SPECIES ASSESSMENT FOR WYOMING TOAD (*BUFO BAXTERI*) IN WYOMING

prepared by

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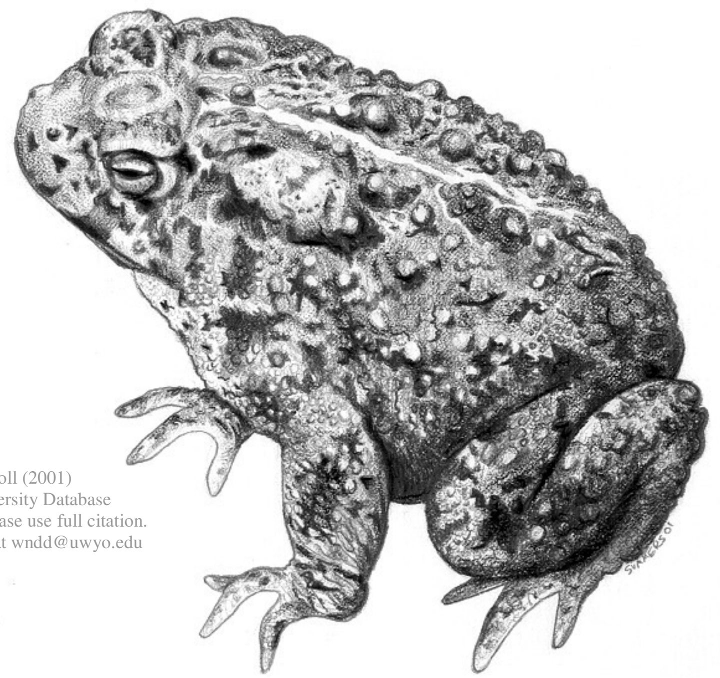

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Introduction

The Wyoming toad (*Bufo baxteri*) is a glacial relict species currently found in one location in Albany County, Wyoming. The toad was once abundant throughout the Laramie Basin and frequented the Big and Little Laramie Rivers and the floodplains associated with it. Habitat modification in the form of irrigation reduced the floodplain habitat and forced the toads to move into the saline lakes of the Laramie Basin. Wyoming toad populations began to decline in the 1970s and the species was listed as federally endangered in January 1984 (49 F.R. 1992, January 17, 1984). At this time, it was also presumed extinct in the wild. However, in 1987 a fisherman reported seeing the toad at a private fishing lake approximately 32.2 km from Laramie. This population began to decline in the early 1990s and individuals from the population were taken into captivity. In 1996, a Species Survival Plan was formed and currently eight accredited American Zoo and Aquarium Association (AZA) facilities participate in the captive breeding program.

Though the program has experienced some amount of success, much of the life history of this toad remains a mystery making it difficult to manage it in the wild and in captivity. Moreover, all studies conducted on the toad since its rediscovery have necessarily focused on the small relict population, which may not represent the biological condition of a healthy population. There are many obstacles that stand in the way of restoring this toad to the Basin, but the amphibian chytrid fungus (*Batrachochytrium dendrobatis*) appears to be the biggest. This fungus has drastically increased the mortality rate of young toads, so recruitment of sexual mature adults is low. In the current state, the population is not self sustainable and would not exist if it were not for the augmentation form the captive breeding facilities.

The current population is managed as a cooperative effort between the U.S. Fish and Wildlife Service (USFWS), Wyoming Game and Fish Department (WGFD), and the University of Wyoming (UW). A Wyoming Toad Recovery Team was appointed in 2000 that contains members from the above groups as well as representatives from Wyoming Natural Diversity Database (WYNDD) and Laramie Rivers Conservation District.

Natural History

Morphological Description

The background color of adult Wyoming toads is variable and can be dark brown, gray, or greenish, with small dark blotches and a distinct light median line. The belly is often spotted and some individuals have well-defined light lateral stripes. The dorsal surface of the body has raised, rounded glands (warts). The warts of the Wyoming toad are intermediate in size between those of the Great Plains toad (*Bufo cognatus*) and the boreal toad (*Bufo boreas boreas).* The cranial crests fuse medially to form an elongate boss, a ridge with a median groove, or paired ridges. The boss is often thick with keratin (cornified). Postorbital ridges are indistinct or absent. The tympanum is round and smaller than the eye. Cutting tubercles on the hind foot are well developed (Baxter and Stone 1985, Stebbins 1985).

The average length of an adult Wyoming toad is 5.5 cm, with females growing slightly larger than males (Baxter and Stone 1985). Males can also be distinguished from females by the presence of raised flesh (nuptial pads) on the forefingers and thumbs. The nuptial pads are often dark brown or orange in color. They appear when the males reach sexually maturity, usually at one year of age. The male Wyoming toad will also develop a throat patch at this time. This patch is solid black in color but can be faint. Females will have dark markings on their throats but they are never solid. These markings are distinct and can be used to identify individual toads.

In addition, male Wyoming toads are able to vocalize while females cannot. There are three distinct calls. The male will confirm his presence during breeding season with a mating call consisting of a short buzzing trill (Baxter 1952). This differs from the deeper pitched guttural vibration the toad will produce when grasped by another male (Baxter 1952, D. Roberts pers.comm.). A short staccato "pip" deemed a protest call has also been documented (Withers 1992) and may occur when the toads are handled.

The Wyoming toad looks similar to closely related Canadian toad (*Bufo hemiophyrs)* except in that it has a narrower mid-dorsal stripe (Baxter and Stone 1985). However, the ranges of these species are separated by a distance of 800km (Parker 2000). Adult Wyoming toads are easily distinguished from other *Bufo* species within its range. Historically, only the boreal toad has occupied the same habitat as the Wyoming toad. However, the toads occupied the habitat during different times. The Woodhouse's toad (*Bufo woodhousei woodhousei)* has been reported in the Laramie Basin in recent years (G. Baxter pers. comm., D. Miller pers. comm.).

The Wyoming toad can be distinguished from the boreal toad based on skin texture, size, skin color, and differences in breeding calls and cranial crests. The boreal toad is larger than the Wyoming toad and has an average length of 7.62 cm (Baxter and Stone 1985). In addition, the skin of the boreal toad tends to be thinner (similar to frog skin) and smoother than that of the Wyoming toad. Boreal toads vary in color from dire-gray to black and are typically darker than Wyoming toads. Furthermore, boreal toads lack fused cranial crests. The male boreal toad rarely calls while in breeding congregations but will produce a chirp when grasped by another male. Lastly, it is worth noting that boreal toads give off a musky odor, which is absent in Wyoming toads (Baxter and Stone 1985).

Differences between the Wyoming and Woodhouse's toad include: body size; stomach coloration; tympanum size; cranial crest differences; postorbital ridge presence; and differences in breeding calls. Adult Woodhouse's toads are much larger than adult Wyoming toads. The average snout vent length of an adult Woodhouse's toad is 8.3 cm (Baxter and Stone 1985). The stomach of Woodhouse's toads typically lacks coloration while the stomach of the Wyoming toad tends to be spotted. Furthermore, the tympanum of the Woodhouse's toad is oval and larger than the eye whereas the tympanums of Wyoming toads are circular and small. The Woodhouse's toad has well-separated, paralleled cranial crests with distinct postorbital bosses which contrast with the medially fused cranial crests of the Wyoming toad. In addition, the Wyoming toad is usually lacking postorbital ridges. Lastly, the call of the Woodhouse's toad is a loud squall (Baxter and Stone 1985).

The Wyoming toad can be distinguished from Ranids (*Rana sp. and Pseudacris sp.)* in its range by the presence of glands (warts), these are lacking in all species of Ranid in its range. It can be easily distinguished from spadefoots (*Spea sp.)* by the presence of cranial crests and paratoid glands.

It is difficult to distinguish between Wyoming, boreal, and Woodhouse's toad juveniles that are younger than six months of age. Identification of the postorbital bosses can make the distinction easier; these are absent in Wyoming toads. In addition, after six months the fused cranial crests of the Wyoming toad will appear making the distinction easier.

Wyoming toad tadpoles are jet black and can be distinguished from other *Bufo* tadpoles by evaluating teeth characteristics, skin color, pigmentation, and snout shape (Altig et. al. 1998). This is almost impossible to do in the field and often requires the use of a microscope. The black coloration of the Wyoming toad tadpoles distinguishes it from frog and spadefoot tadpoles, which can be dark in coloration but never solid black. In addition, they can be identified as *Bufo* tadpoles based on the location of the vent. In *Bufo* tadpoles, the vent is at the midline of the venter while it is found on the right side of the venter in frog species (Livo 1998). Wyoming toad tadpoles will reach a maximum length of 30mm prior to metamorphosis; this will decrease as the tail is absorbed

The eggs of the Wyoming toad resemble a black pearl necklace and are identical in appearance to other *Bufo* eggs in the region (Baxter and Stone 1985). They vary from the large egg masses laid in clumps by local species of frog and spadefoot.

Taxonomy and Distribution

When the Wyoming toad was first reported in 1946, it was classified as *Bufo hemiophrys baxteri* as it was presumed to be a subspecies of the Canadian toad (*Bufo hemiophrys).* The subspecies rank was assigned after evaluation of the following traits: gas and paper chromatography of the paratoid venom, parameters such as snout vent length (SVL) and head width, mating call spectrographs, and reproductive traits. After the above were evaluated, it was found that there were no differences in paratoid secretions. However, the Wyoming toad population had smaller head width and SVL that the contiguous population and had a more prominent cranial crest. It was also observed that the Wyoming toad had a lower dominant frequency and the breeding call was longer in duration. Lastly, no significant differences in fertilization success or rates of development were noted (Porter 1968). However, in 1997, the Wyoming toad was reclassified as *Bufo baxteri* (Smith et al.). This reclassification was based upon additional morphological differences. These differences included: throat and vocal pouch (Blair 1957), mating call (Blair 1957), vertebrae (Porter 1968), and measurements and features of the skull (Porter 1968, Smith et al. 1997).

Before the population crashed in the 1980s, the Wyoming toad was found along the floodplains and seepage lakes of the Big and Little Laramie Rivers (Figure 1). The approximate range was about 24 km north of Laramie, Wyoming and 24 km west (Stebbins, 1985). The Wyoming toad was found at elevations ranging from 7000 - 7500 ft. (G. Baxter pers. comm.). Currently, the only known population is found at Mortenson Lake, Wyoming (Jennings et. al 2000).

Mortenson Lake National Wildlife Refuge (MLNWR) was established in May of 1993 to protect the last known breeding populations of the Wyoming toad. The Refuge encompasses 719 hectares of the Laramie Plains, and is located approximately 24 kilometers southwest of Laramie, Wyoming in Albany County. The Refuge contains four impoundments including Mortenson Lake, Garber Lake, Gibbs Pond, and Soda Lake. All toads present at the lake are thought to be the result of captive propagation and reintroduction.

In addition, toads were historically found at Lake George, which is 20 km from MLNWR within the boundaries at the Hutton Lake National Wildlife Refuge. Captive animals were released at Lake George from 1995-2000 (i.e., in 1995, 1996, 1999, and 2000). Hutton lake NWR is located in Albany County, in southeastern Wyoming, 20 kilometers southwest of Laramie. Established in 1932, it serves as a resting and breeding ground for migratory birds, and to provide a haven for other indigenous wildlife. The Refuge encompasses 796 hectares consisting of 570 hectares of sagebrush-dominated uplands, 227 hectares of open water and marsh. The Refuge has five small lakes (Hutton, Creighton, George, Rush and Hoge) that experience large water level fluctuations and high alkalinities. Generally, Refuge water rights only provide water in early spring and late fall. However, due to dry conditions in the Basin, the head gates have not been opened and therefore Hutton has not received water since about 1999 (USFWS 2000).

Habitat Requirements

General

Wyoming toads appear to be a water dependant species. During research conducted in 1998-1999, they were not found far from the moist margin of the lake during the active season. In addition, when approached by researchers, 70 percent of the wild juveniles used open water as a means of escape (Parker 2000).

Soil moisture appears to be the factor that influences habitat use the most. According to the 1-2-3-4 scale of soil moisture, (dry-moist-saturated-standing water) Wyoming toads in 1990-1991 used soiled that rated 2.0-3.6 (Withers 1992). Similar observations were made in 1998 and 1999 (Parker 2000).

Though slightly different results were obtained, research conducted indicates that a variety of vegetation and a variety of amount of vegetative cover are needed. Wyoming toads require open areas, areas of intermediate vegetation cover, and dense vegetation cover (Withers 1992; Parker 2000). Based on observations and plant measurements, the presence of narrow stemmed plants (diameter less than 7 mm) in the littoral zone is necessary. Low stem densities allow freer movement of amplexing toads. In addition, this will allow more light to shallow areas where embryos develop. The rate at which embryos develop is directly related to temperature (Withers 1992).

Mortenson Lake is approximately 32 hectares and has a well-developed wetland complex of bulrush (*Scirpus aqcutus*) and Baltic rush (*Juncus balticus*). Garber Lake is approximately 9 hectares, has relatively high alkalinity, and an unstable water level. The fluctuating water levels of Garber have limited vegetation development to a narrow band of Baltic rush. Nebraska sedge (*Carex nebrascensis*) and saltgrass (*Distichlis stricta*) occur on seasonally flooded areas adjacent to both lakes. Gibbs pond is approximately 9 hectares surrounded by short grass prairie with very little wetland vegetation. Soda Lake is approximately 28 hectares with minimal wetland vegetation and the water is very alkaline. In recent years, modified irrigation practices have reduced salinity in both Soda Lake and Gibbs Pond. The refuge is currently closed to all public access to minimize impacts to Wyoming toads. Prescribed grazing is the primary land management tool; the Refuge is divided into 4 pastures and approximately 450 Animal Unit Months (AUM's) of grazing occur each growing season. Grazing is also used to manipulate vegetation near Mortenson Lake for the benefit of the Wyoming toad and other wildlife (USFWS 2000)

Spring Shifts

Specific habitats that are used in the spring are utilized by calling males, breeding pairs, and for egg laying. Based on all current habitat research, it appears that the habitat Wyoming toads use for calling and breeding the same as with that which is used for egg laying and rearing (McCleary 1989; Withers 1992). Calling males and breeding (amplexing) pairs utilize similar habitats of shallow water with low stem densities (Withers 1992). On average, this water is 3.5-6.5 cm in the littoral areas of the lake (Withers 1992).

During the summers of 1990 and 1991, eggs were laid and developed in 3.5 - 6.3 cm of water in the littoral areas of the lake. Creeping spike rush (sci name) and American bulrush were the dominant plants in the areas used for calling, breeding, egg laying, and egg development. The mean vertical cover at the 0 - 10cm interval was 25 - 33%. The maximum pH measured at the nest sites was 8.63 (Withers 1992). Eggs at Mortenson Lake have always been laid on the north shoreline where the average water temperature is more than 10°C warmer than other shorelines of the lake (Foster 2001). However, whether the toads prefer the north shore based upon temperature and not habitat structure or water chemistry has not been investigated.

Summer Shifts

Specific habitat for tadpole rearing and metamorphic sites are used during the summer months. Tadpoles have been observed at a mean water depth of 3cm and always seek out the shallowest water in a given area during the day. They typically used water that was 1.7(C warmer and 1.5cm more shallow than surrounding areas presumably in response to the nighttime cooling of the shallows. At night, the tadpoles seek out deeper water (Withers, 1991). In 1991, tadpoles formed four large congregation sites at MLNWR and occupied the warmest water in a given area. Water temperature during development varies greatly. In 1990, the temperature varied between 20.6°C and 23.7°C.

Metamorphosing toadlets utilize open areas (areas with little to no vegetative cover) during the day. These open areas were bordered by dense vegetation dominated by American bulrush (*Scirpus americanus*). In 1991, YOY metamorphosed onto saturated soils in littoral areas with sparse vegetation and shallow water (Withers 1992).

Fall-Winter Shifts

During the fall in late September through October, Wyoming toads begin selecting hibernation sites. Data from early observations indicate that the toads at Mortenson Lake migrate from the north shore to the south shoreline to hibernate (Withers 1992, Chamberlain 1990). However, there was no evidence of this migration in later years (Parker 2000).

Research conducted indicates that the closely related Canadian toad (*Bufo hemiophyrs*) enter hibernacula in mid-September in Alberta (Kuyt 1991), but such movements may begin as early as late August in Minnesota (Breckenridge and Tester 1961). Both adults and juveniles exhibit strong homing abilities to specific wintering sites (>92% of 1,950 individuals over six years; Kelleher and Tester 1969). In northern regions, hibernacula contain several hundred individuals (Kuyt 1991).

Wyoming toads hibernate during the winter. Adult toads enter hibernation sooner than juveniles (Withers 1992). The habitat required for hibernation has not been studied extensively. Based upon personal research and the habits of *Bufo hemiophrys,* Withers (1992) hypothesized that the Wyoming toads require fossorial rodent burrows or holes that border riparian areas within 15 m of littoral areas. Toads were observed using rodent burrows prior to hibernation that ranged in diameter of 3.8 - 5.0m.

During 1999, four captive-released adult Wyoming toads were fitted with radio transmitters and tracked prior to hibernation at Mortenson Lake. Only one of these toads was observed using a rodent burrow (Parker 2000). One toad was using the soft soil remaining from a badger hole digging. This toad stayed underground at a depth of .36m for a total of 35 days. At that time, the weather improved and it another toad reemerged from the hibernation sites to forage. At this time, the batteries in the transmitters failed. Thus, little information was gained about the hibernacula of the Wyoming toad. However, all prospective hibernation sites were in areas of soft, barely moist soil (Parker 2000).

It is presumed that Wyoming toads hibernated in rodent burrows and diggings when they inhabited the Laramie River (G. Baxter pers. comm.). However, there has been no formal investigation or documentation of the hibernation habitat along the Laramie River.

Area Requirements and Landscape Patterns

Currently, no information exists regarding the area required by individual toads. Therefore, it is difficult to estimate the area needed to support a viable population of Wyoming toads.

At the landscape level, the Wyoming toad appears to need and utilize a variety of microhabitats that range from very open areas to areas of dense vegetation (Withers 1992; Parker 2000). Wyoming toad tadpoles also appear to use a variety of water depths for behavioral thermoregulation; congregating in the deeper water at night in response to cooling and utilizing shallow water during the day (Withers 1992). The toad not only requires varying water depths and vegetation cover, it requires these elements to be intermingled within the same wetland or a series of ponds in close proximity, since the Wyoming toad does not appear to disperse far. Given apparently low dispersal distances and the fact that only one population in one pond is the basis for all current studies, it is unclear what type of landscape level network of ponds is most suitable to support a "metapopulation" Wyoming toads.

Movement and Activity Patterns

Migration

Using photograph mark and recapture and personal observation, seasonal migration at Mortenson Lake was observed from 1990-1992 (Withers 1992; Corn 1993, McCleary 1989). These studies suggest that in late summer the toads migrate from the north shoreline to the south shoreline presumably to hibernate. Parker (2000) did not observe the same migration during 1998-2000. There are two possible hypotheses to explain why the migration was not observed. Parker's investigation did not begin until after the breeding season and the migration could have been missed. Another consideration is that the population of toads that Parker researched was very different from those observed by earlier researchers. The population observed in the early 1990s was wild and had not been supplemented by captive propagation. However, the population that Parker observed was the result of years of captive propagation, during which time the migratory behavior of the toads might have been altered . Moreover, selection in captivity favored those individuals who responded well in a non-migratory environment, so captive animals introduced to the wild may lack the instinct to migrate and hibernate successfully in the wild.

Reproduction and Survivorship

Breeding Behavior

The Wyoming toad is polygamous. In captivity, male Wyoming toads will breed more than once during each breeding season. However, this has not been investigated or documented in the wild. Adult toads emerge from hibernation when the daytime temperatures reach 70°F (Withers 1992). Males appear first and attract females to the breeding site with their calls. Reports from Mortenson Lake indicate that male toads form small breeding congregations consisting of 3-5 toads (A. Anderson pers. comm.). However, breeding congregations of 40 toads along the Laramie River have been observed (Baxter pers comm.). Females that are attracted to the calls will swim near the males. The males will then clasp the female around the waist and form a union called amplexus. During amplexus, the male will squeeze the female and encourage her to release eggs. While the eggs are deposited, the male releases sperm. Toads can remain in amplexus for days. Once the eggs are laid, the male and female toads part ways. The characters that Wyoming toads use to select mates are unknown. However, in other Anuran species, males that produce lower pitched calls are often selected by gravid females (Duellman and Trueb 1986). It is unknown whether male toads attempt to mate with the same groups of females every year or if they choose different partners each season. Whether female toads breed each year has not been determined. In addition, whether pairs return to the same site each year is unknown. However, recorded locations of egg masses at Mortenson Lake indicate that the toads prefer to breed along the north shoreline.

Factors that may limit reproduction include: general weather conditions, wind, sex ratios, age structure of the population, and the over all health of the adult toads. Withers (1992) documented that calling was related to temperature more than light. In 1991, calling at Mortenson Lake took place when air temperatures ranged from 17.5°C - 21.1°C; water temperatures ranged from 17.9°C - 21.9°C. Calling continued as air temperatures fell toward 10°C and decreased once the temperatures fell below this point. During cold summers, a drop in the air temperature may delay the calling of males and thus delay the breeding season. During a managed breeding attempt during 1999, captive toads were transported to Mortenson Lake for breeding. During the attempt, there was a snowstorm. Prior to the storm, the animals had been injected with LHRH (Lutenizing Hormone Releasing Hormone) and were in amplexus. After the storm the toads demonstrated no interest in breeding and couldn't be hormonally induced to resume breeding. During breeding seasons in 1990 and 1991, it was noted that strong winds disrupted transmission of calls and reduced the numbers of calling males (Withers 1992).

Skewed sex ratios could also limit reproduction. If the ratio is skewed favoring males, this could potentially reduce reproductive output. Conversely, if the ratio is skewed favoring females, reproductive output may increase but genetic diversity may decrease depending on how skewed the ratio is. Although age structure can also affect breeding, little is known of age dependant breeding status. Male Wyoming toads reach sexual maturity at one year of age (Withers 1992). Mature eggs were collected from yearling females but Withers (1992) thought that most females reach sexual maturity at two or three years of age. Due to the presence of chytrid fungus, many females will not reach their second or third year of development thus reducing the reproductive output of the population. If this pattern persists for consecutive years, the results could be disastrous.

Breeding Phenology

As mentioned above, the adult toads emerge when daytime temperatures reach 70°F (Withers 1992) which is generally from mid May to the beginning of June. The breeding season will take place for approximately one month following emergence of the female toads from hibernation, usually from mid-May to mid-June (McCleary 1989; Withers 1992; Baxter and Stone 1985). Amplexing time required to encourage a female toad to release her eggs varies. In captivity, this time can be a few hours or a few days. Egg masses have been observed in the wild 24 hours after amplexus (McCleary 1989;Chamberlain 1990).

Fertile eggs will appear as solid black spheres and will develop into small tadpoles in approximately 3-6 days. Unfertile eggs will retain one white and black hemisphere (vegetal and animal poles, respectively) and will not elongate. The fertility rate in the wild varies from 0% to 100%. Infertility, fungus, and trampling have all be cited as reasons for the low fertility rate. As the eggs develop, they will elongate and begin to take on an oval appearance. Hatching will take approximately 48 hours.

Upon hatching, the average length of Wyoming toad tadpoles is 5-7 mm. The first day after hatching, tadpoles will fall into the substrate and remain completely inactive. After this, tadpoles become active and spend the majority of their time feeding and growing. The water temperature and chemistry varies little from that of breeding sites. Complete metamorphosis is usually seen 3-4 weeks post hatch (Withers 1992).

At metamorphosis, Young of the year (YOY) have a snout-vent length (SVL) of 12-15 mm. YOY spend much time during their first season eating and grow an average of 15-18 mm by the end of August. No growth has been noted after September 1 (Withers 1992).

Wyoming toads are suspected to enter hibernation between mid-September and mid-October depending upon the weather conditions that year (Withers 1992; Parker 2000).

Fecundity and Survivorship

Mature ova were collected from wild yearling female Wyoming toads however, Withers (1992) thought that most females were not sexually mature until they were two or three years of age. Wild Wyoming toad males appear to reach sexual maturity at one year of age (Withers 1992). Based on observations in the wild and in captivity, it appears as though female Wyoming toads breed only once per year. However, when given the opportunity, captive male Wyoming toads have had more than one successful breeding attempt in a year (D. Roberts, pers. comm.).

The annual number of offspring produced also varies greatly. In the wild, egg masses ranging in size from 1,000 to 6,000 eggs have been documented (Freda et al. 1988; Withers 1992,;McClearly 1989). Nine egg masses each consisting of 4,000 - 6000 eggs were laid in 1988 (Freda et al. 1988). However, only two masses were observed during 1989 and the number of eggs varied from 1,000 - 5,400 (Withers 1992). Four masses were documented in 1990. One of the masses however was 90% infertile. The last wild egg masses laid at Mortenson Lake were seen during 1991. Four masses consisting of 1,000 - 2000 eggs/mass were documented (Withers 1992). The next time eggs were laid at Mortenson Lake without human intervention was 1998. Four masses were found in historic nest sites along the north shoreline. Three masses were found in 1999. Wyoming Game and Fish and U.S Fish and Wildlife Biologists have also observed evidence of natural reproduction in 2002 and 2003.

Based on historical data at Mortenson Lake collected from 1988 - 1991, nearly 30% of 3-year-old females are expected to produce eggs in a given year (Jennings et al. 2001). However, nearly 10% of egg masses are infertile or destroyed prior to hatching. Therefore, only 27% of adult females are expected to breed and produce metamorphs in a given year. On average, a successful adult female will produce about 3000 eggs per mass. Roughly 95% of these eggs hatch and 10% metamorphose successfully. Given this data, the estimated total average reproductive output per adult female is 285 metamorphs per female, with a range of 95 to 570 (Jennings et al. 2001).

A life table was constructed in 1993 by utilizing photographic mark and recapture methods (Corn 1993). At that time, the population at Mortenson Lake consisted of two age classes; yearlings and 2 year olds. There were few or no toads older than 2 years present. Data collected indicated that adult mortality was 80 percent or greater. Due to the presence of chytrid fungus now and then it is reasonable to assume that the mortality rates observed from 1990-1992 are similar to what the population is experiencing now.

During 2001, a mortality schedule was developed based upon historical field data from Mortenson Lake (Jennings et al 2001). This schedule estimated mortality rates in the wild for toads ages 1-3 to be 85% when chytrid is present. When chytrid is not present, the rate was estimated to be 40%. It was assumed that chytrid did not impact metamorphs (Jennings et al. 2001). This schedule concurs with the above-mentioned figures.

Population Demographics

At this time, there are probably a variety of factors working in concert to limit population growth and geographic expansion such as the effects of inbreeding, predation, and habitat fragmentation. However, the amphibian chytrid fungus (*Batrachochytrium dendrobatidis)* is having the most apparent affect. It is estimated that the mortality rate of 2 -year-old toads infected with the fungus is 80% (Jennings et al 2001). Most females do not begin to breed until they are 3 years old. The fungus is reducing the number of available breeding females thus reducing reproductive output and limiting population growth. It is unknown at this time whether the fungus interferes with the ability of the toad to disperse, but it is likely a factor because infected toads tend to be extremely lethargic. Infected toads exhibiting this symptom may also more prone to predation, as they will not attempt to escape.

Metapopulation Dynamics

No research was conducted while the Wyoming toad was abundant within the Laramie Basin. Therefore no documented information exists pertaining to metapopulation dynamics. However, populations within the Basin were observed for nearly 30 years by University of Wyoming professors and students. From what was observed, the overall impression is that by the 1970s the populations within the Basin were discrete and there was little if any genetic exchange between them. This is puzzling, because the floodplain system of the Laramie Basin at that time provided many suitable wetland corridors and opportunities for toad movement between impoundments (G. Baxter pers. comm.; R. Beiswenger pers comm.). However, by many accounts, the Wyoming toad had begun to decline by the 1970s and this may not reflect what would occur when the toad is more abundant. It is speculated that when toad numbers were more abundant there was a lot of movement and interchange along the floodplains corridors (R. Beiswenger pers. comm.).

Genetic Concerns

When the captive propagation program for the Wyoming toad began the last known population had diminished substantially, to the point where the decision was made to bring all wild animals into captivity. Only 25 animals were brought into captivity during 1989 and 1990 (McCleary 1989; Chamberlain 1990). Survivability and fecundity in the ensuing captive population was initially low. However, in recent years, the amount of offspring produced and subsequently released has increased (Table 2).

Though efforts have been made to maximize the genetic diversity of the population, little diversity remained following the population crash preceding commencement of the captive propagation program. Assuming optimistic pedigrees (e.g. the animals taken into captivity were not related), the entire captive and reintroduced wild population is equivalent to approximately 2.9 unrelated animals. The mean inbreeding coefficient (F) of the population is 0.155, which is slightly greater than a first cousin cross (R. A. Odum pers. comm.). Healthy populations are expected to have an F value of zero, and it has been suggested that some *Rana* species are not able to survive once their F value reaches 0.5 (R. A. Odum pers. comm.). Thus, the Wyoming toad population is definitely has a restricted gene pool and is facing a bottleneck with the potential for severe inbreeding depression. However, it is unclear what this implies for the viability of the species, since there has been insufficient research regarding amphibian genetics from which to draw such inferences. We should view the Wyoming toad as an example case of what can happen when amphibian species are faced with extreme genetic restrictions.

Food Habits

Food items

The complete diet of the Wyoming toad in the wild is not known, but limited information exists. Field personnel have observed Wyoming toad juveniles and adults hunting small black flies and mosquitoes on mud flats (A. Anderson pers. comm.). Thirteen opportunistic scat samples were collected in 1998 and 1999 (Odum pers. comm.). The most common insects identified in the scat were two ant species (*Myrmica incompleta, Formica fusca).M. incompleta* was the dominant food source observed in these samples. Three genera of beetle were also identified *Elaphrus sp., Anara* sp., and *Canthon* sp (Odum pers. comm.). Whether this diet was consistent among the thirteen sampled is unknown.

The diet of tadpoles at Mortenson Lake appears to consist of algae (M. VanVleet, unpublished data). However, the species of algae and the nutritional content of the algae are unknown. It is also unknown whether the tadpoles supplement their diet by foraging on invertebrates such as *Daphnia* as many captive anurans do. Furthermore, it is undetermined whether the diet currently seen at Mortenson reflects the diet of the toad when it was more widely distributed and associated with floodplains of the Big and Little Laramie Rivers.

Foraging Strategy and Flexibility

The foraging strategy of the Wyoming toad has not been well investigated or documented. However, field observations at Mortenson Lake indicate that toads are solitary feeders. In addition, observations indicate that they are nocturnal feeders during the dry portions of summer (Stone 1991).

The only variation of foraging behavior observed has been between life stages. As previously mentioned, the diet of the Wyoming toad tadpole consists mainly of algae and detritus. Once the toads metamorphose, the diet switches to an insect based diet. There are likely differences in the consumption rates and prey species between recently metamorphosed toadlets and adult toads. In addition, differences in consumption rates between mature egg bearing females and sexually mature males are likely. Furthermore, seasonal variation is probable. However, there is no documentation to support this and no research has been conducted to evaluation the foraging variation of the Wyoming toad.

Circadian

The daily activities of the Wyoming toad vary according to temperature (Withers 1992; Parker 2000). In the cool temperatures of early morning, there is little activity. However, as temperatures increase toads have been observed foraging and basking. During hot periods of the active season, toads have been observed burrowing into wet, cool mud to escape the heat. During this time, toads appear to be nocturnal foragers (Stone 1991). However at some point during the night when temperatures decrease adult toads will seek night refuge consisting of 85% canopy cover that is moist to saturated (Parker 2000).

Community Ecology

Interactions between the Wyoming toad and other amphibian species are poorly understood. In addition, interactions (i.e. intraspecific competition) between individual Wyoming toads are not understood. Currently, no documentation or field notation exists to provide anecdotal or speculative information.

Predation on the Wyoming toad by other species is somewhat better understood. The toad is preyed upon by many avian and mammalian species at all life stages. Diving beetle (*Dytiscus sp.)* larvae prey upon the eggs of Wyoming toads. There is no evidence of salamander predation at Mortenson Lake, but predation of boreal toad eggs by salamanders has been documented in Colorado. (C. Fetkavich pers. comm.).

Wyoming toad larvae are predated by a variety of animals. Potential predators include but are not limited to pelicans (*Elecanus erythrorhynchos*), black crowned night herons (*Nycticorax nyticorax),* Sand hill cranes (*Grus canadensis)*, and magpies (*Pica pica).* Field personnel have observed tadpoles being eaten by diving beetle larvae and dragon fly larvae. It has been shown that diving beetle larvae can potentially have large effects on the number of boreal toad tadpoles that survive to metamorphosis (Livo 1999). Gray jays (*Persisoureus canadensis)* are known to prey upon boreal toad tadpoles (Beiswenger 1981). In addition, field personnel have documented boreal toad tadpole predation by mallard ducks (*Anas platyrhynchos (C. Fetkavich pers. comm.).* Currently, there is no evidence that the non-native trout stocked at Mortenson feed on tadpoles.

However, non-native trout have been documented to prey on the mountain yellow-legged frogs in California and other frog species worldwide (Fill and Matthews 1998; Liss and Larson 1991). Predation by fish devastated a population of mountain yellow-legged frogs from fish devastated the population (Fill and Matthews 1998). An investigation of Canadian lakes reported that amphibian species declined in naturally fish-less lakes after the introduction of non-native trout (Liss and Larson 1991).

Depredation of adult Wyoming toads was first documented during 1990-1992. During this time, abrasions puncture wounds, broken limbs, and partial consumption were documented (Withers 1992) but the predator was not identified. Potential predators of adult Wyoming toads include raccoons (*Procyon lotor*), weasels (*Mustela sp.),* skunks (*Mephitis mephitis hudsonica*), coyotes *(Canis latrans),* mink (*Mustela vison*), red foxes (*Vulpes vulpes),* badgers (*Taxidea taxus*), domestic and feral cats (*Felis sp.),* hawks (*Buteo sp.),* and many of the species presumed to prey upon tadpoles. During the summers of 1998 and 1999, depredation of adult Wyoming toads by avian and mammalian predators was observed (Parker 2000). Seven of ten toads implanted with active transmitters were lost to predation. Puncture marks indicate that an avian predator took one of the toads. In the other six cases, teeth and claws marks indicate a mammalian predator was involved (Parker 2000).

Conservation

Conservation Status

Federal Endangered Species Act

The U.S. Fish and Wildlife Service listed the Wyoming toad as endangered in January 1984 (Service; 49 F.R. 1992). It was given a recovery priority of 1, which denotes a high degree of threat and a high recovery potential. A federal permit is required to house, transport, research, breed, display, or release the animal.

Bureau of Land Management

The BLM has not issued additional protection for the Wyoming toad.

Forest Service

The Forest Service has not issued additional protection for the Wyoming toad.

State Wildlife Agencies

The Wyoming toad has been given a Native Species Score of one (NSS1), which indicates that the Wyoming toad is critically imperiled because of extreme rarity (often known from 5 or fewer extant occurrences or very few remaining individuals) or because some factor of a species' life history makes it vulnerable to extinction. According to Chapter 10 of the WGFD regulations, a permit is required to import, possess, confine, transport, or sell wildlife. Permits to possess or import the Wyoming toad will not be issued to the general public but rather are given to accredited zoological facilities. Under Chapter 52 (W.S.§23-1-103, §23-1-302) of the regulations it is unlawful to take Wyoming toads unless a Chapter 10 or other permit is valid and current.

Heritage Ranks and WYNDD's Wyoming Significance Rank

The Natural Heritage Network assigns range-wide and state-level ranks to species based on established evaluation criteria (e.g., Keinath et al 2003, Master et al. 2000). Since the Wyoming toad only occurs in Wyoming, the only natural heritage program to give it a rank is the Wyoming Natural Diversity Database. In general, global heritage ranks are assigned based on the assessed risk of extinction of the species, where G1 species are deemed critically imperiled and G5 species are deemed demonstrably secure. *B. baxteri* clearly merits a global rank of G1, which means that rangewide it is deemed by Heritage scientists to be critically imperiled. This is based on a synthesis of state ranks and biological evidence that suggests it has a "very small range and population in a portion of the Laramie Basin, Wyoming; threats are poorly known" (NatureServe Explorer 2003).

State ranks are assigned based on the assessed risk of extinction within a state using the same five-level categorization as for global ranks (i.e., S1-S5). These assessments are based on biological information of population status, natural history, and threats at the state level. The state rank for *B. baxteri* is S1 (Keinath et al 2003). This is because it has a very small Wyoming range, low range occupation, low and unstable abundance, high biological vulnerability, and a high degree of external threats. Additionally, *B. baxteri* has a "very high" Wyoming Contribution Rank because it is endemic to the state of Wyoming.

Biological Conservation Issues

Trends

Abundance Trends

Wyoming toads were abundant in the Laramie Basin until the mid-1970s. At this time the population suffered a dramatic decline and the population was presumed extinct by 1984 (Lewis et al. 1985; Baxter and Stone 1985; Stone 1991) until toads were found during a 1980 survey (Vankirk 1980). In 1981, only two toads (one male, one female) were sighted in the entire Laramie Basin (Baxter and Meyer 1982). Surveys during 1982 yielded no toad sightings. However two juveniles were found in 1983 indicating that reproduction had occurred that year. This was the first occurrence of reproduction recorded since 1975 (Lewis et al. 1985). Extensive cooperative surveys took place in 1984 and 30 male toads were observed. No toads were found during similar surveys conducted in 1985 and 1986 (Stone 1991). A population of toads was discovered at Mortenson Lake in 1987 (Stone 1991).

During 1991, a photographic mark and recapture study was conducted at Mortenson Lake. In 1990, the population at Mortenson Lake was estimated to contain 120 adult toads (Corn 1991). In 1991, the population estimate expanded to 415 toads. However, most of those toads were 1 to 2 years of age (Corn 1992). In 1992, the population estimate was 155 (Corn 1993). Only two toads were found during the fall annual survey in September 1993 (USFWS unpublished data).

In addition, the EPA proposed a pesticide ban in the Basin to protect the Wyoming toad in 1993. The Governor presented a plan to the EPA and the USFWS to protect the toad and mosquito control in the Basin. The plan entailed intensive surveying of the proposed ban area for Wyoming toads and clearing the area for pesticide use if no toads were observed after two consecutive years. From 1994 - 1995 potential toad habitat within the proposed area (approximately 777 square kilometers) was surveyed. No new populations were found during these efforts (Young 1995).

The population at Mortenson Lake continued to be monitored on a bi-annual basis by the U.S. Fish and Wildlife Service, the Wyoming Game and Fish Department, the University of Wyoming, and interested volunteers (Table 1). Numbers from this monitoring should be viewed with caution because from 1995 to the present the population at Mortenson Lake has been augmented with animals produced in captivity.

Formal surveys of HLNWR were conducted sporadically between 1995 and 2001, focusing on Lake George and Rush Lake, where captive-produced toads had been released (Table 1). Survey efforts were minimal during 2002 and 2003 as no toads were released at the lakes and drought conditions made the area unsuitable for toads.

Population Extent and Connectivity Trends

Along with the dramatic decrease in the abundance following the 1980s, the Wyoming toad has experienced a range contraction. Toads were formerly found in wetlands throughout the floodplains and seepage ponds of the Big and Little Laramie Rivers (see Taxonomy and Distribution section), but currently they are restricted to one saline lake (Figure 1). It is speculated that the toads moved from freshwater systems to closed saline systems in response to habitat changes produced by irrigation (G. Baxter pers. comm.). Even when the toad was abundant throughout the Basin (through the early 1970s), it's population was patchily distributed and was mainly associated with the freshwaters of the Big and Little Laramie Rivers and their floodplains (G. Baxter pers comm.).

Habitat Trends

During the past century, the habitat of the toad within the Basin has undergone many changes. Most of the changes in habitat are due to changes in irrigation practices. Irrigation has led to less flooding in riparian areas decreasing potential habitat for the toad. However, irrigation has also "flushed" many of the lakes making them less saline and more hospitable to the toad (G. Baxter pers comm.). Furthermore, flood irrigation has created new wetland habitat, although these wetlands tend to be more saline than river floodplain habitat (Peck and Lovvorn 2001) so it is uncertain how suitable they are for use by Wyoming toads. There were no wetlands inventories in the Laramie Basin prior to irrigation which makes it difficult to assess whether the amount of available wetland habitat has increased or decreased due to irrigation. Diversion of water for irrigation has undoubtly decreased ephemeral wetlands. Irrigation has also created some permanent paulustrine and lacustrine habitats of high quality (J. Lovvorn pers. comm.).Although the only population of Wyoming toads currently exists at Mortenson Lake. It is thought that the toads prefers the fresh water of the river floodplains and only began using saline lakes as the flood plains disappeared (G. Baxter pers comm.).

At both MLNWR and HLNWR, grazing has been used as a management tool. Typically, cattle are allowed to graze the shorelines of the lake in late fall after the toads have entered hibernation. This practice was implemented as all stages of Wyoming toads utilize open habitat for basking and breeding (Withers 1992). Grazing is thought to then the dense grass that tends to grow on the shore lines making more habitat available for the toad. Parker (2000) found that toads used some areas of dense vegetation as night refugia and recommend that grazing at MLNWR cease.

Extrinsic Threats and Reasons for Decline

Anthropogenic Impacts

The use of pesticides has long been suspected as a cause for the decline of the Wyoming toad. Fenthion (Baytex) was applied aerially throughout the Basin for mosquito control. The spraying of Baytex coincided with population declines. However, research using leopard frogs and boreal toads concluded that neither Baytex nor its diesel carrier resulted in immediate deleterious effects (Lewis 1984, Freda 1988). However, this research did not measure the long term consequences exposure to Baytex may have on amphibians. Baytex is no longer used as a pesticide in Laramie and has since been taken off the market. Peremethrin (Biomist) is now being used to control mosquitoes within the city limits of Laramie.

Disease susceptibility and mortality were found to increase in Woodhouse's toads that were externally exposed to field grade Malathion and then challenged with a bacterial (*Aeromonas hydrophila)* injection (Taylor et al. 1998). Malathion is still used on some property adjacent to Mortenson Lake NWR and other sites within the historic range of the toad. In years when the mosquito population reaches large densities the City of Laramie utilizes Malathion (P. Harrison pers. comm.). The increasing interest in mosquito control resulting from fear of west Nile virus has the potential to increase the use of Malathion by municipalities and ranches within the Laramie Basin and is therefore of concern.

Irrigation has modified the habitat of the toad (G. Baxter pers comm.). Increased irrigation has resulted in less flooding in riparian areas and reduced the extent and quality of the floodplain wetlands where the toad formerly resided (G. Baxter pers comm.). However, new wetland habitat has been created by flood irrigation and the construction of reservoirs. The wetlands created by flood irrigation and reservoirs tend to be more saline than the river flood plain habitat (Peck and Lovvorn 2001).

Confounding the loss of habitat is the issue of water rights. Within the Basin, the demand for water far exceeds the supply. Those areas having senior water rights have first access to water that ultimately flows into MLNWR, and only after the needs of the senior water right holders have been met can junior water right holders receive water. In drought conditions, those holding junior water rights may not receive any water. This was the case in 2001 for HNWR (which obtains its water from Sand Creek and holds only junior water rights). At that time, many of the lakes within the Refuge became completely dry and those that did not dry up became increasingly saline (A. Anderson pers comm.). Though this has not been an issue at MLNWR it should be noted that the refuge receives most of its water from seepage from the Pioneer Canal, an irrigation water supply. Irrigation levels in neighboring meadows determine the amount of available water (Withers 1992). In dry years when junior water rights are not met, less irrigation water flows through Pioneer Canal and therefore Mortenson Lake collects less seepage.

Invasive Species

Currently, no invasive species have directly been implicated in the decline of the Wyoming toad. However, bullfrogs (*Rana catesbiana*) have been moving steadily westward from a population at Wheatland, Wyoming, and have been noted to tolerate the higher elevations present in the Laramie Basin (G. Baxter pers comm.). Negative consequences for the Wyoming toad are expected if this species invades the Laramie Basin.

Genetic Factors

Genetic factors have been speculated as a cause contributing to the decline of the Wyoming toad and are definitely having an impact on current recovery efforts, as there is currently a small amount of gene diversity left in Wyoming toads (see the Population Demographics section above). The longer a population stays at a reduced size, the greater the loss of genetic variation and the risks of stochastic demographic or environmental events (Scott et al. 1996). Furthermore, small populations can have their existence jeopardized by genetic problems that include loss of heterozygosity and inbreeding depression. If deleterious alleles are present in the population, close inbreeding can result in inbreeding depression. This can result in reductions in survival and reproductive performance (Scott et al. 1996). Currently, captive breeding efforts are attempting to maximize genetic diversity in the captive stock.

Stochastic Factors (e.g., weather events)

Data suggests the Wyoming toads have a shorter life span than other *Bufo* species, which increases the risk of extinction from natural disasters (Corn 1993). Anecdotal evidence exists to support the theory that weather events in 1988 caused a population decline (Jennings et al 2001; A. Anderson pers comm.). During the fall of 1988, 450 young of the year were observed at Mortenson Lake. No yearlings were found in the spring of 1989. That winter, the Basin was subject to extreme cold weather. During December 1988 and January 1989, there were 10 to 15 consecutive days were the temperatures failed to rise above –15°F (Jennings et al. 2001). In addition, there have been observations to indicate that spring blizzards may delay or possibly halt breeding (A. Anderson pers comm.).

Drought has had consequences for the Wyoming toad at HNWR. During dry years, the refuge is last to receive water and has not received water for the past two years (P. Bilbeisi pers. comm.). This has caused the lake to become shallow and therefore caused an increase in salinity and hardness, making the water chemistry unsuitable for Wyoming toads. Severe drought has not been documented as a significant contributor to population declines at MLNWR.

Natural Predation

It is not known whether natural predation was a factor in the initial decline of Wyoming toad populations, but due to small population size, predation is currently a serious potential threat to the Wyoming toad at MLNWR. Predation has been observed or documented at all life stages (see the Community Ecology section above). Of particular importance are those predators that can have potentially large effects on the number of breeding adults.

Intrinsic Vulnerability

Habitat Specificity and Fidelity

Since the only known population exists on a small, saline lake that is not necessarily indicative of optimal toad habitat, no information exists regarding habitat specificity and fidelity.

Territoriality and Area Requirements

The area requirements for a sustainable, wild population of Wyoming toads are unknown. Male Wyoming toads have not been observed defending breeding territories, nor has this behavior been witnessed in other *Bufo* species (B. Spencer pers. comm.; B. Foster pers. comm.). Therefore, it is unlikely that breeding territory constrains population size in a given area. A more likely mechanism limiting population size is the area of foraging habitat required to sustain individual toads. Here, again, no information is known regarding the extent of foraging habitat necessary to support toads either in their current condition at MLNWR or in their historic habitat within the Laramie Basin.

Area requirements might be roughly derived based on known population size and area of occupation. However, the presence of chytrid fungus at Mortenson Lake since roughly 1989 (A.

Pessier pers. comm.) has made it difficult to estimate a healthy or ideal population size, and abundance has historically not been recorded along the Laramie Rivers.

Susceptibility to Disease

Wild and captive Wyoming toads appear to be very susceptible to disease, especially the amphibian chytrid fungus (*Batrachochytrium dendrobatis*). Amphibian chytrid fungus was found at Mortenson Lake in 2000 (USGS, Dr. David Green) and 2001 (University of Illinois, Dr. Allan Pessier) and is thought to have been present their since at least 1989 (see below). At this time the role the fungus played during the original decline of the toad in the 1980s is unknown. However, recent decline of the Wyoming toad at Mortenson Lake coincides with the latest emergence of the fungus. (A. Pessier, pers. comm.). The chytrid fungus should be a top priority of land managers working with the Wyoming toad.

Chytrid fungi are ubiquitous organisms that live on a variety of substrates including chitin, cellulose, and keratin. Many species are parasites of plants, algae, and invertebrates. At this time, *B. dendrobatis* is the only species of chytrid that is known to infect vertebrate hosts. The chytrid fungus lives within keratin in the superficial skin layers and in heavy infections leads to a thickening of the epidermis. The fungus matures within the cell and eventually forms zoospores, which are expelled through a discharge tube. The spores can then re-infect the same individual or find a new host. As the fungus spreads, the epidermis thickens interfering with the osmoregulatory function of amphibian skin.

In addition, lesions are often formed which provide an ideal environment for other infectious fungi and bacteria (such as *Aeromonas hydrophila*). This can make a proper diagnosis difficult. If one is not looking for chytrid, the infection can easily be mistaken for bacterial red leg or another fungal disease. Such appears to be the case with early diagnoses for the Wyoming toad. The cause of death in the majority of wild and captive toads during 1989 through 1996 appeared to be caused by a fungus *Basidiobolus ranarum* (Taylor et al 1999). *Batrachochytrium dendrobatis* and *Basidiobolus ranarum* have similar morphological features making it difficult to distinguish between them. Recent re-examination of histological sections indicates that the chytrid fungus has been present at Mortenson Lake at least since 1989 (A. Pessier pers. Comm.). The new diagnosis was based on features that are distinct to the amphibian chytrid fungus, such as discharge papillae on fungal thali that orient toward the skin surface, the presence of internally septate (colonial) fungal thali and the presence of thin root-like rhizoids observed extending from fungal thali in silver stained histological sections (Pessier 1999; Longcore 1999; Berger 2000).

Lethal infections of amphibian chytrid fungus have been observed in susceptible laboratory animals without co-factors such as immunosuppression or stress being present (Nichols 2001). However, not all amphibians appear to have the same susceptibility to the disease. For instance, preliminary findings suggest that chorus frogs (*Pseudacris)*, bullfrogs (*Rana catesbiana),* and tiger salamanders are fairly resistant. Resistant species could be carriers for the chytrid fungus and possibly a source of infections for susceptible species, which appears to include many species of *Bufo* (A. Pessier, pers. Comm.).

Dispersal Capability

Current research and observations indicate that the dispersal ability of the Wyoming toad is poor. During 1987, several hundred toads were found at Mortenson Lake. During the next five years only five had dispersed to Meeboer Lake (G. Baxter, pers. comm.). The mean distance that newly introduced captive toads moved in 1999 was 5.05 meters per day (Parker, 2000). In addition, both Withers (1991) and Parker (2000) found that adult Wyoming toads were rarely found far from the moist margin of the lake. This may indicate that Wyoming toads will not disperse in disconnected or closed water systems. The dispersal capability of *B. baxteri* while it inhabited the Big and Little Laramie Rivers is unknown.

Reproductive Capacity

Under the right conditions, the reproductive capacity of the Wyoming toad appears to be similar to other amphibians. However, there are some restrictive factors, including highly variable fertility rates, a potentially high age of first reproduction, a relatively short lifespan, and unknown intervals between reproductive episodes (see below and the above sections on Breeding and Population Demography).

In 1999, a pair of amplexing toads was observed at Mortenson Lake. Though the pair remained in amplexus for more than a day, it did not result in an egg mass (B. Foster pers. comm.). These situations have been observed in captivity as well. It is not uncommon to have failed breeding attempts even after multiple injections of Lutenizing Hormone Releasing Hormone (LHRH). The fertility rate observed in the wild varies from 0-100% (Withers, 1991) and a wide range of fertility has also been observed in captivity (G. Spencer pers. comm.). Based on anecdotal observations, maximal egg production in captivity and the wild may be similar. In recent years, egg masses consisting of 5,000 eggs have been achieved in captivity and a wild-laid egg mass observed in 1998 was estimated to contain 4,000. However, although the reproductive potential in captivity (where a large proportion of eggs survive through metamorphosis) is potentially high, it must be noted that survival of young in the wild is much less and more variable.

Some male Wyoming toads have been able to successfully reproduce as yearlings in captivity (D. Roberts pers. comm.) and mature sperm has been retrieved from wild yearling males (Withers 1992), but it is unknown whether wild toads breed as yearlings. Yearling female Wyoming toads have also been successfully bred in captivity (D. Roberts pers. comm.) and mature ova have been observed in wild yearlings, but Withers (1992) thought that females did not breed until they were two or three years old. Greater reproductive success has been seen in captive female Wyoming toads that are two years of age and older (R.A. Odum pers. comm.).

In captivity, Wyoming toads of both sexes are able to produce egg masses on an annual basis, but it is unknown if wild toads breed annually or every other year, like some other toads in the Rocky Mountains. The percentage of the population breeding in a given year is also unknown. It was once estimated based on past observations (Jennings et. al 2001), but the estimate was unreliable for two main reasons. First, the population at Mortenson Lake has been infected with the chytrid fungus since at least 1989 (A. Pessier pers. comm.) and the high mortality rate witnessed with such infections would undoubtedly have a negative impact on the number of toads breeding in a given year. Second, the survey methods used to estimate the population at Mortenson are not statistically valid and therefore, should not be used to estimate the percentage of the population breeding in a given year.

Protected Areas

The Wyoming toad has been reintroduced on both Hutton and Mortenson National Wildlife Refuges. MLNWR was purchased with the sole purpose of providing habitat for the toad. This refuge is closed to the public and management decisions are made based upon what is best for the toad. Though HLNWR was originally designated as habitat for migratory birds, the habitat needs of the toad are now given high priority when making management decisions regarding land use such as cattle grazing and pesticide use.

The Service is in the process of working with the Laramie Rivers Conservation District (LRCD) to create a Safe Harbor Agreement for Albany County. This voluntary agreement would allow interested private landowners to participate in proactive conservation of the Wyoming toad through habitat protection or reintroductions while allowing day-to-day land operations to continue. This is allowed through a Section 10 permit, which through special terms and conditions allows for take of endangered species. The Service expects this agreement to be in the federal register and available for public comment during the spring of 2004.

At this time, current protection laws appear to be adequate. If Wyoming toads are released in sites other than those contained within the National Wildlife Refuge system, more enforcement of existing laws may be necessary.

Population Viability Analyses (PVAs)

A Population Habitat and Viability Analysis (PHVA) workshop was held in Laramie, Wyoming in February 2001 (Jennings et al. 2001). PVAs are used to determine if populations are viable and what factors contribute to this. Many use this information to suggest management options. The goal of this PHVA was to better understand the factors leading to the Wyoming toad decline and to develop a set of alternative population management options (Jennings et al. 2001). A variety of individuals participated in the workshop. The participants included representation from the USFWS, the WGFD, American Zoo and Aquarium Association (AZA), U.S. Geological Survey (USGS), University of Colorado, University of Wyoming, University of Illinois, Laramie Rivers Conservation District, Big Laramie Mosquito Control District, and the ranching community.

In addition to generating the above list to assist in guiding management, some population models were constructed using VORTEX. VORTEX is a computer-programming model that projects the interactions of multiple parameters used as input to the model. For a more detailed explanation of VORTEX and its use in population viability analysis, refer to Miller and Lacy (1999) and Lacy (2000). This task proved daunting due to the lack of demographic information pertaining to the wild population of Wyoming toads. Furthermore, the survey techniques used at Mortenson Lake do not yield statistically valid results. For these reasons, a quantitative analysis of the individual Wyoming toad population viability was not possible.

Despite lack of quantitative data, the VORTEX model was employed to demonstrate the kinds of analyses that are possible and the ways in which it can be used to guide future research and management efforts (Jennings et al. 2001). The negative effect chytrid fungus has on the population quickly become apparent in these efforts. A model was developed that simulates, with reasonable accuracy, the true toad population trajectory of Mortenson Lake beginning in 1990. 93% of the simulated toad populations declined to zero after five years, while 99% became extinct after 10 years. A "chytrid free" model was also developed that illustrated a rapid increase in abundance and stability at any given site. These simple models, based on the researcher's best estimates of Wyoming toad population and demography, led the Wyoming Toad Recovery Team to conclude that the Mortenson Lake population is not viable in the presence of chytrid fungus (Jennings et al. 2001).

Conservation Action

Existing or Future Conservation Plans

A recovery plan for the Wyoming toad was published in 1991. However, this plan is currently being revised, as the earlier plan does not address current disease and management issues and needs. Though the current recovery plan was written with the best information available at the time, more information has become available that should be included.

The current recovery plan is lacking new information pertaining to the life history and management of the Wyoming toad. For instance, the current plan does not include habitat use information gained from the research of Withers (1992) and Parker (2000). This information is critical as it directly pertains to identifying new release sites. Furthermore, recent information regarding pesticides (Taylor et. Al 1999) and predation is lacking. This recent information is also vital when setting management goals. When the original plan was written (1991) the chytrid fungus had yet to be identified. Now that it has been identified and its presence at MLNWR has been confirmed this information needs to be included and management goals may need to be revised.

The management groups described in the original plan no longer exist. For example, the Wyoming Toad Recovery Management Team is no longer active. Furthermore, the Wyoming Toad Recovery Group was replaced by a USFWS appointed Recovery Team in 2000. New members were added to this team during 2003.

The recovery goals outlined in the original plan are not adequate. The original down-listing criteria for the Wyoming toad were five populations of 100 adults each. This estimate was based upon observations made at MLNWR in the late 1980s. Though the population appeared healthy at the time, it was not. Wyoming toads that died from that time have been retrospectively analyzed and have been diagnosed with chytrid fungus. The population estimated to be 100 at the time crashed in the early 1990s indicating that the size was not adequate to protect the population from rapid decline. Furthermore, field notes from Dr. George T. Baxter indicate that breeding congregations on the Laramie Rivers could easily reach 20 or more adult toads. This exceeds the half a dozen males and a few females that were observed at MLNWR (USFWS 1991), which is further indication that the population observed at MLNWR, was not of adequate size. Dr. Steve Corn (1993) determined that the population was not self-sustaining. For these reasons, the recovery criteria should be re-examined.

The taxonomic status of the Wyoming toad has also changed since the original plan was published. At the time, the Wyoming toad was thought to be sub-species of the Canadian toad but was subsequently determined to be its own species (See Taxonomy and Distribution section).

Lastly, very little of the information contained in the current recovery plan is cited. This makes it difficult to distinguish between anecdotal and documented or scientific evidence and observations.

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Conservation Elements

During 2001, a PHVA (Population Habitat Viability Analysis) workshop was held in Laramie, Wyoming. Participants were divided into working groups to deal with the following topics: disease, captive population management, population dynamics and risk assessment, and wild population management. Within each group, tasks necessary to recover the species were developed and ranked using the paired ranking system. Near the conclusion of the workshop, each working group presented the top five tasks they deemed necessary to insure survival of Wyoming toads. All tasks were ranked using the paired ranking system. Following are the top 10 action recommendations as ranked by the larger group (Jennings et al. 2001):

1. **Development of captive/ wild infectious disease management protocols**. The Wyoming Toad Specie Survival Plan (SSP) Veterinarian and Pathologist have been working on writing formal disease management protocols. These protocols will be used for guidance for captive facilities as well as release sites. These documents have not been completed as of the publication of this assessment.
2. **Gather disease information and begin research to reduce the rate of mortality in the captive population.** All necropsy report information is forwarded to the Wyoming Toad SSP Pathologist. This allows for review of all causes of mortality and early detection of outbreak. This information may also be used to track the spread of infectious diseases throughout the program. In addition, all necropsy reports generated by Wyoming State Veterinary Lab are forwarded to the USFWS Ecological Services office where they are kept in a database.
3. **Increase the long-term survival rate of captive yearlings from 50% to 80%.** The causes for high mortality of the yearling Wyoming toads are unknown. However, the SSP has undertaken nutrition and water chemistry research that may provide some information. The SSP Pathologist and Nutrients have been investigating the possibility of a vitamin A deficiency in the captive population. Preliminary data suggests that there may be a deficiency however, the sample size is small and further investigation is necessary before any action is taken. Plans were underway to conduct further research at the Sybille Wildlife Research Center during 2001, but this never happened. Since then, the SSP Nutritionist collected diet data from all captive facilities. This information included all items (including vitamin dusts or supplements) being fed to all life stages of toads in their Facilities also sent in feeding frequency and weights fed for each of these items. For items that have not been analyzed, facilities sent in samples of the food items for nutritional analysis. These data are still being compiled to determine if there is an obvious portion of the captive diet that is lacking vitamin A. These will also be compared to vitamin A levels collected from wild specimens. Based on stomach and fecal scat analysis, it was determined that a majority of the adult "wild" Wyoming toad diet consists of two species of ants. During 2003, these two species of ants were collected at MLNWR so that a

complete nutritional analysis could be conducted. Particular attention will be spent analyzing the vitamin A levels in these ants to determine if they could be the source of vitamin A in the wild toad diet, and thus one of the reasons that hepatic retinol levels in wild toads are much greater in than captive ones. Also, the Central Park Zoo conducted water chemistry and quality research at Mortenson Lake during the summers of 2000 and 2001. The information gained was used to begin constructing a synthetic mix of water that may be used at facilities with poor water quality/chemistry. In addition, the Saratoga National Fish Hatchery obtained a grant to fund further water chemistry and quality research. Water samples will be taken three key development stages. These results will be incorporated into the breeding success data obtained yearly to determine if there are water quality/chemistry differences between those facilities that are successful and those that are not.

4. **Improved captive larvae rearing success from 25% to 95%.** Larval rearing success rates will be improved by the research performed above.
5. **Assemble existing and collect new, accurate demographic and ecological data from the Wyoming toad in the wild.** Existing data has been collected and compiled at the USFWS Ecological Service office with aid of the Wyoming Toad Recovery Team and SSP. New, accurate data is the focus of a research project being conducted by the USGS, which is not complete. The information gained during this preliminary monitoring project will allow for the establishment of a permanent monitoring program that will yield statistically valid results.
6. **Increase the number of viable (captive) eggs by increasing the egg hatch rate from 27% to 95%.** This will be accomplished by performing the research outlined in 3 and 4 above.
7. **Develop the funding sources necessary to hire a full-time, permanent Recovery Coordinator.** Although the funding for a permanent Recovery Coordinator has never been established, a full-time temporary position is currently funded and this person works out of the Fish and Wildlife Service office in Cheyenne, Wyoming.
8. **Using existing data, determine the appropriate ecological criteria for identifying new release sites for the establishment of wild populations, and develop a detailed process for choosing new sites.** The Wyoming Toad Recovery Coordinator is working on

establishing criteria for new release sites by compiling historical data and information regarding the amphibian chytrid fungus. These criteria were scheduled to be reviewed by the Wyoming Toad Recovery Team during the summer of 2002. 9). Determine mortality-causing disease factors at proposed release sites. Currently, no action has been taken on this goal. However, a grant obtained by the Saratoga National Fish Hatchery will fund a technician to work for the Arapaho National Wildlife Refuge and sample the Laramie Basin for chytrid fungus. This will allow for better assessment of the distribution of the amphibian chytrid fungus. This information will be important when choosing new release sites.

9. **Conduct molecular genetic analyses (DNA fingerprinting and mtDNA) to determine the overall degree of relatedness among those individuals thought to constitute the captive population's founder base, to assess the potential for the identification of new genes within the "B Line" of captive Wyoming toads, and to establish the taxonomic relationship between *Bufo baxteri* and *Bufo hemiophrys.*** No action is currently being undertaken to determent the overall degree of relatedness among the captive population. However, Greg Pauley, a graduate student at the University of Texas at Austin is conducting a phylogentic study of the genus *Bufo* using DNA. The information obtained in his study will provide more information regarding the relationship between *B. baxteri* and *B. hemiophrys.*

As can be seen from this list and the preceding discussions, there are many elements acting in concert that are having negative impacts upon the survival of the Wyoming toad. However, the presence of chytrid fungus and lack of release sites appear to be the biggest obstacles. The current release site, Mortenson Lake, is infected with the fungus. In order to prevent researchers from spreading the fungus to other sites within the Basin, researchers have been advised adopt the protocol in Attachment 1.

Based upon information presented at the PHVA it is inadvisable to re-introduce toads into an area where chytrid fungus is present as it appears as though the toad cannot persist in these areas. When potential release sites are found they should be evaluated for the presence of the fungus.

Using the PCR primers developed by Dr. Seanna Annis at the University of Maine, Pisces Molecular has developed a PCR test that can detect the presence of the *Batrachochytrium dendrobatidis* in amphibian skin samples. Skin scraping and toe samples yield the best results. Dr. John Wood at Pisces Molecular is currently investigating the possibility of testing environmental samples such as mud or algae for the presence of the chytrid fungus. The Service is currently using both PCR and histopathology to diagnose chytrid in resident amphibians at proposed release sties. These methods are also being used to diagnose captive animals with chytrid to ensure that only "clean" animals are being released at new sites. Further, the Saratoga National Fish Hatchery obtained a grant that funded collection of amphibians throughout the Laramie Basin for chytrid fungus analysis. This will assist in determining the distribution of the fungus and may assist in determining which areas are better suited for release of Wyoming toads. The results of this effort are pending.

As noted above, histopathology techniques can supplement PCR test efforts, but there are problems with this technique. Moreover, it is possible to miss low-level infections using this method, particularly when assaying other amphibian species that may have different infection and mortality rates for chytrid fungus than Wyoming toads. This complicates the sampling effort, because until the prevalence of the chytrid fungus is known at a given site, determining the number of resident amphibians to collect to obtain a confident diagnosis is difficult (A. Pessier pers. comm.). Finding uninfected amphibians does not guarantee there is not chytrid at a site. Therefore, although histopathology will find chytrid fungus, it is not an acceptable method to use to determine whether or not a site is “free” of chytrid. However, it still should be used, as it is the best screening technique available to date.

Based upon information presented at the PHVA it is inadvisable to re-introduce toads into an area where chytrid fungus is present. The toad cannot persist in these areas. Therefore, areas that are free of chytrid should be given the highest priority. However, it is possible that chytrid fungus is present throughout the Basin. In that case the advice of the SSP Pathologist should be sought and the possibility of creating habitat should be investigated.

If a site that is free of chytrid fungus is found all available precautions should be taken to maintain the site as chytrid free. For example, all animals that are being released should come from a facility that has not had recent chytrid outbreaks. In addition, a stringent release site protocol should be developed.

However, it is possible that chytrid fungus is present throughout the Basin. In that case, the SSP Pathologist would not recommend rejecting potential release sites that meet all other ecological criteria based upon the presence of chytrid. There may be unique factors present at Mortenson Lake that cause chytridiomycosis to be more prevalent there (A. Pessier pers. comm.). Since Mortenson Lake is currently infected with the chytrid fungus and available data indicates that it has been suffering from outbreaks since 1989, new release sites should be found. The current situation at Mortenson Lake creates a situation in which it will be impossible to restore the species. Finding new release sites presents an entire new set of obstacles. Much of the suitable habitat within the Basin is located on private lands. Furthermore, the issue of water rights will figure prominently in the selection or creation of habitat. Most senior water rights are already owned and would be extremely difficult to obtain. Since the Wyoming toad has never been observed far from water and it’s early life stages are dependant upon water for the first two months of life this poses a problem. The Laramie River Conservation District is currently attempting landowner outreach to increase landowner awareness. This may eventually lead to willing landowner participation in a USFWS sponsored conservation agreement, such as a Safe Harbor agreement that may provide new release sites for the Wyoming toad.

A total of fourteen radio telemetry transmitters were purchased from Halohil Industries in July 2003 using funds obtained through the Fish and Wildlife Serivce's Platte / Kansas Eco-team. These transmitters were custom made to be suitable for Wyoming toads. They do not exceed ten percent of the average toad's body weight. Seven of these transmitters are very light weight and transmit location information only. Due to the late arrival of the transmitters, these will be used in the summer of 2004 to obtain information regarding the habitat use and movements of Wyoming toads. Specifically, the Service, Wyoming Toad Recovery Team and Species Survival Plan (SSP) group hope to obtain information regarding nocturnal movements and monitor the toad's response to habitat modifications at Mortenson Lake National Wildlife Refuge.

The remaining seven transmitters are heavier, have longer lasting batteries and transmit temperature information. These transmitters will be attached to the backs of seven toads during the fall of 2003. These toads will be monitored through the early fall and at least monthly in the winter to obtain hibernacula information. The location of the Wyoming toad's hibernacula is currently unknown, as are the temperatures in which it hibernates. The information obtained will assist the Service and Wyoming Toad Recovery Team better assess potential reintroduction sites for suitability. For instance, if the Wyoming toad has specific needs for hibernation this information could be used to determine that a site is unsuitable due to lack of specific hibernacula. Furthermore, this information will be used by federally permitted SSP captive breeding facilities when designed artificial hibernacula. This in turn, could reduce average hibernation mortality (twenty-five percent) currently observed in captivity.

Inventory and Monitoring

Monitoring of the Wyoming toad population at Mortenson Lake began in 1988. However, the techniques used have not been consistent since that time. Aside from two graduate studies and a single photographic mark and recapture effort, the techniques used do not produce statistically valid information. Since 1994, the U.S. Fish and Wildlife Service organized biannual surveys. The surveys are usually conducted once in the summer after the toads have emerged from hibernation and once in late summer or fall after captive releases have ceased. During these surveys, the volunteers are divided into three groups and slowly walk around Mortenson and Garber Lakes flushing toads. Efforts have been made to make the time of day and weather events consistent but that has not always been possible. The number of surveyors and surveyor experience has varied. Furthermore, the actual areas searched are not consistent from year to year or even within one season. Finally, Parker (2000) demonstrated that there is strong likelihood that not all toads are flushed and many are seeking refuge under dense grasses and therefore not detected.

Though these surveys may offer general information about the population, they are not statistically valid. The data collected do not allow for a meaningful comparison of the population between years. Additionally, the information collected has not and will not be able to offer the following demographic data: estimated sex ratio, estimation of mortality, natural population fluctuations, survivability of captive bred yearling releases, and an estimation of age structure of the population. As the PHVA illustrated (see PVA section above) the lack of this crucial data makes population modeling difficult.

The USGS conducted preliminary mark and recapture research at MLNWR during the toads' active season in 2002 and 2003 to assist the Service in developing a scientifically defensible monitoring program (Muths and Drietz 2003). The mark and recapture sessions utilized 8,145 post metamorphic, captive-reared Wyoming toads. All toads released were marked to differentiate between naturally produced animals and those that were released for the study. Results indicated that over-summer survival in the young-of-the-year cohort was low (~24%) and affected by water temperatures and that capture and recapture probabilities were influenced by wind speeds. Over-winter survival was not estimated, as data was sparse. However, 42 marked post-metamorphic toads were captured during the second summer, so despite the presence of the chytrid fungus some animals are surviving through winter. This information was forwarded to the Wyoming Toad Recovery Team and the Service with the intent that it will be used to develop a monitoring protocol for the Wyoming toad.

Habitat Preservation and Restoration

To date, Wyoming toads have been released on two National Wildlife Refuges, Mortenson Lake National Wildlife Refuge (MLNWR) and Hutton Lake Wildlife Refuge (HLNWR), both of which explicitly take the welfare of the toad into account when considering habitat use and quality. MLNWR is now closed to the public so that the Service can more easily manage the refuge for the toad. At MLNWR, grazing is used as a tool to create more open areas for the toad necessary for breeding and basking (Withers 1991). Grazing was also used as a way to manipulate the habitat at HLNWR. Due to drought conditions, the grazing pasture of HLNWR was split into two areas during 2000. Due to the continued presence of drought, grazing ceased during 2002 to preserve the small amounts of vegetation that remained (G. Langer, pers. comm.).

In addition to grazing, other habitat improvements have been made on behalf of the toad. Historically, lease payments from the Mortenson Lake Fishing Club were used to purchase temporary habitat conservation easements from adjacent landowners. More recently, the Arapaho National Wildlife Refuge staff repaired the dam and dike to Soda Lake on the MLNWR and installed ditch checks to wetlands east of Soda (G. Langer pers. comm.). During 2003, property adjacent to Soda Lake was purchased and included within the boundaries of MLNWR. This land could provide toad habitat in the future.

Captive Propagation and Reintroduction

A captive breeding program was initiated in 1993, which at the time included three AZA (American Zoological and Aquarium Association) accredited zoos. By 1996, the AZA formed a Species Survival Plan (SSP) group. An SSP is a coordinated effort to save an endangered species through captive breeding, public education, habitat preservation, supportive research, and in some cases, the re-introduction of captive bred animals to the wild (G. Spencer, pers. comm.). As of 2003, there are now eleven AZA accredited facilities and two federal breeding facilities involved in the Wyoming Toad SSP . From 1995 to 2003, the Wyoming toad captive breeding program has increased its holdings (Table 3) and released nearly 46,000 Wyoming toads of various life stages (mostly methamorphs) back into the wild (Table 2; USFWS unpublished data).

The Wyoming Toad SSP facilities continue to play an integral role in recovery. Many of the facilities have donated funding to research projects as well as conducting their own. For instance, in 1998, the Toledo Zoo funded a master's thesis (Parker 2000). The Central Park and Houston Zoos collected water chemistry and quality data at Mortenson Lake during 2000. These parameters were analyzed and the Central Park Zoo is currently using these data to experminnt with the possibility of creating a synthetic water blend. This water could potentially be used at facilities with poor water quality.

Information Needs

As this Assessment illustrates, there is a lot of information about the Wyoming toad that is missing. Currently, the lack of information about the chytrid fungus appears to be the biggest obstacle to conserving the toad. The life cycle and history of the fungus is not well understood at this time. Gaining this information would greatly benefit the conservation effort of this and other amphibian species in the Basin. For example, if it is determined that the fungus does not form a cyst while unfavorable conditions persist it may be possible to rid Mortenson Lake of the fungus by not releasing animals there for a period of time. In addition, how the fungus is being dispersed is not fully understood. Precautions are being taken to reduce the possibility of spread by humans however; there may be other methods of dispersal that are currently unknown. The fungus appears to be able to survive the harsh winters in the Basin but how it does is unknown. Some species appear to be carriers but, the role that these reservoir species play in the cycle of the fungus is unknown.

Furthermore, the presence of chytrid fungus throughout the Basin and therefore future release sites is unknown. It has been illustrated that the amphibian chytrid fungus has drastic effects on the survival rate. Therefore, the presence or absence of the fungus should be considered at all potential release sites. This information would also assist in the management of other amphibian species.

The lack of demographic and ecological information makes it very difficult to estimate what numbers are necessary in order to recover the toad and, it makes it difficult to construct population models to further assist recovery decisions. Unfortunately, this information will never be known for pre-decline, wild populations, so we must rely largely on current conditions at MLNWR and anecdotal, historical information.

The role contaminants play regarding chytrid outbreaks is also unknown. It has been shown that Malathion increases mortality in Woodhouse's toads that are exposed to field grade concentrations and then challenged with a bacterial injection (Taylor et al. 1998). There may be contaminants that the toad is exposed to that are making it more susceptible to chytrid infections.

Additional re-introduction sites are lacking. Currently, the only release site is MLNWR, which was determined to be infected with the chytrid fungus. In order to conserve the species, new (preferably chytrid free) sites are necessary. However, current technology limits testing for chytrid at only those sites which have resident amphibian populations. Chytrid can be detected through either histological techniques or through Polymerase Chain Reaction (PCR) testing which can detect small amounts of the DNA of the chytrid fungus in amphibian skin. Currently, the PCR test cannot detect the chytrid fungus DNA in environmental sources such as water or soil. This is problematic as there is a lack of knowledge regarding the life cycle of the chytrid fungus and how it may be able to survive at sites without amphibians. This makes determining the chytrid status of potential sites difficult in the absence of resident amphibians.

We do not have an understanding of the area requirements of the Wyoming toad. For instance, the home range of males and females is unknown. This makes it difficult to determine how large a potential release site should be.

A lack of specific information pertaining to hibernation sites creates problems for wild recovery and captive breeding efforts. While looking for new release sites, hibernacula must be present and adequate in size and structure. However, at this time it is unknown what the toad requires. This also creates a problem within the captive propagation program. Each year, an average of 25% of all animals put into hibernation die (VanVleet, unpublished data). The SSP is currently collecting data from each institution to determine if there is a correlation between successful breeding and hibernation. Lipps and Odum 2001 determined that there was a likely a link between hibernation and successful breeding but recent data has contradicted this. Therefore, clarification of the effect of hibernation on breeding is needed to insure viable reintroduction sites as well as improved captive breeding success.

Presently, little is known about the diet requirements of the Wyoming toad. Currently preferred prey and foraging flexibly are unknown. Again, when evaluating potential release sites this information would prove to be valuable. If the toad were a generalist, then prey would not be an important consideration to make when choosing a site. However, if the toad has a specific prey base or specific nutritional requirements that only few prey species would satisfy this becomes important. In captivity, the toads are feed a variety of foods ranging from crickets and earthworms to pinky mice. However, evident is mounting that this diet is insufficient and may be causing a vitamin A deficiency in the captive population (A. Pessier pers. comm.).

Determining the ideal water chemistry for survival and reproduction of Wyoming toads is another subject that would benefit from further research. Extensive tests have been executed at MLNWR and HLNWR. However, these tests only illustrate the conditions at MLNWR and HLNWR, which may not be ideal habitat. In addition, the USFWS obtained water chemistry and quality results from the rivers and lakes in the Basin from 1988-1993. While this information can be useful, the quality and chemistry of the water while the toad thrived is unknown. Investigation may yield a range of values the toad was found to be successful at, which would aid in SSP efforts and selection of future release sites.

Stochastic weather events have anecdotally been shown to be detrimental to breeding and to emerging toads (USFWS 1991). However, no documentation exists to evaluate this claim. Further understanding the role that weather can play may help aid management decisions, or help explain mortality events.

Tables and Figures

Table 2: Historical Wyoming Toad Releases

Year	Number released	Location
1995	1803	Lake George area
1995	1613	Mortenson Lake
1996	1812	Lake George area
1996	2652	Mortenson Lake
1997	213	Mortenson Lake
1998	3578	Mortenson Lake
1999	56	Lake George area
1999	2082	Mortenson Lake
2000	1550	Lake George Area
2000	6789	Mortenson Lake
2001	8338	Mortenson Lake
2002	8229	Mortenson Lake
2003	4362	Mortenson Lake
2003	2792	Private property

Mortenson Lake total to date: 37,962
Lake George area total to date: 5,221
Private land total to date: 2,792

Table 1a: Mortenson Lake Survey Results. Following is a summary of the results obtained from those bi-annual censuses that took place at Mortenson Lake (USFWS, unpublished data):

Date observed	# of adults	# of yearlings	# of YOY	**Total**
6/2/1994	0	0	0	**0**
8/30/1994	0	0	0	**0**
Spring 1995	0	0	0	0
8/18/ 1995	0	0	54	**54**
6/10/ 1996	0	35	0	**35**
8/30/ 1996	8	0	134	**142**
Spring 1997	17	57	0	**74**
Fall 1997	27	61	0	**88**
6/24/ 1998	20	25	0	**45**
Fall 1998	30	00	295	**325**
6/71999	9	151	0	**160**
8/24/1999	27	153	412	**517**
6/2/ 2000	18	138	00	**156**
8/30 2000	12	26	21	**59**
6/8/2001	4	52	00	**56**
8/31/ 2001	6	8	182	**196**

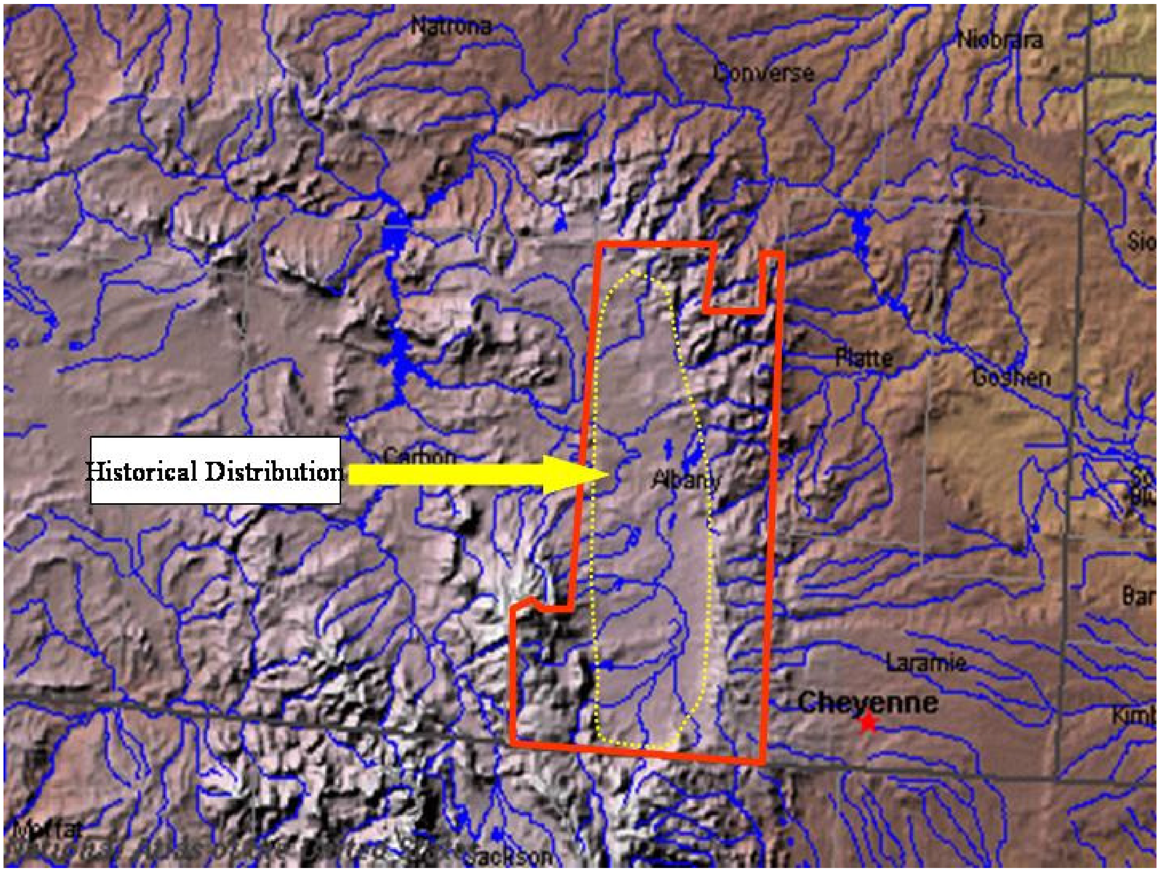
Table 1b: Other Survey Results. Wyoming toads were also released at Hutton Lake Wildlife Refuge from 1995 to 2000. The area was also formally surveyed. Surveys were concentrated around Lake George and Rush Lake. Following are the results of those surveys:

Year observed	# of adults	# of yearlings	# of YOY	**Total**
1995	0	0	31	**31**
1996	1	2	67	**69**
1997	5	16	1	**22**
1998	2	0	0	**2**
1999	3	0	0	**3**
2000	0	0	3	**3**
2001	0	0	0	**0**

Table 3: Numbers of Wyoming toads housed in captivity since 1989.

Year	Males	Females	Unknowns	**Total**
1989	1	2	0	**3**
1990	3	4	0	**7**
1991	14	10	0	**24**
1992	24	19	1	**44**
1993	39	30	14	**83**
1994	37	35	2	**74**
1995	225	149	138	**512**
1996	230	136	182	**548**
1997	152	96	5	**253**
1998	331	226	32	**589**
1999	178	149	7	**334**
2000	141	118	37	**296**
2001	157	152	61	**370**
2002	126	127	210	**463**
2003	110	93	237	**440**

Figure 1. Historical Distribution of the Wyoming toad in Albany County, Wyoming



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Appendix A: Guidelines for Reducing the Spread of Pathogens Between Wyoming Toad Reintroduction Sites

(Taken from Appendix C of the Wyoming Toad Recovery Plan)

The following guidelines will reduce the spread of pathogens between re-introduction sites of the Wyoming toad. Currently, the biggest threat to the health of wild Wyoming toads is the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, thus it is the focus of these guidelines. These may be updated or changed as more information regarding wild mortalities is gathered.

Between sites

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

- Clean all organic debris off boots and all field equipment with a scrub brush and water.
- Disinfect boots, field equipment, and cleaning equipment with a 10% bleach solution away from any nearby bodies of water. Rinse with tap or bottled water away from any watersheds.
- Disposable latex gloves will be used when handling animals. A new pair of gloves will be used between each animal.
- If funds are available, separate field equipment (nets, boots, etc.) should be purchased for each site.
- Tires do not necessarily have to be disinfected but at a minimum should be rinsed off before moving to new locations. Rinsing should remove most of the organic debris which could contain zoospores.

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

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

Re-establishing populations

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

- Resident amphibians at potential reintroduction sites will be collected and screened for chytrid using a combination of PCR testing and histological examination. The use of adult Wyoming toads as sentinel animals (when populations of resident amphibians do not exist) will be considered when the efficacy of this technique has been evaluated.
- All animals to be released will come from facilities that have not had a chytrid diagnosis or other significant mortality event within the last year. Participating facilities will follow disease surveillance guidelines as established by the Wyoming toad SSP Pathologist and Veterinarian. Results of the surveillance need to be relayed to the SSP Pathologist.
- Prior to release, adults will undergo a 10 day prophylactic treatment of a 0.01% solution of Sporanox (.01% suspended itraconazole) diluted with Amphibian Ringer's Solution and chytrid screening. Tadpoles and small toadlets will not be treated as Sporanox is lethal to these life stages.
- In the future, submitting a sub-sample of tadpoles for chytrid screening may be considered as the efficacy of this technique is determined and funding allows.
- Lastly, all SSP facilities will follow the rules and guidelines set forth in the Wyoming Toad Husbandry Manual and their individual facility disease protocols.