</td>
<td>0.23393</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>0.308</td>
<td>0.160</td>
</tr>
<tr>
<td>Conductivity</td>
<td>0.8788</td>
<td>7.14e-05 ***</td>
</tr>
<tr>
<td>Water Temperature</td>
<td>0.366</td>
<td>0.593</td>
</tr>
<tr>
<td>Air Temperature</td>
<td>0.0567</td>
<td>0.703</td>
</tr>
</tbody>
</table>
Table 3.4 P values for general linear model results between conductivity, pond circumference and amphibian abundances.

<table>
<thead>
<tr>
<th></th>
<th>Conductivity</th>
<th>Pond Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray Tree Frog Tadpoles</td>
<td>0.129</td>
<td>0.262</td>
</tr>
<tr>
<td>Leopard Frog Tadpoles</td>
<td>0.442</td>
<td>0.731</td>
</tr>
<tr>
<td>Adult Boreal Chorus Frogs</td>
<td>0.829</td>
<td>0.698</td>
</tr>
<tr>
<td>Adult Northern Cricket Frogs</td>
<td>0.5004</td>
<td>0.695</td>
</tr>
<tr>
<td>Tiger Salamander Larvae</td>
<td>0.3920</td>
<td>0.2800</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY


Wright, M. L. et al. (1994) Anterior Pituitary and Adrenal Cortical Hormones Accelerate or Inhibit Tadpole Hindlimb Growth and Development Depending on Stage of Spontaneous Development or Thyroxine Concentration in Induced Metamorphosis. Journal of Experimental Zoology 279:175–188.