WYOMING TOAD MONITORING ON SAFE HARBOR REINTRODUCTION SITES: 2012

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TABLE OF CONTENTS

SUMMARY

The Wyoming Natural Diversity Database, with the help of the Wyoming Game and Fish Department continued survey and monitoring efforts for the Wyoming Toad at the Buford reintroduction site in the Laramie Plains area of Wyoming in 2012. We conducted timed visual encounter surveys in established search blocks during one week in June (breeding season surveys) and one week in August (post-breeding season surveys). Captive-bred Wyoming Toad tadpoles and toadlets have been released at the Buford site since 2005.

Although a record number of Wyoming Toads and possibly evidence of breeding in the wild were detected at the Buford site in 2011, no toads were detected in 2012. The reason for the lack of toads seen in 2012 presently is unknown, but will hopefully become apparent with continued monitoring. The number and age distribution of toads detected in the next several years will shed light as to whether the lack of toads seen in 2012 was due to severe drought, skipped breeding, chytrid fungus, or other causes. The drought likely significantly impacted toad behavior at Buford, though the extent of those impacts is not fully understood and likely differed between life history stages. Irrigation water was shut off on June 13, rather than in September as is typical, resulting in a loss of preferred habitat for juvenile and adult toads. The shoreline of Porter Lake receded substantially by August, leaving over 50m of dried unvegetated mud flats between cover and open water in places.

Another challenge faced by toads at the Buford site this year was the functional loss of Crescent Pond, the site of most toad activity and possibly of successful breeding in 2011. A muskrat took up residence in the pond early in 2012, digging multiple tunnels and trenches in the retaining wall and causing the pond to drain. Despite constant repairs by Art Anderson, Crescent Pond was unable to retain water once irrigation was curtailed and the pond was completely drained by mid-June.

The fact that no toads were seen in 2012 does not mean that toads no longer exist at the Buford site. Rather, it is possible that the poor conditions caused low tadpole recruitment and caused adults to change behavior, possibly foregoing attempts at breeding. Monitoring in subsequent seasons is necessary to make this determination. Information on returning toads will be crucial to determining why toads were not detected in 2012. We also recommend continuing reintroduction efforts at the Buford site, especially in light of ongoing studies to determine optimal release techniques and tadpole rearing conditions at reintroduction sites. Increasing tadpole survival and recruitment of toads into the population will improve the success of reintroduction efforts and hopefully allow populations to persist through stochastic events such as severe drought.

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INTRODUCTION

The Wyoming Toad (*Anaxyrus baxteri*) is a glacial relict species restricted to the Laramie Plains in southeastern Wyoming and protected under the Endangered Species Act. Conservation efforts for the Wyoming Toad have included reintroductions and/or population monitoring at four sites in the Laramie Plains since the species was rediscovered in the wild in 1987. Most members of this species currently exist in captive breeding facilities and numbers of toads in the wild appear to fluctuate (Estes-Zumpf and Keinath 2012), though the reasons for fluctuations are not understood and may vary among sites and years.

In 2012, the Wyoming Natural Diversity Database (WYNDD) entered into a cooperative agreement with the Laramie Rivers Conservation District (LRCD) to continue monitoring Wyoming Toads at ponds and waterways owned by the Buford Foundation. This reintroduction site is located in the Laramie Plains and is covered by a U.S. Fish and Wildlife Service (USFWS) Safe Harbor Agreement with the LRCD. Monitoring efforts are designed to document breeding in the wild should it occur, provide relative abundance estimates for different age classes of toads across years, and track the prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) and other threats to toads. This report summarizes reintroduction efforts in 2012 and presents survey results for the Buford site.

Project Goals for 2012:

1. Document wild breeding if it occurs.
2. Estimate relative abundance of sub-adult life stages.
3. Estimate abundance of overwintered adults and potential breeders.
4. Determine chytrid fungus infection rates in adult Wyoming Toads.
5. Evaluate the daily and seasonal timing of breeding calls for the Wyoming Toad.

METHODS

Study Areas

Surveys were conducted with the help of the Wyoming Game and Fish Department (WGFD) at the Buford Foundation property in 2012 (Figure 1). The USFWS also conducted survey and reintroduction experiments at Mortenson Lake; however, results for Mortenson Lake studies are not included in this report. The Buford Foundation property is located approximately 8 miles east of Centennial, WY, and includes two reservoirs (Porter Lake and Hardigan Lake) and several small ponds. Porter Lake is connected to the Little Laramie River on the north via the Lake Hattie Supply Canal #2, which is typically closed except in years with high winter snowpack. The supply canal continues south from Porter Lake to Hardigan Lake and eventually to Lake Hattie. Surveys are conducted on the margins of both lakes and the portion of the supply canal connecting them (Figure 2). Captive breeding facilities have released Wyoming Toad tadpoles and toadlets at Porter Lake since 2005, and WYNDD has monitored toad abundance at Buford since 2006.

Due to budget cuts in 2012, the LRCD and USFWS chose to cease standardized monitoring at a former experimental release site located along the Little Laramie River and owned by Fred Lindzey. The Lindzey property is located just north of Porter Lake and includes sections of the Little Laramie River and surrounding floodplains (Figure 1). Although the Lindzey property is not an official Safe Harbor reintroduction site, Wyoming Toad tadpoles and toadlets were released at the Lindzey property from 2003-2005 under a special permit. WYNDD surveyed for Wyoming Toads at the Lindzey site from 2008-2011. Evidence of breeding (e.g., egg strings, tadpoles) has been documented in the past; however, breeding has not been documented for several years. Furthermore, no toads have been found during formal surveys and toads have not been heard calling at the site for several years.

Survey Protocols

Given constraints on time and budget, we use an adaptive sampling scheme (Thompson and Seber, 1996) to survey for Wyoming Toads at the Buford and Lindzey properties. Under this sampling scheme, standardized searches target areas of highest likelihood-of-occurrence, primarily areas next to fixed bodies of water and deemed to be moist through much of the spring-summer season, as confirmed by their vegetation composition. From 2006 to 2008 shorelines and wetlands at all properties were relatively constant between years, with higher shorelines in spring followed by a gradual reduction in water levels over the summer. High winter snowpack in 2009 and 2010, however, resulted in water from the Little Laramie River being released through the supply canal and into Porter Lake to fill Lake Hattie. This flooded all water bodies at the Buford site beyond the maximum level seen in previous survey years, and levels remained above normal for the duration of the summer. Water was not released into Porter Lake in 2011 and 2012 and the shoreline was similar to that of previous non-flood years.

At the onset of monitoring efforts, search areas were stratified into "search blocks" of known size (e.g. Figure 2), within which we conducted the field searches summarized in Table 1 (for detailed description of search types see Griscom et al. 2009, Appendix 1). Standard search blocks were used in 2006-2008, and 2011-2012. In 2009 and 2010, however, flooding at Buford precluded use of previously identified search blocks (Figure 2 inset), and ad hoc search blocks of approximately 0.3 acres were established. In each search block we conducted visual encounter surveys with strict documentation of survey effort (i.e. search time specified by area of block; e.g., Heyer et al. 1994), and survey methodology was consistent across all years. Thus, although results for the ad-hoc search blocks are not directly comparable to individual search block results from non-flooded years, overall results remain directly comparable across all years since search effort was the same (i.e., 30 minutes/acre across all available wetland habitats).

Search efforts were as follows:

1. **Sessions**: Two survey sessions were conducted; one during the breeding season (mid-June) and one at the end of the summer after breeding activity was likely completed (mid-August).
2. **Search Intensity**: We searched all blocks at a rate of 30 minutes per acre. In blocks where at least one adult toad was found we conducted 2 replicate searches (using the same search intensity). Replicate searches were spaced approximately 24 hours apart

when possible because toads can temporarily move out of a search block immediately after being handled (Estes-Zumpf and Keinath 2011). Toads, however, typically returned to the area where they were first captured within one day. Repeated searches conducted in this manner allow use of mark-recapture analysis to estimate population size, as long as enough toads are observed.

3. **Search Blocks**: We surveyed the same blocks in 2012 as in previous non-flood years.
4. **Size Classes**: There are 3 size classes to which surveyed toads were assigned according to the survey session and toad weight; *Young of the Year, Overwintered Adult*, and *Possible Breeder* (Figure 3). Young of the year include metamorphs (recently metamorphosed tadpoles) and toadlets (found only during August surveys) that metamorphosed earlier that summer. Overwintered adults have lived at least a year. Possible breeders have likely overwintered at least twice and are thought to be large enough to breed if conditions are favorable. Weight standards for this class are based on captive breeding observations (J. Palmer, *personal communication*, 2008).
5. **Shoreline Searches**: We searched for eggs strings by conducting an additional un-timed single pass survey of the shoreline in search blocks where toads have been documented in the past. Shoreline surveys were conducted once during the breeding season.

Data collected during surveys in 2012 were very similar to those collected in previous years (Estes-Zumpf and Keinath 2012). During all standard block searches, we recorded the number of egg strings, tadpoles, young of the year (YOY) and adults observed in each block. All adult toads (overwintered and potential breeders) were captured by hand or net. We then photographed each adult toad and recorded the sex, body mass (measured to the nearest 1/10 gram using a digital scale), snout-vent length (SVL), and exact location via Global Positioning System (GPS) receiver. A separate set of sterile latex gloves was used to process each toad and measuring equipment was disinfected between toads in order to prevent disease transmission. We assigned each adult a unique identification number at the time of its first observation and used these numbers to identify all subsequent recaptures.

Individual identification was achieved through analysis of unique dorsal wart patterns using photo recognition software (WILD-ID; http://www.dartmouth.edu/~envs/faculty/bolger.html). We photographed the dorsum of all adults using standardized protocols developed in 2010 (Estes-Zumpf and Keinath 2011). We constructed tubes of flexible translucent plastic (12cm long; 10cm diameter) and placed tubes over toads in holding containers. Use of photo tubes increases the number of high quality photos for analysis and reduces handling time of toads. Photos were then compared to previously photographed toads. During all searches, we also checked adult toads for previously implanted PIT tags using a reader. The tag number of each toad thus identified was recorded. At the site where each adult was found, substrate type, water temperature, water depth, and a habitat photo were recorded.

We also recorded specific information when we found tadpole aggregations. When exceptionally high local densities of tadpoles were found, we recorded the GPS location of the center of these aggregations. We then recorded the average depth and temperature of the water

within the aggregation. Although egg strings have yet to be found, technicians were directed to assign any egg strings a unique identification number, record its exact location, photograph it, and record the water depth and temperature. Temperature loggers also were placed on the north and south sides of Porter Lake to record daily variations in water temperature throughout the summer.

Population Estimates

Numbers of recaptures of adult toads remain too low to effectively estimate abundance with mark-recapture analyses at the Buford site (Griscom et al. 2009). Therefore, despite large associated confidence intervals, adult population estimates were calculated using results from detectability trials with dummy toads (i.e. painted rocks) at the Buford property (Keinath et al., 2007). We estimated abundance of adult Wyoming Toads (overwintered toads and potential breeders) at the Buford site by dividing counts from formal surveys by the mean, minimum, and maximum detectability rates reported in Keinath et al. (2007).

Chytrid Analyses

In previous years, we collected epithelial tissue samples from adult Wyoming Toads found during surveys to determine the prevalence of chytrid fungus in toads at Porter Lake. Sample collection followed established procedures approved by the WTRT (Livo 2003, Boyle et al. 2004, UCB 2004). Toads were systematically swabbed with sterile cotton swabs to collect epidermal DNA. Swabs were only collected from adult Wyoming Toads that were otherwise processed for monitoring purposes. Swabs were immediately stored in sterile microcentrifuge tubes containing 95% ethanol and labeled with unique specimen numbers. We stored samples in a -20°F freezer until shipping. Samples were sent to genetic laboratories for analysis via PCR test to determine if the fungus was present. In the past, samples have been sent to either the Amphibian Disease Laboratory at the San Diego Institute for Conservation Research or to Pisces Molecular, LLC in Boulder, Colorado.

Breeding Calls

Wyoming Toads were heard calling for the first time in several years at Porter Lake in 2009 (Estes-Zumpf and Keinath 2010). Because calling signifies breeding behavior, this event prompted WYNDD and the WTRT to set out acoustic recording devices (frog loggers) in subsequent years to examine patterns of breeding calls and correlations between calling and local habitat and weather conditions. We set out frog loggers at the northwest and southwest corners of Porter Lake, where male Wyoming Toads were heard calling in previous years. Frog loggers were programmed to record for two 10-minute segments every hour from mid-April to mid-July, 2012. We analyzed recordings using a Wyoming Toad call recognizer developed by Heidi Meador (USFWS) and modified to better detect calls recorded in the field.

Microhabitat Analyses

To investigate microhabitat characteristics used by Wyoming Toads in the wild, WYNDD collected water temperature and water depth at locations where adult toads (overwintered or potential breeder) and tadpole aggregations were found during surveys. We summarized water temperature and depth results for Wyoming Toads located during surveys from 2007-2012. We also determined how often toads were detected on a particular substrate. Substrate categories include dry or firm moist ground, soft muddy ground, or water.

RESULTS AND DISCUSSION

Surveys

Breeding season and post-breeding season surveys were conducted from 12-14 June and 14-15 August, respectively. Standard search blocks were used during breeding and post-breeding season surveys; however, the severe drought in 2012 caused the smaller ponds to dry up and the shorelines of Porter and Hardigan Lakes to recede considerably. Crescent Pond, where breeding by Wyoming Toads was suspected to have occurred in 2011, had no open water by June surveys due to damage to the retaining wall by a muskrat and lack of available irrigation water (Figure 4a). South Pond and the two ponds on the north shore of Hardigan Lake were also dry by August surveys (Figure 4b). In August, search blocks were shifted significantly to accommodate the receding shoreline of Porter and Hardigan Lakes, and search blocks along the shore contained extensive mud flats (Figures 5 & 6). Most upland search blocks were completely dry due to lack of irrigation. All blocks were searched for evidence of toads even if dry. A Shoreline Search for egg strings was conducted on 14 June, but no Wyoming Toad eggs were found.

Reintroductions

A total of 13,008 tadpoles and 92 toadlets were released from 9 captive breeding facilities in 2012 (Table 2). Individuals were released at the Buford site (11,700) and into soft release herptariums for reintroduction experiments at Mortenson Lake (1,400). The number of tadpoles and toadlets released at Buford in 2012 was slightly higher than releases in the past two years (Figure 7). Tadpoles were released from 25 May to 3 July, 2012. The last toadlets were released on 14 September. Tadpoles and toadlets are typically released at the northwest corner of Porter Lake. In 2012, however, some toadlets released later in the summer were taken to beaver ponds along the supply canal between Porter Lake and Hardigan Lake because the beaver ponds offered the best habitat for Wyoming Toads remaining at that time of year due to the drought (Jason Palmer, USFWS, *personal communication*).

Counts:

Although surveys in 2011 revealed a record number of adult Wyoming Toads at the Buford site, and possibly the first tadpoles resulting from natural breeding at that site, no Wyoming Toads were detected during breeding season and post-breeding searches in 2012 (Figure 8, Tables 3&4). Visits to the site for frog-logger and temperature logger maintenance, and for tadpole and toadlet releases throughout the summer also yielded no incidental sightings of toads. Although we currently cannot

determine why no toads were found in 2012, several hypotheses could explain their absence from the Buford site.

First, the severe drought could have influenced toad behavior. Anecdotal evidence suggests that amphibians may not attempt to breed, and might not even return to breeding sites, during drought years. Large numbers of boreal chorus frogs (*Pseudacris maculata*) are typically seen at the Buford site during surveys, but we detected only a few adults and tadpoles in 2012. Tiger salamander (*Ambystoma mavortium*) larvae, which are typically detected during surveys, also were not observed in 2012. In Yellowstone National Park, Boreal Toads (*Anaxrus boreas boreas*) were not detected at several known breeding sites in the Lamar Valley during consecutive drought years, but returned to sites the first wet year following the drought (Dr. Melanie Murphy, University of Wyoming, *personal communication*). Similarly, only one adult boreal toad was detected at one of two sites in the Medicine Bow National Forest in 2012, despite adults and tadpoles being documented at both sites in 2011.

Second, skipped breeding (a type of temporary emigration) has been documented in other toad species, including the Boreal Toad. During skipped breeding, individuals of one or both sexes will not breed in consecutive years. Rather, they skip one or more years between breeding events (Muths, et al. 2006, Bull & Carey 2008, Loman & Madsen 2010, Muths et al. 2010). Because detailed monitoring data is required to detect skipped breeding, this life history trait is poorly understood in toads and has not yet been shown to be synchronous among all individuals in a population. However, small populations could develop synchronous breeding if numbers of breeding-age individuals are limited, as with the Wyoming Toad at the Buford site. Preliminary data from six years of monitoring a small population of Boreal Toads at Ryan Park in the Medicine Bow National Forest suggest a two-year breeding cycle (Zack Walker, WGFD, *personal communication*). This trend is disrupted only in 2012, possibly due to the severe drought.

Lastly, the high rate of chytrid fungus infection documented in toads at the Buford site in 2011 could have resulted in high toad mortality between fall of 2011 and spring of 2012. However, individuals infected with chytrid fungus do not necessarily die. Amphibians can control or rid themselves of chytrid fungus if they are able to bask frequently in the sun and dry off. It is likely that mortality due to chytrid fungus was greater between 2011 and 2012 than in past years due to the high infection rate in toads sampled in 2011. However, we have no data to support a loss of all or most Wyoming Toads at the Buford site due entirely to chytrid fungus. Most likely, the lack of toads documented at the site in 2012 resulted from a combination of one or more factors (e.g., high mortality combined with a severe drought year).

Another challenge faced by toads at the Buford site in 2012 was the functional loss of Crescent Pond at the northwest corner of the Buford site. The vast majority of toads detected in 2011 and in previous years were located around Crescent Pond and between the pond and the northwest shoreline of Porter Lake. Crescent Pond also was where tadpoles likely resulting from breeding in the wild were detected in 2011. Unfortunately, a muskrat took up residence in the pond early in 2012, digging multiple tunnels and trenches in the retaining wall and causing the pond to drain. Despite constant repairs by Art Anderson, Crescent Pond was unable to retain water once irrigation was curtailed and the pond was completely drained by mid-June. Muskrats also are omnivores and are known to eat amphibians. Due to the small size of Crescent Pond, toads using that site in the spring of 2012 could have been easy prey for the muskrat.

The fate of tadpoles and toadlets released at the Buford site from captive breeding facilities in 2012 also is unknown. Tadpoles were only detected in one search block (P-01) along the northwest

shore of Porter Lake in June. Unlike past years, no toadlets were detected during surveys in August. We are unsure at this time whether the lack of toadlets in August was due to tadpole mortality, toadlet mortality, or early dispersal from the site due to lack of habitat. Toads and toadlets appear to prefer moist soil with some cover. The western half of Porter Lake typically provides such habitat, and is where most Wyoming Toads of all age classes have been found in previous years. In 2012, however, the severe drought forced irrigation to be curtailed on June 13 rather than in September, as is typical, and caused the shoreline of Porter Lake to recede substantially. This resulted in gaps of over 50m in places between vegetation and open water (Figure 5). Toads and toadlets would have to cross these unvegetated dried mudflats in order to access open water, or even moist soil in places. Toads would be susceptible to both predation and desiccation during such movements. The fate of individuals released in 2012 cannot be determined until surveys are completed in 2013.

Documentation of Breeding

Breeding calls from male Wyoming Toads were not heard during visits to the Buford site and were not detected with either of the two frog-loggers. Also, no egg strings were detected during the breeding season shoreline search, however, the best potential breeding site (Crescent Pond) was dry by mid-June due to muskrat damage to the retention wall and limited irrigation as described above. The LRCD rebuilt the retaining wall in the fall of 2012 and options for muskrat control are being assessed by the LRCD and USFWS.

Chytrid Analyses

No toads were caught in 2012, so no chytrid samples were collected. However, WGFD contributed funds to have duplicate samples analyzed from 14 of the 35 toads swabbed in 2011 to investigate differences between labs in the sensitivity of PCR tests for chytrid fungus. Chytrid samples from the Buford site were sent to the Amphibian Disease Lab at the San Diego Zoo in 2006 through 2008 and in 2011. However, samples were sent to Pisces Molecular in Boulder, Colorado in 2009 and 2010. Due to its close association with the Wyoming Toad Recovery Team, the Amphibian Disease Lab has generally analyzed chytrid samples from Wyoming Toads, while Pisces Molecular is the lab normally used by state and federal agencies in Wyoming. It is therefore valuable to determine if detection rates are similar among these labs. Both labs use similar PCR methods to test for chytrid fungus.

In 2011, chytrid fungus was detected by the Amphibian Disease Lab in 100% of the samples, suggesting a significant and alarming increase in the chytrid infection rate at Buford from previous years (Figure 9) However, chytrid fungus was detected in only 57% the duplicate swabs tested by Pisces Molecular. This is a substantial difference that dramatically affects our ability to draw inference from chytrid infection rates.

The difference in chytrid results between the two labs could be due to 1) differences among laboratories in the sensitivity of PCR tests to detect chytrid, or 2) differences in the quality of DNA on sample swabs. If the PCR tests used by the Amphibian Disease Lab are more sensitive than those used by Pisces Molecular, then the magnitude of the increase in infection rate from 2010 to 2011 likely was exaggerated (Figure 9). If differences in sensitivity exist between labs, the infection rate at Buford might have been consistently higher than indicated by results from 2009 and 2010. Therefore, with the exception of the low infection rate in 2008, Wyoming Toads

might have been surviving at the Buford site despite high chytrid infection rates for the past several years.

Degradation of DNA also could cause the difference in chytrid infection results between labs. Although samples were stored in the same manner, samples were stored for 5-7 months before analysis by the Amphibian Disease Lab but had been stored for 15-17 months before analysis at Pisces Molecular. DNA is known to degrade over time, even under optimal storage (95% ethanol and stored in freezer), however, the rate of DNA degradation and its effects on PCR tests for chytrid fungus are not well understood.

Microhabitat Analyses

Depth of water varied greatly at locations where toads were detected, ranging from dry ground to 30cm (Table 5). Water temperatures where adult toads were found ranged from 57.5 to 89.4 °F (mean = 72 °F; Table 5). Potential breeders were detected most often (47%) on muddy substrate with little or no standing water, while overwintered adults were detected on muddy ground (40%) and in water (40%) more often than on dry ground (Table 6). We detected tadpole aggregations in warmer water than that used by adult toads (range = 76.8 - 83.1 °F) and at an average water depth of approximately 7cm.

At Porter Lake, water temperatures at locations used by both adult and tadpole Wyoming Toads occur most often along the northwest, west, and southwest shorelines, as well as in Crescent Pond and South Pond (Estes-Zumpf and Keinath 2012). These also are the areas at Buford where toads and tadpoles are most often detected during surveys in June and August. Wyoming Toads are capable of moving at least 700m (Estes-Zumpf and Keinath 2012) and, thus, can access all areas surrounding Porter Lake. However, toads appear to prefer the western portion of the lake and the surrounding small ponds over the eastern shore likely due, in part, to the presence of warmer water temperatures.

RECOMMENDATIONS

1. *Continue Monitoring Efforts:* Understanding why no Wyoming Toads were seen at the Buford site in 2012 following the high number of toads and possible first breeding event in 2011 is critical to our understanding of Wyoming Toad ecology and to the recovery effort. The lack of toads detected could result from the severe drought, skipped breeding following a big breeding attempt in 2011, mortality due to chytrid fungus, or other causes. Surveys are needed to establish 1) whether toads reappear post-drought, 2) the number of toads that reappear, and 3) the age distribution of toads that reappear. Because so little is understood about the ecology of Wyoming Toads in the wild, all information uncovered by continued monitoring will inform and advance recovery efforts.

2. *Chytrid analyses*: Due to possible differences in the sensitivity of the PCR tests used by different labs to detect chytrid fungus, we recommend that all samples from Buford, Mortenson, and future reintroduction sites be sent to the same lab for analysis. This will allow for comparability of chytrid infection rates across years and sites. If the

Amphibian Disease Lab used to test samples in 2011 is more sensitive than the lab used in 2009 and 2010, then the increase in the rate of chytrid infection in toads at the Buford site between 2010 and 2011 was not as dramatic as initially thought, and more toads were likely infected with the fungus in both 2009 and 2010. Yet the population persisted. This assumes, however, that the DNA samples from 2011 sent to Pisces Molecular were not degraded. Because we cannot confirm this, and because understanding the influence chytrid fungus has on the persistence of toad in the wild is critical to the reintroduction effort, we recommend sending samples of the same age and from the same toads to both labs. This will rule out the possibility of DNA degradation.

3. *Continue reintroductions as Buford:* Establishing a wild population through reintroduction is a difficult process and set-backs are bound to happen. Prior to 2012, results from the Buford site were promising, clearly suggesting that the site has potential. We recommend that reintroductions of tadpoles and toadlets continue at this site. Improved release techniques are currently under investigation at Mortenson Lake and information on optimal tadpole rearing conditions is being gathered. The results of this study should improve tadpole survival and increase the number of toads recruited into the population. Captive breeding facilities have made significant advances in breeding and rearing captive toads. The recovery team is now conducting rigorous experiments to advance the survival of toads in the wild. Information currently being gathered could contribute significantly to the success of the reintroduction program.

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TABLES AND FIGURES

Table 1. Summary description of Wyoming Toad monitoring searches conducted at the Buford Foundation property in 2012.

Search Type	Purpose	Location	Level of Effort	Timing
Initial Search	To determine where toads and egg masses occur and identify sites for replicate searches.	All search blocks	Each block searched at the rate of about 30 minutes per acre.	Breeding Season Search: June 12 - 14. Post-Breeding Search: August 14 – 15.
Replicate Search	To determine relative abundance of adult toads and obtain population estimates of adults.	All search blocks where over-wintered adult toads were identified from Initial Searches (see Appendix 1 for details).	Each block searched at the rate of about 30 minutes per acre.	Breeding Season Search: June 12 - 14. Post-Breeding Search: August 14 – 15.
Shoreline Search	To document wild breeding, should it occur.	All moist, vegetated shorelines within a block of any previous adult toad observation.	Detailed, un-timed shoreline search.	Breeding season only.

Table 2. Breakdown of release dates, locations, and source for the 13,100 Wyoming Toad tadpoles and toadlets released at the Buford Foundation Property and Mortenson Lake National Wildlife Refuge in 2012.

Breeding Facility	Release Date	Tadpoles	Toadlets	Cohort	Location of release	Notes
Como Zoo	6/20/2012	1744	0	STPA12	Buford	
Como Zoo	6/20/2012	890	0	STPB12	Buford	
Como Zoo	6/20/2012	175	0	STPA12	Mortenson	
Como Zoo	6/20/2012	175	0	STPB12	Mortenson	
Como Zoo	9/14/2012	0	10	STPA12	Buford	Released by Red Buttes
Como Zoo	9/14/2012	0	15	STPB12	Buford	Released by Red Buttes
Detroit Zoo	8/10/2012	0	12	DETC12	Buford	Released by Red Buttes
Kansas City	7/3/2012	725	0	KC12	Buford	Released by Red Buttes
Mississippi River Museum	6/20/2012	661	0	MRMA12	Buford	
Mississippi River Museum	6/20/2012	543	0	MRMB12	Buford	
Mississippi River Museum	6/20/2012	28	0	MRMC12	Buford	
Mississippi River Museum	6/20/2012	175	0	MRMA12	Mortenson	
Mississippi River Museum	6/20/2012	175	0	MRMB12	Mortenson	
Mississippi River Museum	6/20/2012	28	0	MRMC12	Buford	
Mississippi River Museum	9/10/2012	0	1	MRMB12	Buford	Released by Red Buttes
Mississippi River Museum	9/10/2012	0	4	MRMC12	Buford	Released by Red Buttes
Omaha Zoo	6/21/2012	275	0	OMAH12	Buford	
Omaha Zoo	6/21/2012	230	0	OMAB12	Mortenson	
Red Buttes	6/8/2012	268	0	RBEA12	Buford	
Red Buttes	8/7/2012		10	RBEA12	Buford	
Saratoga	5/25/2012	896	0	SARB12	Buford	
Saratoga	5/25/2012	50	0	SARC12	Buford	
Saratoga	6/8/2012	190	0	SARB12	Buford	Released by Red Buttes
Saratoga	6/8/2012	100	0	SARE12	Buford	Released by Red Buttes
Saratoga	6/8/2012	500	0	SARG12	Buford	Released by Red Buttes

Saratoga	6/8/2012	500	0	SARH12	Buford	Released by Red Buttes
Saratoga	6/13/2012	751	0	SARG12	Buford	Released by Red Buttes
Saratoga	6/13/2012	528	0	SARH12	Buford	Released by Red Buttes
Saratoga	6/14/2012	39	0	SARH12	Buford	
Saratoga	6/14/2012	492	0	SARG12	Buford	
Saratoga	6/14/2012	1298	0	SARF12	Buford	
Saratoga	6/18/2012	198	0	SARH12	Buford	
Saratoga	6/18/2012	199	0	SARF12	Buford	
Saratoga	8/14/2012	0	3	SARA12	Buford	
Saratoga	8/14/2012	0	1	SARB12	Buford	
Saratoga	8/14/2012	0	8	SARE12	Buford	
Saratoga	9/10/2012	0	1	SARC12	Buford	Released by Red Buttes
Saratoga	9/10/2012	0	2	SARE12	Buford	Released by Red Buttes
Toledo Zoo	6/21/2012	461	0	TOLB12	Buford	
Toledo Zoo	6/21/2012	230	0	TOLB12	Mortenson	
Toronto Zoo	6/21/2012	51	0	TORB12	Buford	Little movement, appeared stressed
Toronto Zoo	6/21/2012	120	0	TORD12	Buford	Little movement, appeared stressed
Toronto Zoo	6/21/2012	120	0	TORB12	Mortenson	
Toronto Zoo	6/21/2012	120	0	TORD12	Mortenson	
Toronto Zoo	6/29/2012	66	0	TORC12	Buford	
Toronto Zoo	6/29/2012	7	0	TORD12	Buford	
Toronto Zoo	8/8/2012	0	25	TORD12	Buford	
TOTAL		**13008**	**92**			

Table 3. Raw counts of individual Wyoming Toads found during monitoring activities at Buford from 2006-2012. Numbers before parentheses are counts of individual toads observed during formal surveys. Number in parentheses is the count including incidental observations (i.e., toads encountered outside formal search efforts). Because evidence of breeding has not been confirmed at Buford, all young of the year are likely the result of reintroduced tadpoles and toadlets.

	2006		2007		2008		2009		2010		2011		2012	
Size Class	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding
Young of the Year	83 (88)	103 (106)	8 (12)	59 (74)	34 (34)	17 (17)	0 (0)	24 (33)	0 (0)	65 (78)	0 (0)	53 (69)	0 (0)	0 (0)
Overwintered	5 (9)	1 (1)	8 (8)	2 (4)	9 (9)	7 (7)	5 (5)	1 (1)	4 (5)	4 (4)	3(10)	9 (11)	0 (0)	0 (0)
Potential Breeder	1 (2[a])	0 (1)	0 (0)	3 (3)	0 (0)	0 (0)	2 (4[b])	0 (0)	1 (1)	3 (3)	1 (7)	14 (18c)	0 (0)	0 (0)

[a] 1 male toad was heard calling but was never found.
[b] 2 male toads were heard calling but were never found.
[c] 2 female potential breeders were originally caught during the 2011 breeding season as overwintered toads.

Table 4. Abundance estimates for overwintered and potential breeder Wyoming Toads at Buford during monitoring activities. Estimates of abundance were calculated by dividing raw counts (excluding incidental observations) by detection probabilities listed in Keinath et al. 2007).

	2006 Estimate (Range)		2007 Estimate (Range)		2008 Estimate (Range)		2009 Estimate (Range)		2010 Estimate (Range)		2011 Estimate (Range)		2012 Estimate (Range)	
Size Class	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding	Breeding	Post Breeding
Overwintered	10 (7-33)	2* (1-7)	15 (11-53)	4 (3-13)	17 (12-60)	13 (9-47)	10 (7-33)	2* (1-7)	8 (5-27)	8 (5-27)	6 (4-20)	17 (12-60)	0 (0)	0 (0)
Potential Breeder	2* (1-3)	0*	0*	5 (4-8)	0*	0*	3* (3-5)	0*	2* (1-3)	5 (4-8)	2* (1-3)	24 (18-37)	0 (0)	0 (0)

* Cells labeled with an asterisk should be viewed with caution due to extremely low observations that make extrapolation uncertain.

Table 5. Average water temperature and depth where adult (overwintered and potential breeder) and tadpole Wyoming Toads have been found at the Buford Foundation Property from 2007-2012 (n = sample size for each age group).

Toad Age	n	Avg. Water Temp (°F)	Range	Avg. Water Depth (cm)	Range
Overwintered	39	73.0	(57.5 - 89.4)	5.8	(0 - 30)
Potential Breeder	10	70.8	(59.9 - 84.4)	7.0	(0 - 28)
Tadpole	4	80.4	(76.8 - 83.1)	6.6	(2.4 - 14)

Table 6. Percent of adult Wyoming Toads found on different substrate types at the Buford Foundation Property during surveys from 2007-2012.

Toad Age	Dry or firm ground	Mud	Water	Grand Total
Overwintered	20.8	39.6	39.6	100
Potential Breeder	26.5	47.1	26.5	100

Figure 1. Map of the Buford Foundation Property. The Buford Property is under a Safe Harbor Agreement for the Wyoming Toad. Properties are shown with key landscape features identified. Locator map is shown below, with approximate historic range of Wyoming Toad highlighted in green.

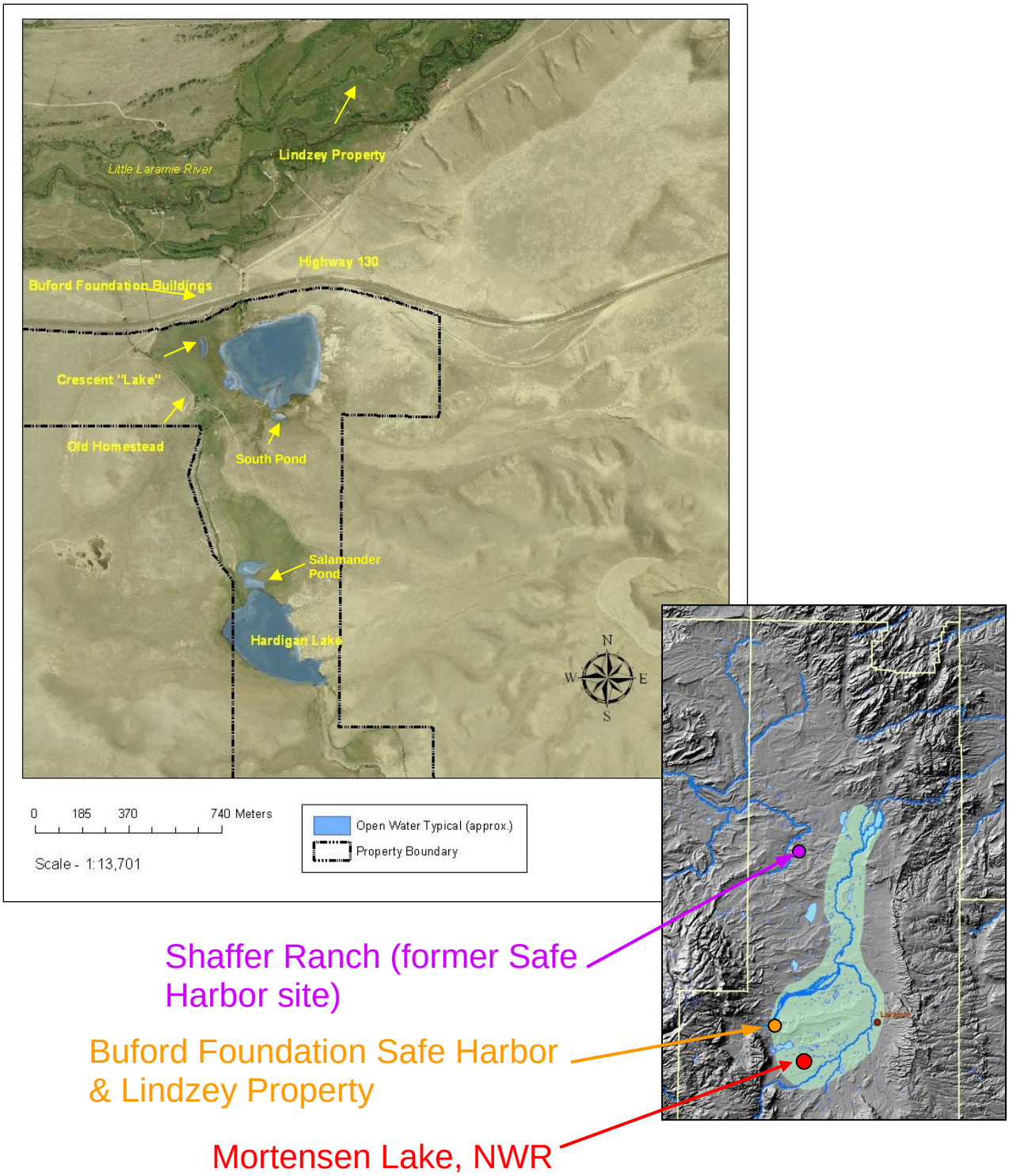

Figure 2. Search blocks used during Wyoming Toad surveys at the Buford Foundation Safe Harbor property in 2006-2008 and 2011-2012. Inset is a view of the approximate extent of flood waters at Porter and Hardigan Lakes in 2009 and 2010.

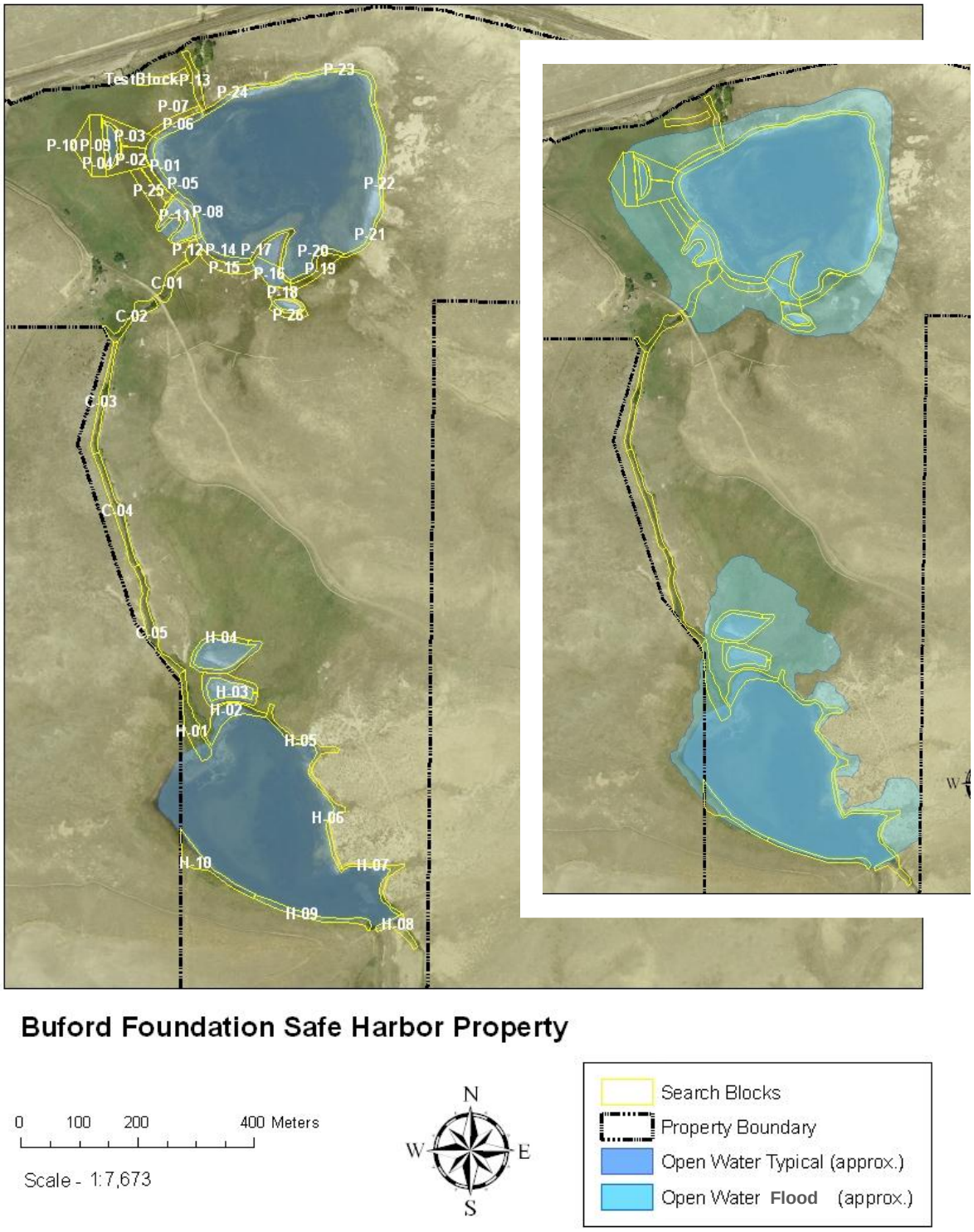

Figure 3. Size classes defined for Wyoming Toads at reintroduction sites in Albany County, WY.

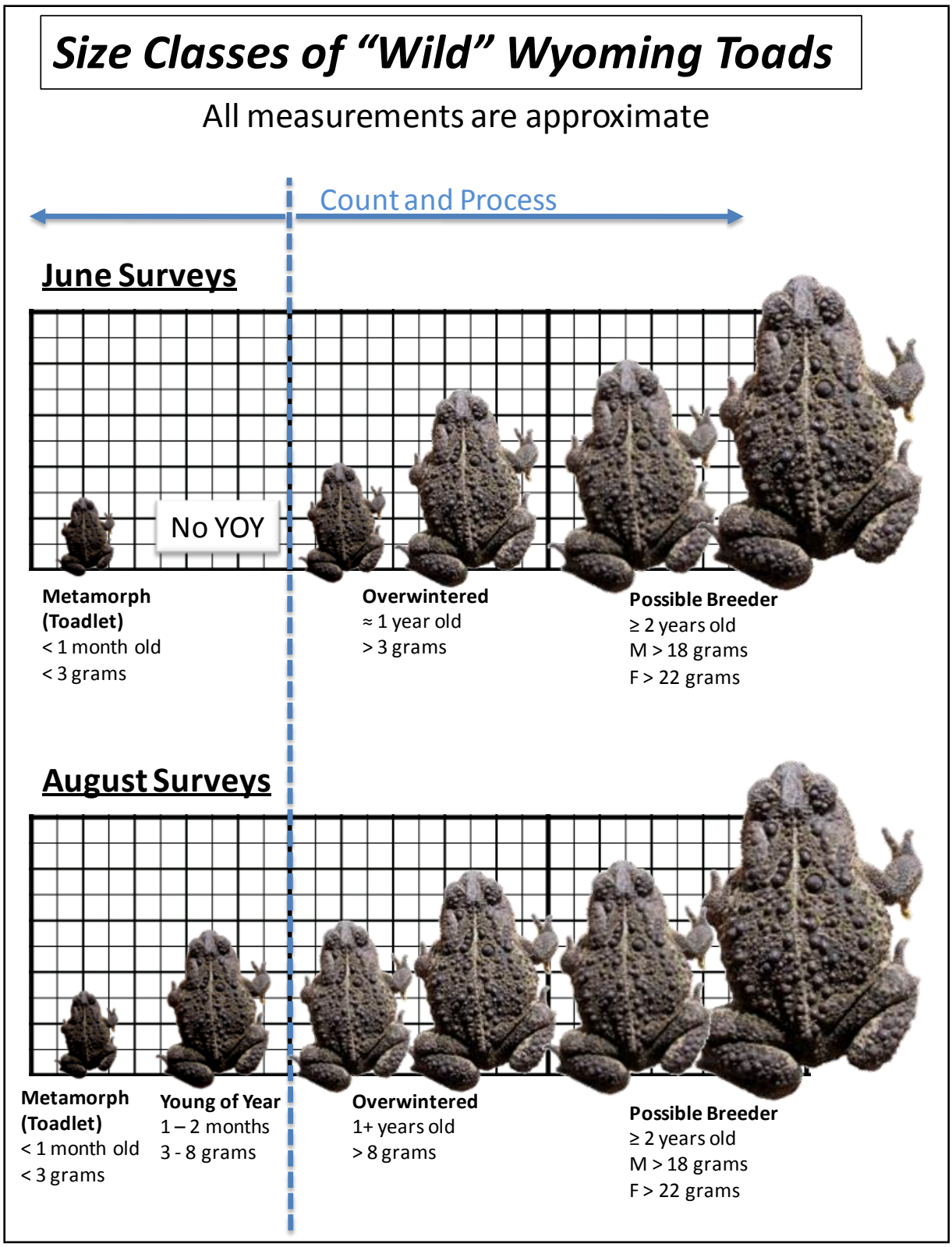

Figure 4. a) Crescent Pond on northwest shore of Porter Lake in mid-June 2012, and b) Salamander Pond (block H-03) on north shore of Hardigan Lake in mid-August 2012.

a)

b)

Figure 5. Western shoreline of Porter Lake in a) June 2012 and b) August 2012 showing extent of receding shoreline due to drought. Photos taken facing north.

a)

b)

Figure 6. Southwest shoreline of Porter Lake in a) June 2012 and b) August 2012 showing extent of receding shoreline due to drought. Photos taken facing west.

a)

b)

Figure 7. Numbers of captive-reared tadpoles and toadlets released from 2005 to 2012 at Buford

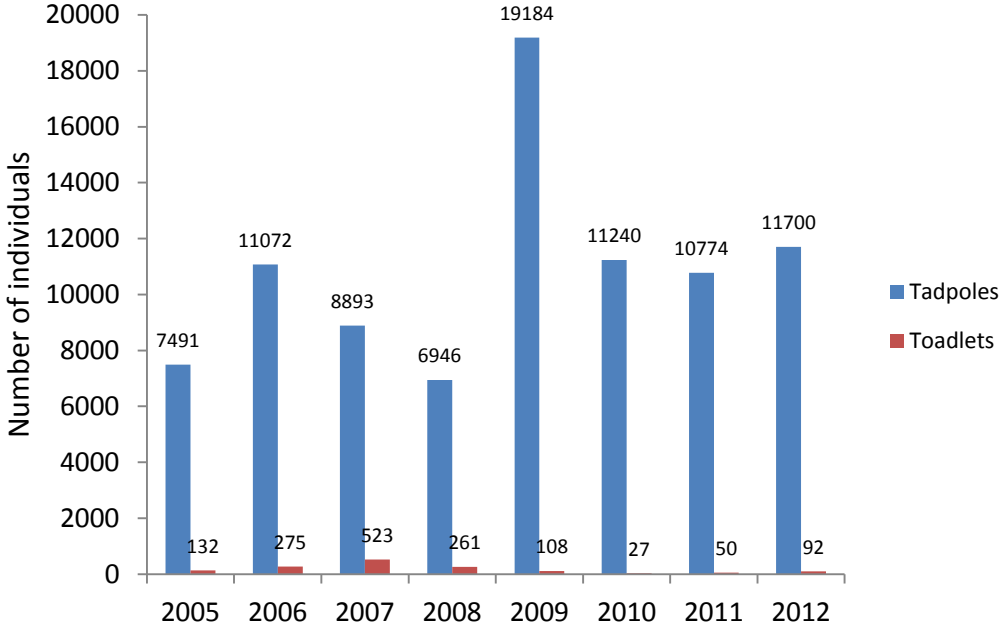

Figure 8. Total number of unique adult toads (including incidentals) detected during breeding and post-breeding season surveys at the Buford Foundation Property from 2006-2012.

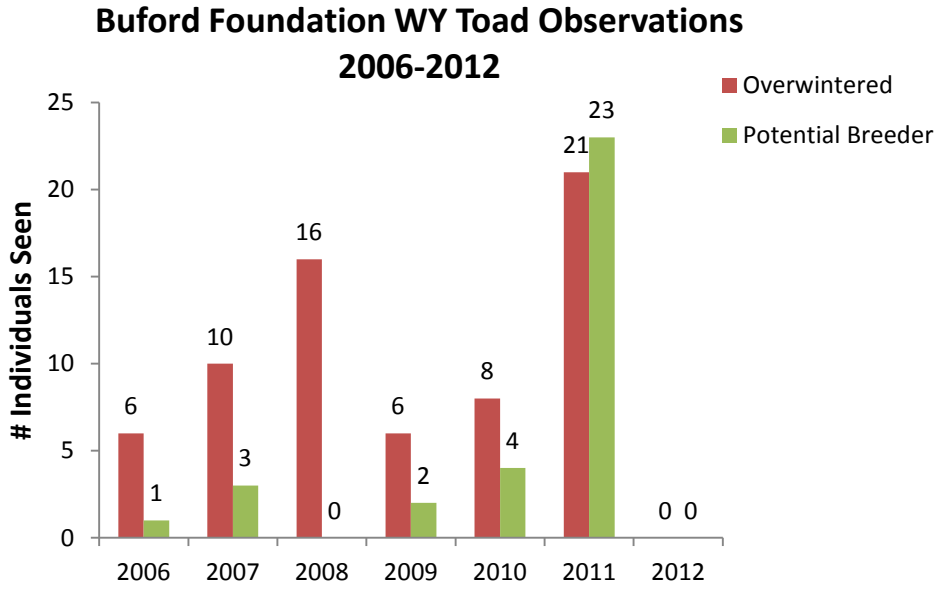

Figure 9. Chytrid fungus infection rates for adult Wyoming Toads at the Buford Foundation property from 2006-2011. Results from two analysis labs are shown. Two samples were taken from a subset of toads in 2011 and sent to the different labs to assess sensitivity PCR tests for chytrid fungus between labs. No toads were captured or sampled in 2012.