

U.S. Fish & Wildlife Service

Wyoming Toad *Bufo hemiophrys baxteri* *now known as *Anaxyrus baxteri**

Draft Revised Recovery Plan 2013

Original Approved Recovery Plan, September 11, 1991



Photo by Sarah Armstrong, Wyoming Toad Studbook Keeper

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for

Mountain-Prairie Region
U.S. Fish and Wildlife Service
Denver, Colorado

December 2013

Wyoming Toad

Bufo hemiophrys baxteri
now known as Anaxyrus baxteri

DRAFT REVISED RECOVERY PLAN 2013

Original Recovery Plan Completed in 1991

Mountain-Prairie Region
U.S. Fish and Wildlife Service
Denver, Colorado

Approved: _____



Regional Director, U.S. Fish and Wildlife Service

Date: 1.15.14

DISCLAIMER PAGE

Recovery plans delineate such reasonable actions as may be necessary, based upon the best scientific and commercial data available, for the conservation and survival of listed species. Plans are published by the U.S. Fish and Wildlife Service (Service or USFWS), sometimes prepared with the assistance of recovery teams, contractors, State agencies and others. Recovery plans do not necessarily represent the views, official positions or approval of any individuals or agencies involved in the plan formulation, other than the Service. They represent the official position of Service only after they have been signed by the Regional Director. Recovery plans are guidance and planning documents only; identification of an action to be implemented by any public or private party does not create a legal obligation beyond existing legal requirements. Nothing in this plan should be construed as a commitment or requirement that any Federal agency obligate or pay funds in any one fiscal year in excess of appropriations made by Congress for that fiscal year in contravention of the Anti-Deficiency Act, 31 U.S.C. 1341, or any other law or regulation. Approved recovery plans are subject to modification as dictated by new findings, changes in species' status, and the completion of recovery actions.

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Additional copies of the draft document can also be obtained through prior arrangement from:

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Recovery plans can be downloaded from: http://ecos.fws.gov/tess_public/SpeciesRecovery.do

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Special Acknowledgement to George Baxter:

The Wyoming toad was named in honor of Dr. George Baxter who discovered the species in the Laramie Basin in 1946. Dr. Baxter maintained a strong interest in the toad until his death in 2005 at the age of 86. Dr. Baxter grew up in Wyoming and completed a Bachelor's and Master's degree at the University of Wyoming (UW). After earning a PhD from the University of Michigan in 1951, Dr. Baxter returned to UW where he served on the faculty of the Department of Zoology and Physiology until his retirement in 1984. He was a popular teacher and mentored many graduate students who went on to successful careers. He achieved a national reputation as a distinguished scholar and an acknowledged expert on the fish, amphibians, and reptiles of Wyoming and the Rocky Mountain Region. He actively participated in the Wyoming toad recovery program until his death. He received numerous awards during his academic career and in 2005 was given the prestigious Conservation Service Award by the Department of Interior.

Dr. Baxter made many important contributions to the Wyoming toad recovery program. His research provides the basis for our knowledge of the toad's natural history. Furthermore, he was an active participant in recovery efforts and provided an invaluable historical perspective to aid in the recovery of the toad. His dedication to this effort was inspiring to his colleagues and to the many young, emerging scientists who were fortunate enough to interact with him.

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EXECUTIVE SUMMARY

Current Species Status: The Wyoming toad (*Bufo hemiophrys baxteri* now known as *Anaxyrus baxteri*) was federally listed as endangered in 1984 under the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq.*). The Wyoming toad is considered one of the four most endangered amphibian species in North America and is classified as “extinct in the wild” (IUCN 2011). Approximately 500 individuals are currently in captivity. Recovery of this species will require both sustained, long-term conservation actions and repeated experimentation to determine the optimal means to reestablish wild populations. The known historic distribution of the Wyoming toad was restricted to approximately 5,000 hectares of habitat consisting of flood plains, ponds, and small seepage lakes in the short-grass communities of the Laramie Basin in Albany County, Wyoming.

Habitat Requirements and Limiting Factors: The Wyoming toad is a glacial relict species known only from Albany County, Wyoming. The toad formerly inhabited floodplain ponds and small seepage lakes associated with the Laramie River. Current distribution is limited to the Laramie Plains, specifically at the Mortenson Lake National Wildlife Refuge (Mortenson Lake) and one Safe Harbor Agreement (SHA) site. Limited habitat use has been studied at Mortenson Lake although specific information about the toad’s life history remains unknown. Primary threats at the time of listing (1984) were identified as follows: a limited distribution, habitat manipulation, disease, and small population size. Currently, primary threats include: a small population size and limited distribution, chytridiomycosis (an infectious disease of amphibians caused by the pathogenic fungus, *Batrachochytrium dendrobatidis*); inadequacy of existing regulatory mechanisms; and a small population size.

Recovery Strategy: The general recovery strategy for the Wyoming toad is to reduce threats to the species and to establish at least five viable, self-sustaining sub-populations. Many of the necessary actions for habitat protection are based on an increased understanding of the relationship of the Wyoming toad to its physical, chemical, and ecological environment. Increased knowledge of the species is crucial for improved science-based management decisions and conservation actions.

Recovery Goal: The ultimate recovery goal is to restore multiple self-sustaining populations within and nearby the historical range, allowing for subsequent downlisting and delisting of the Wyoming toad.

Recovery Objective: This recovery plan’s structure articulates both short and long-term strategies that together comprise the conditions under which the Wyoming toad may be delisted. The ultimate recovery objective is to restore a minimum of five self-sustaining populations within and/or nearby the historical range, and subsequently to delist the Wyoming toad. To do this, captive populations will need to be maintained at a sufficient level, loss of genetic variability in captivity will need to be minimized, suitable habitat will need to be restored and/or identified, and disease will need to be suppressed at a level to which the populations are self-sustaining and viable.

Recovery Criteria

A) Downlisting Criteria: The Wyoming toad will be considered ready for reclassification from endangered to threatened, when all of the criteria below are realized:

- (1) Three self-sustaining, viable populations of the Wyoming toad are established within and nearby the toad's historic range. These three sub-populations will be established as viable for a minimum of seven consecutive years as evidenced by a Population Viability Analysis (PVA).
- (2) The captive assurance population is targeted to a minimum of 500 toads (excluding tadpoles and toadlets) for seven consecutive years during establishment of self-sustaining wild populations. This targeted minimum may fluctuate (by approximately 50 individuals) along with natural fluctuations within a given year or during a naturally unsuccessful year.
- (3) The WTSSP has a peer-reviewed Captive Breeding Plan for 25 years after downlisting. This plan will be comprehensive enough, as determined by the Service and the WTSSP to prevent further loss of genetic diversity in the captive population.
- (4) Chytridiomycosis infection rates do not pose a threat to the long-term sustainability of Wyoming toad populations or are maintained at a level that can ensure long-term sustainability of the populations.

B) Delisting Criteria: The Wyoming toad will be considered recovered and ready for removal from the list of endangered and threatened wildlife (delisted) when all of the criteria listed below are realized:

- (1) Two additional self-sustaining populations of Wyoming toads are established within and nearby the toads' historic range, for a total of five self-sustaining and viable (as determined by a PVA) populations.
- (2) A peer reviewed and Service-approved monitoring plan is in place to assess population viability of each of the five sub-populations for twenty-five years after delisting.
- (3) Chytridiomycosis infection rates continue to not pose a threat to the long-term sustainability of the additional Wyoming toad populations needed for delisting or are maintained at a level that can ensure long-term sustainability of the populations.

Actions Needed: (1) Habitat Protection, (2) Habitat Management, (3) Population Augmentation, (4) Reintroductions, (5) Outreach Cooperation with Stakeholders and Partner Agencies, (6) Research, (7) Monitoring, (8) Adaptive management. Overall, these recovery actions are tied directly to achievement of the recovery criteria for the Wyoming toad (Appendix A). It can be assumed that continued intensive management may be required for several decades. The following is a preliminary timeline for the first 10 years of recovery actions:

- Years 1-3 (2013-2015)
 - Finalize Southern Laramie River Conservation Area (SLRCA)
 - Collect and compile population dynamics parameters
 - Explore *in situ* *Bd* mitigation methods
 - Finalize reintroduction protocol
 - Finalize habitat management protocol

- Years 4-5 (2016-2017)
 - Establish one self-sustaining/breeding population at Mortenson Lake and two in historic toad habitat along the Laramie River.
 - Develop *Bd* mitigation plan

- Years 6-10 (2018-2022)
 - Establish 3 new reintroduction sites
 - Reintroductions to maintain 3 self-sustaining populations
 - Implement *Bd* Mitigation Plan

Total Estimated Cost of Recovery: \$4,260,000 plus any unforeseeable costs is estimated for delisting the Wyoming toad. The first 5-year management period is estimated at \$1,420,000 and costs will be accrued continually for the following 10 years until the 15-year minimum mark estimated for delisting has been reached. Additional costs will accrue if recovery criteria are not met within the estimated 15-year time frame. Downlisting is estimated to require a minimum of 13 years at the same cost as delisting and is therefore estimated to cost approximately \$3,692,000.

Estimated Date of Recovery: If recovery criteria are met, recovery could occur in 15 years or by the end of 2028. The first five years will be needed to establish a minimum of five, self-sustaining breeding populations in the wild. Three additional years will be required to ensure the most recently introduced population has reached breeding maturity, and the subsequent seven years will be required to demonstrate long-term sustainability. Downlisting is estimated to require a minimum of 13 years: three years for establishment of the three self-sustaining populations, three additional years to reach breeding maturity, and a subsequent seven years to demonstrate long-term sustainability.

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1.0 BACKGROUND

Section 4(f) of the Endangered Species Act (ESA) mandates that the Service develop and implement recovery plans for threatened and endangered species, unless such a plan would not promote conservation of the species. The law's ultimate goal is to "recover" species so they no longer need protection under the ESA. Recovery plans describe the steps needed to restore a species to ecological health. FWS biologists write and implement these plans with the assistance of species experts; other Federal, State, and local agencies; Tribes; nongovernmental organizations; academia; and other stakeholders.

1.1 Brief Overview

Federal Conservation Status

Entity listed:	<i>Bufo hemiophrys baxteri</i> (now known as <i>Anaxyrus baxteri</i>)
Common Name:	Wyoming toad
Federal Register:	January 17, 1984 (49 FR 1992) Final Listing Rule
Federal Status:	Endangered, range wide

State of Wyoming Conservation Status

Species of Greatest Conservation Need

Species Status: NSS1 (Aa)

NSS = Native Species Status

1 = High Priority

A = Population Imperiled

a = Extreme limiting factors

The population of Wyoming toads declined dramatically in the 1970s, and by the 1980s, individuals were extremely rare (Baxter and Stromberg 1980, Stromberg 1981, Vankirk 1980, Baxter et al. 1982, Baxter and Stone 1985, Lewis et al. 1985). By 1985, the Wyoming toad was presumed extinct. In 1987, an extant population was discovered at Mortenson Lake (Lewis et al. 1987). Ten Wyoming toads (4 young-of-the-year and 6 yearlings) were first taken into captivity on September 1, 1989 (McCleary 1989) from Mortenson Lake. These toads were the last Wyoming toads known to exist in the wild. Since 1989, all toads at Mortenson Lake have been re-introduced.

A variety of factors have contributed to the Wyoming toad's decline, including infectious disease and habitat changes caused directly or indirectly by human activity. Significant features of the species' life history, behavior, ecological interactions, and habitat needs remain unknown. Due to these uncertainties, detailed long-term recovery planning is difficult, and the exact needs of the recovery program cannot be specified beyond a relatively short time horizon. Recovery of this species will require both sustained, long-term conservation actions and repeated investigations to determine the optimal means to reestablish wild populations.

Table 1 displays the ranking system for determining Recovery Priority Numbers, as established in 1983 (48 FR 43098, September 21, 1983 as corrected in 48 FR 51985, November 15, 1983). Recovery priority numbers, which range from a high of one to a

low of 18, are based on degree of threat, recovery potential, taxonomic distinctiveness, and presence of an actual or imminent conflict between the species and development activities (C represents conflict). The recovery priority number for the Wyoming toad is 2, indicating a **high** degree of threat, a **high** recovery potential, and taxonomic standing as a full **species** (Table 1).

Table 1. RANKING SYSTEM FOR DETERMINING PRIORITY NUMBERS

Degree of Threat	Recovery Potential	Taxonomy	Priority	Conflict
High	High	Monotypic Genus	1	1C
		Species	2	2C
		Subspecies/DPS	3	3C
	Low	Monotypic Genus	4	4C
		Species	5	5C
		Subspecies/DPS	6	6C
Moderate	High	Monotypic Genus	7	7C
		Species	8	8C
		Subspecies/DPS	9	9C
	Low	Monotypic Genus	10	10C
		Species	11	11C
		Subspecies/DPS	12	12C
Low	High	Monotypic Genus	13	13C
		Species	14	14C
		Subspecies/DPS	15	15C
	Low	Monotypic Genus	16	16C
		Species	17	17C
		Subspecies/DPS	18	18C

1.2 Description and Taxonomy

1.2.1 Description

The Wyoming toad (*Bufo hemiophrys baxteri* now known as *Anaxyrus baxteri*) was federally listed as endangered in 1984 under the Endangered Species Act of 1973 (Act). The Wyoming toad is considered one of the four most endangered amphibian species in North America and is classified as “extinct in the wild” (IUCN 2011). The background color of adult Wyoming toads is variable, but can be dark brown, gray, or greenish, with small dark blotches and a distinct light median line. Some individuals have well-defined light lateral stripes. The belly is often spotted. Wyoming toad females are larger than males. Males can further be distinguished from females by the presence of a dark throat patch and nuptial pads, dark raised skin on the front toes which are used to grip females during mating.

Only male Wyoming toads vocalize. Three distinct vocalizations have been identified: the mating call, a short buzzing trill; the release call, a guttural vibration deeper in pitch than the mating call and used when grasped by other

males; and the protest call, consisting of a short staccato “pip” (Baxter and Stone 1985, Withers 1992).

Wyoming toad tadpoles are typically jet-black in color and grow to a length of 5 to 7 millimeters (0.29 to 0.32 inches) (Withers 1992). The species most likely to occur with Wyoming toad tadpoles is the boreal chorus frog (*Pseudacris maculata*) which can be most easily distinguished by the lighter brown pigment of the chorus frog tadpole. Wyoming toad tadpoles can additionally be distinguished from chorus frog tadpoles by eye placement; the eyes of chorus frogs protrude dorsally whereas the eyes of toad tadpoles protrude laterally (Altig et al. 1970).

Individual toads can be identified by unique skin color and wart patterns (Withers 1992) and unique throat patch markings. In 2010, Wyoming Natural Diversity Database (WYNDD) initiated a study using wart and pigment patterns as a unique identifier. Results of evaluations of the use of computer-assisted recognition software to identify individual toads are expected in 2014 (WYNDD 2010).

1.2.2 Taxonomy

George Baxter first reported the Wyoming toad in 1946 (Baxter 1990 pers. comm.). At that time, the Wyoming toad (*Bufo hemiophrys baxteri*) was considered a subspecies of the Canadian toad (*Bufo hemiophrys*), but was later determined to be a distinct species *Bufo baxteri* (Smith et al. 1998). The Canadian toad and the Wyoming toad differ in morphology, venom composition, advertisement calls, and are widely separated geographically (Porter 1972, Smith et al. 1997).

The classification of frogs and toads of the world has changed since the Wyoming toad was listed and since the publication of the 1991 Recovery Plan. New World toad species were divided into a number of new or revised genera and previously North American *Bufo* spp. were placed into the new genus *Anaxyrus* (Frost et al. 2006, Crother 2008, Collins and Taggart 2009).

In The Center for North American Herpetology’s (CNAH) 2009 *Standard Common and Current Scientific Names for North American Amphibians, Turtles, Reptiles & Crocodylians* as well as the Integrated Taxonomic Information System (ITIS) lists the taxon as *Anaxyrus baxteri* (Porter 1972). The Service now uses the scientific name *Anaxyrus baxteri* for the taxon.

1.3 Distribution and Habitat Use

1.3.1 Distribution

Historically, Wyoming toads were commonly associated with the floodplains of the Laramie River (Figure 1) (unless explicitly stated otherwise, *Laramie River* as used throughout this document includes both the Little Laramie and the Big Laramie Rivers). Dr. George Baxter concluded historic use by Wyoming toads of lakes in the Laramie

River Basin was limited, as toads preferred ponds in the flood plains of the Laramie River to the saline seepage lakes (Baxter 1990 pers. comm.). When irrigation was implemented in the Basin, the lakes were flushed out making them less saline and more suitable as toad habitat. At that time, the range of the Wyoming toad was believed to be 2,331 square kilometers (990 square miles) in the Laramie Basin, with the town of Laramie near the eastern border of the range (Figure 1).

Currently, the range of the toad is limited to Mortenson Lake which is a result of reintroductions. No other populations are known to exist in the wild. Despite the fluctuating numbers over the years, until 2008 less than 50 Wyoming toads were reproducing, over-wintering, and persisting at Mortenson Lake and the SHA site. However, the population at Mortenson Lake crashed between 2009 and 2012; only one adult toad was found during 2011 and 2012 surveys (the same toad). No new adult toads were found during surveys at Mortenson Lake or the SHA site in June and July of 2012.

The Wyoming Toad Recovery Team (WTRT) made the decision in April of 2012 to repopulate Mortenson Lake through soft releases. That summer, the Service conducted a pilot study using head start tanks (mesh cages) for protecting tadpoles through metamorphosis. The toadlets were then moved to small corrals for protection, feeding, and to allow them to grow prior to release. Over 800 toadlets were released from the head start tanks at Mortenson Lake in August, 2012 and 28 were recovered during monitoring surveys in September 2012. The University of Wyoming repeated the head start tank soft release in 2013. During 2013 monitoring surveys, 507 toads were found (408 toadlets, 79 juveniles, and 20 adults) at Mortenson Lake.

1.3.2 Habitat Use

Most of the information regarding Wyoming toad habitat use is provided by two studies conducted at Mortenson Lake (Withers 1992, Parker 2000). Both studies illustrate that the Wyoming toad uses a variety of habitats during different lifestages, such as embryo, tadpole, juvenile, and adult. While both studies provide useful information, they are not exhaustive and more research is necessary to fully understand Wyoming toad habitat use to make informed decisions of potential habitat restoration and new reintroduction sites.

Wyoming toad tadpoles and newly metamorphosed individuals tend to prefer warm water and soil. Tadpoles occupy the warmest water in a given area and seek deeper water during the evening in response to the nighttime cooling of the shallows (Withers 1992). Soils on which young-of-the-year metamorphosed into toadlets are saturated and warmer than adjacent areas (Withers 1992). However, both adults and juveniles have been observed burrowing into moist substrate during the warmest part of the day and basking in open areas during cooler days.

The vegetation density where a toad is found tends to increase as the toad ages. Newly metamorphosed Wyoming toads use areas with open vegetation that is often bordered by dense vegetation. Juveniles tend to use habitat that is more open than that used by adults, but is denser than that used by metamorphs. At night, adults seek refuge in vegetation

that is denser than that used during the day (Parker 2000). The varied vegetation in which each lifestage of the Wyoming toad is found signifies the need for a variety of vegetation densities at reintroduction sites

The vegetation immediately around Mortenson Lake consists of a mixture of rush, sedge, and grass communities. Uplands consist of short grass with scattered shrubs. The areas of shoreline with the deepest water are comprised of thick stands of American bulrush (*Scirpus americanus*) and areas of shallower water are dominated by spike rush (*Eleocharis palustris*), a short, low density plant. These areas of low stem-density plants allow for free movement of breeding toads and allows light to reach shallow areas where warm water is required for embryo development (Withers 1992).

This species is closely water dependent and remains nearby the margin of Mortenson Lake or along the moist drainages. Individuals are almost always located on moist soil or in shallow water and toads flee from predators by jumping into water (Withers 1992, Parker 2000).

Rodent burrows are an important component of Wyoming toad habitat, not only for refugia in hot weather, but also for hibernation (Withers 1992). Wyoming toads begin selecting hibernation sites in fall, but the precise factors that signal hibernation remain unknown. No documented studies exist about depths or temperatures at which Wyoming toads hibernate.

1.3.3 Habitat Management

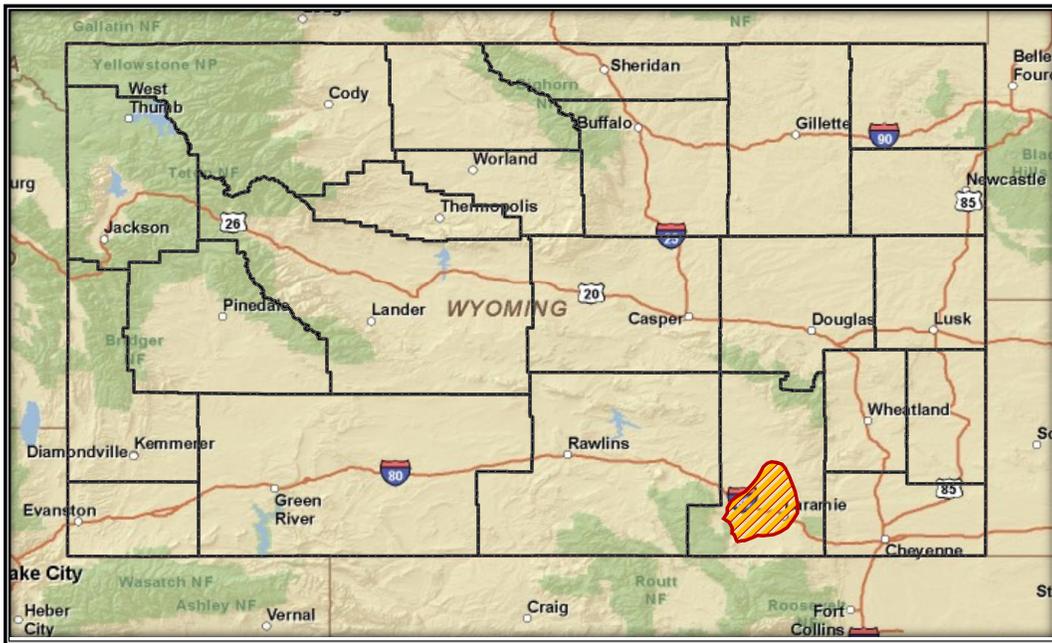
The habitat at Mortenson Lake and within the greater Laramie Basin is adapted to a natural fire regime and was historically grazed by native ungulates and domestic cattle. Without management to reduce build-up of bulrushes, sedges, and grasses, overgrown vegetation shades shallow water resulting in water too cold for Wyoming toad breeding and may not allow for free movement of toads through the vegetation. Since the 1990s, the vegetation at Mortenson Lake has been managed with grazing and prescribed burning.

Grazing has been used as a management tool since Wyoming toads were discovered at Mortenson Lake. Cattle are allowed to graze along the shoreline during the fall when it is drier and vegetation is dense. Arapaho National Wildlife Refuge (Arapaho) is in the process of developing an annual grazing plan that involves keeping cattle restricted from toad breeding areas along the shoreline during spring and summer months. In some years, the fall is wetter than usual or for some other reason cattle are not able to get to the lakeshores to graze down the vegetation. Cattle cannot graze for extended periods in standing water without developing hoof problems. An alternative habitat management tool, prescribed burning, can be used when vegetation cannot be grazed and unavoidably develops a dense layer.

Subsequent to a prescribed burn in 2005, the Wyoming toad population at Mortenson Lake seemed to be rebounding (Table 1). However, populations began to decline in

2009. These declines may have been a result of habitat degradation or increased occurrence of *Bd*. No information is available on habitat conditions before and after the burn. In 2012, another prescribed burn was conducted at Mortenson Lake to reduce the buildup of dead vegetation in toad habitat and is currently on a 3 year prescribed burn rotation. Vegetation surveys were conducted before and after the burn and analysis of the results are ongoing.

Figure 1. HISTORIC RANGE OF THE WYOMING TOAD



1.4 Life History

1.4.1 Reproduction

The Wyoming toad breeding season is from mid-May to mid-June depending on annual weather conditions (McCleary 1989, Chamberlin 1990, Withers 1991). Adult Wyoming toads emerge out of hibernation in May after daytime temperatures reach 23°C (73.4°F) to breed (Withers 1991). Males appear first and begin calling. The mating call of the Wyoming toad is a buzzing trill that lasts a few seconds (Baxter 1952). In 1991, calling at Mortenson Lake took place when air temperatures ranged from 17.5°C to 21.1°C (63.5°F to 70.0°F) and water temperatures ranged from 17.9°C to 21.9°C (64.2°F to 71.4°F). Calling continued until air temperatures fell below 10°C (50°F). Calls could be heard from a distance of approximately 200m (650 feet) when no wind was present (Withers 1992). In the 1950s and 1960s, Dr. George Baxter observed breeding congregations in floodplains of the Laramie River that consisted of at least twenty toads. Breeding congregations during the 1990s consisted of only five to 10 individuals.

Eggs are deposited in gelatinous strings resembling a black pearl necklace and are often intertwined with vegetation. Eggs are laid in shallow water averaging 3.5 to 6.3 centimeters (1.4 to 2.5 in) in depth and in temperatures that ranging from 20.6°C to 23.7°C (69.1°F to 74.7°F) (Withers 1992). Egg masses vary in size and number. The minimum number of eggs observed in wild egg masses during 1990 to 1992 was 1,000 with a maximum of 5,400 (Withers 1992). Fertility in these masses varied from 0 percent to 10 percent (Withers 1992). The numbers of eggs in egg masses produced in captivity have ranged from 1 to 5,000 eggs (Lipps and Odum 2000). Eggs laid in the wild hatch in one week and tadpoles metamorphose in four to six weeks (Withers 1992). Captive Wyoming toads have metamorphosed in shorter periods of time when exposed to higher temperatures (Paddock 2009 pers. comm., Palmer 2010 pers. comm.).

Wild Wyoming toad males have been shown to display secondary sexual characteristics such as a dark throat patch and nuptial pads after one year of age (Withers 1992). These males also vocalized when handled and mature sperm was discovered upon necropsy. However, whether or not wild yearling Wyoming toads breed has not been documented. It is difficult to determine the sexual maturity of female Wyoming toads in the field. Upon necropsy, a small proportion of yearlings had mature eggs in the ovary. However, it is believed that most female Wyoming toads did not breed until their second or third year (Withers 1992). In captivity, both males and females have been successfully bred as yearlings.

1.4.2 Diet

Little information is available about the diet and nutritional needs of the Wyoming toad in the wild, hindering the development of an optimal captive diet and complicates identification of potential reintroduction sites. A limited number of samples have been analyzed to identify prey items. The most common prey items are two species of ant: *Myrmica incompleta* and *Formica fusca*. Dungbeetles (*Canthos* sp.) and two species of beetles in the Carabidae family: *Elaphrus* sp. and *Anara* sp. were identified. Although

this information is valuable, only a couple samples have been examined and these may not be indicative of the food source of the population as a whole. More samples need to be examined to thoroughly understand the diet of the Wyoming toad in the wild.

Tadpoles of the Wyoming toad have mouthparts suited for scraping surfaces and have been observed feeding on unidentified algae in Mortenson Lake. Captive tadpoles are fed a varied diet, including: tropical fish flakes, frozen kale, frozen romaine lettuce, algae cultured in tanks, spirulina, and tetramin tablets.

Captive post-metamorphic Wyoming toads are fed crickets and other invertebrates dusted with supplemental nutrients. At the Wyoming facilities, additional net sweeps for native insects are conducted when possible in order to add a more natural element to captive diets.

1.4.3 Movement

Compared to other frogs and toads, Wyoming toads do not appear to move far within their habitat. Out of several hundred wild toads studied at Mortenson Lake in 1987 only three were observed to have dispersed to Meeboer Lake which is less than 402 meters (0.26 miles) from Mortenson Lake (Baxter 1990 pers. comm.). Some toads were observed to move from the north shore to the south shore of Mortenson Lake over a period of several days (Baxter 1990 pers. comm.). In 2011, Wyoming toads were tracked moving from the north shore to the south shore in 24 hours. However, daily movements of Wyoming toads are limited. The mean distance that newly introduced captive adult toads moved in 2011 was 38 meters (124.7 feet) per day (Hvidsten 2011 pers. comm.). The effect *Bd* has on dispersal ability has not been determined, but it is hypothesized to be a hindrance (Annis et al. 2004).

In 2011, the Service released 13 captive adult Wyoming toads with radio transmitters at Mortenson Lake. The purpose of this research was to document habitat use and movement patterns of captive-raised toads in the wild. Eleven toads either died, dispersed beyond tracking distance, or the battery in the transmitter lost power: that is, transmitter signals were lost and the fate of these individuals remains unknown. The two toads that were tracked into hibernation during October made movements away from the lake or into mammal burrows between August 21 and August 29. The average distance toads moved over a 24-hour time period was 51m and the maximum distance noted was 426m. Toads were often located nestled into the vegetation or soil, and were sometimes completely concealed by vegetation.

1.5 Threats

There is not a definitive understanding of what caused the Wyoming toad to decline. However, declines in amphibian populations have occurred globally during recent years. Factors proposed or identified in amphibian declines include: UV radiation (Blaustein et al. 1997), pollution (Bishop and Pettie 1992, Berill and Bertram 1997), pesticide use (Taylor 1998, Davidson et al. 2001), habitat degradation (Johnson 1992; Wardell-Johnson and Roberts 1991, Gillespie and Hollis 1996, Dubuis 1997, Waldick 1997), grazing (Winegar 1977, Behnke and Raleigh 1978, Kauffman and Krueger 1984, Marlow

and Pogacnik 1986), introduction of exotic predators (Bradford et al. 1993, Lannoo 1998), immune system suppression (Taylor 1998), lack of prey base, and disease (Berger et al. 1998). Below is a discussion of threats that have been identified for the Wyoming toad. Most likely, the cumulative effects of more than one factor rather than single factors have led to declines.

1.5.1 Factor A. The Present or Threatened Destruction, Modification, or Curtailment of Habitat

1.5.1.1 Irrigation Practices

The major use of water in Wyoming is for flood irrigation and reservoir storage, while some is used for municipal and domestic use. As is the case with most of the western United States, the demand for water is greater than the supply, resulting in over-appropriation of the water supply, which means that only the most senior water rights are entitled to water during low water years.

Minimal flows in the Big Laramie River are common from the city of Laramie upstream to the Dowlin ditch diversion during the summer (a distance of about 24 km). Historically—prior to extensive development of irrigation practices and water diversions—backwater wetland and riparian habitats associated with river oxbows and flood events likely provided much of the natural habitat occupied by the Wyoming toad. Lovvorn and Peck (2001) found that flood irrigation is critical to Laramie Basin wetlands, with 65 percent of flows into these wetlands derived directly from irrigation. Flood irrigation of hayfields in the Laramie Basin has also created many wetlands, both ephemeral and permanent, over the last century (Lovvorn and Peck 2001). A high fraction of historically absent wetlands would be lost if land were retired from irrigation or if flood irrigation were replaced by more efficient irrigation practices. However, there are no public plans proposed to upgrade irrigation in Albany County.

Mortenson Lake is a 25 hectare lake with no direct inflow from irrigation ditches. The inflow to the lake is fresh water interflow percolating from ditches, irrigated fields, and groundwater seeps. Mortenson Lake has a control structure at the east end of the lake and high lake levels from precipitation or snowmelt can be manipulated to some degree. Even during drought periods, lake levels in Mortenson Lake do not usually drop drastically. Considering the relative stability of irrigation in the area, the Service believes the threats associated with water diversion and management to be **moderate** at this time.

1.5.1.2 Presence of Livestock in Toad Habitat

Livestock grazing was listed as a threat to recovery in the original listing document of the Wyoming toad (Service 49 FR 1992). Since that time, no specific research has been conducted to demonstrate cattle grazing as a direct threat to the Wyoming toad.

Many anuran species, including the Wyoming toad, have been observed to co-exist with managed livestock grazing. High numbers of California red-legged frogs (*Rana draytonii*) are found on private lands where stock ponds and cattle are prevalent. In many of these areas, the presence of the California red-legged frog (*R. draytonii*) is attributed to grazing and the water source associated with it. In many cases, livestock operators have artificially created ponds for livestock water where there were none before and thus created frog habitats. In such habitats, grazing helps maintain habitat suitability by keeping ponds clear where they might otherwise fill in with cattails, bulrushes, and other emergent vegetation (Bobzien et al. 2000, Fellers *in litt.* 1998).

Cattle can trample and eat emergent vegetation, often eliminating or severely reducing ground cover (Gunderson 1968, Duff 1979). Loss of emergent vegetation can result in negative environmental consequences to toad habitat. It can reduce habitat for insects and other anuran prey items thus reducing the food base (Cordone and Kelley 1961). It also reduces cover at the shoreline making toads more susceptible to predation. Livestock grazing can also create water quality issues because of cattle urination and defecation in congregation areas near wetlands or other water bodies (Doran et al. 1981, Nader et al. 1998).

However, in habitats where grazing by native ungulates has been suppressed such as Mortenson Lake, grazing can be used as a tool to clear overgrown vegetation and restore suitable habitat for the toad. The Refuge has used grazing as a management tool at Mortenson Lake for many years. Vegetation along the lake margins becomes overgrown and shades shallow water areas preventing the water from warming to temperatures required for Wyoming toad breeding. Depending on local weather, cattle can be placed on toad habitat in early spring and late fall to control vegetation.

Arapaho develops annual grazing plans to manage cattle and the vegetation in toad habitat at Mortenson Lake, as does the Laramie Rivers Conservation District with SHA sites. Electric fences are used to keep cattle out of sensitive Wyoming toad habitat during breeding season. The cattle are then used to reduce vegetation cover when toads are not breeding and for the next season. Considering the positive impacts of grazing on vegetation management and that all grazing plans are approved by the Service, we rank the overall threat level of negative grazing effects in occupied Wyoming toad habitat as **low**.

1.5.1.3 Limited Distribution

Currently, the Wyoming toad's distribution is severely limited and is confined to Mortenson Lake and one SHA site. Mortenson Lake is the only lake within the Arapaho lakes complex with suitable habitat. The others lack shoreline vegetation and/or their waters are alkaline and unsuitable for toads. Wyoming toads were released at Lake George and Rush Lake in the 1990s, but no populations were established.

Species with restricted distribution are vulnerable to extinction by natural processes and human disturbance (Levin et al. 1996). Healthy populations are stratified over a variety of locations, where one sub-population can act as a backup to another should a catastrophic event occur at one of the sites. Random events causing population fluctuations or population extirpations become a serious concern when the geographic distribution of the species is very limited.

Three self-sustaining populations are needed for downlisting and an additional two (for a total of five) are needed for delisting. Although the previously described habitat threats are ranked as low to moderate, the lack of protected reintroduction sites within its historical range is a significant barrier to increasing the distribution of the Wyoming toad. To overcome this barrier, reintroduction sites may need to be chosen outside of the toad's historic range, but should remain in close proximity as geographic variability will likely create habitat characteristics beyond what is tolerable for the Wyoming toad.

The Service believes that the limited distribution of the Wyoming toad presents a **severe** threat to recovery of the species.

1.5.1.4 Contaminants

1.5.1.4.1 Heavy Metals and Pollution

The 1991 Recovery Plan identified heavy metals and pollution as a potential contributor to the Wyoming toad's declining population. The role of trace elements in the decline of the Wyoming toad is unknown. The Service conducted a monitoring study in 1989, 1990, and 1991 to determine if trace elements occurred at levels that could affect the Wyoming toad. This study determined there were no elevated concentrations of trace metals at Mortenson Lake that could impact the Wyoming toad (Ramirez 1992).

Heavy metals are specific forms of contamination that have also been suggested as potential contributors to declining toad populations. Except for measurements of metal concentrations in waters throughout the basin by the Service contaminant program in 1992, little research has been done to evaluate their effects on Wyoming toads (Ramirez 1992). However, early studies identified low levels of contaminants in the Laramie Plains Lakes area. For these reasons, the Service assigns the overall threat level for heavy metals and pollution as **low**.

1.5.1.4.2 Mineral Fertilizers

The 1991 Recovery Plan identified mineral fertilizers as a potential threat to Wyoming toads. Mineral fertilizers are materials, either natural or manufactured, containing nutrients essential for normal growth and development of plants. The three plant nutrients applied in large quantities are nitrogen, phosphorus, and potassium. These fertilizers can

be transported from agricultural fields into waterways and ultimately into suitable toad habitat through overland flow.

Schneeweiss and Schneeweiss (1997) found that up to 100 percent of amphibians were dead in pitfall traps located in fields that were augmented with mineral fertilizers. In contrast, no dead or injured amphibians were found during simultaneous monitoring of non-fertilized fields. Marco et al. (1999) found increased mortality of northern red-legged frog (*Rana aurora*) larvae when exposed to nitrite below the levels allowed for safe drinking water. Additionally, Marco and Blaustein (1999) found that Cascades frog (*R. cascadae*) larvae exposed to sub-lethal concentrations of nitrite were more vulnerable to predation because of delayed metamorphosis and increased occupancy in shallow water.

Since the 1991 Recovery Plan was developed, no specific investigation or information on use of mineral fertilizers in Albany County or possible effects on Wyoming toads have been identified. Since there is potential for significant negative impacts to frogs and toads from exposure to mineral fertilizer, the Service will continue to investigate this issue. At this time, the threat to the Wyoming toad from mineral fertilizers is considered to be **moderate**.

1.5.1.4.3 Pesticides (including insecticides and herbicides)

The effects of pesticides on amphibians have been investigated in recent years in response to the major decline of amphibians worldwide. Although not confirmed, pesticides have been suspected as a cause of population declines of the Wyoming toad. Because the response to each chemical is species specific (Blaustein 2002), there is a continuing need to monitor the potential effects of pesticides commonly used in or near habitat of the Wyoming toad.

Pesticides are not applied directly to Mortenson Lake, but are transported through aerial deposition. In California, pesticides from the Central Valley are transported by winds to the Sierra Nevada and have been correlated to population declines of the Sierra Nevada yellow-legged frog (*Rana sierrae*) (Davidson 2004, Fellers and Sparling 2009).

Any and all applications of pesticides in Albany County are closely coordinated and monitored with Albany County Weed and Pest Control (Dickerson 2013 pers. comm.). The following pesticides are currently and historically used in Albany County, Wyoming:

1.5.1.4.3.1 Fenthion (Baytex)

Widespread aerial spraying of fenthion (Baytex) for mosquito control coincided with population declines of Wyoming toads. Research conducted by Freda (1988) and Lewis (1984) with boreal

toads (*Anaxyrus boreas boreas*) and leopard frogs (*Lithobates pipiens*) illustrated that fenthion caused no immediate detrimental effects. However, these studies did not measure the long-term effects of chronic exposure or the indirect effects of fenthion on reduction of prey base were not investigated. Fenthion is no longer used as an insecticide in the Laramie Basin and is thus not considered a threat to the Wyoming toad.

1.5.1.4.3.2 Malathion

Malathion has been shown to have negative effects to amphibians. Taylor 1998 and Taylor et al. 1999e found that disease susceptibility and mortality were shown to increase in Woodhouse toads (*A. woodhousii*) when exposed to malathion and subsequently injected with an pathogen. Malathion was been found to be moderately toxic to six species of amphibians and the toxicity doubled for one species in the presence of a predator (Relyea 2004). In years when mosquito populations are large, malathion is sprayed on properties adjacent to the refuge and other sites within the Wyoming toad's historic range. Low concentrations of malathion due to aerial drift have been documented on Wyoming toad reintroduction sites (Dickerson et al. 2003). The Service believes that the current level of malathion in the Laramie Basin is a moderate threat to the Wyoming toad.

1.5.1.4.3.3 Atrazine

Atrazine was listed as a potential reason for decline in the original listing documents (Dickerson 2013 pers. comm.). Research conducted by Hayes et al. (2003) found atrazine at concentrations as low as 0.1 ppb to cause feminization of male leopard frogs (*Rana pipiens*). In a more recent study, Hayes et al. (2010) found male African clawed frogs (*Xenopus laevis*) exposed to atrazine developed female sex organs and were unable to reproduce (Hayes et al. 2010). Frogs collected on the Platte River near Saratoga, Wyoming in waters containing atrazine exhibited hermaphroditism (Dickerson 2013 pers. comm.).

In 2004, atrazine metabolites (metabolism products) were detected in Mortenson Lake and the Big Laramie River at the Colorado/Wyoming State line at 0.1 ppb and 1.2 ppb respectively. A study of atrazine levels in surrogate amphibian tadpoles in the Laramie Plains Lakes area was conducted by the Service in 2008 and 2009. Concentrations of atrazine were detected at <0.1 ppb in 2008 and <0.01 ppb in 2009. Hermaphroditic individuals were not identified and it is believed that the concentrations detected were not high enough to cause hermaphroditism. However, not all individuals could be positively sexed because the toads' organs

were not completely differentiated yet. Due to the low concentrations of atrazine detected and the unlikelihood of hermaphroditism within the Wyoming toad range, the Service does not believe the current level of atrazine use is a threat to Wyoming toad.

1.5.1.4.3.4 Permethrin (Biomist) and *Bacillus thuringiensis israelensis*

Permethrin (Biomist) and the larvicide *Bacillus thuringiensis israelensis* (BTI) are the primary pesticides currently being used for mosquito control in the town of Laramie (Whitman 2002 pers. comm., Harrison 2002 pers. comm.). However, in years when mosquito populations are large, malathion is used outside of the city limits. BTI, has undergone extensive risk studies both prior to and after registration. It is effective on most mosquito species, black flies, and midges in a very wide variety of habitats and is used to control mosquitoes on private properties adjacent to Mortenson Lake on the western border of the refuge, as well as on nearby Wyoming Game and Fish Department (WGFD) managed property. BTI has been proven to have no detrimental effects to amphibian populations.

Pesticide use varies throughout the Wyoming toad's historic range and potential reintroduction sites need to be monitored for pesticide presence. Due to the varied use within the toad's historic range, the Service believes the overall threat level for pesticides to the Wyoming toad is **moderate**.

1.5.2 Factor B. Overutilization for Commercial, Recreational, Scientific, or Educational Purposes

Overutilization was not identified as a threat in the 1991 Recovery Plan and continues not to be a significant threat to the Wyoming toad. A commercial market for the Wyoming toad does not exist. Recreation is currently prohibited at Mortenson Lake and thus does not pose a threat to the Wyoming toad. The WTRT takes extreme caution to ensure scientific studies do not inhibit recovery of the species, by making sure that conservation benefits out-weigh potential impacts of the research. As long as efforts to minimize impacts while maximizing conservation outcomes continue, impacts from scientific research do not pose a threat to the Wyoming toad. The Wyoming toad is not currently used for educational purposes and thus is not a threat to the species. The Service believes overutilization to not pose a threat to the Wyoming toad.

1.5.3 Factor C. Disease or Predation

1.5.3.1 Infectious Disease

Many previously unexplained amphibian declines worldwide have been linked to outbreaks of chytridiomycosis, the disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) (Berger 1998, Lips et al. 2006, Voyles et al.

2009, Wake and Vredenburg 2008). The Sierra Nevada yellow-legged frog (*R. sierrae*) has experienced precipitous declines as a result of *Bd* infections (Rachowicz et al. 2006, Vredenburg et al. 2010) and the Yosemite toad (*Anaxyrus canorus*) has tested positive for the disease as well (Green and Sherman 2001). In Populations of the California red-legged frog (*R. draytonii*) have been lost or reduced by *Bd* outbreaks (USFWS 2002). Arizona, Bradley et al. (2002) diagnosed dead and dying Chiricahua leopard frogs (*Rana chiricahuensis*) with chytridiomycosis. In the Rocky Mountains, die-offs in remaining populations of the boreal toad (*A. boreas*) were attributed to *Bd* (Muths 2003). Wyoming toads also experienced a *Bd* related die-off in 2003 and possibly in 2010 as well (Pessier 2009 pers. comm.).

Batrachochytrium dendrobatidis was documented in wild Wyoming toads from Mortenson Lake in 2000 (Green 2001 pers. comm.) and in 2001 (Pessier 2001 pers. comm.) and was a recurring problem in captive toads in some facilities during this time period. It is unknown whether *Bd* was the cause of the initial Wyoming toad decline; however, the presence of the disease is an important consideration for recovery efforts. An extensive analysis of Wyoming toad mortality between January 1989 and June 1996 indicated that mycotic dermatitis (inflammation of the skin) due to the fungus *Basidiobolus ranarum*, often with secondary bacterial infection, was a primary cause of death in both wild and captive animals (Taylor et al. 1999a, 1999b, 1999c, 1999d). Many morphologic features of *B. ranarum* can easily mimic features of *Bd*, which was not formally described taxonomically until 1999. Based on strong similarities in the histological appearance of *B. ranarum* infections and *Bd* (Muths 2003), a review of material from Wyoming toads diagnosed with *B. ranarum* infection was performed. The review suggests that many cases diagnosed as *B. ranarum* infection were instead cases of *Bd* (Pessier 2000 pers. comm.). Wyoming toad museum specimens from the Laramie Basin from tested positive for *Bd* as early as 1965. This finding places *Bd* in the Laramie Basin just prior to the major decline of the Wyoming toad and disease introduction must be considered as one potential contributory factor to the major decline. In 2009, 93 out of 125 samples (74 percent) of wild Wyoming toads tested positive for *Bd* and in 2010, two out of six toads (33 percent) tested positive. Nineteen percent of wild toads sampled tested positive at Mortenson Lake in 2013.

Humans are a potential vector for the spread of *Bd*. Equipment used within the habitat during population surveys and other on-the-ground activities could serve as pathways for the introduction of infectious disease into the population. The Service has a strict boot and equipment disinfecting protocol that must be followed for all visitors to Mortenson Lake and SHA sites. This protocol is strictly enforced with visitors prior to entering a Wyoming toad occupied area and upon departing. This Service is confident that *Bd* will not be spread with adherence to this protocol.

Because *Bd* is a high intensity threat with potential for very significant exposure of the species potentially causing significant mortality or extinction of the species, we rank the overall threat level as **severe**.

1.5.3.2 Predation

Mortality from a variety of predators is part of the Wyoming toad's life history. However, because of the small population size, predation is a threat to sustaining populations.

Many bufonid species, including the Wyoming toad, employ noxious or toxic skin secretions to defend themselves against predators (Duellman and Trueb 1986, Flier et al. 1980, Low 1972). The toxic secretion prevents some predation on toads but not all. For example, raccoons, snakes, bullfrogs, predaceous diving beetles (*Dytiscus* spp.), and several species of birds have been identified to prey on several species of toads (Jones and Goettl 1999, Withers 1992, Clake 1977, Fitch 1965, Beiswenger 1981, Hammerson 1989, Olson 1989, Sherman and Morton 1993, Livo 1998). Withers (1992) found the skin of several Wyoming toads remaining after predation.

Predation of the Wyoming toad was documented in studies conducted during 1998-1999 (Parker 2000). Seven out of 10 toads implanted with active radio transmitters were lost to predation. Puncture wounds on one of the toads were indicative of an avian predator, and teeth or claw marks on the transmitters of the remaining toads were indicative of mammalian predators.

Fish have been documented to have significant effects on frog populations, but studies have shown that fish generally avoid preying on toads. Sierra Nevada yellow-legged frog (*Rana sierrae*) populations were devastated by fish predation of non-native trout in the Sierra Nevada (Knapp and Matthews 1998, Knapp and Matthews 2000). In Canada, Liss and Larson (1991) reported decline of Ranid species in naturally fishless lakes after non-native trout were introduced. However, Grasso (2010) found eggs, tadpoles, and metamorphs of Yosemite toads (*A. canorus*) unpalatable to non-native brook trout. Similarly, existing literature, as summarized by Dunham et al (2004), states that the presence of trout is not negatively associated with boreal toads (*A. boreas boreas*). Yet, there have been observations in streams on the Kern Plateau in California of numerous western toad (*A. boreas halophilus*) metamorphs in the stomachs of native golden trout (Knapp 2013 pers. comm.). This observation suggests that although toad tadpoles are typically avoided by trout, metamorphs may in fact be palatable.

At the same time, there is currently no evidence of any trout species inhabiting Mortenson Lake and there is no evidence that the fishes present in Mortenson Lake prey on Wyoming toads. Field observations from 2011 by USFWS employees indicated that golden shiners (*Notemigonus crysoleucas*) and fat head minnows (*Pimephales promelas*) were present at Mortenson Lake. A netting survey conducted by WGFD personnel in April 2013 verified the presence of

fathead minnows (*P. promelas*), white suckers (*Catostomus commersoni*), and Iowa darters (*Etheostoma exile*) (Gelwicks 2013 pers. comm.).

Predator control is very difficult in open reintroduction areas like Mortenson Lake where large and small mammals, birds, fish, and predatory aquatic insects are regularly present in toad habitat. More information on specific predators would be necessary to determine if it would be beneficial or feasible to control these common predators.

The number of potential natural predators on Wyoming toads is likely high. We know of no invasive or introduced predators in the area. Future research is needed to understand the extent of predation on wild populations. At this time we lack specific information on Wyoming toad natural predators and the ability to control them; natural predation is a mortality factor and is primarily a threat in light of the low numbers of toads in the wild. Therefore, we rank the overall threat level from predators as **moderate**.

1.5.4 Factor D. The Inadequacy of Existing Regulatory Mechanisms

The inadequacy of regulatory mechanisms was not listed as a threat in the 1984 listing or the 1991 Recovery Plan. However, in the absence of listing, the existing regulatory mechanisms would not be adequate to protect the Wyoming toad from extinction. Wyoming does not have an endangered species act for plants or animals. Instead, the state abides by the ESA and the Species of Greatest Conservation Need (SGCN) designation process based upon its Native Species Status (NSS) classification system. At this time, other than the ESA and minimal State and County statutes, protection of the Wyoming toad and its habitats is restricted to captive populations, the Mortenson Lake population, and one SHA site. These populations are protected in the sense that they cannot be directly impacted by unregulated activities.

Historic habitat for the Wyoming toad is located in wet meadows adjacent to the Laramie River. We believe this type of habitat provides the best chance for success for reintroduction efforts. However, at this time, private lands dominate the floodplains of the Laramie River. The pursuit of acquisition of additional Federal lands for the benefit of Wyoming toads is not possible within the existing refuge boundary for Mortenson Lake and the Hutton Lake complex. At present, the only avenue of reintroduction of Wyoming toads on private lands is through SHA agreements.

Without additional protection of historical Wyoming toad habitat through Federal acquisition and conservation easements, sufficient reintroductions cannot be accomplished and factors such as disease cannot be controlled in the majority of suitable habitat in the Laramie Basin. Therefore, the Service assigns the overall level of the threat of lack of regulatory protection of historical Wyoming toad habitat as **severe**.

1.5.5 Factor E. Other Factors Affecting the Species' Continued Existence

1.5.5.1 Small Population Size

The Wyoming toad's small population size is a significant threat to its continued survival. Species with small population size are vulnerable to extinction by

natural processes and human disturbance (Levin et al. 1996). Populations of all animals fluctuate depending on food availability, nutrient limitations, pollutants, disease, competition, and predation. Healthy populations can survive natural fluctuations, but a small population is extremely vulnerable to extirpations due to natural fluctuations. Random events causing population fluctuations or population extirpations become a serious concern when the number of individuals of the species is very limited. A single human-caused or natural environmental disturbance could destroy the entire population of the Wyoming toad.

When a population's genetic variability falls to low levels, its long-term persistence may be jeopardized because its ability to respond to changing environmental conditions is reduced. In addition, the potential for inbreeding depression increases, which means that fertility rates and survival rates of offspring may decrease. Although environmental and demographic factors usually supersede genetic factors in threatening species viability, inbreeding depression and low genetic diversity may enhance the probability of extinction of rare species (Levin et al. 1996).

The small population size of the Wyoming toad increases the possibility of extinction. The Service considers the overall level for this threat to be **severe**.

1.5.5.2 Low Genetic Diversity

The 1991 Recovery Plan states that from the late 1970s through 1986, the lack of substantial reproduction hinted that genetic issues could be a factor in declining Wyoming toad populations.

A preliminary genetic study of captive Wyoming toads identified a decrease in genetic diversity during the period 2000 to 2010 as compared to 1989 to 1999 (Martin et al. 2010). This analysis is ongoing and continued understanding of the relatedness of the captive population will be valuable to provide a baseline for future work assessing the relatedness of Wyoming toad.

Bd has decimated amphibian populations globally and may pose increased threat to populations and species with low levels of genetic diversity. There are differences in levels of susceptibility and survivorship following infection among species suggesting a genetic basis. Recently, there have been several papers that have demonstrated a link between variation at a particular gene complex, major histocompatibility complex [MHC], and the ability of an individual to survive *Bd* infection (Savage and Zamudio 2011, May et al. 2011). This link is a significant finding in progress toward understanding *Bd* and genetic influences.

The captive population is currently managed with every attempt to maximize genetic diversity. However, stochastic, or random, changes in a wild population's demography or genetics, can threaten the persistence of populations with little genetic diversity (Brussard and Gilpin 1989, Lacy 1997). A stochastic demographic change such as a skewed age or sex ratio (for example, a sudden

loss of adult females) could negatively affect reproduction, especially in a small population. The disruption in gene flow due to reduction and isolation of populations may create unpredictable genetic effects that could impact the Wyoming toad's existence. Because of the risks involved, the Service considers low genetic diversity to be a **high** level of threat at this time.

1.5.5.3 Climate Change

Analyses under the ESA include consideration of ongoing and projected changes in climate. The terms "climate" and "climate change" are defined by the Intergovernmental Panel on Climate Change (IPCC). "Climate" refers to the mean and variability of different types of weather conditions over time, with 30 years being a typical period for such measurements, although shorter or longer periods also may be used (IPCC 2007, p. 78). The term "climate change" thus refers to a change in the mean or variability of one or more measures of climate (e.g., temperature or precipitation) that persists for an extended period, typically decades or longer, whether the change is due to natural variability, human activity, or both (IPCC 2007, p. 78). Various types of changes in climate can have direct or indirect effects on species. These effects may be positive, neutral, or negative and they may change over time, depending on the species and other relevant considerations, such as the effects of interactions of climate with other variables, like habitat fragmentation, for example (IPCC 2007, pp. 8 - 14, 1 - 19). Expert judgment is used to weigh relevant information, including uncertainty, in considerations of various aspects of climate change.

The magnitude of warming in the northern Rocky Mountains has been particularly great, as indicated by an 8-day advance in the appearance of spring phenological indicators (life cycle events influenced by variations in climate) since the 1930s (Cayan et al. 2001). The rates of river flows and water levels in rivers, lakes, reservoirs, and marshes in the northern Rockies also has changed with global climate change and is projected to change further (Bartlein et al. 1997, Cayan et al. 2001, Stewart et al. 2004). Under global climate change scenarios, the mountainous areas of northwest Wyoming may eventually experience milder, moister winters and warmer, drier summers (Bartlein et al. 1997). Additionally, the pattern of snowmelt runoff also may change, with a reduction in spring snowmelt (Cayan et al. 2001) and an earlier peak runoff (Stewart et al. 2004), so that a lower proportion of the annual discharge will occur during spring and summer.

Although many species already listed as endangered or threatened may be particularly vulnerable to negative effects of climate change, some species may be affected positively or not at all. In any case, the identification of effective recovery strategies and actions for recovery plans, as well as assessment of their results in 5-year reviews, should include consideration of climate-related changes and interactions of climate and other variables. These analyses also may contribute to evaluating whether an endangered species can be reclassified as threatened, or whether a threatened species can be delisted.

Information on the potential threats of climate change on the Wyoming toad is currently unavailable and to date, there is no evidence of direct effects to the species at this time. However, amphibian populations are sensitive to changes and key ecological events are influenced by air and water temperature, precipitation, and the hydro-period (length of time and seasonality of water presence) of their environments (Carey and Alexander 2003). These events are influenced by weather changes, such as air and water temperature and precipitation patterns. The timing of reproduction, metamorphosis, dispersal, and migration may shift in response to higher temperatures and changes in rainfall (Beebee 1995). As temperatures warm and small breeding pools dry, tadpoles are likely to experience lower rates of survival to metamorphosis. Also, because of their affinities to aquatic habitats and their small size, amphibians typically have relatively small home ranges and low dispersal rates (Deullman and Trueb 1986, Wells 2007). Wyoming toads have been shown to stay relatively close to their home range and may not be able to disperse should their home range habitat become unsuitable.

Some studies have predicted that amphibians will be even more susceptible to climate change than birds or mammals because of their dependence on microhabitats, hydrological regimes, and limited dispersal abilities (Blaustein et al. 1994). Susceptibility to diseases may additionally be influenced by climate change (Pounds et al. 2006). Some models predict substantially larger changes in amphibian populations than in birds or mammals based primarily on potential future range contractions and expansions. This multitude of projected impacts could exacerbate the current declines being observed across many amphibian populations (Stuart et al. 2004).

Researchers warn that changes in climatic regimes are likely to increase pathogen virulence as well as amphibian susceptibility to pathogens (Daszak et al. 2003, Fisher et al. 2009). On the other hand, *Bd* is a relatively cool climate pathogen, preferring temperatures between 15°C and 23°C (59°F to 74°F) and warming temperatures may, in fact, have a negative influence on *Bd* if the zoospores dry out in the increased temperatures. Yet, if an amphibian species is highly aquatic, the zoospores may not be able to dry out despite increased temperatures. Even though specific information on the effects of climate change on Wyoming toads is lacking and conclusions regarding direct and indirect impacts are uncertain at this time, the research on amphibians in general indicates they are particularly sensitive to potential long-term changes to weather patterns. Thus, the Service believes the threat of climate change to Wyoming toad survival is **moderate**.

1.5.5.4 Weather Events

Populations of most species are cyclic in nature and fluctuate in response to natural factors such as weather events, disease, fire and predation. Natural events including long-term drought have a less negative overall effect on a species when the species is widely and continuously distributed. Small, fragmented, or isolated

populations are more vulnerable to extirpation by random events. Additionally, changes in weather patterns may result in successive years of reproductive failure. For example, a series of late, cold springs may freeze egg masses before hatching and excessively hot, dry summers could dry small pools where tadpoles congregate before metamorphosis. In June 2012, a permanent weather station was installed at Mortenson Lake. This station will allow for continuous collection of weather data to help inform future management decisions.

As droughts in Wyoming and much of the West continue to occur and human water needs increase with rising populations, the amount of available habitat for the Wyoming toad will decrease. The lakes in the Laramie Basin are naturally alkaline, but as water levels lower, concentrations of minerals increase, resulting in habitat too alkaline for the Wyoming toad. As water levels drop, concentrations of nitrates, pesticides, and other chemicals also increase, resulting in harmful and even lethal impacts to Wyoming toads.

Considering the stochastic nature of weather events and the relatively stable captive population, it is unlikely that one or a series of local weather events could cause extinction of the toads. Short-term, local, quasi-extinctions could be addressed through reintroduction. Balancing the possibility of damage by unpredictable weather patterns on a small population with the ability to reintroduce with captive populations, the Service assigns the overall threat level for local weather events as **moderate**.

1.5.5.5 Captive Diets

A primary concern for captive amphibians is the maintenance of a nutrient sufficient diet. Common diet imbalances include vitamin A and D₃, thiamine, calcium, and phosphorous. These imbalances can lead to a variety of complications that affect toad survival, such as Metabolic Bone Disease (MBD) and squamous metaplasia, or short-tongue syndrome (STS). MBD is associated with an imbalance of calcium, phosphorous, and vitamin D₃ (McWilliams 2008), while STS is caused by a deficiency in vitamin A.

Animals with STS don't actually have shorter tongues, but develop an inability to capture prey as they mature. Histological changes in the tongue tissue have been observed in animals diagnosed with this disorder. Preliminary tests indicate that captive animals with STS have significantly lower levels of liver vitamin A (retinol) compared to 10 wild-caught Wyoming toads and several wild-caught American toads (*Anaxyrus americanus*) and southern toads (*A. terrestris*) (Pessier 2009 pers. comm.). Additional complications caused by a vitamin A deficient diet include immunosuppression and negative impacts to reproductive success (Pessier 2009 pers. comm.).

Considering the negative impacts to the health of the toads and the significant population impacts of nutrient deficiencies, the Service considers this threat to be **high**.

Table 2. SUMMARY OF THREATS AND OVERALL THREAT LEVEL RANKING

List of Threats		Overall threat level			
Factor A: Habitat		Low	Moderate	High	Severe
	Irrigation Practices		X		
	Presence of Livestock in Toad Habitat	X			
	Limited Distribution				X
	Contaminants		X		
Factor B: Over-utilization – No threats identified					
Factor C: Disease or Predation					
	Infectious Disease				X
	Predation		X		
Factor D: Regulatory Mechanisms					
	Inadequacy of Regulatory Mechanisms / Protection				X
Factor E: Other					
	Small Population Size				X
	Genetic Homogeneity			X	
	Climate Change		X		
	Weather Events		X		
	Captive Diet			X	

1.6 Conservation Efforts

In September 1987, a recovery group was formed consisting of representatives from the Service, WGFD, University of Wyoming (UW), and The Nature Conservancy (TNC). This group coordinated protection, research, and recovery efforts. In 2001, this informal group was replaced by the WTRT, which was officially appointed by the Service. This team is composed of representatives from WGFD, University of Wyoming, the Association of Zoos and Aquariums (AZA), Laramie Rivers Conservation District (LRCO), Wyoming Natural Diversity Database (WYNDD), private landowners and ranchers, and the Service’s Wyoming Field Office (WFO) (Ecological Service), the National Fish Hatchery System (Saratoga National Fish Hatchery and Ennis National Fish Hatchery), and Arapaho. This team identifies and recommends to the Service priority research projects and studies to facilitate recovery of the Wyoming toad.

Conservation efforts for the Wyoming toad are a fundamentally collaborative and cooperative process between multiple partners. Without the cooperation and coordination of the WTSSP providing a cohesive captive breeding program none of these efforts would be possible. The LRCO has been a key player in facilitating cooperation with private landowners of privately owned habitat and paving the way for reintroduction for safe harbor sites. Essential to the recovery of this species is close coordination with WGFD. The WGFD has been a part of recovery planning efforts since they began in 1987 and continues to be a key conservation partner in recovery efforts. Specifically, they have played an active role on the WTRT providing key biological input to aid in decision making, reviewing documents prepared within the recovery planning efforts, assisting in planning and participating in survey efforts, and a number of other fundamentally important recovery planning efforts. The WGFD continues to be an active conservation partner in recovery planning and implementation efforts and continued

coordination and cooperation is a priority for the USFWS (See Recovery Action 5.0 “Outreach and Cooperation with Stakeholders and Partner Agencies” for more information). Since 1995, the following actions have been recommended by the WTRT, the WTSSP, and the Service and are in varying stages of completion:

1.6.1 Reintroduction/ Release Efforts

The Wyoming toad captive breeding program started releasing Wyoming toad eggs, tadpoles, and toadlets back into the wild in 1995. From 1995 to 2004, 37,382 tadpoles and toadlets were released at Mortenson Lake and an additional 5,741 were released at Lake George and Rush Lake. During this time period, release efforts were focused on Mortenson Lake. In 2003, a mass die-off of Wyoming toads occurred at Mortenson Lake due to *Bd*. At the recommendation of the WTRT and WTSSP, releases of tadpoles to Mortenson Lake were halted because of the major die-offs associated with *Bd*. In 2003, the Sybille captive breeding facility was thought to be closing and there was no place to hold the toads. Ten adults and 61 subadults were released at Mortenson Lake. In 2004, release of tadpoles and subadults shifted to two SHA sites (see Appendix H for detailed release numbers).

Over a three year period, 18,563 Wyoming toad tadpoles were released at this site. *Bd* was present, but over the 3 years of the study, natural reproduction only occurred for two consecutive years (2007-2008).

Also in 2005, a SHA was reached with a local private land owner to enable the Service to release Wyoming toad tadpoles and toadlets onto a new reintroduction site. To date, over 88,565 tadpoles and toadlets have been released onto this property. In 2011, over 40 adult breeders were found at this site and there was evidence of natural reproduction. Unfortunately, toads were not located during 2012 surveys.

A second SHA was reached on another property in 2006. This site was slightly out of the historic range of the toad. Approximately 8,900 tadpoles and toadlets were released on the second site between 2006 and 2009. During subsequent surveys, no Wyoming toads were found at the site; the habitat was not appropriate for reproduction (little vegetation cover or breeding habitat). In 2009, WTRT recommended no further reintroductions at this SHA site. In 2012, the landowner requested withdrawal of the SHA.

1.6.2 Population Monitoring

Population surveys are intended to estimate the abundance of adult Wyoming toads in the areas of highest likelihood-of-occurrence; primarily areas proximate to fixed bodies of water and deemed to be moist throughout much of the spring and summer seasons, as confirmed by their vegetation composition. These surveys are conducted by USFWS staff, WGFH, SNFH, AZA and other local organizations in the area. In 2008, a systematic monitoring protocol was developed by Wyoming Natural Diversity Database (WYNDD) (see Appendix G) for monitoring toads in permanently established search blocks. Searches in each search block are timed. Time spent in search blocks are based

on Wyoming toad detection rates determined through repeated mock searches with dummy toads. These mock searches establish time/acre rates at which the most toads can be located. This rate is used to allow repeatable surveys over time.

The results of systematic surveys from 2008 through 2013 for Wyoming toads at Mortenson Lake are included in the following table (Table 3). The *Bd* results of the subset of the population tested for *Bd* is included as well.

Table 3. SYSTEMATIC SURVEY RESULTS 2008 THROUGH 2013

Year	People	Days	Hours	Adult Toads	Over-wintered	Young of the Year	Percent <i>Bd</i> +
2008	34	10	476	4 Male	0	24	67% (8/12)
				8 Female			
2009	33	9	540	17 Male	87	1	74% (93/125)
				19 Female			
				2 Unknown			
2010	33	7	282	4 Male	0	0	100% (4/4)
				2 Female			
2011	23	7	174	1 Male	0	0	0% (0/1)
2012	34	12	288	1 Male	0	28	0% (0/1)
2013	27	10	65	9 Males	79	408	16% (30/184)
				10 Females			
				1 Unknown			

1.6.3 Mark and Recapture

At present, all adult toads (weighing 20 grams or more) located during surveys are Passive Integrated Transponder (PIT) tagged. However, many adult toads and toadlets weigh less than 20 grams and cannot be PIT tagged and thus cannot be identified as a recapture.

In 2010, WYNDD, with the support of the Service, initiated a study of different methods of identifying individual toadlets and toads in the field. The WYNDD study focuses on consistency of wart patterns of toadlets in captivity to adulthood to determine how much wart patterns change over time and if they can be readily recognized through photos and pattern recognition software. This method would allow for reliable mark and recapture studies that could significantly contribute to demographic information needed for recovery of the Wyoming toad. Final results of this study are expected in 2014. However, in 2012 WYNDD reported that preliminary results indicate Wyoming toads do retain their wart pattern from toadlets to adults. Eventually, we will be able to track individuals of all terrestrial lifestages of Wyoming toads encountered during surveys using wart patterns (Estes-Zumpf 2012 pers. comm.). However, photographing each individual toad was implemented in the 2013 monitoring surveys and was met with many

challenges. The quality of photos varied significantly and sorting and editing photos was timely and ultimately prohibitive. Unless a clear photo of every individual toad is captured, this is not a reliable technique for mark-recapture analysis.

WYNDD is also investigating the possibility of marking individuals with Visible Implant Elastomer (VIE), a small amount of brightly colored pigmented elastomer (red, green, orange, and yellow liquid plastic) injected under the skin. The elastomers can be difficult to see in normal light, but are visible under ultra-violet light. This method of tagging provides a reliable technique to identify marked individuals throughout the life of the toad and the cost of materials, toad handling time, and ill effects are minimal.

Preliminary results indicate it is most likely possible to use VIE technology reliably with Wyoming toads (Estes-Zumpf 2012 pers. comm.).

1.6.4 Toad Call Monitoring

The Service has been monitoring toad calls with call surveys during the early spring for many years. Previous to 2006, calling surveys were opportunistic and specific results were not recorded. In 2006, the Service began using Frogloggers, recording devices that are sound or interval activated and can be placed in toad habitat to monitor calling during the breeding period (May-June). From 2006 to 2008, the Frogloggers experienced excessive technical difficulties and no calling was identifiable.

In 2009, the Service began a trial using newer Frogloggers and SoundScope software to analyze Wyoming toad calls. The recording devices are placed near known breeding sites and set at different intervals for activation of recording. These recordings are then transcribed to identify Wyoming toad calls and to determine: 1) what time of year peak calling occurs for Wyoming toads; 2) what time of day does peak calling occur; 3) if call intensity increases after flood events and; 4) if there are differences in timing of calls between different sites. Frogloggers were successful in picking up three Wyoming toad calls at Mortenson Lake in 2009. However, no calls were detected in 2010, 2011, and 2012 when the population at Mortenson Lake was low or nonexistent. The Service will continue to monitor calling with Frogloggers and opportunistic surveys.

1.6.5 *Bd* Monitoring

Wyoming toads are monitored for *Bd* by a quantitative Polymerase Chain Reaction (qPCR) test. A *Bd* sample is collected for qPCR through a non-invasive skin swab, similar to swabbing for human genetic material from the inner cheek. A qPCR test is useful to determine not only the presence of *Bd*, but the severity of the infection by examining the number of zoospores per sample.

Captive toads can be successfully treated with antifungal solutions if infected with *Bd*. However, treating wild animals in a natural environment presents challenges. Treating the environment in which the Wyoming toad inhabits with fungicide would be detrimental to the beneficial and naturally occurring fungi. Currently, no evidence exists for an effective chytridiomycosis vaccine for controlling the disease in wild populations (Stice and Briggs 2010).

Wyoming toad management priorities are generally divided between (1) halting pathogen spread and developing survival assurance colonies, and (2) prophylactic or remedial disease treatment. Epidemiological models of *Bd* in amphibians suggest that mitigation strategies can control disease without eliminating the pathogen and existing evidence suggests some amphibians can persist with low or non-lethal levels of *Bd* (Woodhams et al. 2011). Successful disease mitigation must be developed to manage already infected populations by decreasing pathogenicity and host susceptibility. In the future, while it may not be possible to change the biological susceptibility of Wyoming toads to *Bd* without intervention, it may be possible to treat some amphibians in the wild in order to reduce the intensity of infection to a less lethal level with hope that animals could survive with a mild *Bd* infection (Briggs et al. 2010, Vredenburg et al. 2010). A promising area of research is looking at the possibility of introducing symbiotic bacteria that inhibit the growth of *Bd* into wild amphibian populations (Harris et al. 2009) and investigating the immune response of the Wyoming toad to *Bd*. Preliminary research in California has shown that mountain and Sierra Nevada yellow-legged frogs (*Rana muscosa*, *R. sierrae*) can acquire immunity to *Bd* (Knapp 2013 pers. comm.). Individual adult frogs that were previously exposed to *Bd* and subsequently cleared of the infection demonstrated a high degree of resistance to infection and high survivorship upon re-exposure.

1.6.6 Movement and Habitat Use Monitoring

In 1998, a radio-telemetry field study investigated microhabitat use Parker (2000). However, because of equipment failure and high toad mortalities, this study yielded little information on specific habitat use by Wyoming toads.

In the summer of 2011, 13 captive, adult Wyoming toads were implanted with radio transmitters and released at Mortenson Lake as a collaborative effort between the Service, Indiana State University, and the University of Wyoming. The purpose of this research was to document habitat use and movement patterns. This study demonstrated that toads mainly stay within approximately five meters of the water's edge for most of the summer and then migrate into upland habitat sometime in late August. Three toads were observed hibernating; one was observed digging its own burrow and the others were seen using mammal burrows. The average distance toads moved over a 24-hour time period was 51m and the maximum distance noted was 426m. Toads were often located nestled into the vegetation or soil, and were sometimes completely concealed by vegetation.

1.6.7 Maximizing Genetic Diversity

The captive population is currently managed by the WTSSP's Reproductive Manager and Studbook Keeper with every attempt to maximize genetic diversity. Genetic information relating to pedigree and demographic history of captive Wyoming toads are stored in a database called the studbook. Mean kinship analysis is used to measure genetic importance of an individual and how rare an individual animal's unique combination of genes is in the entire population. Animals with a lower mean kinship value have

relatively fewer genes in common with the rest of the population, and are therefore more genetically valuable in a breeding program.

Historically, attempts had been made to manage three subpopulations of captive Wyoming toads separately. The first group of toads, “A”, consisted of toads that could be traced back to the original collections of wild toads from Mortenson Lake in the early 1990s. Group “B” consisted of toads that were collected from Mortenson Lake and known to be offspring from “A”. These were managed separately than “A” in the chance that this group could represent additional gene diversity if there were any remaining wild toads that survived past 1994. Toads in group “B” were considered to have been produced by parents that have demonstrated some level of fitness under natural conditions. Their parents not only survived in the wild for several years after release, but were capable of reproducing once mature. For genetic analysis, wild collected “B” toads were considered the product of a single unrelated wild pair of parents (hypothetical founders). The third subpopulation of toads, “M”, for “Mixturado”, consisted of toads that were progeny of an “A” toad and a “B” toad. They were not included in groups “A” or “B” and as a result their genes were not being utilized. Captive Wyoming toads are now managed as a single group using mean kinship analysis to ensure the greatest genetic diversity. By managing the subpopulations as one group, potentially important genes from group “M” are not excluded. The Service continues to make every attempt possible to maximize genetic diversity of the captive population.

1.6.8 Hibernation

Captive toads are routinely hibernated on a short cycle of 60 days in artificial hibernation units (refrigeration units). This short-cycle hibernation, in addition to hormone injections, is effective in priming toads for breeding and egg production at all captive breeding facilities

In 2008, Red Buttes began experimenting with hibernating toads outdoors. Adult toads were placed in containers in the early fall and were able to dig down and hibernate for the winter. Eighty-five percent of the adult toads in these containers came up from hibernation ready to breed, but most toads still required hormone injections to breed. However, in 2010, two of the toads, upon emerging from the containers, bred without the use of hormone injections. Red Buttes plans to continue hibernating toads in this manner. The Service will also attempt to refine the protocol for hibernating toads outdoors.

1.6.9 Sperm cryopreservation and *in vitro* fertilization

Cryopreservation and *in vitro* fertilization (IVF) offer the possibility of retrieving viable Wyoming toads from a population and a species that are lost from the wild. These tools can be used to manage the genetics of small populations by storing genetic diversity while it is still high and extending the effective generation time through reintroduction of stored genes at a later date (Kouba and Vance 2009). Cryopreservation and IVF offer: 1) protection against reproductive failure and preservation of genetic diversity (stored sperm or eggs allow reproduction of genetically important animals even if they die before reproducing naturally), 2) security against local extinctions (animals can be produced using stored gametes for reintroduction to the wild), 3) additional space (stored eggs and

sperm take up much less space than live animals), and 4) transfer of reproductive cells between breeding facilities (much easier than transportation of live animals).

1.6.9.1 Sperm Cryopreservation

Declining amphibian populations around the world have prompted zoos, aquariums, governments, and conservation non-governmental organizations (NGOs) to begin establishing amphibian gene banks to conserve, in perpetuity, the remaining existing genetic diversity. A genome bank (also called genetic resource bank or biomaterial bank) is a frozen storage area designed to preserve genetic materials from animals and may include eggs, sperm, embryos, tissues, blood, hair, or cells (Amphibian Ark). They allow for the long-term viable storage of genetic materials that would otherwise degrade or become unviable very quickly.

Barton and Guttman (1972) cryopreserved the first amphibian sperm using the American toad (*Anaxyrus americanus*) as a model. In 2001, the Memphis Zoo created the first amphibian genome bank in the United States. The idea behind genome banking is deposition and withdrawal of biological material to facilitate genetic management of threatened species or assist with scientific research (e.g., disease). Between 2004 and 2005, the Memphis Zoo initiated a preliminary study to evaluate the application of sperm cryopreservation protocols for the Wyoming toad. The zoo non-invasively collected live toad sperm through hormone induction and collection of spermatid urine (urine with sperm). Sperm samples from 20 different male Wyoming toads were then placed into straws and frozen in liquid nitrogen, and stored in a dry shipper for transport to the Memphis Zoo (A. Kouba 2013 pers. comm.).

The initial results for this preliminary study were poor with low recovery of motile sperm in post-thaw samples. However, the poor motility was likely due to transportation complications. During inspection by the Transportation Security Administration (TSA), the cryobanked samples had thawed when pulled out of the transport vacuum flask. The samples were carefully repacked, but after evaluation when they arrived in Memphis, less than 5 percent of the sperm were motile. The remainder of the Wyoming toad sperm is owned by the Service and is held in reserve by the Memphis Zoo. The sperm is not likely useable for *in vitro* fertilization (IVF) (see section below), but instead for more sophisticated technologies like intra-cytoplasmic sperm injection into oocytes (A. Kouba 2013 pers. comm.).

1.6.9.2 IVF

IVF is a process by which an egg is fertilized by sperm outside the body (*in vitro*). Since most frog and toad females lay their eggs in water and the eggs are fertilized externally by males as the eggs are being laid, the process for IVF is much less complex than for other species. The sperm and eggs can be mixed in a petri dish with water. In 2004, initial studies by the Memphis Zoo showed that

large numbers of Wyoming toads could be produced by IVF. This method enabled twenty female toads to produce 25 percent (nearly 2000) of the released Wyoming toad tadpoles in 2004 (A. Kouba pers. comm.). The tadpoles spawned from these experiments represent the first time in history that an endangered amphibian has been introduced into the wild that had been produced by artificial fertilization (Browne et al, 2006).

1.6.10 Future Research

Upcoming research projects will include studies on the effects of temperature and the duration of hibernation, field work to identify toad hibernation sites, and a genetic analysis of captive versus wild bred populations. Each facet of the Wyoming toad SSP and recovery program is an integral part in our plan to restore this endangered amphibian to a secure status. In 2011, the WTRT identified research priorities to be used for future investigations (see Appendix I). However, since all research needs identified in this document are critically important to the survival of the toad, the WTRT has not been able to assign specific priorities to the research needs identified.

The WTRT identified seven categories of research needed for Wyoming toad recovery:

- Population Demographics
- Habitat Requirements
- Reintroduction Strategies
- Survey and Monitoring
- Health and Disease
- Captive Husbandry
- Captive Reproduction

2.0 RECOVERY STRATEGY

The general recovery strategy for the Wyoming toad is to reduce threats to the species and to establish at least five viable, self-sustaining sub-populations. Many of the necessary actions for habitat protection are based on an increased understanding of the relationship of the Wyoming toad to its physical, chemical, and ecological environment. Increased knowledge of the species is crucial for improved science-based management decisions and conservation actions.

Implementation of the revised recovery plan will require adaptive management strategies to more effectively manage the Wyoming toad both in the wild, as well as in captivity. Knowledge of genetic variation of the captive population, captive dietary needs, and management of infectious diseases in the wild population will be needed to ensure the survival of the species.

3.0 RECOVERY PROGRAM

3.1 Recovery Goal, Objectives, and Criteria

Recovery Goal

The ultimate goal of this revised recovery plan is to minimize the threats to the Wyoming toad to the point that protection under the ESA is no longer required and the Wyoming toad can be delisted.

Recovery Objectives

The recovery plan articulates both short and long-term strategies that together comprise the conditions under which the Wyoming toad may be delisted. The ultimate recovery objective is to restore a minimum of five self-sustaining populations within and nearby the historical range. To do this, captive populations will need to be maintained at a sufficient level, loss of genetic variability in captivity will need to be minimized, suitable habitat will need to be restored and/or identified, disease will need to be eradicated or suppressed at a level to which the populations are self-sustaining and viable, and there needs to be a continued increase in understanding of the relationship of the Wyoming toad to its physical, chemical, and ecological environment. The accomplishment of these objectives is intended to provide reasonable assurance for the continued survival of the species even if ESA protections are removed.

Recovery Criteria

The ESA requires recovery plans to include “objective, measurable criteria” which, when met, would result in the determination that the species be removed from the list. Recovery criteria describe discrete targets with standards for measurement to determine that species have achieved recovery objectives and may be delisted. Developing precise measurable criteria for recovery of the Wyoming toad is challenging due to a general lack of sufficient data. However, the Wyoming toad is critically endangered and is continually experiencing threats to its recovery. Potential precipitous and drastic population reductions and/or extinction are currently a reality. Many of the recovery actions allow for future development of more specific criteria as more is learned about the requirements of this species and how to eliminate or suppress the threats it is experiencing.

The Wyoming toad will be considered ready for reclassification from endangered to threatened when all of the below criteria are realized:

A. Reclassification to Threatened Criteria

- (1) Three demographically distinct, self-sustaining, and viable populations of the Wyoming toad are established within and nearby the toad’s historic

range. Minimal genetic exchange may occur between any or all of these sub-populations, but each sub-population must be genetically independent from the other. Sub-populations may be established in lotic or lentic habitats. These three sub-populations will be established as viable for a minimum of seven consecutive years as evidenced by a Population Viability Analysis (PVA). Benchmark criteria for viability, including time horizon, quasi-extinction threshold, and exact probability of persistence, will be developed by the WTRT using the abundance-based PVA approach (Dennis et al. 1991, Morris et al. 1999, Morris and Doak 2002).

- (2) The captive assurance population is targeted to a minimum of 500 toads (excluding tadpoles and toadlets) for seven consecutive years during establishment of self-sustaining wild populations. This targeted minimum may fluctuate (by approximately 50 individuals) along with natural fluctuations within a given year or during a naturally unsuccessful year.
- (3) The WTSSP has a peer-reviewed Captive Breeding Plan for twenty-five years after downlisting. This plan is comprehensive enough, as determined by the Service and the WTSSP, to prevent further loss of genetic diversity in the captive population.
- (4) Chytridiomycosis infection rates do not pose a threat to the long-term sustainability of Wyoming toad populations or are managed and maintained at a level that can ensure long-term sustainability of populations.

B. Delisting Criteria

- (1) Two additional self-sustaining populations of the Wyoming toad are established within and nearby the toad's historic range (for a total of five sub-populations) and are viable as evidenced by a current Population Viability Analysis (PVA). Benchmark criteria for viability, including time horizon, quasi-extinction threshold, and exact probability of persistence, will be developed by the Wyoming Toad Recovery Team using the abundance-based PVA approach (Dennis et al. 1991, Morris et al. 1999, Morris and Doak 2002). Sub-populations may be established in lotic or lentic habitats.

It is estimated that approximately 10,000 acres of land will be required to support a total of five self-sustaining populations necessary for delisting. The acquisition of fee title lands (on a willing seller/willing buyer basis only), in conjunction with perpetual conservation easements, will be used to protect Wyoming toad habitat within and nearby its historic range. While fee title land acquisition is preferred over conservation easements due to the increased management control of the lands for Wyoming toad habitat and research opportunities, utilizing both methods in combination,

ensures maximum likelihood of achieving the objective of 10,000 acres of habitat. Fee title lands provide the unique opportunity for the Service to exercise control over habitat management reducing risk of future development: they provide opportunities to conduct research in order to better understand the biology of the species essential to its recovery, and enable the Service to control potentially harmful impacts associated with various land use practices (e.g., housing development, energy development, agricultural production). However, there may not be enough landowner interest in selling fee title to the Service in order to secure 10,000 acres. Conservation easements, therefore, provide an additional means with which to secure the estimated need of 10,000 acres.

Conservation easements allow lands to remain in private ownership, facilitating cooperative conservation efforts in recovery with willing landowners. Conservation easements are a critical complimentary tool to fee title acquisition for the following reasons: (a) they provide perpetual habitat conservation for Wyoming toads enabling the establishment of populations that contribute toward recovery objectives; (b) they contain habitat providing important ecological functions such as suitable water quality, in-tact/un-fragmented watersheds, a mosaic of aquatic habitat types including both lotic and lentic systems; (c) they have the support by many local partners as a preferred alternative to Federal fee title land acquisition; (d) they can be utilized for margin habitats that protect high-quality Wyoming toad habitat from potentially harmful impacts associated with other land uses; and (e) they are one of several tools available (e.g., SHAs, WEAs, HCPs, CCAAs) with which the Service can demonstrate flexibility and creativity in accomplishing recovery of listed species to all of our partners, fostering support and opening doors for future conservation and recovery opportunities.

- (2) A peer-reviewed and Service approved monitoring plan is in place. This plan includes sufficient monitoring data of the rates needed to assess population viability of each of the five sub-populations for twenty-five years after de-listing.
- (3) Chytridiomycosis infection rates continue to not pose a threat to the long-term sustainability of the additional Wyoming toad populations needed for delisting or are maintained at a level that can ensure long-term sustainability of the populations.

3.1.5 Changes to Recovery Criteria

Recovery plans are not regulatory documents, but are instead intended to provide guidance on methods of minimizing threats to listed species and on criteria that may be used to determine when recovery is achieved. There are many paths to accomplishing

recovery of a species, and recovery may be achieved without all criteria being fully met. For example, one or more criteria may be exceeded while other criteria may not be accomplished. In that instance, we may judge that the threats are minimized sufficiently, and the species is robust enough to reclassify from endangered to threatened or to delist. In other cases, recovery opportunities may be recognized that were not known at the time the recovery plan was finalized. These opportunities may be used instead of methods identified in the recovery plan. Likewise, information on the species may be learned that was not known at the time the recovery plan was finalized. The new information may change the extent that criteria need to be met for recognizing recovery of the species. Recovery of a species is a dynamic process requiring adaptive management that may, or may not, fully follow the guidance provided in a recovery plan.

3.2 Recovery Actions

The recovery program for the Wyoming toad is divided into eight areas of action: (1) Habitat Protection, (2) Habitat Management, (3) Population Augmentation, (4) Reintroductions, (5) Outreach and Cooperation with Stakeholders and Partner Agencies, (6) Research, (7) Monitoring, and (8) Adaptive Management. Overall, these sets of recovery actions are tied directly to achievement of the recovery criteria for the Wyoming toad (Appendix A).

Full descriptions of the recovery actions are provided in the Recovery Action Narrative. In the narrative, a priority number of 1 to 3 has been assigned to each action. These priorities are based on the following classifications:

Priority 1a: Actions that must be taken to prevent extinction or to prevent the species from declining irreversibly.

Priority 1b: Actions that by itself will not prevent extinction, but which is needed to carry out a Priority 1a action.

Priority 2: Actions that must be taken to prevent a significant decline in species population/habitat quality or some other significant negative impact short of extinction.

Priority 3: All other actions necessary to provide for full recovery of the species.

Recovery Action Narrative

1.0 Habitat Protection

- 1.1 The Service plans to **expand the boundary of the area around Mortenson Lake** and the Hutton Lake complex to facilitate the development of new reintroduction sites for the Wyoming toad to counteract the primary threat of small population size and limited distribution. The proposed conservation area includes historic, suitable habitat along the Laramie River and adjacent wet meadows and ponds within the floodplain. Acquisition of fee title lands (on a

willing seller/willing buyer basis only) and conservation easements in these areas would allow for reintroductions and protection of the Wyoming toad habitat within and nearby its historic range on public and private lands. Fee title ownership allows the strongest protection for the habitat in order to reach desired recovery objectives with the end goal of delisting the Wyoming toad. The easement contracts would restrict development and specify perpetual protections of habitat for trust species (migratory birds and threatened and endangered species), therefore supplementing recovery efforts and providing additional conservation areas to aid in recovery (**Priority 1b**).

- 1.2 In conjunction with the expansion of the Mortenson Lake boundary (SLRCA), the Service may **pursue the development of a 10(j) rulemaking** for the Wyoming toad. Section 10(j) allows reintroduced “experimental non-essential populations” of endangered species to be managed as if they were threatened. Landowners can engage in lawful activities, such as recreation, forestry, agriculture and are relieved from liability for the unintentional take of a Wyoming toad. This would allow private landowners to continue to manage their lands with reintroduced toads. Populations on Federal lands or lands within conservation easements acquired with the expansion of Mortenson Lake will have full protection of an endangered species and will not be subject to the 10(j) exemptions (**Priority 2**).
- 1.3 **Continued protection of Mortenson Lake** and its Wyoming toad population is essential to preventing wild extinction. Although only one adult toad was found in the wild during breeding season of 2012, 28 toadlets from the Reintroduction Pilot Study were found during the August/September surveys. During search surveys the following spring, 11 toads were found that had survived the winter. In July and August of 2013, an additional 505 Wyoming toads, spanning multiple post-metamorphic lifestages, were located during search surveys. Evidence of breeding has not occurred to date, but the Service is hopeful that the Reintroduction Pilot Study continues to be successful and provides the basis for a new subpopulation at Mortenson Lake (**Priority 1b**).
- 1.4 **Continue pursuing SHA sites** as refugia for Wyoming toads until protected habitat is acquired. Pursuing SHA sites will be the primary mechanism for acquiring new reintroduction sites on private lands until additional protected lands are acquired. LRCD works closely with USFWS Partners for Wildlife and the Natural Resource Conservation Service to identify potential SHA sites. Habitat protection and enhancement at these sites will be fostered through a variety of approaches, including encouraging favorable land use practices and other protective and enhancement measures. Incentives should be provided to encourage participation by individuals (e.g., cost-sharing on projects, compensation for loss of income). Under current Service policy, populations established on SHA sites cannot count toward recovery objectives. However, the current population of the Wyoming toad is so small that the stability of the population is in jeopardy. Until protected habitat is acquired, SHA sites could

provide a mechanism to increase the overall population size, while providing key insight as to the threats and needs of the Wyoming toad in the wild (**Priority 2**).

2.0 Habitat Management (including infectious disease control)

- 2.1 **Investigate the effect of disturbance** in the system as it relates to the needs of the toad. Complete research to determine the effects of various land management methods (e.g., grazing practices, prescribed burning) in reintroduction sites throughout the historical range of the Wyoming toad (**Priority 3**).
- 2.2 **Develop Grazing Plan and Prescribed Burn Plan** on all reintroduction sites based on vegetation conditions. The Wyoming toad is dependent on shallow water and a saturated substrate that allows for varying temperatures for adequate thermoregulation (**Priority 1b**).
- 2.3 **Annually collect and analyze water quality** samples from all reintroduction sites to identify and eliminate potential contaminant sources to aquatic habitats of the Wyoming toad to the maximum extent practicable. Of special concern are potential inputs from pesticide/herbicide applications and mineral fertilizers throughout the Laramie Basin (**Priority 1b**).
- 2.4 **Adhere to currently approved WTSSP guidelines** and protocols for disease control at new reintroduction sites to reduce the spread of *Bd*. This could include releasing and relocating only *Bd*-free animals or other biological secure methods (**Priority 1b**).
- 2.5 **Investigate predation** by other species and incorporate information obtained into management of the sub-population. Predation levels by all co-habiting species should be examined for the Wyoming toad through field investigations (**Priority 1b**).

3.0 Population Augmentation (captive population management)

- 3.1 Develop a plan for and **construct a Wyoming toad Breeding Facility** on Federal lands within the current boundaries at Mortenson Lake (or similar facility). A larger facility will provide a permanent breeding and rearing facility of a size with the capacity to allow for increased reproduction of toads and increased area for growing toads to all lifestages. Increased numbers of animals for reintroduction are required to achieve population level recovery criteria (**Priority 1a**).
- 3.2 **Expansion of the Saratoga NFH solar greenhouse** that will provide a permanent, reliable, disease-free food source for captive Wyoming toads (**Priority 1b**).
- 3.3 Because the Wyoming toad is on the brink of extinction in the wild, continuing appropriate **management of the captive population** is the first and most

important step to recover the species. Retention of the highest possible level of genetic diversity is essential to maintaining recovery potential. Maximum genetic diversity can be retained only by increasing the size of the captive population rapidly and by minimizing mortality risk. Though progress has been made, there are still many factors that are keeping the captive breeding program from reaching its full potential. This will be accomplished by implementing each of the following tasks (**Priority 1a**):

3.3.1 Populations in the wild should be managed together with the captive population as a single population for purposes of **genetic and demographic optimization**. Management of wild sub-populations should aim to prevent genetic drift and sub-population decline. (**Priority 1b**).

3.3.2 **Obtain an increased commitment from Association of Zoos and Aquariums** facilities to hold additional animals and attempt increased pairings (**Priority 1a**).

It is important to note that while new facility participation is encouraged, it is contingent on meeting strict biosecurity requirements and amphibian husbandry experience. The Wyoming toad is a challenging species to keep in captivity and new facilities must be aware of the challenges.

3.3.3 **Reduce disease in captive toads across all developmental stages**. The Wyoming toad SSP Pathologist and WTSSP Veterinarian will continue to develop and implement protocols that will decrease the spread of disease in the captive population. In addition, the WTSSP facilities will implement disease treatment protocols developed by the WTSSP Pathologist and Veterinarian. Furthermore, WTSSP facilities will make an increased commitment to keep accurate and thorough treatment and mortality records and each mortality event will be investigated by representative postmortem examinations (**Priority 1a**).

3.3.4 Continue existing **pedigree and founder relatedness research** using recent DNA analysis. Provide analysis to the WTRT by end of each calendar year (**Priority 1a**).

3.3.5 Should a new Wyoming toad population be discovered, the studbook keeper and reproductive manager will make recommendations on how to **incorporate new genetics into captive populations** (**Priority 1b**).

3.3.6 The recovery coordinator will **annually review captive population status** and new information related to captive propagation, suggest new studies and protocols, and prepare a summary for the WTRT. These activities can be done in consultation with others if requested by the Service (**Priority 1b**).

3.3.7 Ensure any removal of animals from the captive population will not negatively impact the **demographic stability of the captive population** or reduce the ability of the population to achieve growth targets (**Priority 1b**).

3.4 Continue existing and initiate new research aimed at **improving breeding and reproductive success of the captive population**. Continue to explore all possible means to encourage breeding of individuals that have not bred or are under-represented in the captive population (**Priority 1b**).

3.4.1 To accomplish many of the goals related to the captive program, the WTSSP and WTRT must **continue research relating to the Wyoming toad's nutritional needs (Priority 1b)**.

3.4.2 Hibernation requirements for the Wyoming toad are not fully understood and captive breeding facilities and zoos have been trying different methods with varying success. **Continued analysis of captive, short-duration and long-duration (outdoor) hibernation** results would inform and allow for refinement of these protocols (**Priority 1b**).

3.4.3 Continue **experimentation with hibernating toads outdoors** for the winter. Outdoor, underground hibernation was conducted at Red Buttes and the results have been promising. However, more research is needed to determine optimum conditions required for hibernation of Wyoming toads in the outdoor, underground hibernation units and in the wild. Results of this research will supply the WTSSP and the WTRT with information necessary to develop and implement optimal hibernation protocols (**Priority 1b**).

3.4.4 The captive program should continue to develop management strategies to **optimize captive breeding strategies** by using known environmental patterns. Determining physical conditions of wild historical patterns is problematic since very little data was collected historically. However, compiling and analyzing available captive and wild environmental data could provide information that could be useful. Use the data from the weather station at Mortenson Lake to inform temperature requirements for captive management strategies. If enough information is available, protocols to optimize environmental conditions should be developed for captive toads (**Priority 1b**).

3.4.5 The captive program should **pursue sperm cryopreservation** and *in vitro* fertilization optimizing breeding and/or genetic diversity of captive populations. Sperm cryopreservation is an important tool to preserve the genetic diversity of animals in the only remaining Wyoming toads (**Priority 1b**).

4.0 Establishment of New Sub-populations (reintroductions)

- 4.1 **Develop and implement a plan for how to distribute captive-bred stock** among reintroduction sites (**Priority 1b**).
- 4.2 **Develop goals for the numbers of toads to be released annually** and how available stock will be distributed among release sites. When possible, these goals should be based on population viability analyses, modified as necessary to accommodate availability of captive-bred stock and the logistics of reintroduction (**Priority 1b**).
- 4.3 **Develop a plan to determine the optimum age class that should be released at each site** (e.g., tadpoles, metamorphs, adults, etc.), based on PVA and research relative to age-specific survival (**Priority 2**).
- 4.4 **Develop a plan for documenting and reporting release efforts** to the WTRT, including recording release locations, dates, and methods of release (**Priority 2**).
- 4.5 **Review and revise selection criteria for release sites**, which must be prioritized to determine which characteristics are most important for long-term success of reintroduction sites (**Priority 1b**).

5.0 Outreach and Cooperation with Stakeholders and Partner Agencies

- 5.1 **Outreach programs should be jointly developed** through the Service, the WTRT, local community organizations, as well as State and other Federal agencies. Outreach should focus on the rarity of the Wyoming toad and the advantages of habitat protection (i.e., conservation easements) (**Priority 3**).
- 5.2 **Cooperate with stakeholders and partner agencies** to enhance Wyoming toad recovery efforts. Seek and maintain a team relationship with partners and State and other key agencies. Endorse coordination and encourage the partnerships of agencies and stakeholders to continue protection of the Wyoming toad and its habitat. Approval and support governmental agencies and grazing lessees are needed. These entities should be recognized for past land management actions that have allowed the species to persist. See section 1.6 “Conservation Efforts” for details on existing coordination efforts with key agencies (**Priority 1a**).

6.0 Research (includes Genetic Research, *Bd* mitigation, and Population Viability Analysis)

- 6.1 Conduct targeted studies to further knowledge of wild and captive Wyoming toads.
 - 6.1.1 **Identify specific data needed to inform management decisions** (e.g., target levels of vegetation or water levels) and how that data will be collected as part of research and monitoring programs. Design an

adaptive, research-based strategy to refine recovery goals and actions (e.g., release techniques, husbandry). Identify research needs to improve our understanding and management of infectious disease in wild Wyoming toads (e.g., resistance, effects of land management, and environmental variables) (**Priority 2**).

- 6.1.2 **Prioritize new research** based on relevance to implementation plan tasks and modify management actions based on analysis of results (see Appendix I for research priorities) (**Priority 2**).
- 6.1.3 **Perform studies on wild populations** including life history, reproduction, *Bd* prevalence and variables related to infection rates, and habitat preference of tadpoles, toadlets, juveniles, and adults. This information can be used to assist in developing captive breeding techniques for maintaining captive populations and assessing potential competition. This information could also be critical to management of the ecosystem to benefit reproduction of the species (**Priority 1b**).
- 6.1.4 **Determine the population structure of Wyoming toads in the wild to satisfy data needs of Population Viability Analysis (PVA)**. Determine sub-population viability, optimum numbers, and the spatial arrangement of the sub-population, as well as sub-population dynamics including fecundity, age and size class, sex ratio and longevity, through population estimations (see Appendix F for details). This information is critical to determining the viability of the sub-populations through PVA (**Priority 1b**).
- 6.1.5 **Perform studies on captive populations** on genetic relatedness, life history, reproduction, and nutritional requirements for all lifestages. Important factors could be discovered that are currently limiting the captive populations and will be useful to ensure adequate husbandry needs (**Priority 1b**).
- 6.1.6 **Investigate *Bd* management and control in the wild**. Mitigation for disease is an essential component of population management. At this time, there is no known method of eliminating *Bd* in the wild. Recently, there have been several promising discoveries regarding *Bd* mitigation: 1) bacteria on the skin of some amphibians have strong anti-fungal properties, 2) some species of amphibians display an immune response to *Bd* 3) a freshwater microorganism (*Daphnia* spp.) has been identified that may consume the free-swimming stage of *Bd* (Buck et al. 2011) and 4) Woodhams et al. (2009) suggested manipulating habitats to create more basking areas. All potential methods of *Bd* control should be investigated and incorporated into the habitat management plan for Wyoming toad reintroduction sites (**Priority 1a**).

7.0 Monitoring

- 7.1 **Review and analyze previous studies and records** of historic sites in the Laramie Basin to synthesize pertinent information regarding historically occupied habitats. The first step in implementing a defensible monitoring program is to identify what information is needed to satisfy recovery goals for each subpopulation (**Priority 1b**).
- 7.2 Document conditions for all current and potential wild Wyoming toad sites to **identify site-specific threats and data needs**. Conditions should be relevant to toad survival and could include (but not be limited to) water chemistry, habitat composition, vegetative cover, *Bd* presence, predator presence, other amphibian species presence, and forage insects presence (**Priority 1b**).
- 7.3 **Develop a monitoring program** for sub-populations of Wyoming toads by considering site-specific threats and data needs. Suggested demographic data to be collected for re-introduced Wyoming toad sub-populations include survival, growth, and reproductive rates, causes of mortality, and relative population numbers. Environmental factors, habitat characteristics, and known threats at each reintroduction site should be collected to integrate with population demographic data. Targeted research outside the monitoring program may also identify what habitat features correlate with toad survival, thus informing what metrics should be recorded in a monitoring framework. Should the collection of the above detailed data become prohibitive in terms of both efforts and/ or actual cost, the WTRT may determine a subset of data necessary for the creation of a population viability analysis model (**Priority 2**).
- 7.4 **Consistently implement a monitoring program** of reintroduced subpopulations. Adequate monitoring of sub-populations of re-introduced Wyoming toads is necessary to identify when recovery criteria and goals have been achieved, and is thus vital to eventual delisting. Relative consistency of monitoring is necessary to ensure that data is comparable over time and between sub-populations. However, conditions can change and new information may require the alteration of or additions to the basic methodology. A plan should be in place to allow such change while ensuring consistency (to the greatest extent possible) with previously collected data (**Priority 2**).
- 7.5 **Develop a post-downlisting and delisting monitoring plan** for the Wyoming toad. Section 4(g)(1) of the ESA requires that the Service monitor the status of all recovered species for at least ten years following delisting. In keeping with this mandate, a post-delisting monitoring plan should be developed by the Service in cooperation with WGFD, other federal agencies, academic institutions, and other appropriate entities. This plan should outline the indicators that will be used to assess the sub-population status of the Wyoming toad, develop monitoring protocols for those indicators, and evaluate factors that may trigger consideration for relisting (**Priority 2**).

8.0 Adaptive Management

- 8.1 **New information should be evaluated** and used to modify the strategy for recovery of the Wyoming toad, as appropriate. The strategy of this recovery plan is based on the best available science; however, we recognize there are considerable knowledge gaps regarding the species and the ecosystem upon which it depends. As a result of this uncertainty, the process of Wyoming toad recovery will necessitate adaptive management. Throughout the implementation of recovery actions, new information and technologies will become available. With increasing knowledge, some recovery actions will likely become obsolete and other actions will be proposed that cannot be envisioned now. Likewise, the objectives and criteria of this recovery plan may be adjusted in the future as our understanding improves. Through a continual process of planning, doing, monitoring, research and evaluation, and adjusting management, we will learn how to effectively conserve this species (**Priority 2**).
- 8.2 A habitat management plan does not exist for current or future release sites. To ensure progress toward eventual delisting it is necessary **to prepare a long-term adaptive management plan** that will be implemented currently and continue to be implemented following delisting, as per time frames outlined in the Recovery Goals. The plan should be approved and implemented by all participating agencies having proprietorship over the populations of Wyoming toads (e.g., USFWS, WGFD) (**Priority 2**).

The knowledge we gain from implementation of this recovery plan will be incorporated in the future recovery process. The Service periodically reviews approved recovery plans to determine the need for modifications. This recovery plan should be considered a living document that is flexible and consistent with the available, contemporary, scientific information. This may require periodic updates to the plan without full revisions being completed. This flexibility will maximize the usefulness of the recovery plan. The adaptive management concept ensures that all parties who choose to participate will have opportunities to contribute to the Wyoming toad recovery process. The work to accomplish the species' recovery should be coordinated with multiple agencies. Only by working together with different resources, knowledge, and expertise can recovery objectives and criteria be achieved.

4.0 IMPLEMENTATION SCHEDULE

The following Implementation Schedule outlines actions and estimated costs for the Wyoming toad recovery program over the next five years. Costs are expected to continue after the first five years and may vary. This schedule is a guide for meeting recovery objectives discussed in Section 3 of this plan and indicates action priorities, action numbers, action descriptions, links to recovery criteria, duration of actions, and estimated costs. In addition, parties with authority, responsibility, or expressed interest to implement a specific recovery action are identified in the schedule. The listing of a party in the Implementation Schedule neither requires nor implies a requirement for the identified party to implement the action(s) or secure funding for implementing the action(s). However, parties willing to participate may benefit by being able to show in their own budgets that their funding request is for a recovery action identified in an approved recovery plan and, therefore, is considered a necessary action for the overall coordinated effort to recover the Wyoming toad. Also, Section 7(a)(1) of the ESA, as amended, directs all federal agencies to use their authorities in furtherance of the purposes of the ESA by carrying out programs for the conservation of threatened and endangered species. The schedule will be updated as recovery actions are initiated and completed.

Key to Implementation Schedule Priorities (column 1)

Priority 1a: Actions that must be taken to prevent extinction or to prevent the species from declining irreversibly.

Priority 1b: Actions that by itself will not prevent extinction, but which is needed to carry out a Priority 1a action.

Priority 2: Actions that must be taken to prevent a significant decline in species population/habitat quality or some other significant negative impact short of extinction.

Priority 3: All other actions necessary to provide for full recovery of the species.

Key to Responsible Parties (column 6)

USFWS = U.S. Fish and Wildlife Service

LRCD = Laramie Rivers Conservation District

WTSSP = Wyoming Toad Species Survival Plan

WGFD = Wyoming Game and Fish Department

Table 4. IMPLEMENTATION PRIORITIES AND COST BREAKDOWN OF RECOVERY ACTIONS

Priority	Action Reference	Action Description	Related Recovery Criteria	Action Duration	Responsible Parties	USFWS Lead?	Associated Costs Per Year for First Five Years (\$1,000's)						Comments
							Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Total	
1a	3.1	Construction of a Wyoming toad Breeding Facility	(A1), (A2), (A3), (B1)	3 years	USFWS	Y	180	180	140	20	20	540	Three years are estimated for initial construction. Costs are estimated for a 5-year time period, but future annual costs may vary.
1a	3.3.1	Optimization of genetic diversity in captive populations.	(A1), (A2), (A3), (B1),	Continuing	USFWS WTSSP	Y	5	5	5	5	5	25	Wild populations managed with captive populations as single population.
1a	3.3.2	Obtain increased commitment from AZA Facilities	(A2), (A3), (B1)	Continuing	USFWS WTSSP	N	1	1	1	1	1	5	-
1a	3.3.3	Reduce disease in captive toads across all lifestages	(A1), (A2), (A3), (B1)	Continuing	USFWS WTSSP	Y	3	3	3	3	3	15	-
1a	3.3.4	Continue existing genetic research	(A1), (A2), (A3), (B1),	Continuing	WTSSP	N	4	4	4	4	4	20	Including pedigree and founder relatedness research
1a	5.2	Continued cooperation with stake holders and other partner agencies	(A1), (B1),	Continuing	USFWS WTSSP LRCD WGFD	Y	2	2	2	2	2	10	-
1a	6.1.6	Investigate <i>Bd</i> management and control in the wild	(A1), (A4), (B3), (B5)	5 years	USFWS	Y	10	10	10	10	10	50	<i>Bd</i> is currently present and poses a serious threat to existing populations.
1b	1.1	Obtain additional reintroduction sites.	(A1), (B1)	Continuing	USFWS	Y	45	45	35	20	10	155	Facilitated by Mortenson Lake boundary expansion and possible fee/title and/or conservation easements.
1b	1.3	Continued protection of Mortenson Lake	(A1)	Continuing	USFWS WTSSP	Y	2	2	2	2	2	10	Protection of Mortenson Lake is essential to preventing wild extinction.
1b	3.2	Expansion of Saratoga NFH Solar Greenhouse	(A1), (A2), (A3), (B1)	Continuing	USFWS WTSSP	Y	3	3	3	3	3	15	-
1b	4.1	Develop plan to distribute captive-bred stock among reintroduction sites	(A1), (A2), (A3), (B1)	5 years	USFWS WTSSP	Y	2	2	2	2	2	10	-
1b	4.2	Develop goals for numbers of toads to be released annually	(A1), (A2), (A3), (B1)	Continuing	USFWS WTSSP	Y	2	2	2	2	2	10	When possible, should be based on PVA.

Table 4. IMPLEMENTATION PRIORITIES AND COST BREAKDOWN OF RECOVERY ACTIONS

Priority	Action Reference	Action Description	Related Recovery Criteria	Action Duration	Responsible Parties	USFWS Lead?	Associated Costs Per Year for First Five Years (\$1,000's)						Comments
							Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Total	
1b	4.5	Review selection criteria for release sites	(A1), (B1)	Continuing	USFWS WTSSP	Y	2	2	2	2	2	10	Revisions included as necessary
1b	7.1	Review previous studies	(A1), (A4), (B1), (B3)	Continuing	USFWS	Y	2	2	2	2	2	10	-
1b	7.2	Identify site-specific threats and data needs	(A1), (A2), (B1)	5 years	USFWS	Y	3	3	3	3	3	15	-
1b	2.2	Develop a grazing and prescribed burn plan	(A1), (B1)	Continuing	USFWS	Y	2	2	2	2	2	10	All reintroduction sites included
1b	2.3	Annually collect and analyze water quality	(A1), (B1)	Continuing	USFWS WTSSP	Y	3	3	3	3	3	15	-
1b	2.4	Adhere to current WTSSP guidelines and protocols for disease control	(A1), (A2), (B1)	Continuing	USFWS WTSSP	Y	3	3	3	3	3	15	Disease spread avoidance measures are currently in place and strictly followed.
1b	2.5	Investigate predation	(A1), (B1)	5 years	USFWS	Y	3	3	3	3	3	15	-
1b	3.3.5	Incorporate new genetics into captive populations	(A1), (A3), (B1)	Continuing	USFWS WTSSP	Y	3	3	3	3	3	15	-
1b	3.3.6	Annually review captive population status	(A1), (A2), (B1)	Continuing	USFWS WTSSP	Y	3	3	3	3	3	15	-
1b	3.3.7	Ensure captive population removal has no negative impact on population stability	(A1), (A2), (B1)	5 years	USFWS WTSSP	Y	3	3	3	3	3	15	-
1b	3.4.1	Continue research of nutritional needs	(A1), (A2), (B1)	Continuing	USFWS WTSSP	Y	4	4	4	4	4	20	-
1b	3.4.2	Continued analysis of captive hibernation	(A1), (A2), (A3), (B1)	Continuing	USFWS	Y	2	2	2	2	2	10	-
1b	3.4.3	Continue experimentation of outdoor hibernation	(A2), (A3), (B4)	5 years	USFWS WTSSP	Y	2	2	2	2	2	10	-
1b	3.4.4	Optimize captive breeding	(A2), (B1)	5 years	USFWS	Y	4	4	4	4	4	20	Continue to develop management strategies
1b	3.4.5	Pursue sperm cryopreservation and <i>in vitro</i> fertilization	(A1), (A2), (A3), (B1)	5 years	USFWS WTSSP	Y	4	4	4	4	4	20	-
1b	6.1.3	Research wild toad populations	(A1), (A4), (B1)	5 years	USFWS	Y	10	10	10	10	10	50	Life history, reproduction, <i>Bd</i> , habitat preference, etc.

Table 4. IMPLEMENTATION PRIORITIES AND COST BREAKDOWN OF RECOVERY ACTIONS

Priority	Action Reference	Action Description	Related Recovery Criteria	Action Duration	Responsible Parties	USFWS Lead?	Associated Costs Per Year for First Five Years (\$1,000's)						Comments
							Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Total	
1b	6.1.4	Determine wild population structure	(A1), (B1)	Continuing	USFWS	Y	5	5	5	5	5	25	Necessary for a population viability analysis
1b	6.1.5	Research captive toad populations	(A2), (A3), (B1)	5 years	USFWS WTSSP	Y	8	8	8	8	8	40	Genetic relatedness, reproduction, nutritional requirements, etc.
2	1.2	Pursue the possibility of a 10(j) rulemaking	(A1), (B3), (B5)	5 years	USFWS	Y	2	2	2	2	2	10	-
2	1.4	Continue pursuing SHA sites	(A1), (B1)	Continuing	USFWS LRCD	Y	2	2	2	2	2	10	-
2	4.3	Determine the optimal age class for release	(A1), (A2), (A3), (B1)	5 years	USFWS WTSSP	Y	3	3	3	3	3	15	-
2	4.4	Develop plan to document and report release efforts	(A1), (A2), (A3), (B1)	Continuing	USFWS WTSSP	Y	2	2	2	2	2	10	-
2	6.1.1	Identify data needs	(A1), (A4), (B1)	Continuing	USFWS	Y	3	3	3	3	3	15	Data specific to management needs
2	6.1.2	Prioritize new research	(A1), (A2), (A4), (B1)	5 years	USFWS WTSSP	Y	3	3	3	3	3	15	Based on relevance to implementation plan
2	7.3	Develop monitoring plan	(A1), (B1)	5 years	USFWS	Y	2	2	2	2	2	10	-
2	7.4	Implement standardized monitoring of wild toads	(A1), (B1)	Continuing	USFWS	Y	8	8	8	8	8	40	Monitoring through adaptive management.
2	7.4	Obtain demographic data necessary for PVA	(A1), (B1)	Continuing	USFWS	Y	10	10	10	10	10	50	-
2	7.5	Develop post- down- and delisting monitoring plan	(A1), (B1), (B2)	Continuing	USFWS	Y	2	2	2	2	2	10	-
2	8.1	Evaluate new information as it becomes available	(A1), (B1)	5 years	USFWS WTSSP	Y	1	1	1	1	1	5	Adaptive management approach
2	8.2	Prepare long-term adaptive management plan	(A1), (A3), (B1), (B2)	5 years	USFWS	Y	3	3	3	3	3	15	-
3	2.1	Investigate the effects of disturbance	(A1), (B1)	Continuing	USFWS	Y	2	2	2	2	2	10	Including various land management methods.
3	5.1	Jointly develop outreach programs	(A1), (A3), (B1)	Continuing	USFWS	Y	2	2	2	2	2	10	-
Totals (\$1,000's)							370	370	320	185	175	1,420	

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Appendix A. WYOMING TOAD THREATS

Factor	Threats	Criteria A= Downlisting B= Delisting	Specific Associated Actions	Stressor associated with threat	Geographic Extent/ Scope	Timeframe/ Immediacy	Strength/ Intensity	Population Exposure	Physiological/ Behavioral Response	Overall Threat Level
A. The present or threatened destruction, modification, or curtailment of its habitat or range	1. Irrigation Practices	(A1), (B1)	1.3, 2.3, 6.1.1, 6.1.3, 7.2	Modification of water quality	rangewide	historic/ future	low	insignificant	basic needs inhibited	moderate
				Modification of habitat	rangewide	historic/ future	low	insignificant	basic needs inhibited	moderate
	2. Pesticides	(A1), (B1)	4.5, 6.1.1, 6.1.3, 7.1, 7.2	Contamination of habitat	localized	future	moderate	small	Reduced size/ fitness and/ or mortality	moderate
				Decrease in aquatic vegetation	localized	future	moderate	small	basic needs inhibited	moderate
				Reduction in invertebrates	localized	future	moderate	small	basic needs inhibited	moderate
				Alteration in sexual development	localized	future	moderate	small	reproduction inhibited	moderate
	3. Presence of livestock	(A1), (B1)	2.1, 6.1.1	Water quality degradation	rangewide	historic	moderate	moderate	basic needs inhibited	low
Habitat degradation (vegetation trampling, erosion)				rangewide	historic	moderate	moderate	basic needs inhibited	low	
B. Overutilization for commercial, recreational, scientific, or educational purposes	4. Over-utilization	(A1), (A4), (B1), (B3)	2.4, 6.1.1	Reduction in toad numbers	rangewide	future	low	small	mortality	low
				Impaired reproduction/survival	rangewide	historic/ future	low	small	basic needs inhibited	low
				Introduction of disease	rangewide	future	moderate	moderate	mortality	severe
				Reduction in prey abundance/ diversity	rangewide	historic/ future	low	small	basic needs inhibited	low
				Reduction of habitat quantity	rangewide	historic/ future	moderate	small	basic needs inhibited	low
C. Disease or predation	5. Infectious Disease	(A1), (A4), (B1), (B3)	2.4, 6.1.1, 6.1.6	Reduction or extinction through infection with <i>Bd</i>	rangewide	future	high	significant	mortality confirmed (potential extinction)	severe
	6. Predation	(A1), (B1)	2.5, 6.1.1	Insufficient protective measures	rangewide	historic/ imminent/ future	moderate	moderate	various	moderate
D. Inadequacy of existing regulatory mechanisms	7. Protection	(A1), (B1)	1.3, 7.5	Potential for habitat modification/ degradation	localized	historic/ future	low	small	behavioral (avoidance, startle, mortality)	severe
E. Other	8. Heavy Metals	(A1), (B1)	4.5, 6.1.1, 7.1, 7.2	Poisoning, toxin exposure	rangewide	historic	low	insignificant	avoidance/ basic needs inhibited	low
	9. Mineral Fertilizers	(A1), (B1)	4.5, 6.1.1, 7.1, 7.2	Delayed metamorphosis and behavior alteration	localized	historic/ future	low	small	avoidance/ basic needs inhibited	moderate
				Pollution of habitat	localized	future	low	small	basic needs inhibited	low
	10. Climate change	(A1), (B1), (B2)	6.1.1, 7.3, 7.5	Changes in hydrological conditions, habitat conditions	rangewide	imminent/ future	moderate	moderate	basic needs inhibited	moderate
	11. Genetics	(A1), (A2), (B1)	6.1.5, 3.3.7, 3.4.5, 3.3.4	Limited genetic diversity	rangewide	future	moderate	significant	genetic integrity loss	moderate
	12. Captive diets	(A1), (A2), (B1)	3.4.1, 6.1.1, 6.2.2	Disease, impaired reproduction/ survival, reduced fitness	localized	historic/ future	moderate	significant	basic needs inhibited, mortality	high
	13. Small population	(A1), (A2), (B1)	6.1.1, 6.1.2, 6.1.5, 3.3.7, 3.4.5, 3.3.4	Lack of genetic variability	rangewide	future	moderate	significant	genetic integrity loss	severe
				Sensitive to stochastic population extinctions	rangewide	future	moderate	significant	possibility of extinction	severe
14. Weather Events	(A1), (B1)	6.1.1, 6.1.2, 7.2	Changes in hydrology	rangewide	future	moderate	moderate	basic needs inhibited	low	
			Modification of habitat	rangewide	historic/ future	low	insignificant	basic needs inhibited	moderate	

Appendix B. GLOSSARY OF TECHNICAL TERMS

Acaricide: a chemical employed to kill mites and ticks.

Adult Wyoming toad: a toad that is sexually mature. In males, this requires developed testes, motile sperm, secondary sexual characteristics (dark throat patch, nuptial pads) and production of a mating call. Adult females have mature eggs. Adult toads can have a snout-vent length of 45 mm–65 mm (2 in–3 in). Successful breeding is not required to classify a toad as an adult.

Allele: one member of a pair of series of genes that occupy a specific position on a specific chromosome.

Amplexus: breeding grasp.

Anurans: belonging to the Order Anura (frogs and toads).

Aquatic of or in water; streams, lakes, rivers, ponds, and marshes are aquatic habitats.

Boss: a rounded projection at the top of the head, between the eyes.

Bufonidae: referring to the family of toads.

Candidate: species for which the U.S. Fish and Wildlife Service believes there is enough information on status and threats to support a proposal for listing.

Carbamate: class of chemicals that acts on enzymes of the Central Nervous System by inhibiting a specific enzyme, acetylcholinesterase, this damages nerve function. Most chemicals belonging to this class do not accumulate in body tissue.

Chytridiomycosis: an infection in the superficial skin layers caused exclusively by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*).

Cranial crests: bony ridges on the dorsal surface of the head of toads.

Conspecific: of or belonging to the same species.

Diurnal: active during the day.

Dorsal: of or pertaining to the upper surface; top or back.

Electron Microscopy: microscopy involving the use of an electron microscope. It is similar in purpose to a light microscope but has great resolving power by using parallel beams of electrons rather than visible light.

Extinct: a species that is no longer existing; can refer to a species in its entirety, or in a particular part of the range.

Extirpated: extinct in a particular area. For example, the leopard frog is found throughout the state of Wyoming however, is presumed extirpated from the Laramie Basin.

Flagellated: resembling or having the form of a flagellum; whip-like.

Floodplain: almost level land forming the floor on either side of a stream in a valley often subject of flooding.

Genealogy: an account of the descent of a person, family, or group from an ancestor or from older forms; pedigree.

Guttural: sound coming from or formed in the throat.

Habitat: the environment in which a species or populations lives and grows. Different types of habitats may be used for different lifestages.

Habitat fragmentation: breaking up continuous habitat into smaller, isolated pieces.

Histology: a branch of anatomy that deals with the minute structure of animal and plant tissues as discernible with the microscope.

Hypovitaminosis: disease caused by deficiency of vitamins.

Immunosuppression: lowering the body's normal immune response to foreign substances.

Indigenous: having originated in and being produced, growing, living, or occurring naturally in a particular region or environment; native.

Lacustrine: of, relating to, formed in, living in, or growing in lakes.

Listed: species recognized by Federal or State governments as endangered or threatened.

Littoral: of or on a shore.

Medially: relating to situated in, or extending toward the middle; median.

Metamorphs: Toads that have recently metamorphosed and have not over-wintered.

Metamorphosis: the process of changing from a larvae (tadpole) to an adult (frog or toad).

Metaplasia: transformation of cells from a normal to an abnormal state.

Mima mounds: mound-like features of some grasslands of unknown origin, "pimpled prairies".

Morphology: pertaining to body shape or structure.

Mycotic dermatitis: skin irritation caused by fungal infection.

Necropsy: an examination of an animal after death to determine the cause of death or the character and extent of changes produced by disease.

Nocturnal: active during the night.

Organophosphate: class of insecticide causing direct effects to the central nervous system.

Papillae: any minute nipple like projection, as the papillae of the tongue.

Parotid glands: the toxin producing shoulder glands of toads.

PCR (Polymerase Chain Reaction): a procedure that makes it possible to amplify or copy DNA for identification purposes. An *in vitro* technique that involves separating the DNA into its two complementary strands, using DNA polymerase to synthesize two-stranded DNA from each single strand, and repeating the process.

Pesticide: group of substances that have been designed to repel, kill, prevent or regulate the growth of unwanted biological organisms such as insects, weeds, and rodents.

Phylogeny: the evolutionary relationships among a group of organisms.

Population: in the wider sense, can refer to a species throughout its entire range. More specifically, can be used to refer to a species in one particular locality or watershed; a collection of individuals that share a common gene pool.

Posterior: toward the rear or tail.

Proposed: species for which a proposal to list the species as threatened or endangered has been published in the Federal Register.

Postorbital ridges: bony ridges located behind the eyes of toads.

Pyrethroid: a class of chemicals that inhibits sodium and potassium conduction in nerve cells and blocks nerve impulse transmission.

Ranidae (Ranids): referring to the family of true frogs.

Rhizoid: a root-like structure.

Riparian: relating to or living or located on the bank of a natural watercourse (as a river) or sometimes of a lake or a tidewater.

Self-sustaining population: a population that is able to replace itself without outside intervention or captive breeding.

Snout-vent length (SVL): length of an animal from its snout (tip of the nose) to the vent (anus).

Staccato: cut short crisply; detached short sound.

Squamous: scaly.

Squamous metaplasia: change in the structure of normal cells. In the Wyoming toad, the changes are from the normal tongue cells which are mucous producing cells (enable the toad to obtain prey) to epithelial (normal skin cells).

Sub-population: A sub-population is a sub-set of all re-introduced into the wild.

Sympatric: occurring in the same geographical area.

Taxon: a level in the classification system, such as species, genus, family, or order.

Taxonomy: orderly classification of species according to their presumed natural relationships.

Thalli: lacks differentiation into distinct members (as stem, leaves, and roots), and does not grow from an apical point.

Tympanum: a thin tense membrane covering the organ of hearing in an insect or amphibian.

Ubiquitous: existing or being everywhere at the same time, constantly encountered, widespread.

Upland: terrestrial habitats not included in riparian zones, the higher elevations.

Wetland: an area where saturation or repeated inundation of water is the determining factor in the nature of the soils, as well as plants and animals living there. Includes: marshes, swamps, bogs, fens, bay heads, wet meadows, potholes, sloughs, bayous, river flood plains, estuaries, and lake margins.

Vent: the external cloacal opening; the anus.

Venter: the underside of an animal; the belly.

Viability: capability or capacity to survive; for populations, the ability to survive into the foreseeable future.

Zoospore: a motile, asexual flagellated spore.

Appendix C. GUIDELINES FOR SELECTING WYOMING TOAD RELEASE SITES

Developed by USFWS, Wyoming Field Office, 2006

Due to the presence of *Bd* fungus (*Batrachochytrium dendrobatidis*) and the possibility of catastrophic events occurring at Mortenson Lake, locating new release sites for the Wyoming toad is a high priority. Self-sustaining sub-populations of Wyoming toads must be established at more sites in order to achieve recovery goals.

The following guidelines were developed using the best available information. Information was gained from historical site data, current site data, Canadian toad (*Anaxyrus hemiophrys*) and captive Wyoming toad data. New information regarding habitat selection and use will most likely be gained as toads are reintroduced into new areas. This information will be used to update these guidelines.

These guidelines provide biological and ecological guidance when selecting new reintroduction sites. However, these criteria may not apply in every situation (i.e. drought). Therefore, the final decision will be based on the discretion of the Wyoming toad Recovery Coordinator often in conjunction with the WTRT and technical advisory group.

The first consideration for a release site is that it falls within the historic range of the Wyoming toad. This includes all wetlands or wet areas within the Laramie Basin that have an elevation of 2,134 meters to 2,286 meters (7,000 to 7,500 feet). If sites within the historic range do not meet the necessary criteria, sites outside, but nearby the historic range may be considered. Additional criteria of a successful reintroduction site include the following:

Criteria: Soil Moisture values between a rating of 2.0 and 3.6

Withers (1992) found that soil moisture was the factor that influenced habitat uses the most. According to the 1-2-3-4 (dry-moist-saturated-standing water) scale of soil moisture, Wyoming toads in 1990 to 1991 used soils that rated 2.0 to 3.6. Similar results were obtained in 1998 and 1999 (Parker 2000). Measurement: Soil moisture will be visually estimated using the 1-2-3-4 (dry-moist-saturated-standing water) scale of soil moisture.

Criteria: Varying vegetative cover (open, intermediate, and dense) and type.

Breeding, egg laying a development sites should have a mean vertical cover of 29 percent (+/- 15 percent at the 0 to 10 centimeters (3.9 inches)) level. Metamorphic sites should have a mean vertical cover of 61 percent (+/- 15 percent at the 0 to 10 centimeters (3.9 inches)) level and a mean horizontal cover value of 34.9 percent (+/- 10 percent)

Plants with stem diameters ranging from a minimum of 1 millimeter to maximum of 7 millimeters (0.3 inches) and maximum height or 90 centimeters (35 inches) should be present. In littoral areas, plants with 1 millimeter to 2 millimeters (0.04 to 0.08 inches) stem diameters are preferred but need to be at least less than 7 millimeters (0.3 inches).

Cover

Given sufficient moisture, vegetative cover affects habitat suitability more than other indicators (Withers 1992). Any site that is to be considered for future release of Wyoming toads should have open areas, areas of intermediate vegetation cover, and dense vegetation cover (Withers 1992, Parker 2000). Metamorphosing toadlets and young of the year toadlets use open areas during the day. Furthermore, toads of all age classes tend to favor areas that are more open in the fall, most likely due to declining ambient air temperatures and decreased sunlight (Withers 1992). Open areas were typically bordered by dense vegetation (Withers 1992). Parker (2000) found adult Wyoming toads seek refuge during the night in areas of relatively dense (less than 50 percent cover), to presumably avoid predation.

During the time of breeding, vertical cover at the 0 to 10 centimeters (0 to 3.9 inches) level at egg laying and development sites averaged 29 percent with a minimum of 0 percent and maximum of 65 percent. Mean horizontal cover was 12.1 percent. Plant height averaged 34.3 centimeters (13.5 inches) (Withers 1992).

At metamorphic sites, vertical cover at the 0 to 10 centimeters (0 to 4 inches) level averaged 61 percent with a minimum of 37.5 percent and a maximum of 82.5 percent. Mean horizontal cover was 34.9 percent (Withers 1992).

Vegetative type

Eleocharis palustris (spike rush) and *Scirpus americanus* (bulrush) are the two dominant plants at calling and breeding sites (Withers 1992). It is unknown whether these species are required for Wyoming toads; but, plants with similar morphology should be available at potential release sites. *E. palustris* is small in diameter averaging 1 to 2 millimeters (0.04 to 0.08 inches) and standing no more than 40 centimeters (15.8 inches) high. *S. americanus* has a triangular stalk and may reach a maximum diameter of 7 millimeters (0.3 inches) with a maximum height of 90 centimeters (35.4 inches) (Withers 1992). No eggs masses were produced in areas that contained *S. validus* (softstem bulrush) although it is available in breeding areas.

Based on observations and plant measurements, the presence of narrow stemmed plants (diameter less than 7 millimeters (0.3 inches) in the littoral zone is necessary. Low stem densities allow freer movement of amplexing toads. In addition, this will allow more light to shallow areas where embryos develop. The rate at which embryos develop is directly related to temperature (Withers 1992).

Measurement: To maintain consistency, cover will be estimated using the same techniques employed by Withers (1992) and Parker (2000). The proposed release site will be divided into evenly spaced sections. Each of these sections will be randomly sampled. Cover will be estimated by visually centering a modified 20 x 50 Daubenmire quadrant centered on a given point. The quadrant is divided into 10 centimeters x 10 centimeters (4 inches x 4 inches) squares. The quadrant will be set over the sample by extending collapsible legs so that it is 28 centimeters (11 inches) above the ground. Cover will be estimated and recorded as a percentage for each square. The measurements will be made during late June through August.

Vertical cover will also be visually estimated. With the quadrant in place, a modified 50 centimeters (20 inches) Robel pole will be set vertically in the center of the quadrant and viewed from a distance of 25 centimeters (10 inches) from the quadrant's edge. Vertical cover was estimated for each of the five graduations to 50 centimeters (20 inches) above the substrate from both the north and south sides of the pole.

Plant diameter will be measured by using either calipers or a flexible tape measure. Plant height will be measured by using a flexible tape measure.

Criteria: Water chemistry

By combining historical water chemistry values at successful Wyoming toad sites and captive breeding facilities along with general amphibian water chemistry information, the following parameters are developed. As more water chemistry research is conducted, the ranges could be narrowed. In addition, more parameters should be set once water chemistry and metal toxicology tests are conducted.

pH range: 6.64-8.63
Conductivity: 100-1700 ohms/centimeters
Copper: <.01-.01 mg/L
Mercury: <.001
Lead: <.02 mg/L
Arsenic: <.01 mg/L
Phosphate: Less than 10 ppm
Ammonia: <.04 ppm
Nitrite: <.06 ppm
Nitrate: not to exceed 10 ppm
Hardness: not to exceed 350 ppm
Calcium hardness: to be less than total hardness
Alkalinity: 15-50 ppm
Free Chlorine: not to exceed 0 ppm
Total Chlorine: not to exceed 0 ppm
Iron: not to exceed 2 ppm

Measurement: Complete water chemistry tests will be run twice using the Colorado State University Lab. One sample will be collected during early spring (April to May) to reflect optimum chemistry/quality results as most lakes and other bodies of water are full during this time. Another sample should be taken during late summer (August to September) to reflect how drought affects the water chemistry and quality. When possible, more than one sample should be taken at each sampling event. For example, samples collected at one site may include associated wetlands, littoral areas, and deeper areas that are used by tadpoles.

Criteria: Other water characteristics

Withers (1992) found Wyoming toad egg masses to be laid in water averaging between 3.5 centimeters and 6.3 centimeters (1.4 and 2.5 inches). To accommodate breeding and egg laying,

proposed release sites should have shallow margins and varying water depths beyond the levels reported by Withers 1992. During the evening, tadpoles of many species will select areas of warmer water found in shallower depths.

Criteria: Available hibernacula

It is presumed that the Wyoming toad historically hibernated in rodent burrows and diggings when it inhabited the Laramie River (Baxter 1990 pers. comm.). However, to date, there has been no formal investigation of Wyoming toad hibernation along the Laramie River.

Parker (2000) has been the first formal attempt to better understand the winter hibernation of the Wyoming toad at Mortenson Lake. During 1999, Parker (2000) tracked four captive bred adult Wyoming toads fitted with radio transmitters prior to hibernation. One toad was observed in a rodent burrow and another in the soft soil remaining from a badger hole digging. The latter toad remained underground at a depth of 36 centimeters (91.44 inches) for a total of 35 days. Both toads emerged to forage when the weather improved. Shortly thereafter the transmitter batteries failed; thus, little information was gained about the hibernation habits of the Wyoming toad. However, all observed prospective hibernation sites consisted of soft, barely moist soil (Parker 2000).

Tester and Breckenridge (1964) radio tracked the closely related Canadian toad (*Anaxyrus hemiophrys*) throughout the year. The most common hibernacula observed were mima mounds, areas of soft raised dirt, lacking vegetation, and measuring 3 to 15 meters (9.8 to 49.2 feet) in diameter. Some were as tall as 6 meters (19 feet) high and individual burrows were observed up to 117 centimeters (46 inches) deep. Toads moved vertically within the mima mounds in response to the shifting frost line. In areas where mima mounds were unavailable, toads hibernated in areas of raised soft substrate.

Until more specific information is obtained, Wyoming toads are assumed to be hibernating in rodent burrows or mima mounds. Candidate sites should contain rodent burrows or diggings within 15 meters (49.21 feet) of the littoral areas (Withers 1992). In addition, the candidate site should be interspersed with rodent burrows, as they appear to offer summer refuge for the toad. A density of three to four surface holes per square meter, ranging in size from 3.8 to 5.0 centimeters (1.5 to 2.0 inches) in diameter would mimic areas most densely populated with fossorial rodents at Mortenson Lake (Withers 1992).

Criteria: Use of BTI as sole pesticide

Malathion and other pesticides have been shown to have negative effects on some species of amphibians. Malathion has been used in the past to control for mosquitos, but it has been shown to act as an immunosuppressant to the Woodhouse's toad (*Anaxyrus woodhousii*) and may reduce the toads' prey base (Taylor et. al 1999). *Bacillus thuringiensis* var. *isralensis* (BTI) has been an effective larvicide at Mortenson Lake and elsewhere throughout the state of Wyoming with no known side effects to non-target species.

Measurement: With landowner permission, the use of BTI could be a condition of the Safe Harbor agreement. If need be, pesticide strips could be placed on the property prior to mosquito spraying.

Criteria: Preference should be given to sites meeting other criteria and resident amphibians do not test positive for *Batrachochytrium dendrobatidis* (*Bd*). However, sites that test positive for *Bd* should not be overlooked if all other criteria are met.

Resident amphibians can be evaluated for *Bd* by a quantitative Polymerase Chain Reaction (qPCR) test. A *Bd* sample is collected for qPCR through a non-lethal skin swab, similar to swabbing for human genetic material from the inner cheek. A qPCR is useful to determine the presence of *Bd* and infection rate by examining the number of zoospores per sample.

The status of *Bd* should be evaluated at all sites to the extent possible and preference will be given to sites that do not test positive for *Bd*. However, a reliable and accurate method of detecting *Bd* at sites without resident amphibians has yet to be determined. Low-level presences of the pathogen are difficult to detect and may be overlooked. *Bd* has been shown to survive for up to seven weeks in the laboratory without a host present (Johnson and Speare 2003) and may very likely be present in the environment even if it is not detected. Thus, the lack of a positive sample should not be used to declare a site “*Bd* free”. At the same time, *Bd* presence shouldn’t necessarily rule out potential sites. There may be unique factors of Mortenson Lake that make Wyoming toads more susceptible to chytridiomycosis, *Bd* can move between sites and previously “*Bd* free” sites can quickly become infected, and management techniques may be developed to eradicate or keep infection rates below lethal levels.

Measurement: Until it is possible to test the soil and water at a site, screening resident amphibians can aid in detecting *Bd*. Determining the number of animals to test at a given site is difficult at best; but sampling 5 to 10 percent of the resident amphibian population has been suggested as reasonable until more information is available (Pessier 2013 pers. comm.).

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Appendix D. DECONTAMINATION TO PREVENT THE SPREAD OF DISEASE

USFWS Guidelines for Reducing the Spread of Pathogens between Wyoming Toads

Developed by USFWS, Contaminants, Wyoming Field Office, 2005

Reintroduction Sites:

The following guidelines will reduce the spread of pathogens between reintroduction sites of the Wyoming toad. Currently, the biggest threat to the health of wild Wyoming toads is the pathogenic fungus, *Batrachochytrium dendrobatidis* (*Bd*), is consequently the focus of these guidelines. These may be updated or changed as more information regarding wild mortalities is gathered.

Between sites:

The following steps will be taken while moving between Wyoming toad reintroduction sites and should be undertaken regardless of the predetermined disease status of the site:

1. Clean all organic debris off boots and all field equipment with a scrub brush and water.
2. Disinfect boots, field equipment, and cleaning equipment with a ten percent bleach solution away from any nearby bodies of water. Rinse with tap or bottled water away from any watersheds.
3. A new pair or disposable latex gloves will be used when handling each animal.
4. If funds are available, separate field equipment (nets, boots, etc.) should be purchased for each site.
5. Tires do not necessarily have to be disinfected but at a minimum should be rinsed off before moving to new locations. Rinsing should remove most of the organic debris which could contain zoospores.

The above-mentioned protocol will also be implemented at all future reintroduction sites. For reintroductions that occur on private land, the landowner will be provided information regarding amphibian pathogens and how to reduce their spread.

Due to the large captive population, translocations of animals between sites are strongly discouraged and should not be necessary. The Re-establishment Team will only undertake this option after unanimous approval. This team is comprised of the WTSSP Pathologist, WTSSP Veterinarian, WTSSP Coordinator, WTSSP Population Manger, WTRT Leader, and the Wyoming toad Recovery Coordinator. No translocations will be considered without prior knowledge of the health status of the population. This information will be obtained by sampling a small portion of the cohort considered for translocation. No translocations will take place from sites with known *Bd* infections.

Re-establishing populations:

The following guidelines will be followed when re-establishing the Wyoming toad throughout suitable habitat in Albany County:

- Resident amphibians at potential reintroduction sites will be collected and screened for *Bd* through a combination of qPCR testing and histological examination. The use of adult Wyoming toads as sentinel animals (when populations of resident amphibians do not exist) will be considered when the efficacy of this technique has been evaluated.
- All animals to be released will come from facilities that have not tested positive for *Bd* or other significant mortality event within the last year. Participating facilities will follow disease surveillance guidelines as established by the Wyoming toad SSP Pathologist and Veterinarian. Results of the surveillance need to be relayed to the WTSSP Pathologist.
- Prior to release, adults will be screened for *Bd* and undergo a 10 day prophylactic treatment of a 0.01 percent solution of Sporanox (0.01 percent suspended itraconazole) diluted with Amphibian Ringer's Solution. Tadpoles and small toadlets will not be treated with Sporanox as it is lethal to these lifestages.
- In the future, submitting a sub-sample of tadpoles for *Bd* screening may be considered as the efficacy of this technique is determined and funding allows.
- Lastly, all WTSSP facilities will follow the rules and guidelines set forth in the Wyoming toad Husbandry Manual and their individual facility disease protocols.

Appendix E. POTENTIAL CONTAMINANTS OF WYOMING TOAD HABITAT

Following is a list of chemicals that have been reported to have been used (historically or currently) and detected within Albany County and may have had or continue to have a negative impact on the survival of the Wyoming toad as well as other wildlife species. The following list also includes a brief summary of the known effects of each chemical. Some chemicals have been studied in detail while the effects that others may have on amphibians are largely unknown.

Atrazine
Azinphos-methyl
Carbaryl
Carbofuran
Chlorpyrifos
Diazinon
Famphur
Glyphosate (Round-up, Rodeo)
Malathion
Methomyl
Methyl Parathion
Naled
Permethrin (Biomist)
Picloran
Rotenone
Thrichlorofon

Units Glossary

LC50—lethal concentration to 50 percent of test organisms
Mg/kg—milligrams per kilograms
Mg/L—milligrams per liter
Ng/l—nanograms per liter
Ug/l—micrograms per liter
PAH—Polycyclic Aromatic Hydrocarbons

Toxicity Analysis for These Chemicals

Atrazine is one of the most commonly used herbicides, primarily used to control weeds in corn fields. In 1999, 64 million acres were treated with atrazine making it the second most frequently detected water contaminant and is commonly found at concentrations of 21 ppb in ground water. It is a restricted-use pesticide and is classified as “slightly toxic” by the EPA. Until recently, atrazine was not considered to be a threat to the survival of amphibians. However, work by Hayes et al. (2002, 2010) demonstrates that feminization of male frogs can occur with exposures as low as 0.1 ppb.

Azinphos-methyl (AZM) is classified by the U.S. Environmental Protection Agency (EPA) as an organophosphate insecticide and miticide. It is used on fruits, nuts, vegetables, field crops, ornamentals, and forest trees. AZM is considered one of the most toxic of this class (Kamrin 1997). Mulla (1962) described a 100 percent mortality of adult bullfrogs (*Rana catesbeiana*) at a

rate of 1.8 kg/ha of field applications and Sanders (1970) found LC50 levels for Woodhouse's toads (*Anaxyrus woodhousii fowleri*) to be 0.13 mg/L.

Carbaryl is a wide spectrum carbamate insecticide, acaricide, and molluscicide. It is used on vegetative crops such as citrus, fruit, cotton, forests, forage crops, rangelands, lawns, nuts, ornamentals, and shade trees; as well as on poultry, livestock, and pets. It is non-to-moderately persistent in the environment and is considered to be slightly volatile and slightly soluble in water (Pimentel 1971). It is highly toxic to fish and reduces sex hormones, increases vulnerability to predation, and lowers swimming capacity (Hudson et al. 1984; NIOSH 1989). Marian et al. (1983) found carbaryl to be lethal to Indian bullfrog (*Hoplobatrachus tigerinus*) tadpoles, and further found that growth, production efficiency, and weight at metamorphosis were negatively affected by increasing carbaryl levels in surviving tadpoles.

Carbofuran is also a carbamate and is used as an insecticide, nematicide, and acaricide. It is soluble in water, slightly volatile, and non-to-moderately persistent in the environment. The majority of toxicology data on the carbamate class as a whole involves birds, fishes, and mammals. It has been found to be acutely toxic, particularly when ingested, but does not accumulate in body tissue. The World Wildlife Fund (1994) called for an overall ban on this insecticide because of its severe effects on wildlife. Little toxicological work has been done on carbofuran with amphibians, but existing literature suggests that carbofuran exposure was correlated with physical deformities and genetic damage in juvenile Green frogs (*R. clamitans*) in agricultural areas in Quebec (Lowcock and Ouellet 1997). Carbofuran may also have negative secondary effects through the reduction and/or contamination of prey (Brunninger et al. 1994, Forsyth and Westcott 1994, Dietrich et al. 1995, Hill 1995, Kring 1969, Mullie et al. 1991, Wayland and Boag 1995).

Chlorpyrifos is an organophosphate insecticide. It is moderately persistent in the environment, slightly soluble, and demonstrates a species-specific toxicity. Toxicological data are limited, but existing literature has found, 24-hour and six day LC50 values for Indian bullfrog (*Hoplobatrachus tigerinus*) tadpoles to be 177 ug/L and 10 ug/L respectively. LC50 values for American toads (*Anaxyrus americanus*) tadpoles were found to be 1 ug/L. Sparling and Fellers (2007) found the 24-hour LC50 for the foothill yellow-legged frog (*R. boylii*) to be 3.00 mg/L.

Diazinon is an organophosphate insecticide and nematicide. It is slightly soluble in water and is not found to be persistent in the environment. However, its immediate toxicity is very high in birds, fish, amphibians, bees, and aquatic insects.

Famphur is an organophosphate used primarily as an insecticide on cattle. Birds are killed indirectly by ingesting the insects that are killed with famphur. Cases of eagle deaths have been attributed to the ingestion of small birds that ate the insects poisoned with famphur. Little is known regarding the effects this chemical has on amphibians.

Glyphosate is well known as the active ingredient in the commercial formulation Roundup®. As of 2007, it was the most commonly used active ingredient in the United States and in the world (Duke and Powles 2008). It is a broad-spectrum organophosphate herbicide, is moderately persistent in the environment, and is soluble in water. Glyphosate alone has been shown to be

only slightly toxic to amphibians. However, glyphosate is mixed with surfactants before application either in a commercial formulation such as Roundup®, or by the end user after purchase. The toxicity of the final formulation varies widely depending on the toxicity of the surfactant added (Folmar et al. 1979, Geisy et al. 2000). Rodeo® and Aquamaster® are commercially available mixtures of glyphosate and water. If used without mixing with a surfactant, these herbicides would only be slightly toxic to amphibians. However, the labels recommend mixing with a surfactant and the resulting formulation can be significantly more toxic than Rodeo® or Aquamaster® alone.

Malathion is an organophosphate insecticide primarily used to control mosquitoes. It is not persistent in the environment and is water soluble. According to a survey conducted by Ferrell et al. (1995) malathion was the most reported pesticide used in association with managing grass hay fields and improved pasture in Wyoming in 1995. Both the Little and Big Laramie Mosquito Control districts utilize malathion for the control of mosquitoes (Anderson 2012 pers. comm.). Malathion is reported as medium to very highly toxic to birds, fish, bees, amphibians, crustaceans, aquatic worms, earthworms, and aquatic insects. Malathion acts as a neuro-toxin in amphibians (Kowsalya et al. 1987) and teratogenic effects to African clawed frog (*Xenopus laevis*) larvae were reported in Bonfanti et al. (2004). Taylor (1999) reported immunosuppression and subsequent death of Woodhouse's toads (*Anaxyrus woodhousii*) following malathion exposure.

Methomyl is a carbamate insecticide and nematicide. It is non-to-moderately persistent in the environment and is water soluble. It is considered to be highly toxic to birds, fish and aquatic insects. There is little information regarding specific effects to amphibians.

Methyl Parathion is an organophosphate insecticide which is moderately persistent to persistent in the environment and is slightly soluble in water. Alvarez et al. (1995) reported skeletal deformities in Perez's Frog (*R. perezi*) tadpoles exposed to low doses (0.25 mg/L) for 14 weeks. Deformities were more marked with increased dosages and appeared in 100 percent of treated animals that survived to metamorphosis. Alvarez et al. (1995) also reported a negative effect on the ability to forage, escape predation, and reproduce. Deviations of the spinal column and/or tail were observed at dosages lower than those found in natural ponds (Honrubia et al. 1993).

Naled is an insecticide and acaricide, which belongs to the organophosphate class. It is not soluble in water and little is known about its persistence in the environment. It is slightly volatile and is slightly toxic to amphibians.

Permethrin is a pyrethroid pesticide reported to be used in Albany County to control mosquitoes. It is not persistent in the environment and is slightly soluble in water. It is available in dusts, emulsifiable concentrates, smokes, ultra-low volume sprays, and wettable powder formulation. There is insufficient information on toxicity of permethrin to amphibians. Toxicity studies on invertebrates have shown that permethrin contaminated sediments are toxic to midge larvae (Fleming et al. 1998).

Picloram is a herbicide which falls into a miscellaneous chemical class. It is soluble in water, moderately persistent in the environment, and is slightly volatile and flammable. It acts as an

herbicide by uncoupling oxidative phosphorylation. There is little information regarding the effects picloram has on amphibians.

Rotenone is a botanical extract used as an insecticide, acaricide, and piscicide. It is slightly soluble in water. Rotenone interferes with normal cell functions and nerve transmission and is highly toxic to amphibians and fish. It is not expected to accumulate appreciably in aquatic organisms as its highly toxic nature allows little survival of the organisms in which it would accumulate. Fontenot et al. (1994) reviewed the hazards of rotenone to amphibians and found that larvae were more sensitive than adults.

Trichlorfon is an organophosphate insecticide. It is moderately persistent in the environment and is very soluble in water. It is considered to be highly toxic to birds, fish, aquatic insects, crustaceans, aquatic worms, and bees.

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Appendix F. POPULATION VIABILITY ANALYSIS

Use of Population Viability Analysis and Population Viability Management to Establish Recovery Goals

Traditionally, most recovery plans have defined goals and benchmarks with hard numbers, usually of the number of separate sub-populations or of total wild individuals. A frequently-mentioned problem with this approach (Morris et al. 2002) is that these firm numerical criteria are usually not tied to any clear measure of population viability – that is, safety from extinction. For many species and recovery plans, this disconnect between recovery criteria and estimated sub-population safety occurs because at the time the recovery plan is drafted, insufficient data exist to rigorously make this tie. This is certainly the case for the Wyoming toad.

An alternative approach is to set criteria in terms of population viability. Field measurable indicators of population health are used to estimate viability, and are used to indicate that a sub-population has reached a sufficient level of safety in order for downlisting or delisting. This approach has recently been used in the recovery plan for the island fox and other species (Bakker et al. 2009; Bakker and Doak 2008). While this method of setting recovery goals requires more analysis of ongoing data collected as part of monitoring and management activities, it has a clear advantage of not locking a plan to outdated information or even mere guesses as to what safe numbers or viable dynamics might be.

Below, we outline the steps to the approach for setting and then using this type of viability criteria:

1. First, the WTRT must decide on a level of population safety that is deemed acceptable. Since any sub-population has some chance of extinction, this process must balance relative safety versus the realities of an uncertain world. At least five different numbers will govern the levels of safety that make up a recovery goal:
 - A. The quasi-extinction threshold is the lower limit on acceptable population sizes, below which a population is assumed to be at drastically increased risk of complete extinction.
 - B. The time horizon over which to evaluate future viability, is the probability that a sub-population will not fall below the quasi-extinction threshold. For example, the WTRT could suggest that recovery requires that a sub-population be large and stable enough that there is little chance of hitting the quasi-extinction threshold over the next 10 years or over the next 1,000 years. The longer the time horizon, the more stringent the recovery criteria.

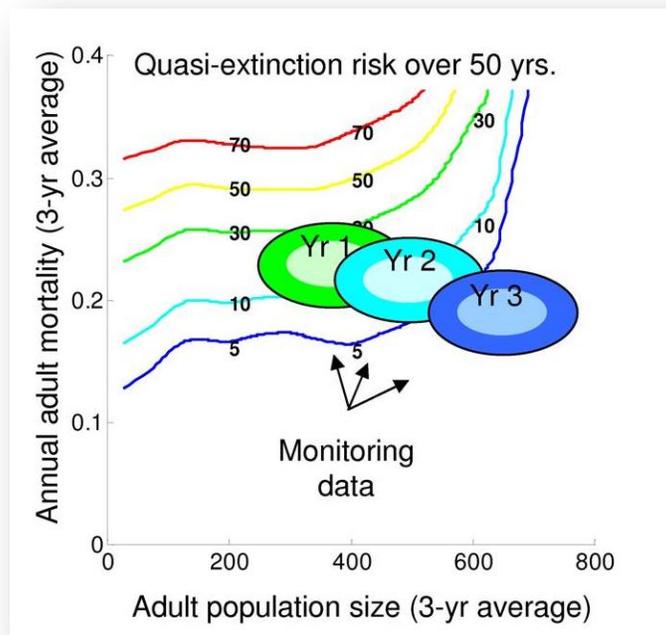
- C. Given the quasi-extinction threshold and the time horizon, the team must decide what risk of “quasi-extinction” is acceptable for recovery, with lower acceptable risks means more stringent criteria.
- D. The certainty of the estimated extinction risk that will be used to trigger recovery must also be decided. All estimates of future extinction risk are based on population models that are in turn parameterized with values that come from field data. Estimates from real data always have uncertainty and this, in turn, creates uncertainty in model outputs, including estimates of extinction risk. In addition, uncertainty about important aspects of population dynamics (e.g., the effects of weather on survival rates, the strength of density dependence, etc.) add more uncertainty to estimated extinction risks. Incorporating this uncertainty into outputs of viability models and their interpretation is thus important (Bakker et al. 2009) and recovery criteria should therefore include some decision of how certain viability must be.
- E. Finally, the team must decide how many sub-populations must each meet the viability criteria that these other values define, in order for downlisting or delisting to occur.

Putting each of these components together for the Wyoming toad, the team arrived at the following baseline criteria for recovery: each of the five, independent populations should meet formal criteria for population viability, assessed using demographic estimates gained from release and monitoring activities. Specifically, each sub-population should, for each of seven years, meet the following criterion: the 80 percent confidence limits for risk of quasi-extinction over 50 years must encompass only values less than 5 percent, using a quasi-extinction threshold of 50 adults (toads greater than three years of age).

- 2. After viability criteria have been established, a monitoring plan that collects data useful in understanding the basic population biology of the target species must be established and continued over time. The information collected can then be used to estimate the basic information needed to establish whether a sub-population has reached the recovery threshold, as embodied in the viability criteria established in the recovery plan. Common types of useful information from monitoring include: the mean and variance of stage-specific survival, growth, and reproductive rates; effects of weather events on survival and reproduction, disease and density-dependent dynamics and how they are influenced by environmental factors. It is expected that as a monitoring plan continues through time, estimates of all these key rates and effects will be improved, reducing uncertainty in viability estimates.
- 3. Pre-existing information and information from the monitoring plan will be used to develop and update one or more population models for the species (see Morris and Doak 2002 for reviews of different viability models). At its most basic, this model can then be used to estimate future extinction risk: given parameter and model uncertainty, in practice, the models will be used to estimate a mean risk of extinction given different starting conditions

or other model assumptions. To make the model most useful for assessing extinction risk, these different scenarios should be tied to observable features of the population in the field: for example the current adult population size and survival rates from the most recent years of data. The estimated extinction risk (from model simulations) for a population with different current sizes and mortality rates can then be estimated into the future, allowing assessment of future viability from currently observed data. An example of this connection is shown below, taken from the island fox recovery plan and from Bakker and Doak (2008):

FIGURE 2: POPULATION VIABILITY ANALYSIS ILLUSTRATION



In the illustration above, estimated mean future viabilities of populations with different initial adult population sizes and adult mortality rates are shown as isoclines of risk (e.g., the 10 percent isocline indicates a 10 percent risk of quasi-extinction over the next 50 years). The confidence limits of field-observed data on mortality and population size for three years are shown, indicating movement of a population towards lower and lower risk. A series of isoclines figures such as that shown above are the key connection between monitoring data and recovery criteria developed for the island fox. A similar numerical and graphical approach to tie viability criteria and field data will be developed for the Wyoming toad as part of this recovery plan.

This appendix gives only an outline of the general approach of using viability criteria to set and evaluate recovery. Particularly in the case of the Wyoming toad, only after several years of field data have been collected will it be possible to better define the exact form of the model that will be used to estimate viability.

Literature Cited for Appendix F

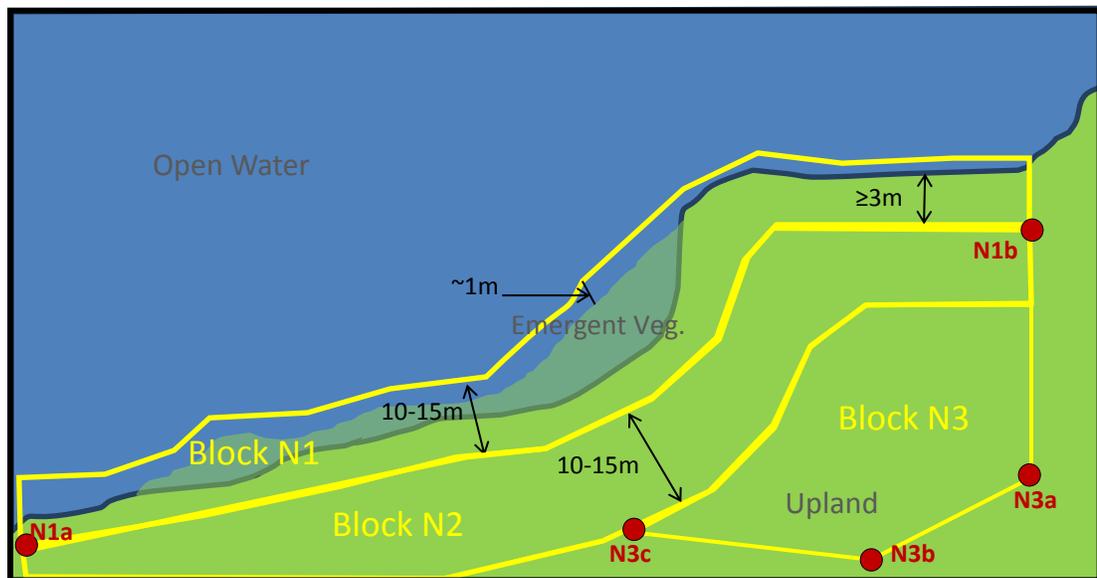
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Appendix G. WYOMING TOAD MONITORING PROTOCOL

Developed by WYNDD and WTRT, 2008, updated 2013

General Information: The intent of this survey protocol is to estimate the abundance of adult Wyoming toads in the areas of highest likelihood-of-occurrence, primarily areas proximate to fixed bodies of water and deemed to be moist through much of the spring-summer season, as confirmed by their vegetation composition.

1. **GPS:** Datum should be NAD 1983 and projection should be UTM, Zone 13.
2. **Survey Sessions:** Surveys should occur at least two times per year
 - a. Breeding Season: Depending on weather, roughly between June 7 and June 20.
 - b. Mid- Season: Depending on weather, roughly between July 7 and July 20.
 - c. Post-Breeding / Pre-Hibernation: Depending on weather, roughly between August 7 and August 20.
3. **Search Blocks:** To delineate search effort, each property is stratified into “search blocks” of known area.



- a. *Shoreline search blocks* (e.g., Block N1) should be roughly 10 to 15 meters wide, extending about 1 meter into open water (depending on depth and vegetation) and at least 3 meters onto shore past the water's edge. The end points of the block are defined by navigation points. The upland and open-water boundaries of the block are defined relative to the shoreline and will therefore change with water levels.
- b. *Near-shore search blocks* (e.g., Block N2) should be roughly 10 to 15 meters wide, extending upland from the highest point of the adjacent shoreline block. The end points of the block are defined by navigation points. The other boundaries of the block are defined relative to the shoreline and will therefore change with water levels.

- c. *Upland search blocks* (e.g., Block N3) can be virtually any shape and are defined by navigation points.
4. **Search Types:** Three classes of amphibian searches are conducted during this monitoring effort: initial searches, replicate searches, and egg mass searches. Unless otherwise noted, searches are visual encounter surveys with strict documentation of survey effort (e.g., Heyer et al. 1994).
- a. *Initial Search:* Purpose is to determine where toads are dispersing and select sites for replicate searches.
 - i. Search in all delineated search blocks.
 - ii. Conduct all initial searches at a site within 5 days of each other to minimize migration of toads between blocks and minimize the effects of variable weather conditions.
 - iii. Two technicians will search each block at a rate of approximately 30 minutes per acre. Since each block is a different size, refer to the search time chart to determine how long to spend in each block.
 - iv. Count all toads, toadlets and metamorphs and collect additional information for each adult (as discussed below).
 - v. Use a countdown timer (e.g., Robic SC-502) to record search time, stopping the timer when not actively searching (e.g., when processing adult toads).
 - vi. Only conduct searches when wind is ≤ 4 on the Beaufort scale (Table 1)
 - vii. Only conduct searches when weather conditions are ≤ 3 on the weather scale (Table 2).
 - b. *Replicate Searches:* Purpose is to search blocks occupied with adults two more times in order to get a depletion rate that can be used to determine abundance of toads.
 - i. All methods same as initial search, except as follows.
 - 1. Conduct two additional searches in each block where adult-sized toads were observed during initial searches.
 - 2. Conduct both replicate searches within 2 days of the associated initial search.
 - c. *Shoreline search for Egg Masses:* The purpose of egg-mass searches is to increase our chance of documenting wild breeding, should it occur. Most shorelines should be searched using the above initial and replicate searches. For the breeding season surveys, technicians should conduct an additional search looking specifically for egg masses. This is an un-timed search wherein technicians carefully search all moist, vegetated shorelines within one search block of any previous toad observations.
5. **Sanitation:** To reduce the spread of *Bd* fungus the following things procedures must be employed.
- a. Sanitize boots when traveling between survey sites by removing all mud and vegetation and then saturating the exterior of the boot with a 10 percent bleach solution.

- b. Sanitize nets used to capture toads between survey sites and if possible between capture of individual toads within a site. This can be done with alcohol (e.g., rubbing alcohol) or with a 10 percent bleach solution.
- c. Use a new pair of non-powdered latex gloves for handling each new toad.
- d. Rinse or wipe plastic containers and photo grids with an alcohol swab or 10 percent bleach solution between each toad.
- e. Follow field sterilization protocols when implanting PIT tags.

Search Protocol

1. Check to make sure you have all necessary equipment.
2. Sanitize equipment as noted above.
3. Searches must be done by TWO technicians working simultaneously.
4. Locate the defining ends of search block using GPS coordinates. Place pin flags to visually define the corners of the search block.
5. Mentally plot each person's search path through the block, trying to cover all available habitat. Zig-zag paths are used, with one person focusing on the "water-side" of a block and the other person focusing on the "land-side" of a block.
6. Set countdown timer to the recommended time for the given search block.
7. Start timer and begin search.
8. Count all toads seen as you search.
9. For adult toads that are large enough to be processed, stop the timer and process the toad, recording all data suggested below. After processing is complete, release the toad and resume the search from the positions when the timer was stopped.
10. Continue till end of block is reached, or until time is up. Try to reach the end of the block EXACTLY as time expires. If you have not completed the block when time is up, finish the block and record how much additional time was taken.
11. Remove pin flags.

Data Collection

Example data sheets are attached to this protocol.

1. **Basic:** The following basic data must be recorded during each day of survey.
 - a. Date (dd-mm-yy)
 - b. Survey Site (e.g., Buford, Shaffer, Mortenson, etc.)
 - c. Names of all observers
 - d. Datum and projection of GPS coordinates (e.g., NAD 1983, UTM Zone 13)
2. **Search Data:** The following additional data is recorded for each search block.
 - a. Search Type being conducted. The following are possible search types: Initial Search (L1), Replicate Searches (L2 and L3), and Shoreline search for egg masses (S). In addition, record the location of all incidentally observed toads and list the search type as "Incidental (I)".
 - b. Block Identification Number: Each block is assigned a unique identification number (e.g., M-01).
 - c. Start time: Time that the search was initiated on each search block.

- d. Duration: The actual length of time the block was searched, which is particularly important if the suggested time was exceeded.
 - e. Wind Code: using the Beaufort Scale (Table 1).
 - f. Ambient Temperature in degrees Fahrenheit.
 - g. Sky code: Use scale to designate percent cloud cover (Table 2).
 - h. Categorical estimate of tadpole abundance in the search block using the following scale: N = None, F = Few (e.g., < 5), S = Some (e.g., 6-20), M = Many (e.g., 21-50), V = Very many (e.g., >50).
 - i. Number of toadlets/ metamorphs observed in search block.
 - j. Number of young of the year observed in each search block.
 - k. Number of adults observed in each search block.
 - l. Notes.
3. **Adult information:** The following additional information is collected at each adult's first incidence of capture.
- a. Take a dorsal photograph using a standard centimeter grid as background. Record picture numbers for later reference, making sure there are no duplicate numbers between cameras or sites. Make the photograph as close-up as possible.
 - b. Sex (M = male, F = female or U = unknown)
 - c. Estimated Age Class, where Y = Young of Year (hatched this year); O = Overwintered (first year after hibernation), and B = potentially breeding adult (2nd year or older).
 - d. Individual identification number.
 - i. Check larger toads (>15 grams) for a PIT tag and record the tag number. If no tag is present, implant a PIT tag and record the tag number.
 - e. Exact easting and northing from a Global Positioning System (GPS) receiver. This should be in UTM coordinates, Zone 13 and based on the NAD 1983 map datum.
 - f. Snout-vent length (SVL) to the nearest millimeter.
 - g. Weight to the nearest tenth gram using a digital scale.
 - h. Collect a *Bd* swab per Bolye (2004).
4. **Habitat Information:** For all adults, tadpole aggregations, and egg masses, be sure the following information is recorded:
- a. Substrate: Record the moisture level of the substrate on which the toad was found using the following categorical scale: G = dry or firm, moist ground; M = soft, muddy ground; W = water.
 - b. Water Depth: Record depth of water to the nearest centimeter using a standard ruler. Do not insert the ruler into muck at the bottom of the pond. Ideally, record the average depth of the water based on at least 5 measurements near the toad or egg mass, or within the tadpole aggregation.
 - c. Water temperature: Record the temperature in the middle of the water column in which the aggregation, egg mass or adult was found. Use degrees Fahrenheit. Ideally, record the average temperature of the water based on at least 5 measurements.

- d. Habitat photograph: Take a photograph of the habitat that the toad, tadpole aggregation, or egg mass was found in. This should be taken from above at a height of 2 meters (approximately six feet) above the surface of the ground or water. Be sure that shadows from the observer are not in the picture.
- 5. Egg Mass and Tadpole Aggregation Information:** In addition to GPS coordinates and habitat data, record the following for each egg mass or tadpole aggregation found.
- a. Egg Mass
 - i. Assign each egg mass an identification number (EM01, etc).
 - ii. Take at least one close-up photograph of the egg mass and record the picture number.
 - b. Tadpole Aggregation
 - i. Assign each aggregation an identification number (T01, etc).
 - ii. Estimate the number of tadpoles in the aggregation by counting the number of tadpoles in a confined area (e.g., 1 square foot) and multiplying by the size of the aggregation. For example, if there are 35 tadpoles in one square foot and the aggregation covers 8 square feet, then the aggregation contains roughly 280 tadpoles ($35 \times 8 = 280$).

Literature Cited

- Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T., and Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* **60**: 141-148.
- Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.-A.C., & M.S. Foster, (eds.) 1994. Measuring and monitoring biological diversity. Standard methods for amphibians. Washington & London, Smithsonian Institution Press, 364 pp.

Equipment List

1. General Searches

- a. GPS unit preloaded with coordinates of navigation points defining the search blocks. Unit must be set to Projection = UTM, Zone 13 and Datum = NAD 1983.
- b. Map of search blocks and navigation points.
- c. Table listing the search times for each search block.
- d. Survey datasheets.
- e. Copy of the following references:
 - i. Toad size chart
 - ii. Wind Scale
 - iii. Sky chart
 - iv. Toad identification guide
- f. Extra pens or pencils for recording data.
- g. Countdown timer.
- h. Digital camera to take pictures of toads, egg masses and habitat.
- i. Photographic background for taking pictures of the dorsum of toads, which is divided into a metric grid.

- j. Digital scale to weigh adult toads.
- k. Holding container for toads. A medium-sized plastic container is good. The container must be sanitized for each new toad.
- l. Rubber gloves for handling toads.
- m. Garbage bag to store used supplies (rubber gloves, swap wrappers, etc.)
- n. Rubber boots, preferably hip boots since some search blocks contain water that is over knee-high.
- o. Thermometers for measuring ambient air temperature and water temperature.
- p. Yard-stick or ruler for measuring height of camera when taking habitat photographs and for measuring depth of water.
- q. Pin flags, for marking the boundaries of search blocks prior to searches.
- r. Small aquarium dip nets to facilitate capture of toads and tadpoles.
- s. Spare batteries for all electronic equipment (e.g., GPS unit, camera, scale).
- t. Handheld counter for counting toadlets and/or young of the year (optional).

2. Supplies for implanting PIT tags.

- a. Surgical Glue
- b. PIT tags
- c. PIT tag injectors
- d. PIT tag reader
- e. Sterilization materials for tags and tag injectors
- f. 70 percent ethanol and sterile water or saline
- g. Gloves (Latex or Nitrile)

3. Supplies for collecting *Bd* swabs.

- a. Specimen datasheet
- b. 70 percent EtOH
- c. Cotton tipped application (“swabs”)
- d. Powder-free latex or nitrile gloves
- e. Alcohol sanitizer for hands (Purell brand is recommended)
- f. Water and alcohol-proof pens (VWR brand is recommended)
- g. Waterproof notebook (Rite in the Rain® brand is recommended)
- h. Sharp-pointed dissecting probe
- i. Waste bag or container
- j. Collection bags
- k. Closeable bags (Zip-Loc® or Whirl-Pac® style recommended)
- l. Vial storage boxes
- m. Bleach Solution: 1 part commercial bleach (5 percent sodium hypochlorite) to 9 parts water

TABLE 1: Beaufort Wind Scale

Scale (force)	Wind (MPH)	WMO Class	Effect on Water	Effect on Land
0	under 1	Calm	Sea surface smooth and mirror-like	Calm, smoke rises vertically
1	1 – 3	Light Air	Scaly ripples, no foam crests	Smoke drift indicates wind direction, still wind vanes
2	4 – 7	Light Breeze	Small wavelets, crests glassy, no breaking	Wind felt on face, leaves rustle, vanes begin to move
3	8 – 12	Gentle Breeze	Large wavelets, crests begin to break, scattered whitecaps	Leaves and small twigs constantly moving, light flags extended
4	13-18	Moderate Breeze	Small waves 1-4 ft. becoming longer, numerous whitecaps	Dust, leaves, and loose paper lifted, small tree branches move
5	19-24	Fresh Breeze	Moderate waves 4-8 ft. taking longer form, many whitecaps, some spray	Small trees in leaf begin to sway
6	25-31	Strong Breeze	Larger waves 8-13 ft., whitecaps common, more spray	Larger tree branches moving, whistling in wires
7	32-38	Near Gale	Sea heaps up, waves 13-20 ft., white foam streaks off breakers	Whole trees moving, resistance felt walking against wind

TABLE 2: Sky Cover / Weather Scale

Scale	Condition
0	0-15% cloud cover
1	16-50% cloud cover
2	51-75% cloud cover
3	76-100% cloud cover
4	fog
5	drizzle
6	light rain
7	heavy rain

Appendix H. WYOMING TOAD CAPTIVE BREEDING

TABLE 1: Wyoming Toad Captive Breeding Summary: Total Wyoming Toads Released by Breeding Facility (1995-2012)

Breeding Facility	TOTAL
Saratoga NFH	62,484
Sybille/Red buttes	54,412
Mississippi River Museum	17,503
Cheyenne Mountain Zoo	6,938
Como Zoo	4,368
Toronto Zoo	3,524
Toledo Zoo	3,011
Omaha Zoo	2,610
Memphis Zoo	1,700
Kansas City Zoo	1,129
Detroit Zoo	901
Houston Zoo	800
Central Park Zoo	512
Cincinnati Zoo	429
Prospect Zoo	200
St. Louis Zoo	150
Totals	160,671

Appendix I. RESEARCH PRIORITIES FOR THE WYOMING TOAD

Compiled by the Research Committee of the Wyoming Toad Recovery Team
February 17, 2011

Doug Keinath
Connie Keeler-Foster
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1. Population Demographics

Research is needed to determine the demographic parameters limiting reintroduced populations of Wyoming toads, thereby providing a better understanding of what is preventing successful establishment in the wild and how these factors vary across habitats. Estimates of demographic parameters should be used to inform a revised Population Viability Analysis of the Wyoming toad that explores the efficacy of various management alternatives.

a. Establish a standardized list of demographic parameters.

In order to target reintroduction activities, monitoring efforts and management actions, monitoring should target the estimation of age and sex specific vital rates of toads in wild populations. This data collection should include demographic components for all age classes, including survival rates, growth rates, reproductive rates, causes of mortality, and relative population numbers. To make these data most useful, a subset of the WTRT should establish a standard list of demographic rates to collect. In particular, the age durations of different survival and growth rates should be clarified, based on biological relevance and practicality.

b. Develop dataset of annual demographic parameters for wild toad populations

To understand the bottlenecks that are limiting recovery of wild populations, data are needed on both the basic vital rate of wild toads, including their variation in time and space, and also on the environmental factors driving these rates. Standard protocols to estimate survival, growth, and reproductive rates at all existing and many future wild population sites should be established. It is important that rates be estimated across multiple years and at multiple populations as only this replication will give understanding of the consistency or differences of limiting factors for toads. In addition, environmental factors and causes of death need to be determined across time and space. Among the key factors that should be measured are: environmental factors (e.g., stream flow, rainfall), habitat features (e.g., vegetation, water chemistry), known threats (e.g., disease, predation).

Reaching these goals will require coordination with the development of an effective monitoring program that can provide data on individual survival and growth rates in the wild. It is also likely that some demographic rates will be difficult to estimate without experiments

that alter the release ages of some captive toads. In particular, holding some toads until metamorphosis and then releasing them may be needed to clearly estimate and thus understand post-metamorphosis survival and growth patterns.

c. Develop dataset of annual demographic parameters for captive toad populations

In order to better understand wild toad ecology, it will be useful to have clear estimates of survival, growth, and reproductive rates in captivity. These data will not only serve as a point of comparison with data from wild animals, but will also allow projection of animals for release.

d. Identify causes influencing specific parameters in wild toads

Targeted efforts to establish cause of death for wild toads should be undertaken. Particular variables to be considered are: local predators, disease, pollution, lack of hibernacula, or other factors. Intensive surveys for dead and dying animals should be conducted to establish cause of death, and repeated survey efforts to establish timing of death will also be useful in understanding causal factors. A plan to conduct a series of such focused analyses is needed to ensure that data on different mortality sources can be directly compared. It is also important to emphasize that while establishing cause of death is more difficult and cannot be conducted as comprehensively as estimation of overall mortality rates, the mortality rate estimates will be useful even when not coupled with detailed cause-of-death data at all sites and times.

e. Use above information to inform a new Population Viability Analysis for the Wyoming toad

To synthesize the data described above, a new PVA (population viability analysis) should be developed. The particular goals of this model development and analysis should be:

1. To prioritize ongoing data collection efforts. A PVA can be used to most effectively target new data collection by establishing the importance for population growth and extinction estimates of uncertainty in different vital rates.
2. To determine how population growth rates vary with habitat factors, leading to identification of the most suitable sites for releases.
3. To understand the relative importance of different lifestages and vital rates in limiting population recovery.

4. Habitat Requirements

This section largely deals with habitat as it directly affects toads. Habitat components that are linked to pathogen assessment are covered under Environmental Factors in the section on Health and Disease.

The goal of habitat research is to establish a site suitability profile that will ultimately define a set of parameters that optimize survival of Wyoming toads, including those released from captivity. Thus, it will inform selection of release sites as well as management of existing toad populations. Since the focus of habitat research is to determine environmental features foster and/or hinder toad survival and recruitment in the wild, this section links directly to item 4 of Population Demographics (i.e., identify causes influencing specific parameters in wild toads).

Currently, habitat research is hampered by the limited number of sites containing “wild” populations. If data were obtained from a sufficient set of different release sites, it could be possible to isolate variables that impact survival. Thus, future release sites could be chosen to intentionally capture new environmental variables.

Habitat information will also play a critical role in managing this species in the face of global climate change. Having well-defined habitat requirements will allow managers to buffer the effects of a changing environment and provide conditions favorable to Wyoming toads in the long term.

a. *Physical Habitat Characteristics*

Habitat structure is an important component to toad survival. Changes in structure may affect toad predation, thermal gradients, humidity levels, and prey availability. A better understanding of cover is needed to determine its effect on toad populations. This could include research focused on both microhabitat and landscape scales. Research could also be conducted examining pathogen/cover interactions.

Examples of Key Research Questions

1. Does decreased cover result in higher predation rates?
2. Does increased cover result in non-preferred thermal regimes?
3. How much aquatic cover is necessary for tadpole survival?
4. Is *Batrachochytrium dendrobatidis* (*Bd*) transmission increased in cooler microhabitats?
5. Do toads prefer a specific water chemistry or temperature?
6. Do toads prefer specific soil characteristics for breeding or hibernation?
7. Are invasive floral species directly affecting toad populations present?
8. In areas of high toad mortality are specific environmental contaminants present?

b. *Hydrology*

Hydrology affects many aspects of the Wyoming toad lifecycle, so knowledge of preferred hydrologic regimes will foster our ability to establish and maintain toad populations on the landscape. One way hydrology could be addressed is to perform dimensional analyses of flow, connectivity and runoff patterns at sites and determine their influence on toad habitat selection and ultimately population dynamics.

Examples of Key Research Questions

1. Do specific water features or hydrologic patterns (e.g., flooding cycles) result in increased toad survival?
2. Do toads prefer palustrine or lacustrine habitats, and is *Bd* more prevalent in either habitat?
3. Do specific flow regimes change transmission rates in *Bd*?
4. How does water management impact habitat suitability?
5. Do toads need a specific juxtaposition of aquatic habitats to thrive?
6. How would global climate changes affect current water resources? What can be done to ensure availability of suitable aquatic habitat for Wyoming toads?

5. Predator-Prey Relationships

a. *Predators*

Predation is a potentially important factor affecting wild populations and can often drive population dynamics. Therefore, understanding (and potentially reducing) natural mortality rates could be important in maintaining or expanding Wyoming toad populations. This topic should address what is currently preying on Wyoming toads, how predator populations can be managed, and how toads can generally be protected after release. Predation is integrally related to items 1 – 5 regarding Population Demographics.

Examples of Key Research Questions

1. What is preying upon Wyoming toads?
2. How is predation affecting population demographics?
3. Is it necessary to reduce predatory populations to increase toad survival, and is this feasible?
4. What mechanical techniques can be applied to reduce predator/ toad interactions?

b. *Prey*

Prey availability is important to questions regarding Wyoming toad persistence. If insufficient prey items are available, toad populations may be negatively affected through resulting increases in emigration, increases in mortality, and decreases in reproduction.

Examples of Key Research Questions

1. What are Wyoming toads feeding upon in the wild?
2. How abundant are preferred prey within reintroduction sites?
3. How do diets vary between reintroduction sites? Does this correlate with Population Demographics?
4. Are there specific, limiting items of which increase toad survival?

5. If shortcomings in prey populations are identified, can they be increased through management?
6. Does grazing influence the ability of toads to successfully find prey?

6. **Reintroduction Strategies**

Captive management, including propagation, rearing, and maintenance, plays a critical role in the recovery efforts for the Wyoming toad and other species by 1) producing animals for reintroduction and recovery efforts, 2) maintaining genetically robust populations to reduce extinction risk, 3) providing animals for research purposes, 4) maintaining populations until short-term stochastic events have passed, such as drought or disease outbreaks and 5) providing animals for education and outreach purposes.

Improvement of activities associated with reintroduction efforts should be a continual goal. The Wyoming toad program has established protocols to rear and release toads. Research should now focus on how to improve reintroduction to maximize survival and successful adaptation to wild environments. The process of “stocking” captively-propagated animals is seductive, and in many instances the involved parties walk away with a feeling of a job well done, but fail to follow through with monitoring to determine the success of the program.

a. ***Release Methods***

Captive propagation of poikilotherms and subsequent release has for the most part been based on a fishery model wherein the animals are transported to the release site, released en-masse into a single location, and left to sink or swim (literally). This method is defined as a hard release. Captive propagation programs targeting warm-blooded animals have increasingly focused on optimizing the release effort by establishing an enclosure, and acclimating animals to the area prior to release, effectively “softening” the transition period. A soft release typically includes providing the basic necessities for the duration of the release period, and beyond, including food, water and shelter. Though potential benefits are large, it is unclear whether Wyoming toad could benefit by a soft-release approach. Research should therefore be focused on evaluating the potential effectiveness of hard versus soft release strategies.

Examples of Key Research Questions

1. What is the efficacy of ‘soft’ release strategies?
2. How can soft release methods improve survival?
3. What is the optimum soft release strategy?
4. What is the best option for duration of release period, feeding and monitoring of released individuals?

b. ***Toad Release Profile***

The goal of research in this section is to define what traits of released toads enhance wild survival and reproduction, ultimately resulting in self-sustaining wild populations. In addition to suggesting specific research, development of a complete release profile will incorporate information on Host Factors described in the section on Health and Disease.

Research should be conducted to determine selection criteria that can be used to identify which individuals or cohorts are most suitable for release based on criteria such as age at release (e.g., tadpole, toadlet, adult), physiology, genetics, and disease resistance. These issues could be investigated, in part, by establishing release strategies at multiple lifestages (i.e. tadpoles, toadlets, young adults, etc.), utilizing *in-situ* research enclosures, using available genetic information to genetically tag cohorts for study, and/or establishing a unified data collection requirement for each stock release.

As part of establishing a toad release profile, it will be necessary to evaluate the fate of released toads, as suggested in the sections on Population Demographics and Survey and Monitoring. Similarly, causes of mortality are largely hypothetical and their relative importance at specific reintroduction sites is unknown. To determine possible management actions, it is necessary to determine the fate of released animals. One way this could be accomplished is by conducting controlled releases in enclosures to assess the fate of released animals. Additionally, genetic markers are now available, and by selectively mating individuals for research in release strategies, it will be possible to collect animals in the field, swab their buccal cavity, and determine which cohort trial they were associated with.

Examples of Key Research Questions

1. What is the best lifestage, or combinations of lifestages, at which toads should be released?
2. What are the best time/ season to conduct releases?
3. What are the key parameters associated with increased survival after release?
4. Can we enhance toad resistance to environmental stress (including disease resistance) through selective breeding or other methods? (For specifics, see the discussion on Host Factors in the Section on Health and Disease.)

7. Survey and Monitoring

We should strive for continual improvement of activities associated with survey and monitoring efforts. Investigation should focus on how we can improve methods to find, monitor, and study Wyoming toads in the wild.

a. *Population Monitoring*

Population monitoring is an essential component for assessing the success of Wyoming toad releases, and determining population health. Recently, a great improvement over previous monitoring efforts has been made through the implementation of standardized visual encounter surveys for all Wyoming toad populations. However, additional and/or

modified techniques may increase our knowledge of toad populations. The following are examples of research that could be undertaken to increase our ability to monitor toad populations.

- i. Frogloggers: We should investigate how to best employ Frogloggers for monitoring, including refining our understanding of when toads call relative to weather and time of day, what percent of toads call, and how old/large toads are when they start calling.
- ii. Tracking Individuals: We should investigate the use of technologies that allow location of hibernacula, analysis of toad movement, relocation of marked toads and/or mortality recovery. Promising technologies in this regard might be PIT tag telemetry or harmonic radar. This type of tracking will also facilitate examination of Population Demographics.
- iii. Mark Recapture: Current monitoring efforts use visual encounter surveys methods to estimate wild populations. If mark-recapture surveys are deemed necessary, marking technologies should be refined to be minimally invasive to toads and maximally efficient in terms of cost and field effort. Adult toads are currently PIT tagged, but until 2013, numbers of toads are so low that mark-recapture analysis is not possible. PIT tags also have several shortcomings including: 1) invasive operations and the risk of infection, 2) extensive time and effort in the field that has heretofore resulted in not all animals being tagged, and 3) many animals are left unmarked because they are not large enough for tag implantation.
- iv. Genetic monitoring: As wild populations increase, it is important to assess the genetic makeup of wild individuals. Although effort has been targeted toward captive populations to ensure genetic diversity, wild populations may represent a subsection of captive genetics. It is necessary to ensure that further bottlenecks do not occur in wild populations.

Examples of Key Research Questions

1. When do Wyoming toads breed?
 - a. When are males calling in the wild?
 - b. As climate changes, do males breed during different times of year?
2. Where are toads hibernating?
 - a. Are suitable hibernation sites limited in reintroduction sites?
3. What is the best way to mark wild toads?
 - a. Do current methods increase toad mortality?
 - b. Do current marking methods satisfy the criteria of mark-recapture analyses?
4. What is the genetic composition of wild toads?
 - a. Are only a subsection of captive genetics present in any given toad population?

b. Is there a specific phenotype better adapted to toad survival in the wild?

b. ***Environmental Monitoring***

We have the capacity to collect environmental data in association with toad monitoring efforts, such as temperature, water chemistry, vegetative cover and other habitat characteristics. Research recommendations outlined in sections on Habitat Requirements (discussed above) and Health and Disease (discussed below) should be used to identify the factors that should ultimately be incorporated into an environmental monitoring program. Once environmental factors have thus been identified, we can implement environmental monitoring research to standardize and optimize collection of such data and refine long-term analysis thereof.

Examples of Key Research Questions

1. What levels of pesticides and herbicides exist in waters of reintroduction sites, and how do these levels change both within seasons and across years?

8. Health and Disease

This category includes studies investigating the diagnosis, characterization, pathogenesis, control, treatment and impact of factors on toad and toad population health. For organizational purposes questions are separated into the traditional epidemiologic categories of Environmental, Host and Agent factors. There are certain infectious agents that are currently considered high threats to anuran populations including *Bd* and ranavirus. Non-infectious agents including glyphosate with POEA surfactants, triazine, organophosphates, and nitrogen fertilizers are also considered to have high potential for disruption of anuran populations. These agents are currently sufficiently prominent to be given particular priority.

a. ***Host Factors***

Host factors relate to the robustness of the Wyoming toad in the face of exposure to an infectious agent or suboptimal environmental conditions, including nutritional deficiencies. Functional variation in physiology, immune function and metabolism can result in differential survival in the face of specific insults.

Examples of Key Research Questions

1. What is the feasibility of developing disease resistant Wyoming toads using captive breeding of individuals that have remained healthy in pathogen positive environments?

- a. Research bioaugmentation methodology to treat *Bd*
 - i. **Inoculations of *Janthinobacterium lividum*:** Research conducted by Harris et al. (2009) found that by adding a naturally occurring antifungal bacterium, *Janthinobacterium lividum* found on the skin of several species of

amphibians, to the skin of the mountain yellow-legged frog (*Rana muscosa*) prevented mortality caused by *Bd*.

1. Is *J. lividum* an effective treatment against *Bd* for Wyoming toads?
 2. Are toads inoculated with *J. lividum* able to pass the bacteria on to tank mates or other toads in the wild?
 3. What is the longevity of the inoculation with *J. lividum*?
 4. Can *J. lividum* be passed on to subsequent generations in the wild?
- ii. ***Daphnia* spp. treatments:** Research conducted by Buck et al. (2011) found that *Daphnia* spp. will consume the zoospores of *Bd* in a laboratory setting.
1. Can *Daphnia* spp. be raised and released at reintroduction sites to increase the naturally occurring native *Daphnia* spp. populations?
 2. What concentration of *Daphnia* spp. would be necessary to make a significant impact on *Bd* prevalence and infection rates?
 3. What species of *Daphnia* spp. are currently present at Mortenson? Only native species should be considered to avoid introducing a non-native species.
- iii. **Increasing immunity to *Bd* through pre-exposure:** Preliminary research has shown that mountain yellow-legged frogs (*Rana muscosa*, *Rana sierrae*) can acquire immunity to *Bd* (Knapp 2013 pers. comm.). Individual adult frogs that were previously exposed to *Bd* and subsequently cleared of the infection demonstrated a high degree of resistance to infection and high survivorship upon re-exposure.
1. Will Wyoming toads treated with *Bd* and cleared of the infection before release have a higher rate of survivorship than *Bd* naïve individuals?

c. *Environmental Factors*

Environmental factors relate to the impact of external conditions on the health of the Wyoming toad. These can impact dose response and endpoint of response to both infectious and noninfectious diseases.

Examples of Key Research Questions

1. What are the impacts of natural waterbody characteristics (e.g., pH, pO₂, ion content, nitrate, organics content) that might affect the biology and survival of infectious agents of interest?
2. What are the impacts of weather (temperature, rainfall) that might affect the biology and survival of infectious agents of interest?
3. Manipulation of the environment to impact survival such as modifying water chemistry, chemical, or even biological control of agent availability or pathogenicity such as external applications of probiotic bacteria to modify environmental conditions.
4. Creation of open basking areas through physical manipulation of the environment to allow toads to clear *Bd* infections.
5. Development of techniques to detect *Bd* in the environment.

c. *Agent Factors*

Agent factors relate specifically to the persistence, life cycle, and pathogenicity of the agent of disease. This includes examination of dose response (for either physical or infectious agents) and evaluation of the seasonal/ environmental impacts of Agent Factors that inform toad management.

Examples of Key Research Questions

1. What density of zoospores of *Bd* in natural water bodies can infect toads?
2. How does dose correlate with intensity of infection?
3. How does the agent move? What are the vectors and persistence of the agent?

9. **Captive Husbandry**

The success of maintaining captive Wyoming toad populations and promoting captive breeding to support reintroduction efforts has improved markedly in the past decade, but there are several areas that should be considered for additional research. All research should be undertaken to meet and enhance the considerations of the joint U. S. Fish and Wildlife Service and National Oceanic and Atmospheric Administration Controlled Propagation of Species Listed under the Endangered Species Act (FR 2000), which states:

“Our policy is that the controlled propagation of threatened and endangered species will be used as a recovery strategy only when other measures employed to maintain or improve a listed species' status in the wild have failed, are determined to be likely to fail, are shown to be ineffective in overcoming extant factors limiting recovery, or would be insufficient to achieve full recovery. All reasonable effort should be made to accomplish conservation measures that enable a listed species to recover in the wild, with or without intervention, prior to implementing controlled propagation for reintroduction or supplementation.”

a. *Chemical Contaminants*

Wild populations of amphibians are exposed to chemicals incidentally, including pesticides, herbicides, and other forms of surface and ground water contamination. Captive populations may be exposed through this avenue via a contaminated water source, but perhaps more important are the long-term impacts of chemical applications specific to the husbandry of Wyoming toads. Wyoming toads are artificially stimulated to reproduce using hormone injections and are treated in many cases with chemicals used to control pathogens. Increased exposure to hormones, chemicals, and byproducts of the captive environment, such as organic waste, urea, ammonium, cleansers, and disinfectants, may impact longevity, reproductive success, release survival, and progeny. Research should focus on specific chemicals currently used in the captive program and any new chemicals that may impact the species, such as 1) water quality in recirculation systems, 2) hormone impact on fecundity, longevity, and metabolic bone disease, 3) short and long-term effects of chemical disease

treatments, 4) presence of contaminants such as environmental estrogens in domestic water supplies, and 5) other chemicals to which captive animals may be exposed.

Key Research Questions

1. Does the use of hormonal intervention into the reproductive cycle of toads reduce longevity?
2. Does the use of hormonally induced reproduction increase, decrease, or impact fecundity?
3. Does the use of hormonally induced reproduction play a role in short tongue syndrome or metabolic bone disease?
4. What is the effect of environmental estrogens on Wyoming toad?
5. What are the long-term impacts of treatments for *Bd*?

b. *Nutrition*

Nutrition for captive reptiles and amphibians continues to be a major impediment to the success of captive maintenance, reproduction and longevity. Specifically, metabolic bone disease, short tongue syndrome, vitamin supplementation and delivery methodology are all poorly understood. Current procedures and protocols are based on successful programs, but all facilities recognize deficiencies still exist. Categories for research consideration should minimally include:

1. **Diet Optimization:** Develop a better understanding of optimal toad diets. Specifically, diets should meet all nutritional needs of toads to facilitate growth, fecundity, and disease resistance, as well as eliminating the occurrence of morbidity resulting from nutrient-deficiencies. Are probiotics delivered in food beneficial?
2. **Short-tongue Syndrome:** Short-tongue syndrome is still a concern and should be further studied. For example, the following are priorities for investigation:
 - a. Develop a better understanding of the vitamin A/ carotenoid requirements of toads and how they relate to the development of squamous metaplasia, or short-tongue syndrome.
 - b. Develop adaptive management strategies that minimize the potential for short tongue syndrome in captive toads. This should begin with a strategy for supplementing toad diets and not using expired supplements, but should eventually incorporate the results of research investigating the optimal method for meeting the vitamin A/ carotenoid requirements of toads.
3. **Metabolic bone disease:** Identify key vitamin and micronutrient requirements as well as optimal ratios. Identify optimally efficient method for supplementation

4. Supplemental lighting: Evaluate the role of supplemental UVB in metabolism, including calcium/ phosphorus ratios and calcium availability.
5. Nutritional studies are usually placed in the Host Factors category and would include studies related to vitamin A deficiency and the use of probiotics.

Examples of Key Research Questions

1. What are the nutrient requirements of wild toads, and how do captive diets differ?
2. What is the optimum method of vitamin supplementation and with what vitamins?
3. What readily available food items are the closest to toad's natural diet?
4. How can we prevent short-tongue syndrome?
5. How can we prevent metabolic bone disease?
6. Does nutrition play a role in hibernacula death?

10. Captive Reproduction

Captive reproduction plays a critical role in the recovery of the Wyoming toad. However, captive reproduction is not without pitfalls, and should not be relied on as the only means of recovery. Concurrent efforts to mitigate the circumstances that caused the decline of the Wyoming toad are essential for true recovery. Problems that may and in some instances have occurred in the captive program include: 1) problems with reliable and predictable reproduction, 2) unknown success in reintroductions, 3) high costs, 4) domestication, 5) preclusion of other recovery techniques, 6) disease outbreaks, and 7) programmatic continuity.

Captive reproduction remains imprecise and inconsistent at most facilities. Thus, research is needed to optimize the success of reproduction in terms of viable egg-masses, number of offspring produced, increased metamorph success, growth rate of progeny and the health and genetic composition of offspring.

a. *Breeding Strategies*

More research is needed to ensure stable, predictable reproduction. Currently, several facilities have good reproductive rates, while others do not. Even facilities with generally good rates exhibit large fluctuations in reproductive success from year-to-year and pairing-to-pairing. Further, no facility has a predictive protocol that allows managers to choose two parents and reliably mate those individuals.

Examples of Key Research Questions

1. Can the toads be cued to reproduce by altering the physical environment (e.g., water temperature, diurnal patterns, humidity artificial/natural hibernation)?
2. What is the optimum hibernation method and timing to enhance breeding?
3. What are the main causes of hibernacula mortalities?
4. How can we better synchronize reproduction and increase rates of fertilization?

5. How can we increase the number of successful tadpoles and toadlets?
6. How can we improve the number of toads that survive metamorphosis?
7. Is colony breeding more productive than one-on-one pairings?
8. What role can the cryopreservation of sperm play in optimizing breeding and/or genetic diversity of captive populations?

b. *Founder Genetics*

Conduct a genetic assessment of founders and captive populations that can be used to further assess inbreeding coefficients of a given toad population and functional genomics (e.g., disease susceptibility). Research is needed to determine: 1) MHC polymorphisms and their role in disease resistance, 2) assess toads resistant to *Bd* to determine if genetics plays a role (for example, when challenged with *Bd*, assess whether gene expression contributes to improved immune response), and 3) DNA sampling and extraction from buccal swabs.

Examples of Key Research Questions

1. Do Wyoming toads have multiple MHC polymorphisms, and does this play a role in disease resistance?
2. Can we test toads that survive a *Bd* outbreak to determine if genetics plays a role?
3. How are conserved genes changing over the duration of the captive propagation program?