U.S. Fish & Wildlife Service

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

Revised Recovery Plan 2015

Original Approved Recovery Plan, September 11, 1991

Photo by Sarah Armstrong, Wyoming Toad Studbook Keeper

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U.S. Fish and Wildlife Service
Denver, Colorado
May 2015

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REVISED RECOVERY PLAN 2015

Original Recovery Plan Completed in 1991

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

Approved: ______________________________
Deputy Regional Director, U.S. Fish and Wildlife Service

Date: 7·16·15

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U.S. Fish and Wildlife Service. 2015. Wyoming Toad *Bufo hemiophrys baxteri* now known as *Anaxyrus baxteri* Revised Recovery Plan, May 2015; Original Approved September 11, 1991. U.S. Fish and Wildlife Service, Cheyenne, Wyoming.

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

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

ACKNOWLEDGMENTS

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. 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 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 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 and provided an invaluable historical perspective. His thesis provides the basis for our knowledge of the toad's natural history. His dedication to this effort was inspiring to his colleagues and to the many emerging scientists who were fortunate enough to interact with him.

Recovery Team and Primary Authors

The Service gratefully acknowledges the commitment and efforts of the Wyoming Toad Recovery Team (WTRT) members and those active in the recovery of the Wyoming toad. Without their assistance and valuable input, this recovery plan would not have been possible. The following individuals are either members of the 2015 WTRT or had either a primary role in preparing and reviewing the final versions of this document:

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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.*) and is considered one of the four most endangered amphibian species in North America (IUCN 2011). The last ten toads believed to exist in the wild were taken into captivity in 1989 for breeding. The captive breeding program, currently consisting of eight zoos, one fish hatchery, and the University of Wyoming Red Buttes Environmental Laboratory, has been successful in producing tadpoles and toadlets for annual releases. However, releases of captive bred tadpoles and toadlets have resulted in tenuous wild populations and very little wild breeding to date. 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 450,000 acres (1,820 km^2 or 700 mi^2) of habitat consisting of flood plains, ponds, and small seepage lakes in the shortgrass communities of the Laramie Basin in Albany County, Wyoming.

Habitat Requirements and Limiting Factors: The Wyoming 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 two release sites created under a Safe Harbor Agreement. Limited habitat use has been studied at Mortenson Lake although specific life history remains unknown. Primary threats at the time of listing (1984) were identified as a limited distribution, habitat manipulation, disease, and small population size. Primary concerns today include limited distribution and a lack of suitable habitat available for reintroductions, disease (specifically chytridiomycosis, an infectious disease of amphibians caused by the pathogenic fungus, *Batrachochytrium dendrobatidis*), and small population size.

Recovery Strategy: 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. An adaptive management approach, which allows for the continual inclusion of updated research and information, will be the main strategy guiding the management of the species. The captive program maximizes genetic diversity in its annual breeding and continuously develops husbandry strategies to maximize the health of captive populations. Increased knowledge of the needs of wild toads is crucial for improved science-based management decisions and conservation actions. Many of the necessary actions for species protection are based on an increased understanding of disease dynamics and the relationship of the Wyoming toad to its physical, chemical, and ecological environment.

Recovery Goal: The ultimate recovery goal is to allow downlisting and ultimately delisting of the Wyoming toad.

Recovery Objective: The recovery objectives are to reduce threats to the Wyoming toad, allowing for the establishment of self-sustaining wild populations. For this to occur, captive populations with maximized genetic variability will need to be maintained at a sufficient level, suitable habitat will need to be restored and/or identified, and disease will need to be suppressed to a level to which it is not a threat to the viability of the wild populations.

Recovery Criteria:

A. Reclassification to Threatened Criteria

(1) Three self-sustaining and viable populations of the Wyoming toad (as evidenced by a Population Viability Analysis (PVA)) are established within or nearby the toad's historic range and remain viable for a minimum of seven consecutive years. 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 and Doak 2002) when the data are available (e.g. reproduction and overwinter survival are occurring).

(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) A peer-reviewed, long-term adaptive management plan is in place to guide conservation efforts of captive and wild populations for 25 years after downlisting. This management plan will provide a framework to maximize the health of and minimize genetic loss in the captive population and maintain the viability of wild populations. It will address the threats identified in the factors section of this plan (Section 2) and any potential threats that may arise that have not been identified to allow for continued recovery of this species.

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 populations) and are viable as evidenced by a current PVA. Benchmark criteria for viability are the same for delisting as for downlisting and include time horizon, quasi-extinction threshold, and exact probability of persistence (Dennis et al. 1991, Morris and Doak 2002).

(2) The long-term adaptive management plan created for downlisting will be updated and peer-reviewed to guide conservation efforts for 25 years after delisting. This comprehensive plan will include detailed monitoring protocols to ensure the continued viability of the five populations established to warrant delisting of the species. It will also address the

threats identified in the factors section of this plan (Section 2) and any potential threats that may arise that have not been identified to allow for continued persistence of this species.

Recovery Actions: The recovery program for the Wyoming toad is divided into the following areas of action:

- Captive Population Management (4.3.1) and Research (4.3.2),
- Wild Population Management (4.3.3), Monitoring (4.3.4), and Research (4.3.6),
- Identify New Release Sites (4.3.5),
- Planning and Adaptive Management (4.3.7), and
- Outreach and Cooperation (4.3.8).

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 2030. 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.

GLOSSARY OF TECHNICAL TERMS

Amplexus: breeding grasp.
Anuran: frogs or toads
Arid: little or no rain; very dry.
Carbamate: class of chemicals that acts on enzymes of the central nervous system by inhibiting a specific enzyme, acetochlolinesterase, damaging nerve function.
Chytridiomycosis: is an infectious disease of amphibians, caused by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*.
Cranial crest: bony ridge on the dorsal surface of the head of some species of toads.
Dorsal: of, on, or relating to the upper side or back of an animal, plant, or organ
Emergent: plants that are rooted below the water surface but extend out of the water.
Extirpation: when a species ceases to exist in an area, but still exists elsewhere; local extinction.
Hard release: a reintroduction of a species directly into the environment with no intermediary protection, such as a mesh enclosure to protect tadpoles.
Histology: a branch of anatomy that deals with the minute structure of animal and plant tissues as discernible with the microscope.
Listed: a species recognized by Federal or State governments as endangered or threatened.
Metaplasia: transformation of cells from a normal to an abnormal state.
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.
Nuptial pad (thumb pad): darkened and swollen thumbs present on the front limbs of sexually mature male toads which aid in gripping females during amplexus.
Parotoid glands: the toxin producing shoulder glands of toads.
Ranidae (Ranids): referring to the family of true frogs.
Shortgrass prairie: A semi-arid rangeland ecosystem dominated by short grass species.
Soft release: a reintroduction involving intermediary protection during a release before being introduced directly to the environment, such as a mesh enclosure to protect tadpoles.
Taxon: is a group of one or more populations of an organism seen by taxonomists to form a unit.
Taxonomy: orderly classification of species according to their presumed natural relationships.
Throat patch: area on the chin of a toad where the vocal sac is located.
Tubercles: Small dark patches on the bottom of the back feet that aid in digging.
Vent: the external cloacal opening; the anus.
Zoospore: a motile, asexual flagellated spore.

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1. 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. Recovery plans describe the steps needed to restore a species to ecological health, with the ultimate goal of "recovering" species so they no longer need protection under the ESA. This plan was written and implemented by Service biologists 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:	*Bufo hemiophrys baxteri* (now known as *Anaxyrus baxteri*)
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 Wyoming toad, originally discovered in 1946 by Dr. George Baxter, was common in the Laramie Basin from the 1950s through early 1970s. Populations crashed precipitously in the mid-1970s and by the 1980s, individuals were extremely rare (Baxter and Stone 1985). The species was federally listed as endangered in 1984 under the Endangered Species Act of 1973 (Act) and by 1985, the Wyoming toad was presumed extinct (Baxter and Stromberg 1980, Stromberg 1981, Vankirk 1980, Baxter et al. 1982, Baxter and Stone 1985, Lewis et al. 1985). In 1987, a small, extant population (estimated as 100-150 adults) was discovered at Mortenson Lake (Stone 1991). This population was reproducing successfully and all lifestages were represented until 1989. On September 1, 1989, the last ten Wyoming toads (four young-of-the-year and six yearlings) believed to remain from the Mortenson Lake population were taken into captivity (McCleary 1989). Since 1989, all Wyoming toads are a product of reintroductions. Current distribution is limited to the Laramie Plains, specifically at the Mortenson Lake National Wildlife Refuge (Mortenson Lake) and two release sites created under a Safe Harbor Agreement.

While the precise causes of the Wyoming toad's population decline are unknown, a variety of factors have likely contributed to the decline. Infectious disease, habitat alterations, and contaminants have been suggested as top contributors to the decline. Significant features of the species' life history, behavior, ecological interactions, and habitat needs remain unknown and limit effective management. Recovery of this species requires a standardized scientific approach to determine limiting factors and the species' needs and sustained, long-term implementation of conservation actions. An adaptive management approach, which allows for the continual inclusion of updated research and information, will be necessary for successful management of the species.

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 1C1C 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*) is considered one of the most endangered amphibian species in North America. It is a small toad, measuring approximately 1.75 to 2.75 in (4.5 to 7.0 cm) when fully grown. The life-span of wild Wyoming toads remains unknown, but the oldest Wyoming toad known in captivity is a currently living individual, that as of September 16, 2014 is 9.25 years old (2014 Armstrong pers. comm.). There are 13 instances in the captive breeding history of individuals living eight years or more (2014 Armstrong pers. comm.).

The dorsal ground color of adults may be dark brown, gray, or greenish with small dark blotches and a distinct light median line. Wyoming toads greater than sixth months of age can be identified from other toads in the region by their small size and fused cranial crests (Geraud and Keinath 2004). Cutting tubercles on the hind on the hind feet are well developed in adults.

Wyoming toad males have a dark throat patch, nuptial pads (dark raised skin on the front thumbs which aid in gripping females during amplexus), and are smaller than females. Only male Wyoming toads vocalize. Three distinct vocalizations have been identified: 1) the mating call, a short buzzing trill; 2) the release call, a guttural vibration deeper in pitch than the mating call and used when grasped by other males; and 3) 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 0.29 to 0.32 in (5 to 7 mm) (Withers 1992). Boreal chorus frog (*Pseudacris maculata*) tadpoles can be found alongside Wyoming toad tadpoles, but are lighter brown in color and the eyes protrude laterally, whereas the eyes of toad tadpoles protrude dorsally (Altig et al. 1970).

1.2.2 Taxonomy

The Wyoming toad was originally taxonomically grouped together as the same species as the Canadian toad: *Bufo hemiophrys* (Baxter and Meyer 1982). George Baxter first reported the Wyoming population in 1946 (Baxter 1990 pers. comm.), but it was not designated as a subspecies, *Bufo hemiophrys baxteri* until 1968 (Porter 1968). Porter did not recognize the Wyoming population as a separate species from the Canadian population due to the absence of genetic incompatibility (Porter 1968). Packard (1971) later recommended that the two populations be treated as separate species rather than a subspecies based on two assertions: 1) genetic incompatibility is simply one of several criteria that should be used in determining specific status of a group of organisms, especially given that *Bufo* (now *Anaxyrus*) spp. are notorious for their ability to produce viable and fertile offspring in the laboratory (Blair 1941) and 2) the phenotypic divergence caused by the geographic isolation could appropriately be regarded as coequal with isolation mechanisms having a genetic basis (Packard 1971). The morphological differences between the two populations were later detailed (skull structure, the width of the vertebral line, and parotoid length), and the Wyoming population was given a distinct species rank and renamed *Bufo baxteri* while the Canadian population remained *Bufo hemiophrys* based on these morphological differences (Smith et al. 1998).

The general 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 2012, Collins and Taggart 2009). The taxon is listed as *Anaxyrus baxteri* in the Center for North American Herpetology's 2009 *Standard Common and Current Scientific Names for North American Amphibians, Turtles, Reptiles & Crocodilians* and in the Integrated Taxonomic Information System.

The Service recognizes the full species status designation and now uses the scientific name *Anaxyrus baxteri* for the taxon. A revision to the official Endangered and Threatened Wildlife list of 50 CFR 17.11 should be revised when time allows.

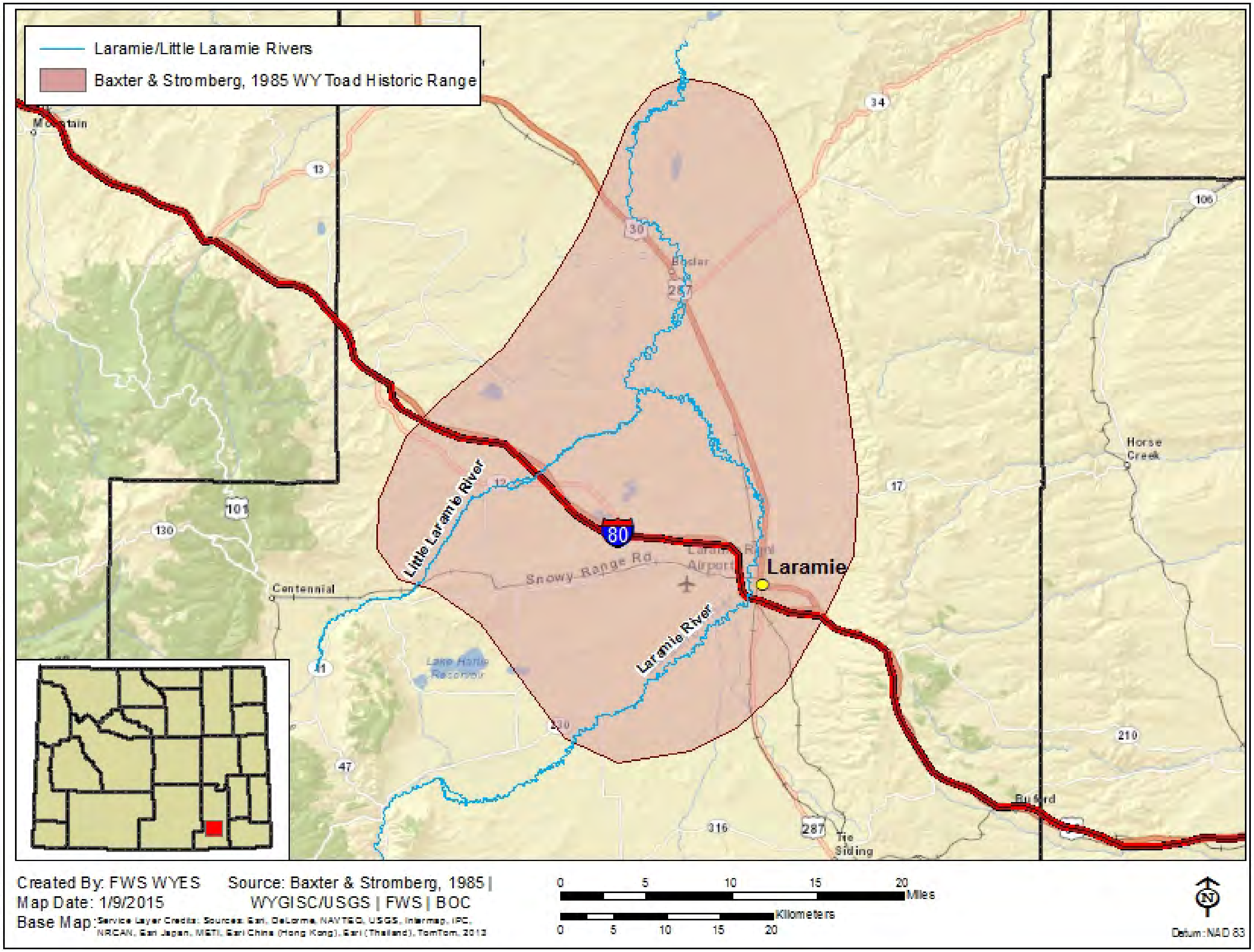
1.3. Distribution and Habitat Use

1.3.1 Distribution

Until the 1970s, the Wyoming toad was common throughout the floodplains of the Laramie River and the margins of ponds and small seepage lakes in the Laramie Basin (Baxter and Stromberg 1985). (Unless explicitly stated otherwise, *Laramie River* as used throughout this document includes both the Little Laramie and the Big Laramie Rivers). The best estimate of the Wyoming toad's historic range is depicted in Figure 1 and was derived from field notes in Baxter and Stromberg (1985). This estimated historic range encompasses approximately 450,000 acres (1,820 km^2 or 700 mi^2) in the Laramie Basin.

Currently, all Wyoming toads are the product of captive bred releases and can be found in the Laramie Basin at Mortenson Lake located on the U.S. Fish and Wildlife Service Mortenson Lake Wildlife Refuge and on two properties covered under the Wyoming Toad Safe Harbor Agreement (SHA). No other populations are known to exist in the wild. Populations have fluctuated over the years, but until 2008, approximately 50 Wyoming toads were reproducing, over-wintering, and persisting at Mortenson Lake and the Buford Safe Harbor 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. Populations at Mortenson Lake have been rebounding in 2013 and 2014 due to the successful implementation of a soft release strategy (See Section 3.3 for more information on soft releases).

Figure 1. HISTORIC RANGE OF THE WYOMING TOAD



The Wyoming toad's historic range was loosely sketched from field notes from the 1960s published in Baxter and Stromberg (1985).

1.4. Habitat Use

Mortenson Lake is a 61 acre (0.25 km^2) lake situated in the shortgrass prairie ecosystem of the Laramie Basin. The vegetation immediately around the lake consists of a mixture of rush, sedge, and grass communities. 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. Uplands are arid and consist of grass with scattered shrubs. While it is frequently thought that Mortenson Lake may not be ideal habitat for the Wyoming toad, this site has had more breeding success and overwinter survival than any other site to date.

Most of the information regarding Wyoming toad habitat use is provided by two studies conducted at Mortenson Lake (Withers 1992, Parker 2003). Both studies illustrate that the Wyoming toad uses a variety of habitats during different lifestages, i.e. embryo, tadpole, juvenile, and adult. While both studies provide useful information, they are not exhaustive and more research is necessary to make informed decisions of potential habitat restoration and new reintroduction sites.

Wyoming toads use a variety of vegetation density throughout their lifecycle and the density tends to increase with age. The smallest toads seek habitats with only limited vegetative cover (Withers 1992) and ample sunlight is required for healthy young-of-the-year growth (Seymour 1972). Additionally, adults seek refuge at night in vegetation that is denser than that used during the day (Parker 2003). 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). Withers (1992) found that Wyoming toad tadpoles tend to prefer warm water and seek deeper water during the evening in response to the nighttime cooling of the shallows. 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 late summer/early fall, but the precise factors that signal hibernation remain unknown. Withers (1992) documented toads hibernating in pocket gopher or ground squirrel burrows between 1.5 and 3 in (3.8 and 5 cm) in diameter on the south side of Mortenson Lake in the boundary between moist and dry soils. Withers (1992) also noted Wyoming toads in the same holes with pocket gophers and chorus frogs. The depths or temperatures at which Wyoming toads hibernate has not been documented.

Despite a wide geographical separation and differences in morphology, the habit use of Wyoming toads is similar to that of Canadian toads: they both frequent the edges of lakes and ponds of the shortgrass prairie ecosystem and are rarely found more than 30 ft (9.1 m) from water (Roberts and Lewin 1979). Canadian toads have also been reported to hibernate in mima-mounds, small mounds of loose soil, by burrowing under the loosened soil. Mima-mounds have also been suggested as possible hibernacula for the Wyoming toad.

1.5. Life History

Reproduction

The Wyoming toad breeding season is from mid-May to mid-June depending on annual weather conditions (McCleary 1989, Withers 1992). Males appear first and begin their mating call, a buzzing trill that lasts a few seconds (Baxter 1952, Withers 1992). In 1991, Withers monitored calling at Mortenson Lake and was found to take place when air temperatures ranged from 63.5°F to 70.0°F (17.5°C to 21.1°C) and water temperatures ranged from 64.2°F to 71.4°F (17.9°C to 21.9°C). 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 early 1990s consisted of only five to 10 individuals (Withers 1992).

Typical of *Anaxyrus* spp., Wyoming toad eggs are deposited in gelatinous strings resembling black pearl necklaces and are often intertwined with vegetation. Characteristics of Wyoming toad breeding and reproduction have only been documented after the major decline of the mid-70s when breeding was minimal and habitat may have been marginal. Withers (1992) documented characteristics of egg masses found at Mortenson Lake between 1988 and 1991. Water temperatures ranged from 69.1°F to 74.7°F (20.6°C to 23.7°C); the fertility of egg masses varied from 0-100%, but was approximately 80% fertile on average over the years; and the number of eggs per mass reported ranged from 1,000 to 6,000 (Freda et al. 1988, Withers 1992). Egg masses were laid in late May to early June and covered with approximately 1.4 to 2.5 in (3.5 to 6.3 cm) of water (Withers 1992). Eggs hatched within a couple days and tadpoles metamorphosed in four to six weeks (Withers 1992). Tadpoles were found in water that was warmer and shallower than that of adjacent water (on average, approximately 35 ºF [1.7ºC] and 1 to 1.7 in [2.5 to 4.2 cm] in depth). The numbers of eggs per egg mass reported from captivity range from 1 to 5,000 eggs (Lipps and Odum 2000) and tadpoles emerged in shorter periods of time when exposed to higher temperatures than found in the wild (Paddock 2009 pers. comm., Palmer 2010 pers. comm.).

In captivity, both males and females have been successfully bred as yearlings. Wild one-year-old males have displayed secondary sexual characteristics such as a dark throat patch and nuptial pads, beginning mid-summer and had mature sperm upon necropsy (Withers 1992). Females do not have obvious external morphologic changes that indicate sexual maturity, but upon necropsy, a small proportion of yearlings had mature eggs in the ovary (Withers 1992). Withers believed that most wild female Wyoming toads did not breed until their second or third year.

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. Withers (1992) documented young-of-the-year toads attempting to catch small black flies common to the open saturated soils of Mortenson Lake. Thirteen scat samples were opportunistically collected in 1998 and 1999 to analyze prey items (Odum 2014 pers. comm.). The most common prey items found were two species of ant: *Myrmica incompleta* and *Formica fusca*. Three genera of beetles were additionally identified: dung beetles (*Canthon* sp.), ground beetles (*Elaphrus* sp.), and *Anara* sp. Although this information is valuable, this limited sample size may not be indicative of the food source of the population as a whole. 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 dusted with supplemental nutrients and other invertebrates that are cultured in-house. At the Service facilities in Wyoming that do not have the same strict biosecurity regulations that prohibit outside food sources as zoos, additional net sweeps for native insects are conducted when possible in order to add a more natural element to captive diets. All Wyoming toad breeding facilities are in constant pursuit of better insect diets and supplements that will enhance nutritional value.

Movement

Compared to other anurans (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 0.25 mi (402 m) 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, the Service released 13 captive adult Wyoming toads with radio transmitters at Mortenson Lake. The north to south migration noted in 1987 was again noted, but this time the migration was made within 24 hours. Daily movements, however, were generally limited. The mean distance that newly introduced captive adult toads moved in 2011 was 124.7 ft (38 m) per day (Hvidsten 2011 pers. comm.). Wyoming toads are rarely found more than 30 ft (9.1 m) from water mostly likely due to the arid environment surrounding their habitat. Two of the 13 toads were able to be tracked through October into hibernation made movements away from the lake or into mammal burrows between August 21 and August 29. The fate of the other 11 toads tracked remains unknown because transmitter signals were lost. Withers (1992) noted that toads moved to the south of Mortenson Lake by late August and September and seek hibernation sites in early September and few are visible after October 1.

2. FIVE FACTOR ANALYSIS

Amphibian populations have declined globally in recent years (Stuart et al. 2004). Factors proposed or identified in these declines include: habitat degradation (Johnson 1992; Gillespie and Hollis 1996, Dupuis 1997), disease (Daszak et al. 2003, Skerratt et al. 2007), pesticides (Boone and Bridges 2003, Davidson 2004, Sparling and Fellers 2009), climate change (Pounds et al. 2006), UV radiation (Blaustein et al. 1997), grazing (Winegar 1977, Behnke and Raleigh1978, Kauffman and Krueger 1984), and introduction of exotic predators (Bradford et al. 1993, Lannoo 1998, Knapp 2005).

The specific causes of the Wyoming toad population declines are unknown, but the decline was most likely caused by the cumulative effects of more than one factor. Below is a discussion of factors that have been identified as affecting the Wyoming toad.

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

2.1.1 Irrigation Practices

Backwater wetland and riparian habitats associated with river oxbows and flood events provided much of the natural historical habitat occupied by the Wyoming toad. The extensive development and channelization of waterways to provide the infrastructure for irrigation practices and water diversions, has thus likely resulted in a loss of historical Wyoming toad habitat along the banks and naturally flooding areas of the waterways.

Flood irrigation and reservoir storage comprise the majority of current water use in Wyoming. Ironically, flood irrigation creates wetlands in areas that were historically arid, creating potential new habitat for the Wyoming toad. Specifically in the Laramie Basin, flood irrigation now accounts for 65 percent of flows into the wetlands and is critical to maintaining the remaining wetland habitats (Peck and Lovvorn 2001). A high fraction of historically absent wetlands would be lost if land were retired from irrigation or if flood irrigation was replaced by more efficient irrigation practices. Because of the channelization of the irrigation canals, habitat would likely not restore naturally without significant restoration efforts if irrigation was discontinued.

Mortenson Lake is a 61 acre (0.25 km^2) 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. 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). The North Platte River Decree of 2001 has caused curtailment of irrigation on many ranches with junior water rights, decreasing the amount of wetland habitat available to toads (2014 Walker pers. comm.).

While development for irrigation may have been the cause of historical habitat loss, current irrigation practices create previously unavailable habitat, are relatively stable, and allow for the control of water levels at breeding sites. Considering both the positive aspects to current irrigation practices and the ability for irrigation to drastically change Wyoming toad habitat when altered, the Service believes the threats associated with water diversion and management to be **moderate** at this time. Irrigation practices are considered seriously for each reintroduction site.

2.1.2 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. However, many anuran species, including the Wyoming toad, have been observed to co-exist with managed livestock grazing. Cattle have the potential to be an effective vegetation management tool, clearing areas that have become too thickly overgrown.

Cattle also have the ability to degrade habitat when not managed properly and are allowed to overgraze (Gunderson 1968). While thickly overgrown vegetation limits breeding habitat and movements, some vegetation is necessary for protective cover. Overgrazing can result in the loss of emergent vegetation entirely due to trampling or feeding (Cordone and Kelley 1961, Jansen and Healy 2003). Livestock can create water quality issues due to urination and defecation (Doran et al. 1981, Nader et al. 1998). Existing studies on the impacts of cattle grazing on amphibians have had mixed results: some studies showing negative impacts (Healy et al. 1997, Jansen and Healy 2003, Schmutzer et al. 2008) and some showing no impacts (Bull and Hayes 2000, McIlroy et al. 2013, Roche et al. 2012). McIlroy et al. (2013) suggested the conflicting results of the studies could be due to the differences in grazing intensity of the systems studied.

Cattle were allowed to graze Mortenson Lake before the listing of the Wyoming toad in 1984. Wyoming toad populations were reportedly stable, as observed by a local ranch owner, until the cattle were removed (Swanson 2013 pers. comm.). While the exact cause of the decline of the population at Mortenson Lake in the late 1980s is unknown, the decline was noted a couple years following cattle removal (Swanson 2013 pers. comm.). It was believed that vegetation along the lake margins became overgrown without either native ungulates or domestic cattle to graze. The overgrown vegetation shades shallow water areas preventing the water from warming to temperatures required for Wyoming toad breeding. Withers (1992) noted an observation where an egg mass was trampled by a horse and remained viable despite the breaking up of the mass and tadpoles hatched days later, suggesting evidence that egg masses may survive livestock trampling. Cow hoof prints may also create small pools suitable for breeding for the Wyoming toad as they do for other amphibian species (Crump 1991).

Grazing by domestic cattle has now been recognized as a useful vegetation management tool to clear overgrown vegetation and restore suitable habitat for the Wyoming toad. Care is taken to manage grazing responsibly and avoid overgrazing. Given the fact that cattle can only graze Mortenson Lake for a short period of time due to seasonality and substrate moisture restraints, i.e. cattle do not graze in the winter and can only graze wet areas for short period of time before developing hoof problems, overgrazing at Mortenson Lake is highly unlikely. The Arapaho National Wildlife Refuge 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 covered sites. Electric fences have been used to keep cattle out of sensitive Wyoming toad habitat during breeding season. Considering the positive impacts of grazing on vegetation management and the highly unlikely nature of overgrazing in Wyoming toad habitat, the Service ranks the overall threat level of domestic cattle grazing in occupied Wyoming toad habitat as a **non-issue**.

2.1.3 Limited Distribution

Currently, the Wyoming toad's distribution is severely limited and is confined to Mortenson Lake and two sites protected under the Wyoming Toad Safe Harbor Agreement (SHA). Breeding has not been documented in recent years at any of these sites; and thus, none of these populations is currently self-sustaining. The land adjacent to the Laramie River is mostly privately owned and not available for reintroductions without protection from the SHA or the Wyoming Toad Conservation Area; see Section 4.3.5.2 for more details.

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. 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.

2.1.4 Contaminants

Heavy Metals

The 1991 Recovery Plan identified heavy metals as a potential contributor to the Wyoming toad's declining population, but acknowledged that evidence was lacking for this claim. The role of heavy metals in the decline of the Wyoming toad remains unknown, but heavy metal contamination has been shown to have negative effects on other species of amphibians (Lefcort et al 1998, Khangarot and Ray 1987). After monitoring heavy metals from 1989 to 1991, the Service concluded that there were no elevated concentrations of trace metals at Mortenson Lake that would negatively affect the Wyoming toad (Ramirez 1992). Events likely to increase heavy metal contamination have not occurred since the 1989-1991 study (Ramirez 1992). Likely contributors to heavy metal contamination, such as mining or energy development, are not currently present in the Laramie Basin. Roads are few and automobile travel is light. For these reasons, the Service assigns the overall current threat level for heavy metals and pollution as a **non-issue**.

The current absence of heavy metal contamination in the Laramie Valley poses no threat to the Wyoming toad; yet if levels increase, threats may be possible. If heavy metals are anticipated to increase, the effects specific to the Wyoming toad should be examined further.

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 nutrients applied in large quantities are nitrogen, phosphorus, and potassium. These fertilizers can be transported from agricultural fields into waterways through overland flow and ultimately into Wyoming toad habitat.

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. Northern red-legged frog (*Rana aurora*) larvae exposed to nitrite below the levels allowed for safe drinking water showed increased mortality compared to unexposed larvae (Marco et al. 1999). Cascades frog (*R. cascadae*) larvae are also more vulnerable to predation because of delayed metamorphosis and increased occupancy in shallow water when exposed to sub-lethal concentrations of nitrite (Marco and Blaustein 1999).

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. While fertilizer application is recommended for rangelands, the overall application is lower than for active crop agriculture. However, because significant negative impacts to amphibians from exposure to mineral fertilizer have been documented, the threat to the Wyoming toad from mineral fertilizers is considered by the Service to be **moderate**.

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 et al.

2002), there is a continuing need to monitor the potential effects of pesticides commonly used in or near habitat of the Wyoming toad.

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 either currently or historically used in Albany County, Wyoming:

Fenthion (Baytex)
Widespread aerial spraying of fenthion (Baytex) for mosquito control coincided with population declines of Wyoming toads. Research conducted by Freda et al. (1988) and Lewis (1985) et al. (*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.

Malathion
Malathion has been shown to have negative effects to amphibians. Taylor et al. (1999a) 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 2005). Malathion is applied aerially on properties adjacent to the refuge and other sites within the Wyoming toad's historic range when adult mosquito populations are high. 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.

Atrazine
Atrazine was listed as a potential reason for decline in the original listing documents. 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, male African clawed frogs (*Xenopus laevis*) exposed to atrazine developed female sex organs and were unable to reproduce (Hayes et al. 2010). Frogs collected approximately 100 mi (161 km) from Mortenson Lake in waters containing atrazine from the Platte River near Saratoga, Wyoming 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 conducted by the Service of atrazine levels in surrogate amphibian tadpoles in the Laramie Plains Lakes area detected concentrations of atrazine at <0.1 ppb in 2008 and <0.01 ppb in 2009. Hermaphroditic individuals were not identified; 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, the Service does not believe the current level of atrazine use is a threat to Wyoming toad.

Permethrin based insecticides and *Bacillus thuringiensis israelensis*
Permethrin based adulticides and the larvicide *Bacillus thuringiensis israelensis* (BTI) are the primary pesticides currently being used for mosquito control in the town of Laramie (Wardlaw 2014 pers. comm.). In rural areas adjacent to Laramie, BTI is applied aerially when larval counts are high. BTI 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. There is no evidence that BTI has any detrimental effects to amphibian populations and is considered to be a non-toxic biocontrol agent.

Pesticides are not applied directly to Mortenson Lake, but could be transported into waterways and ultimately into suitable toad habitat through overland flow or 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, Sparling and Fellers 2009). Pesticide use is relatively low in the Laramie Basin compared to active agricultural areas, but varies throughout the Wyoming toad's historic range and is difficult to monitor on private lands. Potential reintroduction sites should 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**.

2.2.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 and recreation is currently prohibited at Mortenson Lake. The WTRT takes great 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.

2.3. Factor C. Disease or Predation

2.3.1 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). Examples of species susceptible to *Bd* are numerous. The mountain and Sierra Nevada yellow-legged frog (Rana muscosa and *R. sierrae*) have experienced precipitous declines as a result of *Bd* infections (Vredenburg et al. 2010). Chytridiomycosis is responsible for deaths of Yosemite toads (*A. canorus*) in the Sierra Nevada (Green and Sherman 2001). Populations of the California red-legged frog (*R. draytonii*) have been lost or reduced by *Bd* outbreaks (USFWS 2002). In Arizona, Bradley et al. (2002) diagnosed dead and dying Chiricahua leopard frogs (*R. chiricahuensis*) with chytridiomycosis. In the Rocky Mountains, die-offs in remaining populations of the boreal toad (*A. boreas boreas*) were attributed to *Bd* (Muths 2003). Wyoming toads also experienced a *Bd* related die-off in 2003 (Pessier 2009 pers. comm.). Wyoming toad museum specimens from the Laramie Basin tested positive for *Bd* as early as 1965. It is not known if all older samples would test negatively, but if this is the earliest case of *Bd* in the Basin, this arrival of *Bd* occurs just prior to the major decline of the Wyoming toad in the mid-1970s and could have potentially played a role.

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*, was a primary cause of death in both wild and captive animals (Taylor et al. 1999b, 1999c). However, many morphologic features of *B. ranarum* can easily mimic features of *Bd* (Muths 2003), which was not formally described taxonomically until 1999. Wyoming toads originally diagnosed with *B. ranarum* were reexamined and later determined to be infected with *Bd* and not *B. ranarum* (Pessier 2000 pers. comm.). *Bd* was detected on 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 some captive facilities during this time period. In 2009, 93 out of 125 samples (74 %) of wild Wyoming toads tested positive for *Bd* and in 2010, four out of four (100 %) tested positive (Table 2). The population at Mortenson Lake crashed the following couple years; one toad was located in 2011 and 29 were located in 2012. *Bd* was not detected at Mortenson Lake in 2011 or 2012. Thirteen percent (21/159) of toads sampled at Mortenson Lake tested positive in 2013. Potential reservoirs for the disease have not been specifically identified at Mortenson Lake, but boreal chorus frogs, tiger salamanders, and crayfish are present at the site. It is currently believed that *Bd* cannot be eradicated from an environment and the disappearance and reappearance of *Bd* at Mortenson Lake over the years is evidence demonstrating *Bd* will be present in the future, whether or not Wyoming toads test positive or not.

Table 2. BD SAMPLE RESULTS MORTENSON LAKE, 2008-2013

Year	Number Bd + Toads	Number Bd - Toads	% Bd + Toads
2013	21	138	13%
2012	0	29	0
2011	0	1	0
2010	4	0	100%
2009	93	32	74%
2008	8	4	67%

The ability to study the needs of the Wyoming toad is limited by the short lifespans of wild individuals and the lack of viable populations present on the ground. The Service is interested in limiting the spread of *Bd* to allow for critical data collection of wild toads. Allowing toads to live as long as possible, i.e. chytridiomycosis-free, presumably increases the time to collect much needed information to aid management of this species. Contaminated gear could prematurely infect an individual, limiting the time available for data collection. 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 is strictly enforced with visitors prior to entering a Wyoming toad occupied area and upon departing. Disinfection of equipment moving from Mortenson Lake to captive breeding facilities is also crucial to maintaining the health of captive populations.

Because *Bd* is a high intensity threat with the potential for the extinction, we rank the overall threat level as **severe**.

2.3.2 Predation

Predation is a natural part of the Wyoming toad's life history. However, because of the small population size, high predation can pose 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. 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 Stiles 1999, Withers 1992, Clake 1977, Beiswenger 1981, Hammerson 1989, Olson 1989, Sherman and Morton 1993, Livo 1998). Withers (1992) found the skin of several Wyoming toads remaining after predation.

Fish have been documented to have significant effects on frog populations, but studies have shown that fish generally avoid preying on toads. Populations of Mountain and Sierra Nevada yellow-legged frogs (*R. muscosa* and *R. sierrae*) were devastated by fish predation of non-native trout in the Sierra Nevada (Knapp 2005, Vredenburg 2004). In North Cascades National Park, USA, Liss and Larson (1991) reported decline of Ranid species in naturally fishless lakes after non-native trout were introduced. However, existing studies indicate toads may be unpalatable to fish. Grasso et al. (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.

While an exhaustive survey of fish species inhabiting Mortenson Lake has not been completed, 2011 field observations by USFWS employees indicated that golden shiners (*Notemigonus crysoleucas*) and fat head minnows (*Pimephales promelas*) were present at Mortenson Lake. A small netting survey conducted by WGFD personnel in April 2013 verified the presence of fathead minnows (*P. promelas*), and also found white suckers (*Catostomus commersoni*) and Iowa darters (*Etheostoma exile*) (Gelwicks 2013 pers. comm.). No evidence currently exists that the fishes present in Mortenson Lake prey on Wyoming toads. Populations of Wyoming toads were reportedly stable at Mortenson Lake during the mid-1980s when the lake was stocked with fish for the active fishery. Tadpoles may have been in shallow along the emergent vegetation of the lake edge where fish may not have been present.

Predation of the Wyoming toad was documented in studies conducted during 1998-1999 (Parker 2003). 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.

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 mesh enclosures used in the soft release study (detailed in Section 3.3) provide protection from most predators and have increased survival compared to hard releases (tadpoles released directly into waterbodies).

The number of potential natural predators on Wyoming toads is likely high, but no invasive or introduced predators are known to exist in the area. Future research is needed to understand the extent of predation on wild populations and understand what specific predators pose a threat. Because of the impact predators may have on a small population, the Service ranks the overall threat level from predators as **moderate**.

2.4.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 and the Service continues to see this factor as a non-issue. An overview of some of the major laws and regulations in place to protect the Wyoming toad, aside from the ESA, follows.

National Environmental Policy Act (NEPA): NEPA requires all Federal agencies to participate in evaluations of Federal projects and their potential significant impacts to the human environment. Agencies must include a discussion of the environmental impacts of the various project alternatives, any adverse environmental effects which cannot be avoided, and any irreversible or irretrievable commitments of resources. Activities on non-Federal lands are also subject to NEPA if there is a Federal nexus such as federal permits and funding. Cooperating agencies and the public can provide recommendations to the action agency for project modifications to avoid impacts or enhance conservation of the Wyoming toad or other wildlife species. NEPA provides an opportunity to negotiate conservation measures. However, NEPA is a disclosure law, and does not require subsequent minimization or mitigation measures by the lead Federal agency. Evaluation of Wyoming toad conservation needs under NEPA would occur regardless of the species' listing status.

State Mechanisms: 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 designation process based upon its Native Species Status classification system. Wyoming also has regulations in place to prevent take of species under the Wyoming Game and Fish Commission's Chapter 52 Regulations concerning Nongame Wildlife and Chapter 10 regulations concerning the importation, possession, confinement, transportation, sale and disposition of live wildlife. These regulations do not allow the take of numerous species of wildlife in Wyoming, including the Wyoming toad. If a Wyoming toad is taken without a permit, the consequences could include: $120-750 fine and/or up to six months incarceration and/or up to two years loss of fishing and hunting privileges.

Safe Harbor Agreements: The Wyoming Toad Safe Harbor Agreement (SHA) has provided an avenue for releases to occur on interested landowners' properties. Landowners with suitable habitat have entered into agreements under the programmatic SHA known as Landowner Cooperative Management Agreements (LCMA) specific to the property of interest. This is a voluntary agreement involving private or other non-Federal property owners whose actions contribute to the recovery of species listed as threatened or endangered under the ESA. In exchange for actions that contribute to the recovery of listed species on non-Federal lands, participating property owners receive formal assurances from the Service protecting them against liability in the event a listed species is "incidentally taken." These agreements have been instrumental in the establishment of additional populations outside of Mortenson Lake.

The Service believes that the inadequacy of regulatory mechanisms is not a current threat to the Wyoming toad. The threats that the Wyoming toad currently faces, such as lack of reintroduction sites, inappropriate habitat, Bd, etc. will not be mitigated by regulatory mechanisms, but instead by conservation management actions. For example, the conservation action of the expansion of the Mortenson Lake and Hutten Lake complex could provide potential habitat through conservation easements or fee title purchases (with willing sellers only); and vegetation management can help provide suitable habitat. For these reasons, the Service assigns the current overall threat level of lack of regulatory protection of historical Wyoming toad habitat as a **non-issue**.

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

2.5.1 Small Population Size

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. The Wyoming toad's small population size is a significant threat to its continued survival.

Small, fragmented, or isolated populations are more vulnerable to extirpation by random events. In addition, the potential for inbreeding depression increases, which means that fertility and survival rates of off-spring, 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). Because of realistic vulnerability to extinction, the Service considers the overall level of the Wyoming toad's small population size to be **severe**.

2.5.2 Low Genetic Diversity

The captive population is currently managed with every attempt to maximize genetic diversity. However, the genetic diversity of the breeding population is based on only 25 individuals originally brought into captivity (McCleary 1989, Chamberlain 1990) and there may be unknown and unavoidable consequences of earlier genetic bottlenecking. 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).

Threats that may affect only a few individuals in a genetically diverse population can pose a threat of extinction to a population with low genetic diversity. For example, if only a few individuals of a genetically diverse population are susceptible to particular disease, the population will not be wiped out in a disease event. But if the entire population is susceptible to the disease because of genetic homogeneity, a disease event may cause extinction of the species. Populations with low genetic diversity are also particularly susceptible to stochastic changes in a wild population's demography (Brussard and Gilpin 1989, Lacy 1997). For example, a skewed sex ratio (e.g., a sudden loss of adult females) could negatively affect reproduction, causing lower recruitment. The disruption in gene flow due to reduction and isolation of populations may create unpredictable genetic effects.

Because of the risks involved, the Service considers low genetic diversity to be a **high** level of threat at this time.

2.5.3 Climate Change

Analyses under the ESA include consideration of ongoing and projected changes in climate. "Climate", as defined by the Intergovernmental Panel on Climate Change (IPCC), 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" 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). Changes in climate can affect species directly and indirectly. These effects may be positive, neutral, or negative and they may change over time. Effects can be species specific and act synergistically with other variables, such as habitat fragmentation (IPCC 2007, pp. 8 - 14, 1 - 19).

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.

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, existing evidence shows that some amphibian species may be negatively affected by climate change (Pounds 1999). 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 1994). As temperatures warm and small breeding pools dry, tadpoles are likely to experience lower rates of survival to metamorphosis. Drought events cause water levels to lower, potentially causing concentrations of minerals in waterbodies to increase. The resultant habitat would likely be too alkaline for the Wyoming toad. As water levels drop, concentrations of nitrates, pesticides, and other chemicals could also increase, potentially resulting in harmful impacts to Wyoming toads. Wyoming toads have been shown to stay relatively close to their release spot and may not be able to disperse should their 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). 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, Pounds et al. 2006).

Research on amphibians in general indicates that they are particularly sensitive to potential long-term changes to weather patterns. The most significant impacts of climate change on the Wyoming toad may be the exacerbating effect it has on current limiting factors for the toad. For example, survival, reproduction and recruitment may decline due: to changes in aquatic habitat availability, increased concentrations of chemicals associated with fertilizers or pesticides, limited opportunity for dispersal, or increased virulence of pathogens. Specific information regarding effects of climate change on the Wyoming toad is lacking, and conclusions regarding direct and indirect impacts—and the extent to which they pose a threat to the toad—are uncertain at this time.

2.5.4 Captive Diets

A primary concern for captive amphibians is the maintenance of a nutrient sufficient diet. Common diet imbalances include vitamin A and D_3, thiamine, calcium, and phosphorous. These imbalances can lead to a variety of complications that affect toad survival, such as Metabolic Bone Disease and squamous metaplasia, or short-tongue syndrome. Metabolic Bone Disease is associated with an imbalance of calcium, phosphorous, and vitamin D_3 (McWilliams 2008), while short-tongue syndrome is caused by a deficiency in vitamin A.

Many captive Wyoming toads are affected by short-tongue syndrome and have trouble feeding and putting on healthy weight as a result. Larger toads are better breeders, so the inability to put on sufficient weight is a hindrance to an annual sufficient output of tadpoles. Animals with short-tongue syndrome do not 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 Wyoming toads with short-tongue syndrome 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.).

The Service has taken several steps to address the issue of nutritional deficiency including: feeding adult toads a more nutritionally balanced and diversified diet that includes a variety of insects; supplementing the diet of young-of-the-year metamorphs with wild-caught insects; and adding vitamin supplements to food. While these actions have met with some measureable, although limited, success, the Service has identified the need for further research to better understand and manage this potential threat (see Recovery Action 4.3.2.2). Considering the significant impacts of nutrient deficiencies to the health of the toads and consequently the captive population that is crucial to the recovery of this species, the Service considers this threat to be **high**.

Table 3. SUMMARY OF THREATS AND OVERALL THREAT LEVEL RANKING

List of Threats		Overall threat level				
Factor A: Habitat		**Non-issue**	**Low**	**Moderate**	**High**	**Severe**
	Irrigation Practices			X		
	Presence of Livestock in Toad Habitat	X				
	Limited Distribution					X
	Contaminants – Heavy Metals	X				
	Contaminants – Mineral Fertilizers			X		
	Contaminants – Pesticides			X		
Factor B: Over-utilization		X				
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
	Low Genetic Diversity				X	
	Captive Diet				X	

3. CONSERVATION EFFORTS

In September 1987, an informal recovery group, consisting of representatives from the Service, Wyoming Game and Fish Department (WGFD), University of Wyoming (UW), and The Nature Conservancy (TNC), was formed to coordinate recovery efforts. In 2001, this group was replaced by the more formal, Service-appointed Wyoming Toad Recovery Team (WTRT). This team is composed of representatives from WGFD, UW, the Wyoming Toad Species Survival Plan (WTSSP), Laramie Rivers Conservation District (LRCD), Wyoming Natural Diversity Database (WYNDD), private landowners and ranchers, and the Service's Wyoming Ecological Services Field Office (WFO), the National Fish Hatchery System (Saratoga National Fish Hatchery and Ennis National Fish Hatchery), and Arapaho National Wildlife Refuge (ANWR).

Conservation efforts for the Wyoming toad are a fundamentally collaborative process. Without the cooperation and coordination of the WTSSP providing a cohesive captive breeding program none of these efforts would be possible. The LRCD has been a key player in facilitating cooperation with private landowners of privately owned habitat and paving the way for reintroduction for Safe Harbor Agreement (SHA) sites. The WGFD has been a part of recovery planning efforts since they began in 1987 and continue to play an active role providing key biological input to aid in decision making, reviewing recovery planning documents, participating in survey efforts, and a number of other fundamentally important recovery planning efforts. (See Recovery Action 4.3.8 "Outreach and Cooperation with Stakeholders and Partner Agencies" for more information). Below is a description of the conservation efforts to date and the Section 4, Recovery, describes the work intended for future recovery.

3.1. Captive Breeding and Release Efforts

The Wyoming toad captive breeding program started releasing Wyoming toad tadpoles and toadlets back into the wild in 1995 (Release numbers detailed in Appendix B) and are currently a very successful aspect of the Wyoming toad recovery program. Participating facilities currently consist of eight volunteer based zoos from around the nation and two USFWS managed facilities in Wyoming (Red Buttes Biological Laboratory and the Saratoga National Fish Hatchery). Since 2005, over 10,000 tadpoles have been consistently released annually into the wild by participating facilities. This success can be attributed to improved health and husbandry practices (e.g. nutrition and supplementation) and updated hibernation techniques. The captive population is managed to maximize genetic diversity by the WTSSP's Reproductive Manager and Studbook Keeper (See Section 3.9 for more details on maximizing genetic diversity).

Mortenson Lake Releases

With the exception of minimal releases at Lake George and Rush Lake, almost all releases occurred at Mortenson Lake until 2005. From 1995 to 2003, 37,382 tadpoles and toadlets were released at Mortenson Lake. In 2003, the Sybille captive breeding facility was thought to be closing and there was no place to hold the toads, so an additional ten adults and 61 subadults were released. That same year, a mass die-off of Wyoming toads occurred due to *Bd* (Pessier 2009 pers. comm.); therefore, the WTRT recommended halting releases of tadpoles to Mortenson Lake and shift efforts to two SHA sites. Releases did not occur at Mortenson Lake again until the 2012 UW soft release study (See Section 3.3 for more information**)**. Both hard and soft releases occurred in 2013 and only soft releases occurred in 2014.

Buford Foundation Releases- Landowner Cooperative Management Agreement (LCMA)

The major release effort shifted to the Buford Foundation in 2006. As of 2013, over 98,500 tadpoles and toadlets have been released onto this property.

Shaffer Releases- LCMA

Approximately 8,900 tadpoles and toadlets were released at this LCMA between 2006 and 2009, but no toads were found in subsequent surveys. No releases occurred after 2009.

Miscellaneous Release Sites

In 1995 and 1996, 520 tadpoles were released into Rush Lake, but no toadlets were found in subsequent searches. Between 1995 and 2000, 5,221 tadpoles were released into Lake George, but again but no toadlets were found in subsequent searches.

3.2. Identifying New Release Sites

One of the biggest threats to the recovery of the Wyoming toad is its limited distribution and the lack of available reintroduction sites confounds this issue. Currently, the Wyoming toad's distribution is confined to Mortenson Lake and two sites protected under the Wyoming Toad Safe Harbor Agreement (SHA), none of which currently have self-sustaining populations. To satisfy the recovery criterion of five self-sustaining populations, the Service and its partners are eager to identify new release sites to either add to the existing populations should they become self-sustaining, or replace the current release sites if new sites prove successful while the current sites do not. Our partners, (WGFD, LRCD, and NRCS) play significant roles in identifying potential reintroduction sites and success would not be possible without their efforts.

Safe Harbor Agreement

The LRCD has been a key player in facilitating cooperation with private landowners of privately owned habitat and paving the way for reintroduction for SHA sites. In 2004, a programmatic SHA was created for the Wyoming toad. An SHA is a voluntary agreement involving private or other non-Federal property owners whose actions contribute to the recovery of species listed as threatened or endangered under the Endangered Species Act. In exchange for actions that contribute to the recovery of listed species on non-Federal lands, participating property owners receive formal assurances from the Service protecting them against liability in the event a listed species is "incidentally taken." When a landowner is interested in participating in the SHA, they contact the Service's Partners in Fish and Wildlife program to create a Landowner Cooperative Management Agreement (LCMA), specific to the property of interest.

The Buford Foundation entered into an LCMA under the Wyoming toad SHA in 2005 and has since been the reintroduction site with the most Wyoming toad releases. A second LCMA was created in 2006 on a property nearby the first LCMA. Approximately 8,900 tadpoles and toadlets were released on this site between 2006 and 2009, but during subsequent surveys, no Wyoming toads were found at the site. It was suggested that perhaps the habitat was not appropriate for reproduction (due to the presence of thick vegetation); so in 2009, the WTRT recommended no further reintroductions at this site. In 2012, the landowner requested withdrawal from the SHA. A third LCMA was signed in 2014 with local landowners, Fred and Stephanie Lindzey, with an emphasis on identifying appropriate habitat, or creating it where possible. The Service is hopeful that through the efforts of the LRCD and the USFWS Partners for Fish and Wildlife program, more LCMAs will be created under the existing SHA to provide additional reintroduction sites.

Mortenson Lake and the Hutton Lake Complex Boundary Expansion
The Service has proposed to expand the boundary around Mortenson Lake and the Hutton Lake complex to facilitate the development of new reintroduction sites. The proposed conservation area includes suitable habitat within and nearby the historic range along the Laramie River and adjacent wet meadows and ponds within the floodplain. This effort is further detailed in the Recovery Narrative, Section 4.3.5.2.

3.3. Soft Releases

During the summer of 2012, UW began a Service funded study placing tadpoles within head-start tanks (mesh cages) to protect them through metamorphosis. This technique is known as a “soft release”, contrary to a “hard release” where tadpoles are released directly to water bodies without protection. After metamorphosis, most toadlets were released, but some were moved to small corrals for additional protection, feeding, and to allow them to grow prior to release. Over 800 toadlets were released from the soft release tanks at Mortenson Lake in August 2012. UW repeated the soft releases the following two summers, releasing 2,511 toadlets in 2013 and 1,953 in 2014. Numbers of toads have increased dramatically in recent years at Mortenson Lake, most likely due to the protection received from the soft releases. The study also investigated the impacts of different vegetation schemes, water depth, and temperature to growth and survival. The results of this study can be found in Polasik (2014a, 2014b).

3.4. Population Monitoring

Mortenson Lake
From the early 1990s to 2008, monitoring of Wyoming toad populations at Mortenson Lake consisted of non-standardized, informal searches by volunteers sometimes lacking experience. However, an accurate assessment of the Wyoming toad population at Mortenson Lake is essential to allow for informed management decisions and to understand population dynamics. To address the need to collect better quality population data, the Wyoming Natural Diversity Database (WYNDD) developed standardized timed visual encounter surveys (VES) in permanently established search blocks around the water edge of Mortenson Lake. The protocol

was implemented in 2009 and has been used since. While the overall survey design of the VES was not changed, significant efforts were made in 2013 to increase the scientific rigor of the surveys and improve the data collection quality. These surveys are conducted by trained volunteers from Bureau of Land Management, WGFD, WTSSP, USFWS, various universities, and other local organizations.

Prior to annual VES, recording devices known as Frogloggers are placed in areas of breeding congregations to identify when calling has begun for the year. Egg mass searches are conducted shortly after calling is detected. Frogloggers experienced technical difficulties from 2006-2008; but with new devices and Sound Scope software, call monitoring with Frogloggers has become a reliable technique to identify the beginning of breeding season and timing for subsequent egg mass searches.

Daytime VES are repeated three times over the course of the summer and occur mid-month in June, July, and August. Habitat and morphological data, and *Bd* samples [collected according to protocols identified in Boyle (2004) and UCB (2004)] are collected for the first five young-of-the-year encountered in each block, for the first ten overwinters (juveniles), and every adult encountered. Toads 20 grams or more in weight are tagged with 8 mm passive integrated transponders (PIT) tags by trained individuals. All adult toads are photographed for wart pattern recognition to provide a backup to PIT tagging. The results of the VES surveys from 2008 through 2013 for Wyoming toads at Mortenson Lake are included in the Table 4 below.

Table 4. TOTAL WYOMING TOADS BY LIFESTAGE, MORTENSON LAKE 2008-2013

Year	Young-of-the-Year	Over-wintered	Adults	Total Toads
2013	409	79	20	508
2012	28	0	1	29
2011	0	0	1	1
2010	0	0	6	6
2009	1	87	38	126
2008	24	0	12	36

Buford Foundation

The Buford Foundation is currently monitored by WYNDD with a protocol almost identical to the Mortenson Lake VES, but they occur once in June and once in August. In 2011, over 40 adults were found at this site and there was evidence of natural reproduction. Six post-metamorphic toads were found during 2013 monitoring surveys, but no toads were found in 2012 (Estes-Zumpf and Keinath, 2014).

Shaffer

No toads were found following 2006 and 2009 releases at the Shaffer property.

3.5. *Bd* Monitoring

Bd remains a serious threat to wild populations of Wyoming toads and the recovery of this species is dependent on managing *Bd*. A better understanding of disease dynamics and the immune response of the Wyoming toad to *Bd* is necessary to progress towards combatting this devastating disease. The Service currently tests a large portion of wild Wyoming toads located during VES at Mortenson Lake for *Bd* and sends samples to the Amphibian Disease Laboratory at the San Diego Zoo for quantitative Polymerase Chain Reaction (qPCR) analysis (See Table 4 for results from 2008-2013). A *Bd* sample is collected for qPCR through a non-invasive skin swab, similar to swabbing for human genetic material from the inner cheek (Boyle 2004, UCB 2004). 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.

Because of the persistence and devastation of this disease, researching the dynamics of *Bd* and the potential for treatment to the Wyoming toad has become a priority for the Service. Testing for *Bd* of the Mortenson Lake population was expanded to all lifestages in 2013 (see Table 2 for results) and research is underway to determine if *Bd* can be treated in Wyoming toads.

3.6. Habitat Management

The habitat surrounding Mortenson Lake and within the greater Laramie Basin was historically grazed by native ungulates and is adapted to a natural fire regime. Without management to recreate historical conditions, the bulrushes, sedges, and grasses become thick and shade adjacent water. The result is a habitat with water too cold for Wyoming toad breeding and the thick vegetation may inhibit free movement of toads.

Domestic cattle historically grazed Mortenson Lake while populations of wild Wyoming toads were present and reportedly stable. The overgrown vegetation at Mortenson Lake has been periodically managed with grazing and prescribed burning since the 1990s. However, cattle cannot graze for extended periods in standing water without developing hoof problems. Mortenson Lake is flooded in the spring and summer; hence, seasonal timing is an important factor of grazing management at Mortenson Lake. Fall is generally dry enough for grazing, but in wet years, the habitat may still be too saturated. Avoidance of grazing on larkspur (*Delphinium* spp.) has been recommended due to the toxicity to cattle, but patches at Mortenson Lake are currently small and easy to avoid.

Prescribed burns can also clear out overgrown vegetation. Subsequent to a prescribed burn in 2005, the Wyoming toad population at Mortenson Lake seemed to be rebounding (Table 4); but began to decline again in 2009. These declines may have been a result of overgrown vegetation, increased occurrence of *Bd* (Table 2), or other unknown factors. Another prescribed burn occurred at Mortenson Lake in 2012; and, while toads rebounded the following year, the results of toad response are confounded by the UW soft release study and it is unclear how much of an effect the prescribed burn had on a population rebound. Prescribed burns are currently occurring on a three year rotation and toad response will continue to be monitored.

Mechanical removal of vegetation is another option for vegetation management and can serve as a viable option in years when grazing and burns are not a possibility. The effectiveness of mechanical removal is being examined by UW in 2015.

3.7. Movement and Habitat Use Monitoring

In 1998, a radio-telemetry field study investigated microhabitat use (Parker 2003). 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 bred, adult Wyoming toads were surgically implanted with radio transmitters and released at Mortenson Lake as a collaborative effort between the Service, Indiana State University, and the UW to document habitat use and movement patterns. This study demonstrated that Wyoming 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-hr time period was 167 ft (51 m) and the maximum distance noted was 1,397 ft (426 m). Toads were often located nestled into the vegetation or soil, and were sometimes completely concealed by vegetation.

A study was initiated by Florida International University during the summer of 2014 to track Wyoming toads. Due to the invasive nature of surgically implanted tracking devices and the cumbersome, inhibitive telemetry backpacks that have been used in the past, efforts were made to minimize the weight of the tracking devices. Results from this study are pending, but are expected to provide information on optimized tracking devices as well as habitat use and movement of reintroduced Wyoming toads.

3.8. Hibernation

Captive toads are currently hibernated on a short cycle (generally 35-50 days) in artificial refrigeration units. This short-cycle hibernation, in addition to hormone injections, has successfully primed toads for breeding and egg production. However, the Red Buttes laboratory has been experimenting with hibernating toads outdoors and has seen positive results in reproductive success. Eighty-five percent of outdoor hibernated individuals move into amplexus without hormone injections. While this is an improvement, most outdoor hibernated female toads still need hormone injections to lay eggs. Due to strict biocontamination protocols, zoos cannot hibernate animals outside, but conditions recorded during outdoor hibernation have informed indoor hibernation techniques.

3.9. 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.

3.10. Sperm Cryopreservation and *In Vit*ro Fertilization

Wyoming toads in captivity rarely ovulate without hormonal priming (Browne et al. 2006). Cryopreservation and *in vitro* fertilization (IVF) offer the possibility of 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).

Sperm Cryopreservation

Declining amphibian populations around the world have prompted the establishment of amphibian genome banks to preserve the remaining genetic diversity. 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. In 2004, the Memphis Zoo initiated a preliminary study to evaluate the application of sperm cryopreservation protocols for the Wyoming toad. Twenty male Wyoming toads were induced with hormones to produce spermatic urine and samples were collected in straws, frozen in liquid nitrogen, and stored in a dry shipper for transport to the Memphis Zoo (Kouba 2013 pers. comm.). However, the samples had low recovery of motile sperm (less than 5 percent motile), most likely due to thawing during transportation inspections. The remainder of the Wyoming toad sperm is owned by the Service and is held at the Memphis Zoo.

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. 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 (Kouba 2013 pers. comm.).

3.11. Commitment to Standardized Science

The Service has recently adopted a proactive approach with a commitment to standardized science to determine the needs of the Wyoming toad. The lack of wild Wyoming toads makes understanding habitat needs and limiting factors difficult and makes the certainty that the needs identified are optimal, almost impossible. Repeated trials and an adaptive management approach will be key to the recovery of this species. Future research and recovery actions are outlined in Section 4. Each facet of the recovery program is an integral part in our plan to restore this endangered amphibian to a secure status.

4. RECOVERY

Recovery Strategy: 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. An adaptive management approach, which allows for the continual inclusion of updated research and information, will be the main strategy guiding the management of the species. The captive program maximizes genetic diversity in its annual breeding and continuously develops husbandry strategies to maximize the health of captive populations. Increased knowledge of the needs of wild toads is crucial for improved science-based management decisions and conservation actions. Many of the necessary actions for species protection are based on an increased understanding of disease dynamics and the relationship of the Wyoming toad to its physical, chemical, and ecological environment.

Recovery Goal: The ultimate recovery goal is to allow downlisting and ultimately delisting of the Wyoming toad.

Recovery Objective: The recovery objectives are to reduce threats to the Wyoming toad, allowing for the establishment of self-sustaining wild populations. For this to occur, captive populations with maximized genetic variability will need to be maintained at a sufficient level, suitable habitat will need to be restored and/or identified, and disease will need to be suppressed to a level to which it is not a threat to the viability of the wild populations.

4.1.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) As evidenced by a Population Viability Analysis (PVA), three self-sustaining and viable populations of the Wyoming toad are established within or nearby the toad's historic range and remain at viable levels for a minimum of seven consecutive years. 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 and Doak 2002) when the data are available (e.g. reproduction and overwinter survival are occurring).

(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) A peer-reviewed, long-term adaptive management plan is in place to guide conservation efforts of captive and wild populations for 25 years after downlisting. This management plan will provide a framework to maximize the health of and minimize genetic loss in the captive population and maintain the viability of wild populations. It will address the threats identified in the factors section of this plan (Section 2) and any potential threats that may arise that have not been identified to allow for continued recovery of this species.

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 populations) and are viable as evidenced by a current 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 and Doak 2002) when the data are available (e.g. reproduction and overwinter survival are occurring).

(2) The long-term adaptive management plan created for downlisting will be updated and peer-reviewed to guide conservation efforts for 25 years after delisting. This comprehensive plan will include detailed monitoring protocols to ensure the continued viability of the five populations established to warrant delisting of the species. It will also address the threats identified in the factors section of this plan (Section 2) and any potential threats that provide for the may arise that have not been identified to allow for continued persistence of this species.

4.2.Recovery Actions

The recovery program for the Wyoming toad is divided into the following areas of action:

- Captive Population Management (4.3.1) and Research (4.3.2),
- Wild Population Management (4.3.3), Monitoring (4.3.4), and Research (4.3.6),
- Identify New Release Sites (4.3.5),
- Planning and Adaptive Management (4.3.7), and

- Outreach and Cooperation (4.3.8).

Overall, these sets of recovery actions are tied directly to achievement of the recovery criteria for the Wyoming toad.

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.

4.3.Recovery Action Narrative

4.3.1. Captive Population Management

4.3.1.1. Maintain Genetic Diversity of Captive Population (Priority 1a)

Continue to maximize genetic diversity in pairings and ensure removal of animals from captive population does not negatively affect genetic diversity captive population or reduce the ability of the population to achieve growth targets. Continue to explore all possible means to encourage breeding of individuals that have not bred or are under-represented in the captive population.

Manage both Captive and Wild as One Population
Populations in the wild should be managed together with the captive population as a single population for purposes of genetic and demographic optimization, aiming to prevent genetic drift.

Incorporate Genes of Newly Discovered Populations
Should a new population of wild Wyoming toads be discovered, those genes should be incorporated into the captive population.

4.3.1.2. Improve Captive Survival and Reproductive Success (Priority 1b)

The captive program should continue to develop management strategies to optimize captive breeding strategies by using known environmental patterns. Mimicking physical conditions of wild populations has been problematic since very little data was collected while populations were still viable. Research should aim to understand physical needs of the toad in the wild to inform optimal conditions in captivity.

4.3.1.3. Develop and Implement Plan for Captive Distribution (Priority 1b)
Develop and implement a plan for how to distribute captive-bred stock among reintroduction sites maximizing genetic variability and optimizing population capacity of each site.

4.3.1.4. Continue Outdoor Hibernation (Priority 1b)
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.

4.3.1.5. Pursue Sperm Cryopreservation and In Vitro Fertilization (Priority 1b)
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).

4.3.1.6. Continue to Reduce Disease in Captive Toads (Priority 1a)
Bd can have devastating impacts to captive populations. The WTSSP pathologist and veterinarian continue to develop protocols that will decrease the spread of disease in the captive population and WTSSP facilities continue to implement disease treatment protocols.

4.3.1.7. Expansion of Breeding Facility Capacity (Priority 1b)
Captive propagation of Wyoming toads is currently limited by space and breeding facilities have utilized all available space. The majority of releases are currently tadpoles (few toadlets) due to space limitations of older lifestages, but older individuals have a higher chance of survival than tadpoles. The ability to house older lifestages in breeding facilities also provides opportunities for research on post-metamorphic individuals and allows for additional maximizing of genetic diversity.

Encourage New Participation of Zoos, Aquariums and other Federal Facilities
Additional participating facilities would allow for the expansion of the breeding program. In 2015, the Service's Leadville National Fish Hatchery will be added to the group of participating facilities under the WTSSP. 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.

Continue Saratoga NFH Facility Expansion
The Saratoga NFH received funding and began construction in 2014 to expand their breeding facility to house up to 900 toads.

Expansion of the Saratoga NFH Solar Greenhouse
The Saratoga NFH Solar Greenhouse currently provides a diverse and substantial food source for the Saratoga NFH Wyoming toad captive population. A permanent facility will provide increased capacity for a reliable, disease-free food source for captive Wyoming toads.

Construct Additional Breeding Facility
The Red Buttes Biological Laboratory is one of the highest producing facilities in the Wyoming toad program, but its future is unknown. The facility may need to downsize to an amphibian pod, or shipping container if the facility can no longer house Wyoming toads. The construction of a new facility will provide a permanent breeding and rearing facility, eliminating the labor involved with moving breeding facilities.

4.3.2. Captive Population Research

4.3.2.1. Continue Genetic Research (Priority 1b)
Pedigree and founder relatedness research should be continued using recent DNA analysis.

4.3.2.2. Research Nutritional Needs of Captive Population (Priority 1b)
Nutritional needs have been identified as a limiting factor of captive population health and reproductive success. Research should continue to identify ways to optimize toad nutrition.

4.3.2.3. Research Toad Health Captive Population (Priority 1b)
Various health issues have been identified by the captive breeding program, including, but not limited to skin and kidney problems. Research should continue to identify ways to optimize toad health.

4.3.2.4. Research Captive Hibernation (Priority 1b)
Due to strict biocontamination protocols, zoos do not have the ability to hibernate outside of their facilities, but preliminary results have shown greater reproductive success of toads that have hibernated for longer durations outdoors at the Red Buttes Biological Laboratory. Research to optimize outdoor hibernation should continue to optimize success for facilities that have this capacity and inform artificial conditions for facilities that do not.

4.3.3. Wild Population Management

4.3.3.1. Habitat Management (Priority 1b)
Implement Optimal Management Techniques

Based on current vegetation conditions, reintroduction sites should be managed to optimize Wyoming toad habitat. Breeding pools should be shallow to allow for the warm waters Wyoming toads require for sufficient tadpole growth and thick vegetation should be thinned to allow adequate solar heating. The effectiveness of cattle grazing, prescribed burns, and mechanical vegetation removal as land management tools to create suitable habitat for the Wyoming toad is being examined by UW beginning in 2014. The optimal techniques for manipulating habitat should be implemented.

Implement Existing Prescribed Burn Plan
Arapaho National Wildlife Refuge currently implements prescribed burns at Mortenson Lake on a three year cycle.

4.3.3.2. Minimize Spread of Bd in the Field (Priority 1b)

The ability to learn Wyoming toad needs, preferences, and limiting factors to survival is hindered by extremely small populations. Data collection is impossible without wild Wyoming toad survival and the longer toads survive, the more opportunities are available for data collection. Even if Bd is not currently controlled and toads will eventually succumb to the disease, minimizing the spread of Bd will allow the opportunity for data collection by presumably keeping toads alive for longer. A strict decontamination protocol for boots and other field gear is therefore implemented at all sites on arrival and departure.

4.3.3.3. Continue Soft Releases (Priority 1a)

Placing tadpoles within head start tanks (mesh cages) to protect them through metamorphosis is a type of release technique is known as a “soft release”, contrary to a “hard release” where tadpoles are released directly to water bodies to fend for themselves. The UW soft release study (described in detail in Section _) has been identified as an effective management strategy to increase survival of toadlets and increase the population size. The Service plans to expand the use of soft releases at additional reintroduction sites to increase toad survival through metamorphosis.

4.3.3.4. Develop Optimal Release Numbers (Priority 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.

4.3.3.5. Continue to Document and Report Releases (Priority 2)

Release efforts need to be properly documented and reported to the WTRT, including recording release locations, dates, and methods of release.

4.3.3.6. Review and Revise Selection Criteria for Release Sites (Priority 1b)

When new information on the habitat needs of the Wyoming toad is available, it should be integrated into existing selection criteria. Selection criteria characteristics should be prioritized to determine which are most important for long-term success of reintroduction sites.

4.3.3.7. Water Quality Analysis (Priority 1b)

Water samples should be collected and analyzed on an annual basis from all reintroduction sites to identify and eliminate potential contaminant sources to aquatic habitats of the Wyoming toad. Of special concern are potential inputs from pesticide/herbicide applications and mineral fertilizers throughout the Laramie Basin.

4.3.4. Wild Population Monitoring

4.3.4.1. Develop and Implement a Monitoring Program (Priority 2).

A standardized monitoring protocol needs to be developed and implemented at all reintroduction sites. Adequate monitoring 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 reintroduction sites. However, conditions can change and new information may require the alteration of or additions to the basic methodology. Monitoring should include information to inform a population viability analysis, once that information is available.

4.3.4.2. Integrate Previous Studies to Current Management (Priority 1b)

Previous studies and records should be considered when making management decisions. Future records need to be thorough, accurate, and available to WTRT members so they can be included in future decision making.

4.3.4.3. Identify Site-specific Threats (Priority 1b)

Because the specific limiting factors of Wyoming toad survival are not known, conditions at various release sites need to be closely monitored. Conditions monitored could include Bd dynamics, water chemistry, habitat composition, vegetative cover, predator presence, and breeding pool characteristics.

4.3.4.4. Develop a Post-downlisting and -delisting Monitoring Plan (Priority 2)

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 status of Wyoming toad, develop monitoring protocols for those indicators, and evaluate factors that may trigger consideration for relisting.

4.3.4.5. Implement Effective Toad Identification Techniques (Priority 1a)

Marking allows for toads to be followed over time, providing valuable information, such as population size, habitat use, and disease dynamics. Mark recapture analysis is a technique used to accurately assess the current size of a population and is loosely based on the ratio of individuals that were marked on the first visit to the number of unmarked individuals located on a subsequent visit. For a successful mark-recapture, toads can be batch marked (i.e. individuals do not need to be differentiated from each other), but all toads located need to receive a mark. Identifying toads to the individual level, e.g. passive integrated transponder (PIT) tagging or wart pattern recognition, allows for additional analyses such as growth (SVL and weight changes over time) and disease dynamics on individuals. Minimizing negative impacts of marking techniques is and should continue to be a priority for the WTRT.

Passive Integrated Transponder
Passive integrated transponder (PIT) tags are small chips with a unique code inserted subcutaneously through a small incision near the dorsal lateral line. Each adult Wyoming toad over 20g in weight is PIT tagged by trained biologists in captivity and in the wild at Mortenson Lake during monitoring surveys. The Service began using 8mm PIT tags in 2014.

Wart Pattern Recognition
Due to concerns over potential negative impacts of PIT tags and potential tag loss, WYNDD is investigating the use of wart pattern recognition for individual recognition. WYNDD has identified that Wyoming toads maintain their unique wart patterns throughout their life, but software tested for automated recognition has not yet been successful (Morrison et al. 2014). Manual wart pattern recognition can be successful for small populations (wild or captive), but field testing in larger populations (i.e. Mortenson Lake) has proven to be too time consuming and infeasible. WYNDD is hopeful different software may make automated recognition feasible in the future.

Visible Implant Elastomer
A visible implant elastomer (VIE) mark consists of a small amount of colored liquid plastic injected under the skin of each individual toad and is not harmful to toads if marked correctly by an experienced biologist. It is retained over time, visible under ultra-violet light, and can be used to batch identify recaptured toads. In 2010, WYNDD tested marking with VIE on the front and hind feet of Wyoming toads and found that the retention rate in a captive setting was approximately 89% overall (Estes-Zumpf 2013 pers. comm.). VIE could be used for mark-recapture analysis because toads do not need to be identified at the individual level.

4.3.5. Identify New Release Sites
Currently, only Mortenson Lake and two sites covered under the Safe Harbor Agreement are available for releases. More sites need to be identified to satisfy de- and down-listing criteria of three (required for down-listing) or five (required for de-listing) self-sustaining populations. While the minimum size of a self-sustaining population is unknown, Mortenson Lake encompasses approximately 2,000 acres. If each of the five populations needs to be this size, approximately

10,000 acres of land will be required to support a total of five self-sustaining populations necessary for delisting.

4.3.5.1. Continue Pursuing SHA Sites (Priority 1b)

LRCD works closely with USFWS Partners for Wildlife and the Natural Resource Conservation Service to identify potential SHA sites. Sites protected under the SHA 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.

4.3.5.2. Continue to Pursue Mortenson Lake Boundary Expansion (Priority 1b)

Conservation easements and fee title lands (of willing sellers only) cannot be created outside the current refuge boundaries surrounding Mortenson Lake and the Hutton Lake complex. These agreements can only be created with willing landowners if the property exists within the boundaries. The Service plans to expand the boundary around Mortenson Lake and the Hutton Lake complex to facilitate the development of new reintroduction sites. The proposed conservation area includes suitable habitat within and nearby the historic range 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 the expanded area would protect suitable Wyoming toad habitat on public and private lands and provide urgently needed additional reintroduction sites. Fee title ownership allows the strongest habitat protection, but conservation easements also protect suitable habitat from habitat loss. While fee title land acquisition is preferred over conservation easements due to the increased ability to implement research and management, utilizing both fee titles and conservation easements in combination ensures maximum likelihood of achieving the objective of 10,000 acres of habitat available for the Wyoming toad. Additional reintroduction sites are absolutely necessary for the recovery of this species.

4.3.5.3. 10(j) Rulemaking (Priority 3)

Section 10(j) allows reintroduced "experimental non-essential populations" of endangered species to be managed as if they were threatened. Landowners are relieved from liability for the unintentional take of Wyoming toads while engaging in lawful activities, such as recreation, forestry, agriculture. Populations on Federal lands or lands within conservation easements would have full protection of an endangered species and will not be subject to the 10(j) exemptions. However, while the Service believes this could be beneficial for the Wyoming toad, the labor resources involved in a 10(j) rulemaking is beyond current capabilities of the Service (based on the requirements of the black footed ferret 10(j) estimated at $80,000 of salary costs are necessary) and is therefore assigned a lower priority at this time.

4.3.6. Wild Population Research

Wyoming toad recovery is hindered by the lack of general knowledge of current limiting factors and needs of the Wyoming toad. While the captive breeding program is still facing challenges, it has been successful at producing tadpoles and toadlets for release for years. Reintroductions; however, have not been as successful as hoped for and research to understand the limiting factors of toad survival in the wild is crucial.

4.3.6.1. Investigate Bd Dynamics in Wild Populations (Priority 1a)

Because of the persistence and devastation of this disease, researching the dynamics of Bd is a priority for the Service. Understanding disease dynamics is the first step in understanding what may be done about it. Testing for Bd of the Mortenson Lake population was expanded to all lifestages in 2013.

4.3.6.2. Research Bd Mitigation in Wild Populations (Priority 1a)

Captive toads can be successfully treated with antifungal solutions if infected with Bd. However, treating wild animals in a natural environment presents challenges. Treating reintroduction sites with fungicides in attempt to rid the habitat of Bd would be detrimental other species of naturally occurring fungi and is therefore not an option. Captive breeding facilities can be effectively contained and the spread of Bd within and between facilities can be limited. Wild environments on the other hand, are not containable. Examples of how Bd can be spread are through inflow and outflows from waterbodies and carriers of the disease (e.g. other species of amphibians, crayfish).

Existing evidence suggests some amphibians can persist with low levels of Bd (Woodhams et al. 2011). Research has also shown it may be possible to treat amphibians in the wild in order to reduce the intensity of infection allowing some species to persist with a mild Bd infection (Briggs et al. 2010, Vredenburg et al. 2010). Potential areas of research are looking at the possibility of introducing symbiotic bacteria that inhibit the growth of Bd into wild amphibian populations (Harris et al. 2006), looking into effects of habitat manipulation on Bd, 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. The Service plans to investigate the potential for treating Wyoming toads for Bd in the near future.

4.3.6.3. Research Release Techniques (Priority 1b)

Techniques that better the survival rate of released individuals and the optimal lifestage for release should be identified.

4.3.6.4. Research Wild Hibernation (Priority 1b)

Hibernation habits and needs of Wyoming toads are poorly understood. Information regarding hibernation needs can inform decisions about reintroduction sites; for example if the presence of mammal burrows or mima-mounds was identified as a habitat need, sites could be selected that have mammal burrows or mima-mounds. Alternatively, habitat manipulation could also integrate burrow/ mima-mound creation if they are not present to begin with. Information on wild hibernation may also inform better captive hibernation techniques.

4.3.6.5. Investigate Predation (Priority 2)

Because excessive predation has been suggested as a potential cause of decline for the Wyoming toad in the past, predation should be considered in current research when possible and investigated in the future.

4.3.6.6. Research Movement and Habitat Use (Priority 1b)

Very little information is available documenting historical Wyoming toad habitat needs and the lack of toads in the wild has hindered further efforts to understand habitat preferences. Data should continue to be collected during monitoring surveys as well as through research projects to identify optimal habitat characteristics.

Minimize Impacts from Tracking Devices

Due to the invasive nature of surgically implanted tracking devices and the cumbersome, inhibitive telemetry backpacks of the past, efforts were made by a graduate student from the Florida International University to minimize the weight of the tracking devices during the summer of 2014. Results from this study are pending, but are expected to provide information on optimized tracking devices as well as habitat use and movement of reintroduced Wyoming toads. This information should be implemented in future efforts to track Wyoming toads.

4.3.6.7. Identify Specific Criteria for Population Viability Analysis (Priority 1b)

Optimum numbers, the spatial arrangement of the populations, and population dynamics including fecundity, age and size class, sex ratio and longevity, through population estimations need to be identified specifically for the Wyoming toad. See USFWS (2015) for details.

4.3.7. Planning and Adaptive Management

4.3.7.1. Adaptive Management (Priority 1a)

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, the Service recognizes considerable knowledge gaps regarding the species and the ecosystem upon which it depends. As a result of this uncertainty, the process of recovery will necessitate adjustments to management when new information becomes 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, researching, executing, monitoring, and adjusting management, we will learn how to effectively recover this species.

4.3.7.2. Prepare Long-term Adaptive Management Plan (Priority 2)

To ensure progress toward delisting long-term adaptive management plan should be prepared and implemented before and after delisting, as per time-frames outlined in the Recovery Criteria. The plan should be approved and implemented by all participating agencies having proprietorship over the Wyoming toads (e.g., USFWS, WGFD).

4.3.7.3. Implement Current Recovery Plan and Adjust as Needed (Priority 1b)

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.

4.3.8. Outreach and Cooperation with Stakeholders and Partner Agencies

4.3.8.1. Cooperate with Stakeholders and Partner Agencies (Priority 1a)

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. The Service endorses coordination and encourages 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 3, Conservation Efforts for details on existing coordination efforts with key agencies.

4.3.8.2. Outreach (Priority 3)

Programs should be jointly developed through the Service, WGFD, the WTRT, and local community organizations. Outreach should focus on the rarity of the Wyoming toad and the advantages of habitat protection (i.e., conservation easements).

5. 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 5. 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)					Total Cost to Delisting ($1,000's)	Comments
							Yr 1	Yr 2	Yr 3	Yr 4	Yr 5		
1a	4.3.1.1	Maintain Genetic Diversity of Captive Population	A1, A2, A3, B1	ongoing	USFWS WTSSP	N	6	6	6	6	6	90	Because the genetic diversity of the breeding population is originally based on so few individuals, it is imperative to continue to maximize genetic diversity in pair breeding.
1b	4.3.1.2	Improve Captive Survival and Reproductive Success	A1, A2, A3, B1, B2	ongoing	USFWS WTSSP	N	8	8	8	8	8	120	Research should aim to understand physical needs of the toad in the wild to inform optimal conditions in captivity.
1b	4.3.1.3	Develop and Implement Plan for Captive Distribution	A1, A2, A3, B1, B2	ongoing	USFWS WTSSP	Y	2	4	4	4	4	54	Plan should aim to maximize genetic variability and optimize the population capacity of each site.
1b	4.3.1.4	Continue Outdoor Hibernation	A2	ongoing	USFWS	Y	3	3	3	3	3	45	Facilities that have the capabilities of outdoor hibernation should continue to do so, and others should adapt current hibernation techniques to new information.
1b	4.3.1.5	Pursue Sperm Cryopreservation and In Vitro Fertilization	A2	ongoing	USFWS WTSSP	N	4	4	4	4	4	60	Facilitates the ease of shipping genetic material
1a	4.3.1.6	Continue to Reduce Disease in Captive Toads	A1, A2, A3, B1, B3	ongoing	USFWS WTSSP	Y	4	4	4	4	4	60	Precautions should continue to be taken to avoid disease in captive populations.
1b	4.3.1.7	Encourage New Participation of Zoos and Aquariums	A1, A2, A3, B1	ongoing	WTSSP	N	4	4	4	4	4	60	The captive breeding population is limited by space and additional zoos will allow for expansion.
1b	4.3.1.7	Continue Saratoga NFH Facility Expansion	A1, A2, A3, B1	1 year (plus maintenance)	USFWS	Y	20	135	35	5	5	600	The proposed NFH construction will allow for expansion of the captive program.
1b	4.3.1.7	Construct Additional Breeding Facility	A1, A2, A3, B1	ongoing	USFWS WTSSP	Y	80	55	55	5	5	600	The captive breeding population is limited by space.
1b	4.3.2.1	Continue Genetic Research	A1, A2, A3, B1	ongoing	WTSSP	N	2	2	2	2	2	30	Pedigree and founder relatedness research should be continued using recent DNA analysis.
1b	4.3.2.2	Research Nutritional Needs of Captive Population	A2, A3	ongoing	USFWS WTSSP	N	6	6	6	6	6	90	Nutritional needs affect the health of the captive population and consequently reproductive success.
1b	4.3.2.3	Research Captive Toad Health	A2	ongoing	USFWS WTSSP	Y	10	10	10	10	10	150	Toad health continues to be a priority and research should continue to optimize toad health.
1b	4.3.2.4	Research Captive Hibernation	A2	ongoing	USFWS WTSSP	Y	2	2	2	2	2	30	Research to optimize outdoor hibernation should continue for facilities that have this capacity and inform artificial conditions for facilities that do not.

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)					Total Cost to Delisting ($1,000's)	Comments
							Yr 1	Yr 2	Yr 3	Yr 4	Yr 5		
1b	4.3.3.1	Implement Optimal Habitat Management Techniques	A1, B1	ongoing	USFWS WTSSP	Y	10	10	10	10	5	135	Identify and implement vegetation management techniques, including Arapaho NWR's 3-year burn cycle.
1a	4.3.6.2	Research Bd Mitigation in Wild Populations	A1, A4, B1, B3	4 years	USFWS	Y	60	5	50	5	0	360	Understanding disease dynamics is the first step in understanding what may be done about it.
1b	4.3.6.3	Research Release Techniques	A1, B1	ongoing	USFWS	Y	2	2	2	2	2	30	Previous research has shown some success with treating *Bd* on some species of amphibians.
1b	4.3.6.4	Research Wild Hibernation	A1, B1	ongoing	USFWS	Y	5	5	5	5	5	75	Understanding wild hibernation could inform habitat manipulation techniques and captive hibernation.
2	4.3.6.5	Investigate Predation	A1, B1	ongoing	USFWS	Y	0	5	5	5	5	60	Excessive predation has been identified in the past as a potential cause of Wyoming toad decline.
1b	4.3.6.6	Research Movement and Habitat Use	A1, B1	ongoing	USFWS	Y	5	5	5	5	5	75	The WTRT continues to support efforts to understand habitat use and preference while minimizing impacts from tracking devices.
1b	4.3.6.7	Identify Specific Criteria for Population Viability Analysis	A1, A4, B1, B3	3 years	USFWS WTSSP	Y	0	0	5	5	5	45	PVA is required for downlisting and delisting to definitively describe viability of populations.
1a	4.3.7.1	Maintain Adaptive Management Approach	A1, A2, A3, A4, B1, B2, B3	ongoing	USFWS WTSSP	Y	2	2	2	2	2	30	New information and research needs to be implemented into existing program.
2	4.3.7.2	Prepare Long-term Adaptive Management Plan	A1, A2, A3, A4, B1, B2, B3	ongoing	USFWS	Y	2	2	2	2	2	30	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.
1b	4.3.7.3	Implement Current Recovery Plan and Adjust as Needed	A1, A2, A3, A4, B1, B2, B3	ongoing	USFWS WTSSP	Y	4	4	4	4	4	60	This recovery plan is a flexible document and should be consistent with available, contemporary, scientific information.
1a	4.3.8.1	Cooperate with Stakeholders and Partner Agencies	A1, A2, A3, A4, B1, B2, B3	ongoing	USFWS WTSSP	Y	2	2	2	2	2	30	Only by working together with different resources, knowledge, and expertise can recovery objectives and criteria be achieved.
3	4.3.8.2	Outreach	A1, B1	ongoing	USFWS WTSSP	Y	2	2	2	2	2	30	Outreach should focus on the rarity of the Wyoming toad and the advantages of habitat protection (i.e., conservation easements).
1b	4.3.3.2	Minimize Spread of Bd in the Field	A1, A4, B1, B3	ongoing	USFWS WTSSP	Y	1	1	1	1	1	15	Minimizing the spread of *Bd* will allow the opportunity for data collection by presumably keeping toads alive for longer.
1a	4.3.3.3	Continue Soft Releases	A1, B1	ongoing	USFWS WTSSP	Y	20	15	15	5	5	180	The use of mesh enclosures to provide tadpoles a "head start" has been identified as an effective management tool.
2	4.3.3.4	Develop Optimal Release Numbers	A1, A2, A3, B1, B2	ongoing	USFWS WTSSP	Y	2	2	2	2	2	30	Develop goals for the numbers of toads to be released annually and how available stock will be

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)					Total Cost to Delisting ($1,000's)	Comments
							Yr 1	Yr 2	Yr 3	Yr 4	Yr 5		
													distributed among release sites.
2	4.3.3.5	Continue to Document and Report Releases	A1, A2, A3, B1, B2	ongoing	USFWS WTSSP	Y	3	3	3	3	3	45	Releases need to be documented and reported to the WTRT, including recording release locations, dates, and methods.
1b	4.3.3.6	Review and Revise Selection Criteria for Release Sites	A1, B1	ongoing	USFWS WTSSP	Y	2	2	2	2	2	30	When new information on the habitat needs of the Wyoming toad is available, it should be integrated into existing selection criteria.
1b	4.3.3.7	Water Quality Analysis	A1, B1	ongoing	USFWS WTSSP	Y	5	5	5	5	5	75	Water samples should be collected and analyzed on an annual basis.
2	4.3.4.1	Develop and Implement a Monitoring Program	A1, B1	ongoing	USFWS WTSSP	Y	4	4	4	4	4	60	Consistency of monitoring is necessary to ensure comparability over time and between sites.
1b	4.3.4.2	Integrate Previous Studies to Current Management	A1, A2, A3, A4, B1, B2, B3	ongoing	USFWS WTSSP	Y	4	2	2	2	2	36	Future records need to be thorough, accurate, and available to WTRT members so they can be included in future decision making.
1b	4.3.4.3	Identify Site-specific Threats	A1, B1	ongoing	USFWS WTSSP	Y	10	10	10	10	10	150	Because the specific limiting factors of survival are not known, conditions at various release sites need to be closely monitored.
2	4.3.4.4	Develop a Post-downlisting and -delisting Monitoring Plan	A1, A2, A3, A4, B1, B2, B3	3 years	USFWS WTSSP	Y	0	0	5	5	5	45	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.
1a	4.3.4.5	Implement Effective Identification Techniques	A1, B1	ongoing	USFWS WTSSP	Y	5	5	5	5	5	75	The benefits of the information gathered by marking should be balanced by impacts to the species.
1b	4.3.5.1	Continue pursuing SHA sites	A1, B1	ongoing	USFWS WTSSP	Y	5	5	5	5	5	75	SHA sites provide additional reintroduction sites.
1b	4.3.5.2	Mortenson Lake Boundary Expansion	A1, B1	ongoing	USFWS	Y	5	5	5	5	5	75	Acquisition of fee title lands (on a willing seller/ willing buyer basis only) and conservation easements would protect suitable habitat.
3	4.3.5.3 3	10(j) Rulemaking	A1, B1	ongoing	USFWS	Y	40	10	10	10	10	240	Section 10(j) allows reintroduced "experimental non-essential populations" of endangered species to be managed as if they were threatened.
1a	4.3.6.1	Investigate *Bd* Dynamics in Wild Populations	A1, A4, B1, B3	ongoing	USFWS	Y	20	10	10	10	10	180	Understanding disease dynamics is the first step towards the possibility of overcoming this disease.
						Totals ($1,000's)	**370**	**370**	**320**	**185**	**175**	**4,260**	

Appendix A. WYOMING TOAD RELEASES (1995-2013)

NUMBER OF WYOMING TOADS (TOADLETS AND TADPOLES) RELEASED, BY INSTITUTION (1995-2013)

Breeding Facility	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	TOTAL
Saratoga NFH	0	0	0	184	896	7,383	7,089	2,004	2,594	313	2,597	5,182	7,025	4,091	9,520	5,065	2,865	5,756	4,996	**67,560**
Sybille/ Red Buttes*	2,406	3,300	13	2,814	1,242	956	0	6,206	4,433	6,000	11,920	6,870	5,208	807	2,077	359	247	278	6,761	**61,897**
Mississippi River Museum	0	0	0	0	0	0	0	0	0	0	0	0	0	3,441	7,211	4,778	492	1,615	7,253	**24,790**
Cheyenne Mountain Zoo	0	0	0	0	0	0	0	0	0	1,015	50	0	0	2,672	400	1,000	1,801	0	2,244	**9,182**
Como Zoo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1,359	3,009	4,007	**8,375**
Toronto Zoo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3,011	509	0	**3,520**
Toledo Zoo	270	0	0	0	0	0	1,239	0	250	0	0	0	0	0	339	21	0	691	0	**2,810**
Detroit Zoo	0	0	0	0	0	0	10	89	0	0	0	0	0	38	152	0	562	12	1,603	**2,466**
Henry Doorly Zoo	311	352	0	614	0	0	0	0	0	0	0	0	700	0	0	0	0	0	0	**1,977**
Memphis Zoo	0	0	0	0	0	0	0	0	0	1,700	0	0	0		0	0	0	0	0	**1,700**
Kansas City Zoo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44	360	725	23	**1,152**
Houston Zoo	0	800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**800**
Central Park Zoo	0	12	0	0	0	0	0	0	0	250	0	0	0	0	500	0	0	0	0	**762**
Omaha Zoo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	127	505	40	**673**
Cincinnati Zoo	429	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**429**
Prospect Park	0	0	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**200**
St. Louis Zoo	0	0	0	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**150**
Totals	**3,416**	**4,464**	**213**	**3,762**	**2,138**	**8,339**	**8,338**	**8,299**	**7,277**	**9,278**	**14,567**	**12,052**	**12,933**	**11,049**	**20,200**	**11,267**	**10,824**	**13,100**	**26,927**	**188,443**

*The facility currently known as Red Buttes Biological Laboratory was known as Sybille before 2007.

NUMBER OF WYOMING TOADS (TOADLETS AND TADPOLES) RELEASED, BY SITE (1995-2013)

Release Site	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	TOTAL
Buford	0	0	0	0	0	0	0	0	0	0	7,623	11,347	9,416	7,207	19,292	11,267	10,824	11,700	9,799	**98,475**
Mortenson Lake	1,093	2,652	*213	3,762	2,082	6,789	8,338	8,299	4,433	0	0	0	0	0	0	0	0	1,400	17,128	**56,189**
Lindzey	0	0	0	0	0	0	0	0	2,844	9,278	6,944	0	0	0	0	0	0	0	0	**19,066**
Shaffer	0	0	0	0	0	0	0	0	0	0	0	705	3,517	3,842	908	0	0	0	0	**8,972**
Lake George	1,803	1,812	0	0	56	1,550	0	0	0	0	0	0	0	0	0	0	0	0	0	**5,221**
Rush Lake	520	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**520**
Totals	**3,416**	**4,464**	**213**	**3,762**	**2,138**	**8,339**	**8,338**	**8,299**	**7,277**	**9,278**	**14,567**	**12,052**	**12,933**	**11,049**	**20,200**	**11,267**	**10,824**	**13,100**	**26,927**	**188,443**

*Only toadlets were released in 1997.

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PERSONAL COMMUNICATIONS

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Appendix C. COMMENTS TO DRAFT RECOVERY PLAN AND RESPONSES

The draft Revised Recovery Plan for the Wyoming toad was open for public comment from February 20, 2014 to April 10, 2014. Public comments were provided by the Wyoming Game and Fish Department, the Laramie Rivers Conservation District, and Anadarko Petroleum Corporation. We also requested independent peer review and received comments from three experts, including a private landowner in Nevada, an aquatic species specialist from Colorado Parks and Wildlife, and a research scientist specializing in amphibian conservation research in California. All comment letters are on file in the USFWS Ecological Services Office in Cheyenne, Wyoming. The diverse comments received have significantly improved the quality of this document and ranged from editorial suggestions to new ideas. We have incorporated all applicable comments into the text of the final revised recovery plan as appropriate. Following are our responses to comments that warranted further explanation or were not addressed in the text. Comments have been organized into categories below.

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COMMENTS AND RESPONSES

<u>Predation</u>

> **Comment**: One peer reviewer suggested completing a comprehensive fish study at Mortenson Lake to determine what species are present and then to determine if those species are a threat to the Wyoming toad through a palatability study.
>
> **Response**: Because the Mortenson Lake Wyoming toad population was reported to be thriving during the active fishery at Mortenson Lake in the 1980s and additional evidence that toads are not palatable to fish (Grasso 2010), the Service has made a determination that fish do not pose a major threat to Wyoming toads at Mortenson Lake. Fish are not currently stocked into Mortenson Lake, but comprehensive fish and palatability studies could be considered if an active fishery is proposed at any reintroduction site.

Comment: One peer reviewer asked why are only five populations required for delisitng and suggested that because predators could wipe out entire populations, more than five populations would be warranted.

Response: The five populations referenced are the minimum necessary to create a stable and viable population of Wyoming toads as determined by a population viability analysis. While more populations are not discouraged, they are not necessary for delisting of the species. These five populations need to be viable and self-sustaining, determined by a formal population viability analysis- meaning they are not so vulnerable to be wiped out by a predator event. It is likely that more than five populations will be started and some wiped out (i.e. not viable and self-sustaining) before five populations are declared viable enough to warrant delisting.

Reintroduction Sites

Comment: One peer reviewer asked why tadpoles were released at the SHA site if there was no appropriate breeding habitat.

Response: At the time the tadpoles were released at the SHA site, it was unknown that the habitat was not appropriate for the Wyoming toad. This species is unique in that it disappeared before data was collected on habitat preferences and the Service consequently recognizes the need to continue to try new ideas and to learn from failed attempts. Language was adjusted to reflect that it was determined that the SHA site did not have the proper habitat through this attempt.

Comment: One commenter suggested 10(j) programs need to be better emphasized and invoked in the recovery process.

Response: While the Service agrees that a 10(j) rulemaking could be beneficial to the Wyoming toad and is included as possible recovery action (4.3.5.3), it is low priority due to the staff time required. Many of the same landowner assurances are currently provided by an existing programmatic Safe Harbor Agreement, which we anticipate will continue to help in developing new reintroduction sites.

Comment: One commenter asked where the 10,000 acres to support five self-sustaining populations (indicated in the delisting criteria) came from.

Response: The need for 10,000 acres of land for the establishment of five self-sustaining populations was estimated based on the need for five populations for delisting and the size of the Mortenson Lake population. Mortenson Lake is approximately 2,000 acres in size and would be considered one population. While it is not known how large of an area a self-sustaining population would actually need, the area of Mortenson Lake multiplied by five for five populations would result in 10,000 acres. This number is not viewed as a requirement for change of listing status in and of itself, but is offered as a starting point for the acreage of what may be required for the toad. The discussion of the 10,000 acre estimate was moved to Section 4.3.5 to more clearly indicate that it is not a requirement for delisting.

Comment: One peer reviewer asked why the protection of the Mortenson Lake population was essential if populations can be established elsewhere.

Response: Mortenson Lake is the site of the last population of Wyoming toads in existence and was developed into a National Wildlife Refuge for the purpose of recovering the toad. It is currently the only site out of three reintroduction sites where the population has demonstrated overwinter survival. The recovery of the Wyoming toad is currently limited by the lack of reintroduction sites and knowledge about the species' needs. The Mortenson Lake population serves as an opportunity to learn about habitat preferences, life history, disease dynamics, and other areas. While this population does not need to be one of the five populations that would count towards recovery, it would be detrimental to this program if that population was lost without a replacement. Language was updated and clarified to reflect such.

Comment: One peer reviewer asked if the vegetation at other reintroduction sites could be artificially manipulated by management practices.

Response: Yes. The Service is currently funding a UW study to determine what management practices are most beneficial to creating suitable habitat for the Wyoming toad. Once these techniques are identified, they can be applied to additional sites. However, the Service does not have access to habitat manipulation on privately owned land unless the landowner signs up for protections under the Safe Harbor Agreement and agrees to habitat manipulation.

Research and Study Suggestions

Comment: One peer reviewer suggested that the example research questions are too narrowly focused to be useful and suggested targeting research at the most likely cause of low toad survival (i.e., chytridiomycosis).

Response: Current research is focusing on the highest priority research questions (including chytridiomycosis), but there are many aspects of Wyoming toad survival which need to be addressed. The Wyoming toad is unique in that little research was conducted on the species before it was considered "extinct in the wild." By the time the species was listed and the Service began the recovery process, there were too few individuals to determine what optimal habitat and preferences supporting survival really were. In addition, this species is notoriously difficult to keep in captivity and challenges continue in optimizing captive toad health. The Service is currently focusing on high priority research questions, but recognizes there is much to be learned.

Comment: One peer reviewer suggested that without proven methods by which to re-establish Wyoming toad populations in the wild, the captive propagation effort will be for naught. To determine successful reintroduction methods, the peer reviewer suggests conducting a carefully designed study of toads that are released into Mortensen Lake, including the following: release as many adult toads as possible, all of which are PIT tagged (with 8 mm tags) and fitted with radio-transmitters (external transmitters have been used successfully on other toads), determine the locations of these toads on a regular basis (every week) and collect skin swabs for qPCR Bd assays to allow quantification of Bd infection loads through time, habitat use, and causes of mortality.

Response: The recent increase of Wyoming toads at Mortenson Lake has allowed for the collection of more data that was not possible previously. The revised recovery plan has been updated to reflect this effort. The Service is interested in expanding on previous efforts and better understanding the needs of the Wyoming toad in the wild through means such as those suggested. The Service is currently investigating Bd dynamics at Mortenson Lake with PIT tagged adults and qPCR analysis of Bd samples collected during annual monitoring visual encounter surveys.

Comment: One peer reviewer suggested studying a surrogate toad species similar to the Wyoming toad and further suggests that if the Wyoming toad and the Canadian toad are closely related, the Canadian toad could be very useful as a surrogate species for studying habitat preferences and responses to Bd.

Response: Recent population growth of the Wyoming toad at Mortenson Lake has now made it possible to determine habitat needs and preferences in a local setting. If the Service believes the population at Mortenson Lake cannot provide the answers needed, the Canadian toad may serve as a surrogate.

<u>Contaminants</u>

Comment: One peer reviewer notes that contaminants and fertilizers will be found if you look hard enough, but asks given such an expensive endeavor, how the information will impact management decisions.

Response: Because the decline of the Wyoming toad is correlated with the use of Fenthion, the Service believes monitoring for contaminants to be an important aspect of Wyoming toad management. Human activities, even in a rural community such as the Laramie Basin, can have very direct impacts on the toad. Because contaminants may be directly applied to Wyoming toad habitat, the Service believes it is important to monitor the presence and potential impacts to the Wyoming toad on release sites. Management decisions are made with this in mind; for example, water quality is tested at potential release sites to make sure it is within the range that has been identified as acceptable for the Wyoming toad.

<u>Chytridiomycosis/ Bd</u>

Comment: One peer reviewer suggests that the approach taken by the Recovery Plan to deal with Bd is largely one of separating toads from Bd, but the outcome of this approach is the release of Bd-naïve toads into the wild that are highly susceptible to Bd and quickly succumb to chytridiomycosis. The peer reviewer urges that a better understanding of the toad immune response to Bd and the effectiveness of probiotic treatments is critical.

Response: The Service recognizes the need to understand the effects of this disease on the Wyoming toad and also recognizes recent research showing success treating other anuran species for Bd through both exposure to Bd (invoking an immune response) and probiotic treatments. The Service is funding trials in 2015 to test whether these treatments will be successful for the Wyoming toad. Understanding the susceptibility of naïve toads and the virulence of Bd in a naïve host can benefit future recovery efforts if treatments are successful with the Wyoming toads and they can be treated before release.

Comment: One peer reviewer suggested that the idea that Bd can be eradicated is not realistic and the effort to locate Bd-free sites should be halted. The peer reviewer further suggested finding ways to work with Bd, that this pathogen that will be present at these sites for the indefinite future.

Response: The Service agrees and now recognizes that finding Bd-free sites is not a realistic approach to avoiding infection from Bd. The final recovery plan has been updated to reflect this change.

Comment: One commenter asks if it is realistic to make the eradication or suppression of disease (presumably chytridiomycosis) an objective of this program, further commenting that Bd is a worldwide epidemic and its eradication seems outside of the scope of this program.

Response: While the Service recognizes that chytridiomycosis is a devastating problem for the Wyoming toad and other amphibians worldwide, the recovery of this species is most likely dependent on the control of this disease (i.e., the ability for the toad to develop immunity to the disease on its own is unlikely). Promising research has shown that chytridiomycosis can be successfully treated on other anuran species and the Service is currently funding research to determine the possibilities of treating this disease with the Wyoming toad.

Maps and Historical Range

Comment: One peer reviewer requests for a more detailed map of the historic range (Figure 1) and asks if populations outside of the historic range count towards recovery. If so, the peer reviewer asks if all populations can be outside historic range or is there a minimum number of populations that must be in historic range.

Response: A more detailed map has been provided in the final recovery plan. The historic range represented in Figure 1 was loosely complied from field notes and survey data collected by George Baxter and reported in Baxter and Stromberg (1985). The polygon was hand-drawn and may not be a precise portrayal of the historic habitat, but it is the best estimate to date. It is because of this uncertainty that there are no requirements for populations to occur within the presumed historic range (as defined by Figure 1, Baxter and Stromberg 1985) and there is no minimum number that needs to be within the "historic range." However, we expect that reintroduction sites would likely need to be reasonably close to the historic range to provide the habitat conditions suitable for Wyoming toad survival.

Field Techniques

Comment: One commenter states that recent presentations by WYNDD at WY Toad Recovery Team Meetings seemed more optimistic about the use of photos to individually identify toads than the authors of the plan are and further comments that it is interesting that the authors of the plan have given up on the technique before a report has been submitted.

Response: Our partners at WYNDD shared the protocol for wart pattern recognition before the final report was available so the Service could field-test the technique at Mortenson Lake in 2013. After field-testing the technique, the Service determined that it is not feasible for large populations due to the time-intensive nature. The Service has, however, continued to report that wart pattern recognition may still be a useful technique for small populations and continues to record photographs of wart patterns as a back-up for PIT tagging (only toads over 20 g in weight).

Comment: A peer reviewer asks why the Service bothers with using natural pigment patterns when 8 mm PIT tags are innocuous, relatively cheap, and highly effective.

Response: The Service tags all toads 20 g in weight or greater with 8 mm or 12 mm PIT tags during field surveys (phasing out 12 mm tags). The time-consuming nature of wart pattern recognition limits this technique to use in captivity or in small populations. Photos of wart patterns are still collected for all PIT tagged individuals in the field even in larger populations to be used as a backup for PIT tagging (i.e. equipment failure, loss of PIT tags). Other species of toads have shown that tags can be expelled from the body cavity. During the 2014 summer, multiple Wyoming toads were found with PIT tag scars, but no tag was detected. It is unknown if the Wyoming toad is losing PIT tags after marking in the field, but the Service is currently investigating the loss of tags.

Life History and Diet

Comment: One peer reviewer suggested including information regarding the longevity and age of sexual maturity in the life history section to understand whether the proposed timeline is realistic in allocating 2 years to establish populations and 3 years to collect population dynamics parameters.

Response: Much of the basic life history about this species is missing because very little information was collected before the major population crash in the 1970's. However, additional information, albeit minimal, was added to the section to make it more thorough and provide information to aid in understanding generation times as they would relate to PVA.

Comment: One peer reviewer stated that concerns about limited diet information should not hinder identification of potential reintroduction sites because toads will eat any invertebrate that moves and invertebrates are generally plentiful enough to support toads.

Response: Language in this section has been updated to focus on how the lack of information on wild Wyoming toad diet inhibits the development of a sufficient captive diet. There is currently no evidence that lack of prey items are inhibiting Wyoming toad survival in the wild, but has not been ruled out as a possibility.

General Comments

Comment: One peer reviewer suggests that the captive breeding appears to be the dominant current management direction, but an equally important aspect of recovery is the development of methods by which to successfully reestablish toad populations in the wild. The peer reviewer further suggests that an improved scientific foundation for understanding toad reintroductions is necessary.

Response: The Service recognizes that understanding limiting factors of survival in wild populations is essential for recovery of this species. Language in the final recovery plan has been updated to include recent research focusing on habitat management, reintroduction strategies, and chytridiomycosis of wild toads. Understanding how to improve the health and reproduction of captive toads is ongoing as we simultaneously recognize the importance of a healthy captive population.

Comment: One peer reviewer asks if the Service can expect the $4 million considering declining Congressional budgets and asks if inflationary costs were included.

Response: Recovery Actions of a recovery plan are developed in an ideal situation and the Service cannot predict whether or not all the funds outlined will be received. Inflationary costs have not been included in the total.

Comment: One peer reviewer questions the statement "high recovery potential", stating that out of the tens of thousands of tadpoles, toads, and toadlets released into the wild over the past decade (at great taxpayer expense), there are still very few live animals in the wild. The reviewer asks the Service to expand on how such a statement could be made.

Response: Recent research is making significant progress towards providing information that can inform management on how to overcome limiting factors of wild toad survival and progress towards self-sustaining populations in the wild. The Wyoming toad is unique in that little research was conducted on the species before there were very few left and it was considered “extinct in the wild”, making recovery a less than straightforward process. While continuing to address the health needs of captive populations, the Service has recently increased their focus on understanding limiting factors of wild toad survival and adopted an increased commitment to a standardized scientific approach to address the lack of information regarding survival in the wild. Repeated trials are necessary to determine how to ideally support this species and an adaptive management approach is critical to this process by integrating new information and progressing towards survival and viability.

Comment: One peer reviewer suggests that the challenges in developing a Population Viability Analysis (PVA) for the Wyoming toad are many and need to be acknowledged. The peer reviewer then suggests that given the difficulties, the available options are to (1) use what little information is available for the Wyoming toad and augment that with published data on other toad species, or (2) collect information from a surrogate species such as the Canadian toad.

Response: The Service recognizes that it will not have access to the information needed for a PVA until the species is relatively stable and reproducing, but will be necessary to determine the stability of the population into the future. The current preference of the Service is to conduct a PVA on data specific to the Wyoming toad, but may consider using the Canadian toad as a surrogate if data with the Wyoming toad are not available.

Comment: One commenter suggests that the Adaptive Management section should state the Service's commitment to monitoring and further states that the LRCD has been paying for consistent and coherent monitoring at the Buford SHA since toads were first released. The commenter would like to see monitoring as an integral part of any new release site with a formal statement and financial commitment from the Service written into this document.

Response: Consistent standardized monitoring is a priority for the Service at all release sites. The Service continues to provide funding for monitoring at Buford SHA (matching current LRCD funding) and while Service cannot formally allocate future funding, consistent monitoring remains a top priority for the Wyoming field office. The Service continues to work with WYNDD to develop standardized monitoring for all reintroduction sites in order to determine success of reintroductions, population sizes, as well as collect data to inform management decisions.