

NORTH DAKOTA GAME AND FISH DEPARTMENT

Final Report

Establishing Amphibian Population Levels in Local Sites of North Dakota

Project T-26-R

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State Wildlife Grant Final Report 2011

Establishing Amphibian Population Levels in Local Sites of North Dakota

Species of Conservation Priority: Amphibians, Anurans, *Bufo hemiophrys* (the Canadian Toad), *Spea bombifrons* (the Plains Spadefoot)

State Agency: North Dakota Game and Fish Department (NDGFD)

Cooperating Partners: The Amphibian Growth Project at Minot State University, The University Of North Dakota, Fort Berthold Community College, Turtle Mountain Community College and the Wolford Outdoor Learning Site (OWLS)

Report prepared by: Kenneth C Cabarle and Christopher K Beachy



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Activities:

Funding for this project was allocated and approved in May 2008 and the project officially began June 2008. Project activities included coordination of cooperating partners, on-site training, development of drift fence arrays and collections at chosen sampling sites.

The focus for this project has been outreach, recruitment and training of basic amphibian identifications and field sampling protocols. Each of the four cooperating groups (Fort Berthold Community College, Turtle Mountain Community College, Minot State University and the University of North Dakota) had visits from the P.I. (Christopher K Beachy). These visits included basic training in amphibian identification (in class 4 hours at each location), field training in sampling for amphibians (including the use of seines, large funnel traps, small minnow traps, dip netting, and field identifications) fulfilling the proposed visits to cooperators as outlined in the grant. Additionally, time was spent on web site and database development. Development of these resources allows the partners to add and query data from the AGP. This web data portal allows all cooperating partners to communicate and report progress without additional travel.

Sampling was a major focus for the cooperating partners of this grant (Figure 1). The following sites were proposed and surveyed:

Fort Berthold Community College, four potential sites were scouted and surveyed, two were chosen for continued sampling including

- 1) a site adjacent to a part of the Van Hook Wildlife Management Area (WMA),
- 2) Danks dam and cattle pond, a dam site and adjacent cattle pond that have been the focus of cooperative efforts for the North Dakota Game and Fish Department and the Game Department of the Three Affiliated Tribes.

Turtle Mountain, four potential sites were scouted two were chosen for continued sampling including:

- 1) a site in the Wakopa WMA
- 2) a pond on the turtle mountain reservation known as dump pond.

UND cooperating partners scouted four potential sites including sites in Charles C Cook WMA, sites at Turtle River State Park and sites close to Park River. The Charles C Cook and Park River sites were deemed economically unfeasible for continued study. So the following sites will be included for year two:

- 1) Turtle River State Park pond
- 2) additional areas possibly in Prairie Chicken WMA and Oakville Prairie Biological Station.

Additional sites were scouted in cooperation with the Wolford School Outdoor Wildlife Learning Site (OWLS) and the following were chosen for continued sampling:

- 1) Wolford OWLS Drift Fence and cemetery pond
- 2) NDGFD Conservation Plots lands just south of Rolette.

Three undergraduate tribal college students from each of the cooperating partners were recruited and participated in the project in 2010. Additionally, one researcher from Wolford participated through volunteer time in cooperation with the Wolford OWLS site. All of the main objectives of the project were attained in the 2010 season, outreach and training was performed at all of the cooperating locations, sample locations were surveyed for continued sampling potential. Kerry Hartman (Fort Berthold Community College), Scott Hanson (Turtle Mountain), Dr. Robert Newman and Mr. William Langer (Long term Wolford OWLS Drift Fence site) will continue to serve as points of contact for the coordination, and implementation of this project. To date 22 interns have been trained in amphibian identification and basic amphibian sampling through the project.



Figure 1. Google map of current sampling sites.

For the 2010 season the project continued to recruit summer workers. We dedicated time to visit and train workers in basic amphibian biology and field survey techniques (according to the guidelines in the approved grant) and encouraged cooperating partners to keep voucher specimens for collection and identification.

Results:

The current project is directed at the quantification, identification, and monitoring of amphibians at selected sites in North Dakota in cooperation with regional partners. In particular the NDGFD is concerned with two priority species *Anaxyrus hemiophrys* (the Canadian Toad), and *Spea bombifrons* (the Plains Spadefoot). The project is also concerned with the effectiveness of sampling techniques leading to cost effective methods for monitoring amphibians and in particular supporting the North Dakota Comprehensive Wildlife Conservation Strategy (CWCS) 10 year assessment in 2015 (Hagen et al. 2005). Field seasons for this project consisted of collections and sampling efforts utilizing the following techniques:

Basic Un-baited Minnow Trap sampling

Large Funnel Trap Sampling (Traps provided by Dave Mushett, USGS Jamestown)

Visual Encounter Surveys (go to the sight and record all amphibians encountered at that site)

Seines

Additionally, drift fence arrays were constructed at the tribal colleges (Below, Figure 2).



Figure 2. Development of directional drift fence arrays at cooperating tribal colleges.

Spadefoot toads are currently collected at the Simcoe long term drift fence pond in Simcoe North Dakota and at the Ag Site long term drift fence site at the NDSU agricultural experiment station in Minot. During the past three years Spadefoot toads and *Anaxyrus* (formerly Bufo) species (designated as species because of the high degree of hybridization of toads in this region) have been collected at these sites with fluctuating abundances. Numbers of these two species have high fluctuations and are driven by environmental and demographic stochasticity.



Plains Spadefoot
Spea bombifrons (Cope, 1863)

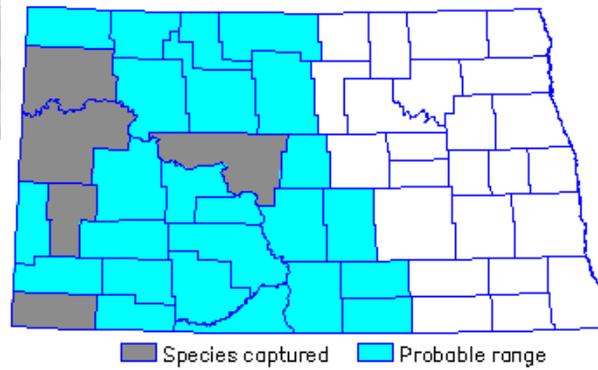
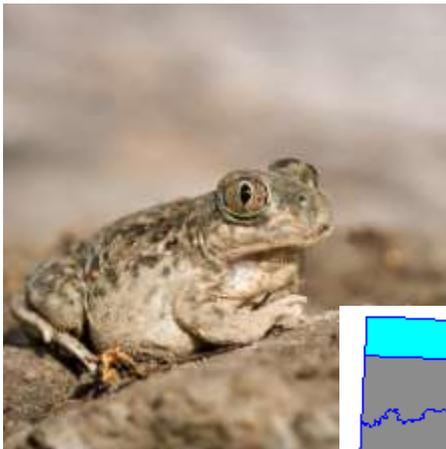


Figure 3. Distribution of the Plains Spadefoot toad in North Dakota.



Canadian Toad
Anaxyrus hemiophrys (Cope, 1886)

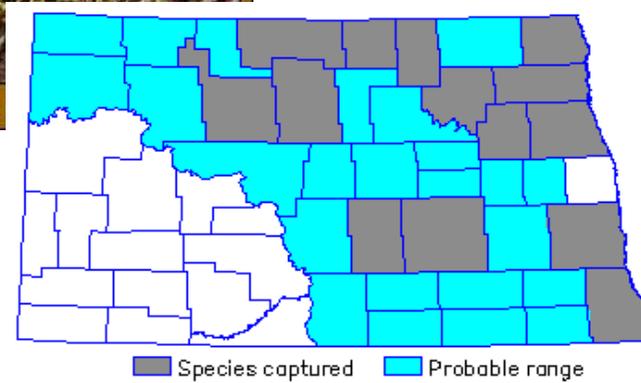


Figure 4. Distribution of the Canadian toad in North Dakota.

Spadefoot toads and *Anaxyrus* (formerly Bufonid) species were collected at several sites in 2009 with some sites showing high abundances of the Great Plains toad. Until the last year of the study the Canadian toad was not verified at any of the sampling sites in this project. This is somewhat surprising as several of the collection locations are well within the boundaries of historic distributions for both Canadian and Spadefoot toads.

In 2010 Canadian toads were collected at Oakville Prairie Biological Station. A total of 3 toads were collected and these are the only verified records for Canadian toads at any of the monitored sites during the three years of the project.

Development of the database and web data entry has gone as planned with a comprehensive data portal located at <http://amphibiangrowthproject.org/>. A snapshot of the current web database portal can be seen in Figure 5.

Current overall captures, relative abundances and capture efforts can be seen in figures 6 thru 9.

Management Implications:

Trends for amphibians in North Dakota fluctuated from year to year with no one species ever falling into levels for conservation concern except for species targeted for conservation. However, this is not a black and white situation. For the species of concern it is the opinion of this study that each of the concern species warrants the current conservation status with the following caveats:

1) Spadefoot toads, Canadian toads and Northern Leopard frogs were never more than 5%, 0.4% and 4% respectively. Determining if these numbers are a reflection of sampling location, temporal distribution, or sampling bias was beyond the scope of this study and requires additional effort.

2) Effort was not equally distributed among and within all years of the study and this can cause bias in the number and presence/absence at sampling locations. Further comprehensive study is needed.

Drift fence arrays are by far the most effective capture method for sampling amphibians in this study. However, drift fences incur man/hr and maintenance costs. The information on year round presence/absence and resource utilization (first arrival, site fidelity) is invaluable and we encourage the NDGFD to support the implementation of additional drift fence arrays especially on NDGFD owned land. A network of drift fences maintained by the NDGFD and checked on a bi-annual basis by technicians and by supervising biologists would promote ownership, conservation efforts and amphibian stewardship throughout the department and set a standard for future conservation efforts.

Amphibians are abundant in the North Dakota landscape and the results from this study along with the long term studies such as the one at Prairie Biological Station (USGS) are critical to understanding fluctuations in population dynamics in amphibians in North Dakota.



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Online data entry is a critical aspect of our amphibian tracking initiative. With our online databases, AGP partners and independent enthusiasts separated by geographic location can bring their amphibian data together in a standardized format that can be accessed online.

Geography is no longer a limitation in effective amphibian tracking, and with global amphibian populations steadily and rapidly declining, our amphibian tracking initiative could not have come to occur! AGP standardized databases give us the ability to monitor and record crucial information including but not limited to relative and absolute species densities, individual and average sizes at specific locations, and migration patterns.

Our web forms are also designed to accept non-standardized data, as well, reducing limitations inherent with rigid adherence to format. In this way, the AGP, its dedicated partners and independent fields are building a potentially limitless amphibian data pool dedicated to northwest North Dakota but available everywhere!



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 Questions or problems? Please contact us directly at cdp@water.health@minnstateu.edu.

<http://fenris.pdsinfo.com/amphibianresearchproject/Data/DataEntry.aspx>

5/4/2010

a.



b.

Figure 5. Web based data entry portal and network communication portal for this project. Site allows for secure data entry and access and allows remote reporting for tribal and cooperating partners. a. Is the web based data entry portal, b. is the front page showing network communications and coordination possibilities for state amphibian reporting including links for all cooperating partners for the project.

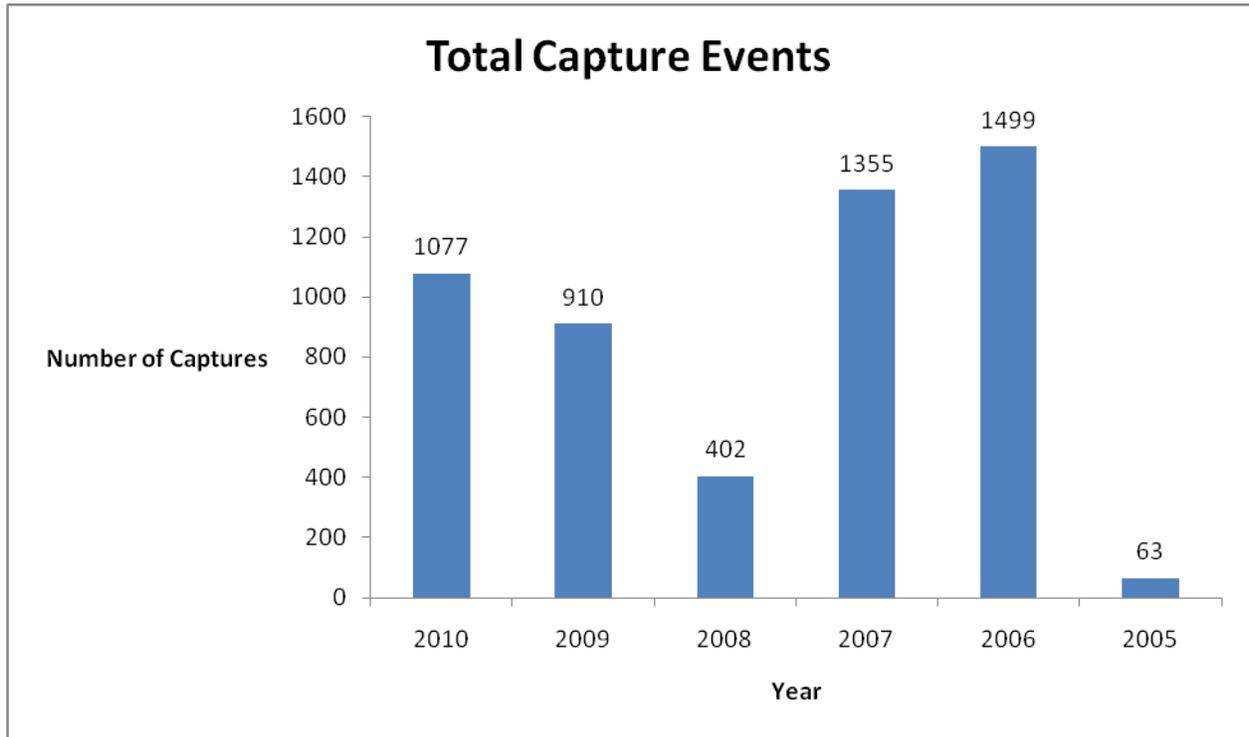


Figure 6. Total capture events of amphibians in North Dakota for the project period and three additional years. Capture events represent amphibians noted in logbook for year of study.

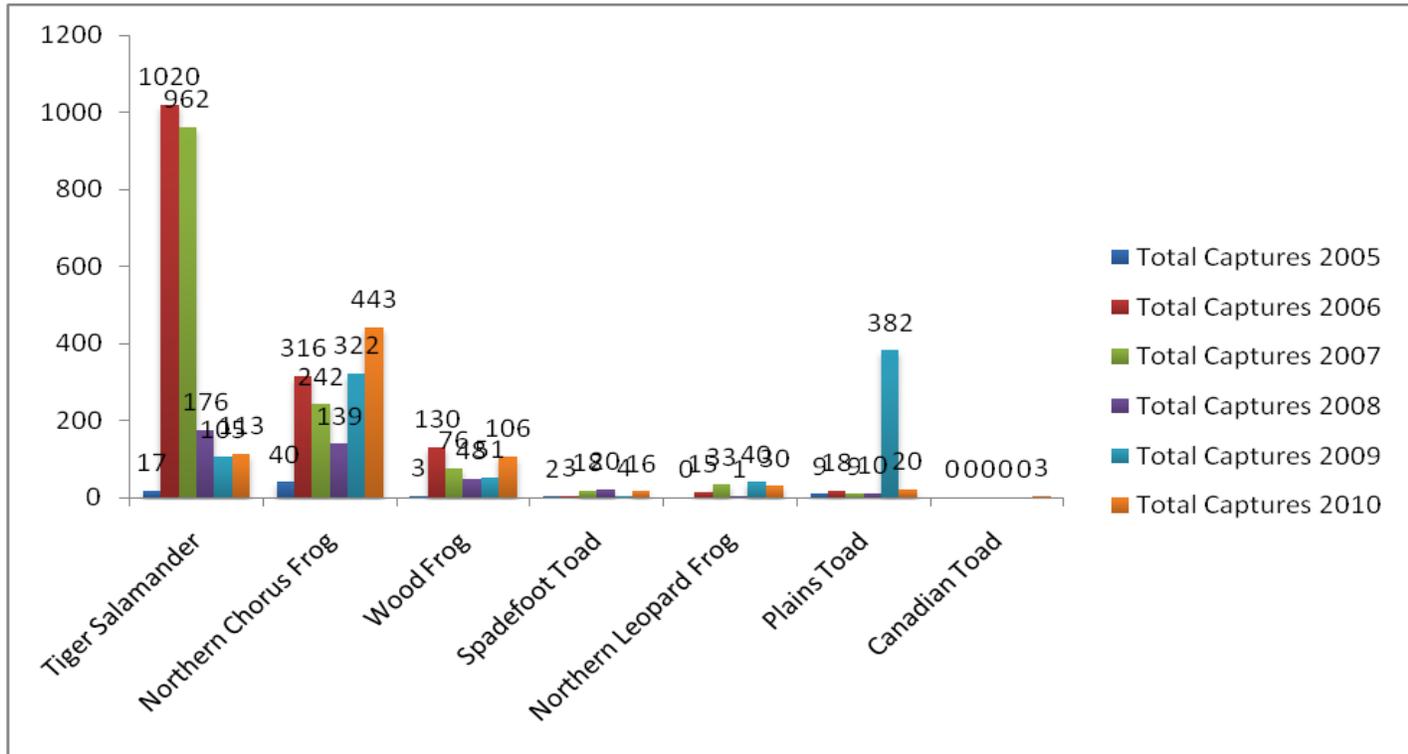


Figure 7. Total Captures by species for project years plus three additional years.

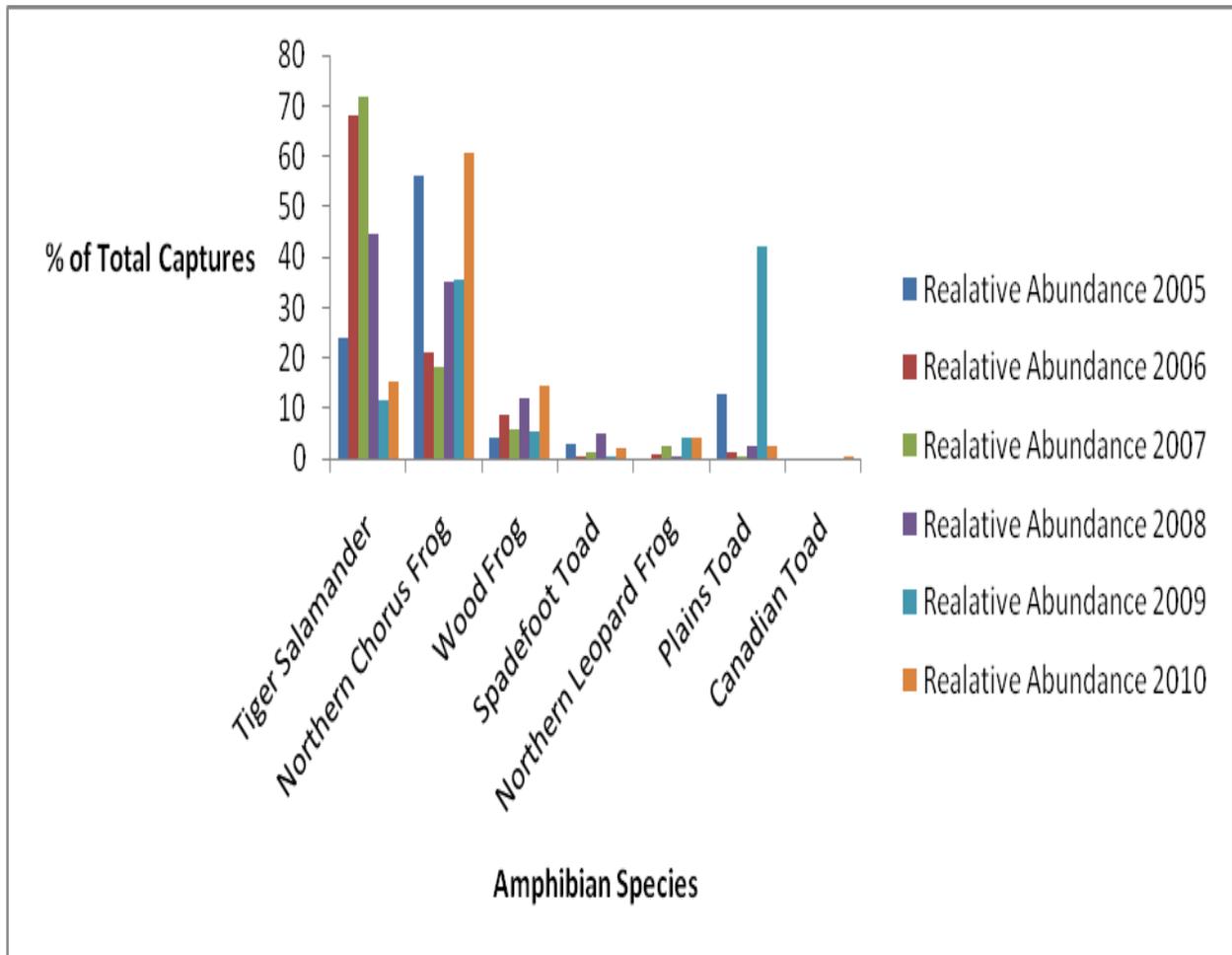
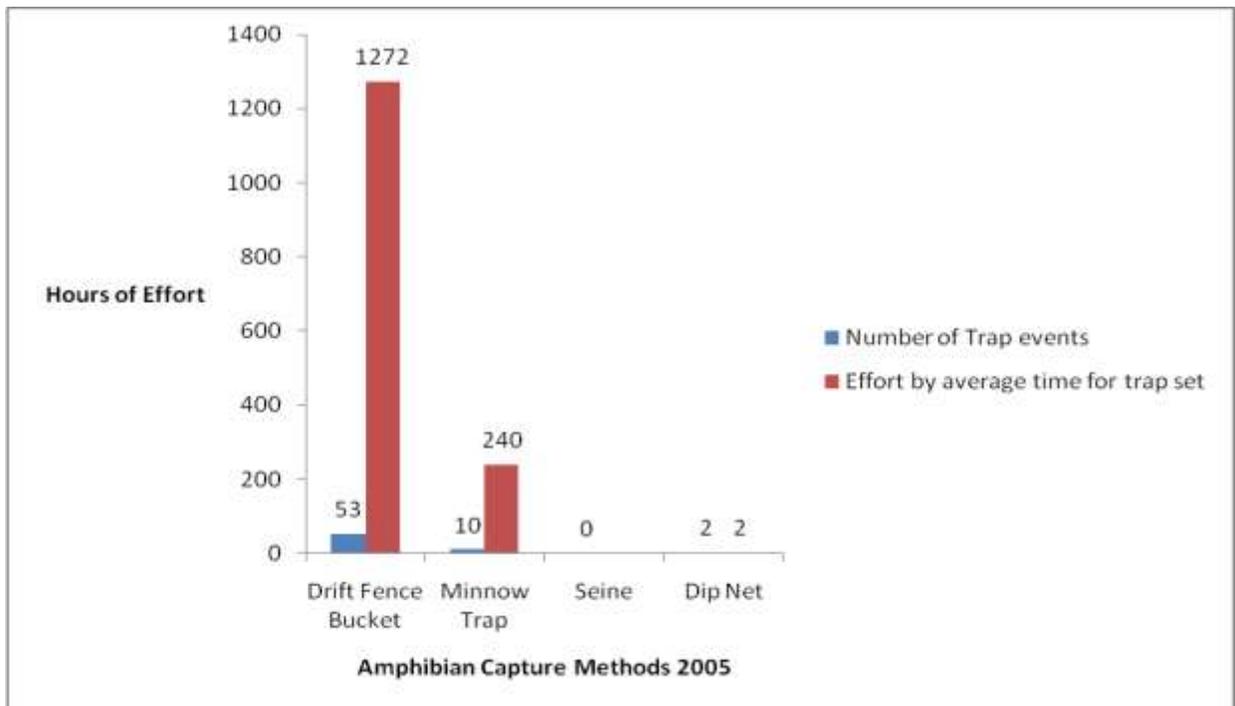
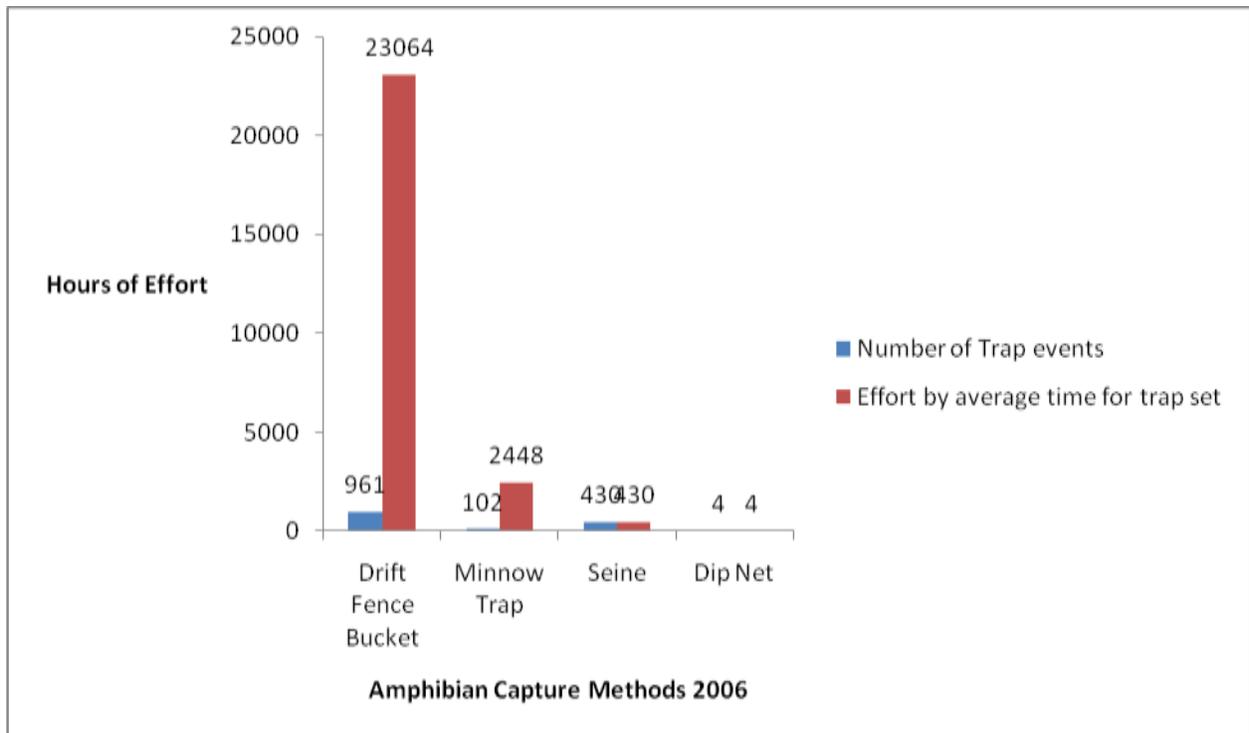


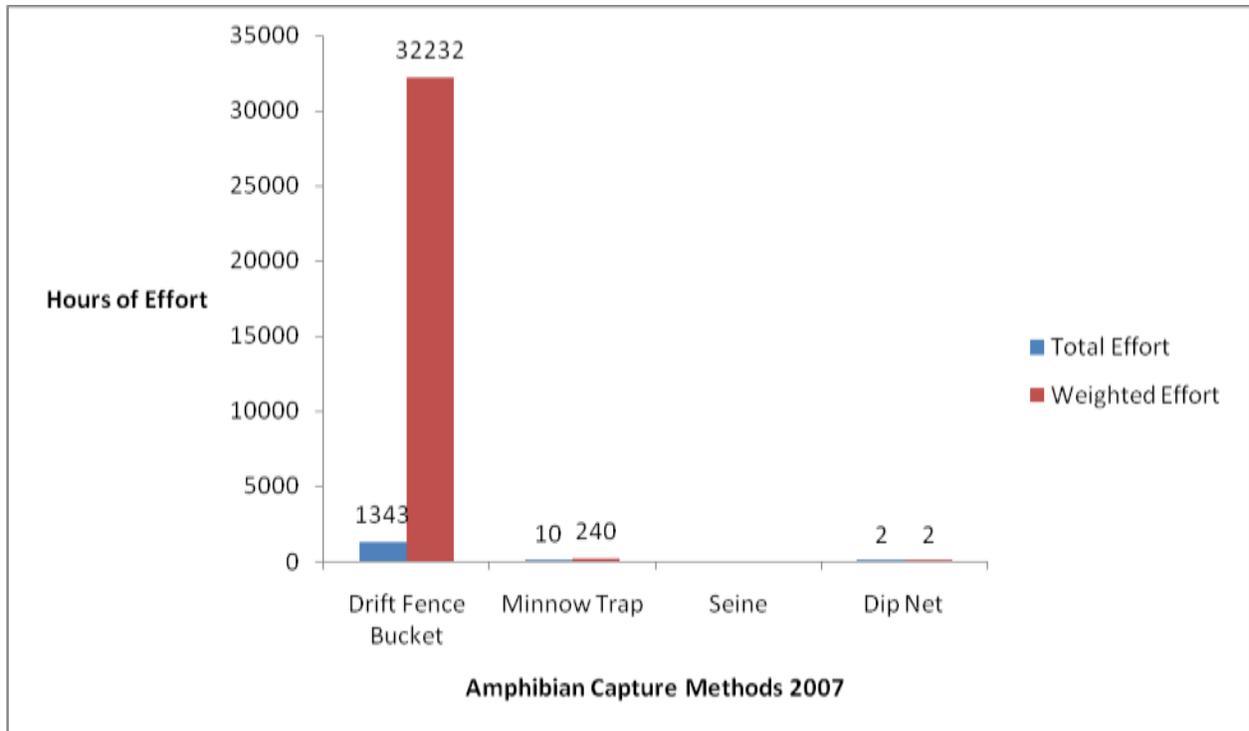
Figure 8. Relative abundance by species for amphibians captured during project years and three additional years. Figure shows % of total catch by species.



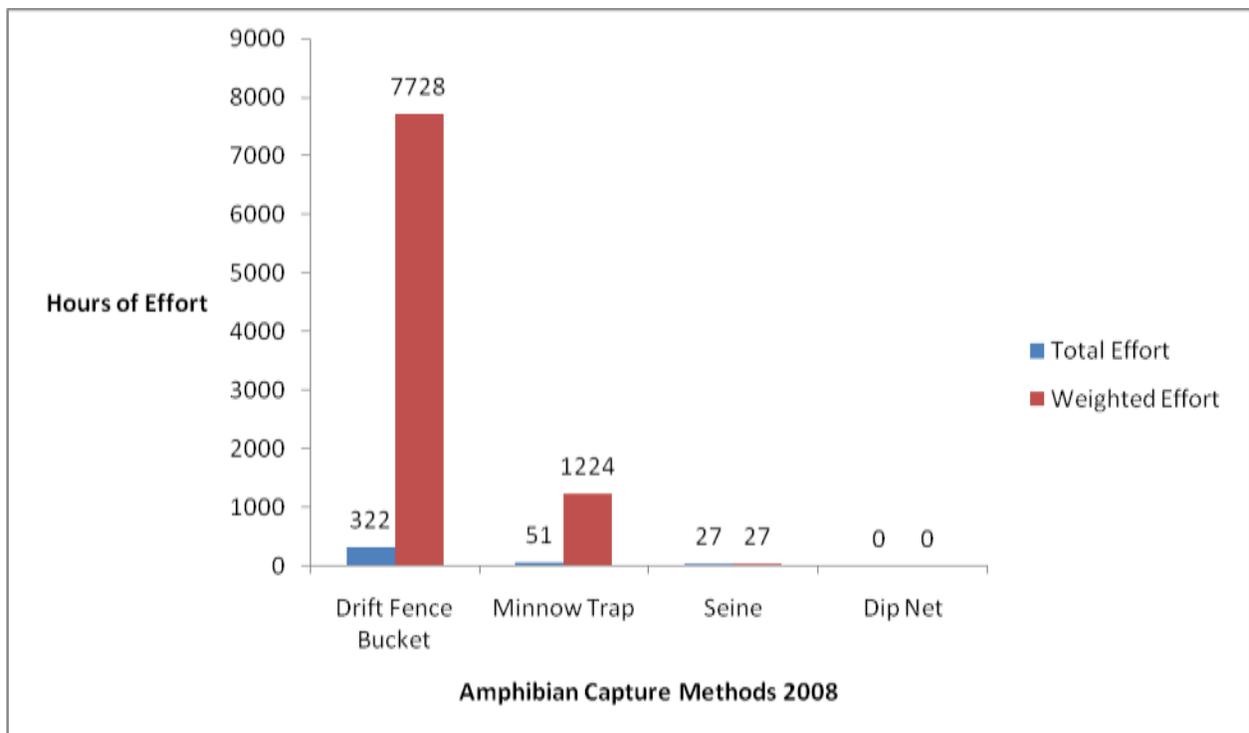
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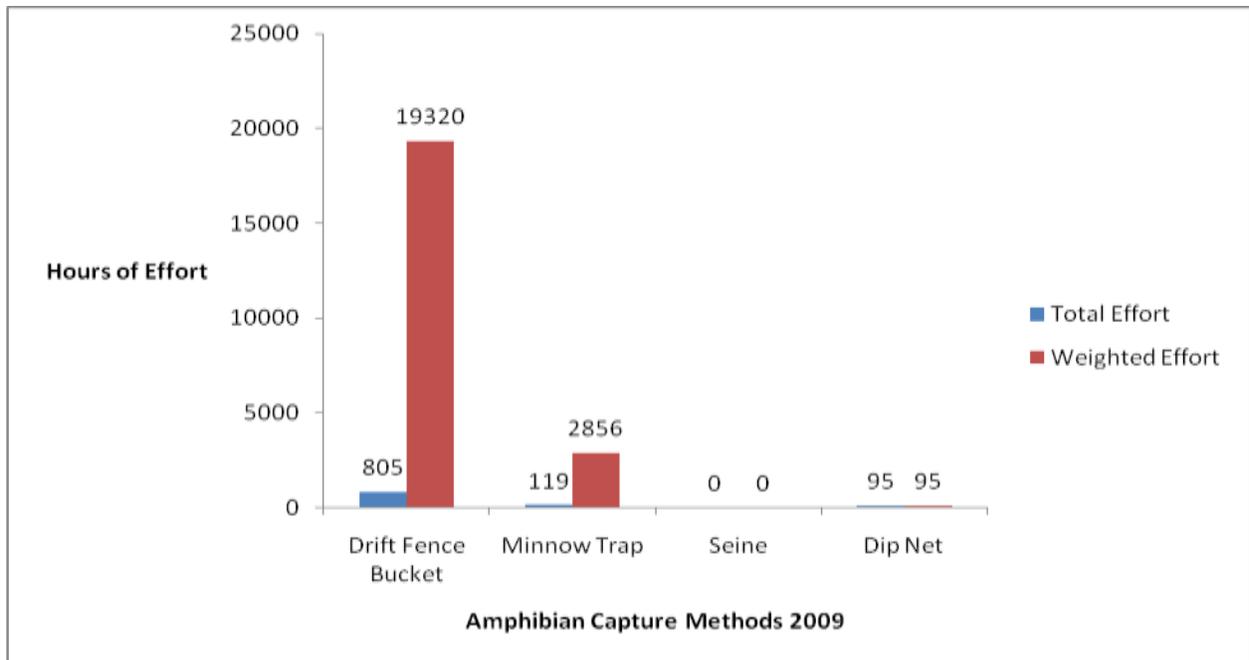
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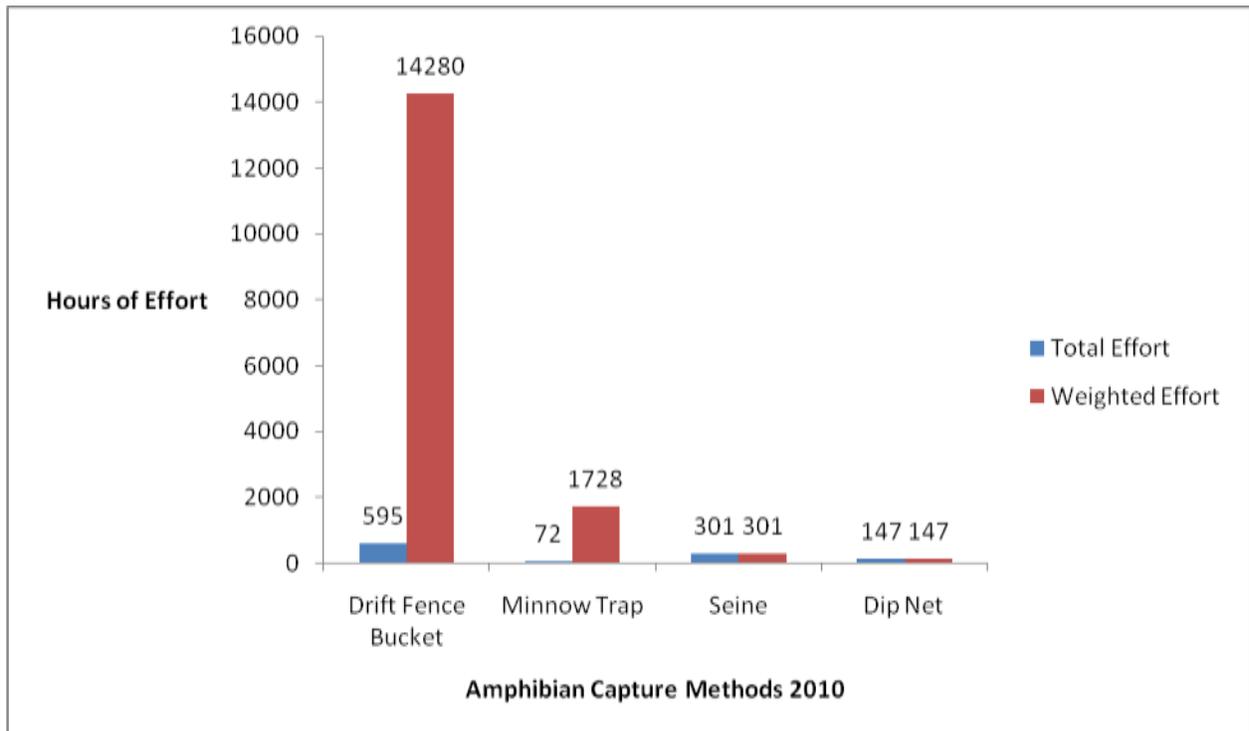
c.



d.



e.



f.

Figure 9. Bar graphs of Capture effort for each year of the project plus three additional years. Graphs are shown sequentially by year.

Spadefoot Toad Pilot Project:

Objective:

Assess the evolutionary phylogeny of the populations of spadefoot toads at project sampling sites utilizing the cytochrome-b mitochondrial DNA region. This region was studied with samples from North Dakota (McKenzie County) in 2008.

Methods:

Primers were designed to sample a 300 base pair region of the cytochrome-b mitochondrial DNA region using analyzed by Rice and Pfenning in 2008. DNA was extracted from 24 spadefoot toads from sites in North Dakota utilizing toe clips (non-destructive sampling). Qiagen DNEasy kits were used for DNA extractions according to (add qiagen protocol). Samples were then sequenced (Functional Bio, 2011) for cytochrome-b haplotype analysis and Blasted in the NCBI nr database for sequence similarity. Protocol for this analysis followed Rice and Pfenning (2008). This analysis had two objectives:

- 1) to analyze the cytochrome-b mitochondrial DNA sequence of spadefoot toads at long term drift fence research sites, as baseline reference for future conservation management assessments
- 2) to describe mitochondrial haplotypes that may be distinctive of local populations of spadefoot toads in North Dakota.

The sequence region was sequenced forwards and backwards in all 24 samples utilizing two sets of primers; one set targeting 450 base pairs of the cytochrome-b region and a shorter nested region of about 150 base pairs. Sequences were aligned by eye in Sequencher (get sequencher site) and consensus sequences were blasted for nucleotide similarity in the NCBI non-redundent database.

Results:

Seventeen samples showed 100% identity to NCBI accessioned sequence gi|169126167|gb|EU499435.1| *Spea bombifrons* voucher DBS 845 haplotype 6 cytochrome b (cytb) gene, partial cds.

Five samples revealed a unique North Dakota haplotype which included one SNP (position 71, change from G to A) representing three local populations at Simcoe, Upham and Carr North Dakota.

Two samples revealed a unique North Dakota haplotype which included one SNP (position 194, change from T to C) representing one local population at Burning Coal Mine, North Dakota. This haplotype may be population specific.

All samples showed identity to haplotype 1 from Rice and Pfenning (2008) which represents samples from Callaway County, MO (1); Carbon County, MT (8); Cheyenne County, KS (1); Clark County, KS (3); Curry County, NM (1); Custer County, SD (2); DeBaca County, NM (1); Dickens County, TX (1); Dunn County, ND (9); Edwards County, KS (1); Ellis County, OK (1); Johnson County, KS (2); Lamb County, TX (11); Lincoln County, CO (19); McKenzie County, ND (5); Meade County, KS (1); Otero County, CO (8); Payne County, OK (2); Quay County, NM (1); Roger Mills County, OK (1); Slope County, ND (4); Washington County, CO (16); Winkler County, TX (1), 100 samples total. This analysis places the five populations from this study closely related to the haplotype 1 clade with two new ND specific haplotypes discovered.

Management Recommendations:

Further study of these populations is needed to document additional population specific genetic variability in North Dakota and to test additional questions of population isolation and potential conservation actions. The populations of spadefoot toads in this study (all from North Dakota) showed relatedness to a mid-western clade which may be at the northern and western extents of spadefoot species distribution.

Specifically, a study including an assessment of 15 samples at 10 populations of spadefoot toads spread across the major geophysical provinces of the state including mitochondrial DNA assessment and microsatellite markers/nuclear markers would provide an informative snapshot of genetic distribution within the state. Such a project could be funded by the state and could support a Masters Research program for 1 or 2 students.

Continued study of this species should remain a priority for the NDGFD as little information is known on the extent and abundance of populations of this toad species.

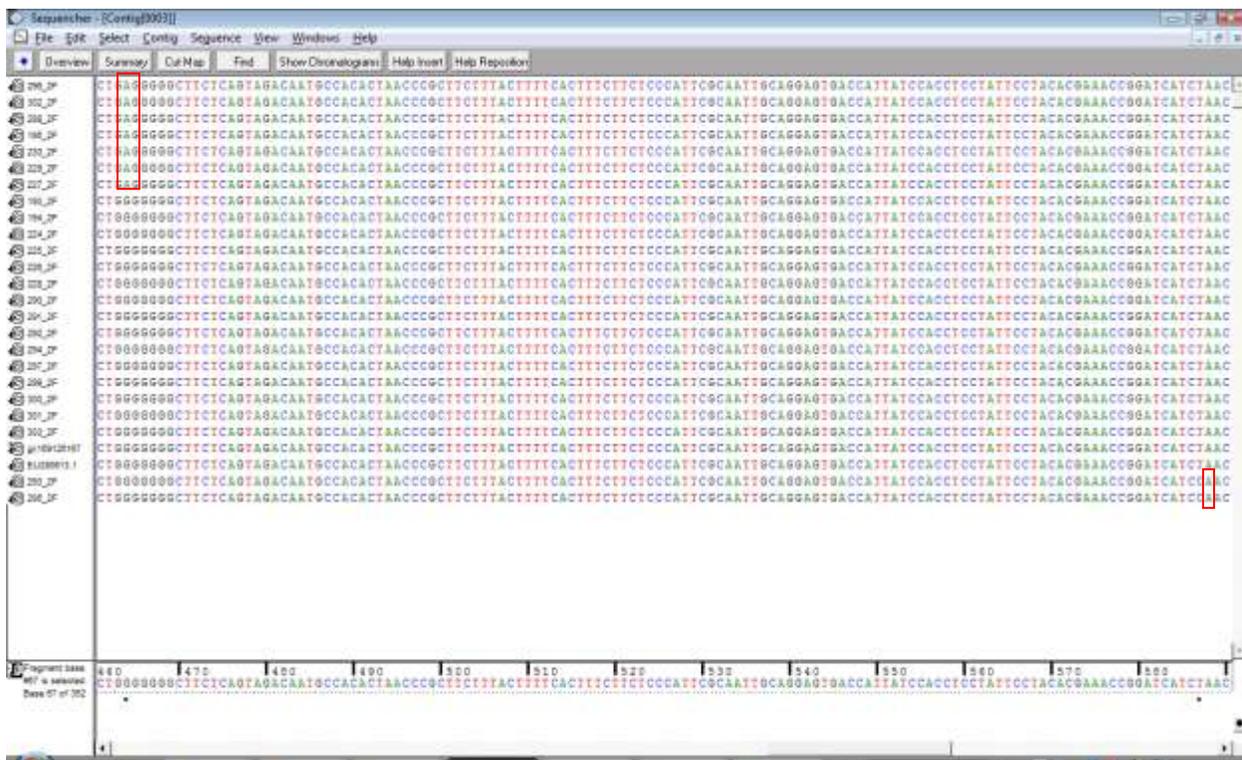


Figure 10. Sequencher alignment of mitochondrial DNA from Spadefoot toads in North Dakota.

Red boxes show two North Dakota specific haplotypes represented by single nucleotide polymorphisms (SNP).

These haplotypes represent new informative mitochondrial changes and require further study to quantify genetic relationships in spadefoot toads in North Dakota. This data indicates a comprehensive study of populations of spadefoot toads in North Dakota is appropriate to support conservation efforts and to guide management for the conservation biology of the species.

Acknowledgments:

We would like to thank the NDGFD for continued support of this project and look forward to the NDGFDs continued support of amphibian monitoring projects throughout the state and the new interns it will expose to the wonders of field work related to amphibian monitoring.

We would like to extend special mention and deep gratitude to Sandy Johnson and Steven Dykes for supporting this project from its inception. Additionally, we would like to thank the 22 interns who shared our passion for amphibians and we thank all of our cooperating partners at the Tribal Colleges, Tribal Game Departments and State Universities.

The Native American culture observes that learning from within the circle allows the observer to feel and identify with the spirit that moves through all things. In sponsoring this grant the NDGFD has given the Tribal schools ownership in the valuable resources that they view as part of the connectedness of all things scattered by the four winds. Funds for this project were administered through Minot State University but the direction of the collections and the coordination of effort were left to the Tribal colleges and cooperating partners. We hope that the NDGFD will continue to support the Tribal schools as they deal with issues of conservation and natural resource management decisions on reservations in North Dakota. In addition, several of the research site in this study were blessed in traditional ceremony by Tribal medicine men and we hope that this tradition will be continued as projects for amphibian monitoring expand into other Tribal and state lands. Amphibians need all the help that they can get.

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