Wyoming Toad Monitoring on Safe Harbor Reintroduction Sites: 2011

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SUMMARY

The Wyoming Natural Diversity Database (WYNDD), with the help of the Wyoming Game and Fish Department (WGFD) continued survey and monitoring efforts for the Wyoming Toad at 2 reintroduction sites (Buford and Lindzey) in the Laramie Plains area of Wyoming in 2011. 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 but reintroductions at the Lindzey site only occurred from 2003 to 2005.

Unlike the past two years when flooding at the Buford site necessitated the use of modified search blocks, we were able to again use the original standard search blocks in 2011. We detected a record number of overwintered and potential breeder-sized adult Wyoming Toads in 2011. Although only 4 adults were detected during formal breeding season surveys, we detected 23 adult toads during formal surveys in August. Fifteen potential breeder-sized toads were detected in 2011. The majority of potential breeders were found near the northwest corner of Porter Lake, however, three (2 female, 1 male) were on the north shore of Hardigan Lake.

Breeding by wild toads may have occurred for the first time in 2011. Tadpoles detected in Crescent Lake in mid-July likely were not the result of captive releases based on their age and location. Furthermore, most adult toads were concentrated in and around Crescent Lake where breeding calls by adult males were heard, suggesting that adults were exhibiting breeding behavior at that location.

We also recorded breeding calls by adult male Wyoming Toads using acoustic recording devices (frog loggers) placed at the northwest and southwest corners of Porter Lake. Calling began on 3 June and continued through 28 June, occurring in 2 pulses. Toads typically began calling in late afternoon/early evening and calls peaked at 10-11pm. Calling ceased by 4am. Onset of calling may have been triggered by periods of warmer weather, however, analyses are preliminary.

The rate of chytrid fungus infection in toads at Porter Lake increased from 41.7% in 2010 to 100% in 2011, the highest rate documented at this site. The ability of an individual toad to survive a chytrid infection depends on a number of variables; and amphibians can control or rid themselves of chytrid fungus if they are able to bask frequently in the sun and dry off.

WYNDD also conducted preliminary analyses of shoreline temperatures and depths at the Buford site using shoreline profile data collected since 2009. We also conducted an evaluation of a proposed Safe Harbor Reintroduction Site at property owned by the University of Wyoming's Red Buttes Environmental Biology Laboratory. A habitat assessment is provided along with a map of potential search blocks should reintroductions occur. General recommendations for reintroduction efforts and field logistics also are provided.

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 4 sites in the Laramie Plains since the species was rediscovered in the wild in 1987. Most members of this species, however, currently exist in captive breeding facilities and the only known breeding population in the wild as of the spring of 2011 was at Mortensen Lake National Wildlife Refuge. Unfortunately, evidence of breeding (i.e., egg strings or toadlets) has not been documented at Mortensen Lake since 2009.

In 2011, the Wyoming Natural Diversity Database (WYNDD) entered into a cooperative agreement with the Laramie Rivers Conservation District (LRCD) to continue monitoring Wyoming Toads at 2 locations in the Laramie Plains. One site is covered by a U.S. Fish and Wildlife Service (USFWS) Safe Harbor Agreement with the LRCD and includes ponds and waterways owned by the Buford Foundation. The second site is located along the Little Laramie River and is owned by Fred Lindzey. Wyoming Toad tadpoles and toadlets have been reintroduced at both sites in the past, and reintroductions are continuing at the Buford Foundation property. Reintroductions at a 3rd site, the Shaffer property, were stopped by the Wyoming Toad Recovery Team (WTRT) in 2010 due to the apparent lack of overwinter survival of toadlets at this site (Estes-Zumpf and Keinath 2011). WYNDD no longer monitors this site. 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 at the 2 sites. This report summarizes reintroduction efforts in 2011 and presents survey results for the Buford and Lindzey sites.

Project Goals for 2011:

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. Conduct a single-pass survey of the Buford property in late summer to look for and collect toad mortalities.
6. Evaluate the proposed Safe Harbor site associated with the University of Wyoming's Red Buttes Environmental Biology Laboratory and map search blocks for potential monitoring efforts.
7. Evaluate the daily and seasonal timing of breeding calls for the Wyoming Toad.

METHODS

Study Areas

Surveys were conducted with the help of WGFD at the Buford Foundation property and the Lindzey property in 2011 (Figure 1). The USFWS also conducted surveys at Mortensen Lake; however, survey results for Mortensen Lake are not included in this report. The Buford Foundation property is located approximately 8 miles east of Centennial, WY, and includes 2 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.

The Lindzey property is located just north of Porter Lake and includes sections of the Little Laramie River and surrounding floodplains (Figure 3). 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 has surveyed for Wyoming Toads at the Lindzey site since 2008.

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 the shoreline was similar to that of previous non-flood years.

At the onset of surveys on each property, search areas were stratified into "search blocks" of known size (e.g. Figures 2 & 3), 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 in 2011. 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, with the exception of 2 blocks at the Lindzey property where survey effort was increased to 60 minutes per acre to compensate for difficulties surveying extremely dense willow thickets. 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 2011 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 4). 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.
6. **Mortality Searches**: At the end of summer, we conducted a single pass un-timed survey for dead toads in all search blocks where toads have been documented in the past. Any dead toads were to be collected for necropsy and testing to determine the cause of mortality.

Data collected during surveys in 2011 were very similar to those collected in previous years. 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) and no adult toads have been found during surveys at the Lindzey site since the onset of WYNDD's monitoring efforts. 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

To determine the prevalence of chytrid fungus in toads at Porter Lake, we collected epithelial tissue samples from adult Wyoming Toads found during surveys. Sample collection followed established procedures approved by the WTRT (Boyle et al. 2004, UCB 2004, Livo 2003). Toads were systematically swabbed with sterile cotton swabs to collect epidermal DNA. 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 the Amphibian Disease Laboratory at the San Diego Institute for Conservation Research for analysis via PCR test to determine if the fungus was present. Swabs were only collected from adult Wyoming Toads that were otherwise processed for monitoring purposes.

Shoreline Temperature Profiles

Wyoming Toads require emergent vegetation for breeding, shallow warm water for tadpole development, and open areas for basking (Geraud and Keinath 2004). The shoreline around Porter Lake varies from greatly from relatively flat wet meadows on the northwest and west edges to sharply sloping bare cut banks on the western shore. WYNDD collects data on water depth and temperature wherever adult toads or tadpole aggregations are found to better understand microhabitat needs of the species. In 2009, WYNDD also began collecting shoreline water temperature and depth profiles in order to better understand shoreline characteristics preferred by the Wyoming Toad. Profiles were collected at the center of each search block and wherever adult toads were found. Profiles are perpendicular to the shoreline and start on dry ground at the edge of standing water. Water temperature and depth is measured along a transect every 2m until water depth exceeds 20cm.

Shoreline temperature profiles have been recorded for the past 3 years. Wyoming Toad Recovery Team members expressed interest in this data at the WTRT meeting in Cheyenne in October 2011. Information on shoreline temperature and water depth may prove helpful in reevaluating release methods for captive-bred tadpoles. Thus, WYNDD has included in this report a preliminary analysis of shoreline water depth and temperature profiles for the WTRT and LRCD to assist in reevaluation of reintroduction and recovery efforts.

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 an acoustic recording device (frog logger) in 2010 to examine patterns of breeding calls and correlations between calling and local habitat and weather conditions. No calling occurred in 2010, however, and analysis of call data by the USFWS was delayed until the following winter because WYNDD did not own the analysis software, Song Scope (Wildlife Acoustics, Inc., Concord, MA). In 2011, WYNDD purchased the software with funds from the LRCD and USFWS. We set out 2 frog loggers at the northwest and southwest corners of Porter Lake, where male Wyoming Toads were heard calling in 2009. Frog loggers were programmed to record for 2 10-minute segments every hour from 27 May through 28 June, 2011.

We analyzed recordings using a Wyoming Toad call recognizer developed by Heidi Meador (USFWS) and modified to better detect calls recorded in the field. We recorded whether Wyoming Toad calls were detected in each 10-minute segment and examined daily and seasonal patterns of calling behavior. We also looked at correlations between calling and local air temperature.

Evaluation of Proposed Safe Harbor site

Currently, the Buford Foundation property is the only Safe Harbor site where Wyoming Toad reintroductions are occurring. The LRCD and the WTRT are looking into additional reintroduction sites. One possible location is the University of Wyoming's Red Buttes Environmental Biology Laboratory (hereafter referred to as Red Buttes). WYNDD zoologists were asked to evaluate the suitability of this proposed Safe Harbor site for Wyoming Toad

reintroductions. Thus, WYNDD visited this site in June 2011 during the toad breeding season to determine if Red Buttes contains habitat for the toad. WYNDD also mapped search blocks for potential monitoring efforts should reintroductions occur.

RESULTS AND DISCUSSION

Surveys

Breeding season and post-breeding season surveys were conducted from 15-18 June and 15-17 August, respectively. Standard search blocks were used at the Buford Foundation Property during breeding and post-breeding season surveys. Flooding along the Little Laramie River at the Lindzey Property, however, precluded breeding season surveys at that site because search blocks were inaccessible. Post-breeding surveys were conducted at the Lindzey Property in August. A Shoreline Search for egg strings was conducted on 24 June, but no Wyoming Toad eggs were found. We also conducted a Mortality Search on 29 September and found no dead toads.

Reintroductions

A total of 10,774 tadpoles and 50 toadlets were released from 9 different captive breeding facilities in 2011, all at the Buford site (Table 2). The number of tadpoles and toadlets released in 2011 was similar to the number released in 2010 (Figure 5). The number of breeding facilities producing toads for reintroduction increased in 2011 from the 6 facilities producing toads in 2010. Tadpoles were released from 8 June to 6 July, 2011. Based on patterns of breeding calls of wild toads in 2011 (see Breeding Calls), tadpole releases in early June may have occurred before conditions were suitable for breeding and tadpole development. Tadpoles released too early may have been exposed to suboptimal water temperatures. Low average water temperatures have been shown to slow tadpole development and delay metamorphosis in other amphibians (Smith-Gill and Berven 1979, Hayes et al. 1993, Olsson and Uller 2002). However, impacts on Wyoming Toad tadpoles remain purely speculative at this point.

Counts:

Although the number of adult toads documented at the Buford property during the breeding season in 2011 was slightly lower than previous years, we detected a record number of adult toads during the post-breeding season (Table 3). More toads are typically detected during the post-breeding season, which is largely due to young-of-the-year (YOY) toads from spring releases, but may also reflect greater mobility of toads in late summer. In 2011, the discrepancy may have been exacerbated due to weather conditions during June surveys. Weather was suitable for breeding season surveys (i.e. no rain, moderate wind), but a storm system moving through the area on 15 June may have influenced toad behavior on the first day of surveys. The majority of adult toads observed during June surveys were incidental toads detected outside of formal survey activities after 15 June.

Formal post-breeding season surveys yielded 9 overwintered toads and 14 potential breeders (Table 3). The total number of adult toads including incidentals seen during the two survey seasons in 2011 was the highest since standardized surveys began at the Buford Property (Figure 6). In addition to

the toads detected during survey sessions, 12 or more toads were seen or heard calling during maintenance and tadpole release visits to the site between June and August. Abundance estimates for 2011 at the Buford Property range from 12 to 60 overwintered toads and 18 to 37 potential breeders (Table 4). We also detected almost 70 YOY toads, including incidental toadlets. All YOY toads were recorded during post-breeding surveys and were concentrated along the northwest, west, and southwest shorelines which were moist and slightly flooded even in August due to irrigation. Although YOY toads likely metamorphosed from captive-bred tadpoles released at Porter Lake, it is possible that some may have resulted from breeding in the wild (see *Documentation of Breeding*). We documented no Wyoming Toads of any age class at the Lindzey property in 2011.

Adult toads at the Buford Property were found from the northwestern corner to the south side of Porter Lake, along the supply canal south of Porter Lake, and on the northwestern shore of Hardigan Lake (Figure 7). The majority of adult toads were found in or near Crescent Lake at the northwest corner of Porter Lake (Figure 8). The 3 potential breeders (2 females, 1 male) on the north shore of Hardigan Lake were found during post-breeding season surveys and mark only the second time toads have been seen in the vicinity of Hardigan Lake. The first record of a toad near Hardigan Lake was of a male calling from the flooded hay meadow north of Hardigan in 2009. Interestingly, one overwintered female detected along the north end of the supply canal in June 2011 was later detected on the north shore of Hardigan Lake during surveys in August. Not only did this female move approximately 700m in 2 months, but also gained 22.3g over that time, resulting in her reaching potential breeder size with a final weight of 32.6g by August. A second overwintered adult female was documented during both June and August surveys. This female also gained 21.6g, increasing from 5.4g in June to 27g in August. Assuming these females were first year overwintered toads in June based on their weights, they reached potential breeder size during the summer and could be capable of breeding the next spring, at 2 years-of-age.

Documentation of Breeding

Although no egg strings were detected during the breeding season shoreline search, it is possible that breeding occurred at Porter Lake for the first time. On 14 July, WYNDD technicians found 3 aggregations of 12-18 very young Wyoming Toad tadpoles in Crescent Lake. Tadpoles were younger (smaller) than those typically released from captive breeding facilities and the last release of captive-bred tadpoles that season was over a week earlier (6 July; Table 2). Discussions with biologist at the Red Buttes and Saratoga breeding facilities confirmed that all tadpoles were released directly into Porter Lake, never into Crescent Lake. Although irrigation maintains several inches of water between Porter and Crescent lakes, it is unlikely that tadpoles from Porter Lake moved through the wet meadow in sufficient numbers to result in the 3 aggregations found at Crescent Lake. However, we cannot rule out this possibility.

The fact that these tadpoles were in the same area as the majority of breeding-size adults (i.e., Crescent Lake; Figures 7 and 8) provides further evidence that they may be from wild reproduction. Wyoming Toad breeding calls were heard at Crescent Lake by WYNDD and USFWS biologists on several occasions during the breeding season in 2011. Lastly, a frog logger positioned on the northwest corner of Porter Lake recorded periods of high intensity calling by Wyoming Toads throughout the breeding season (see *Breeding Calls*).

Chytrid Analyses

We collected tissue swabs from 35 adult toads at the Buford site (Table 5). Chytrid fungus was detected in all toads sampled (100%). The chytrid infection rate in 2011 is a sharp rise from the past 3 years, and surpasses the 80% infection rate documented in 2007, when the fungus was first detected at the Buford Property (Figure 9). The 2 adult females captured during both the breeding and post-breeding season tested positive for chytrid fungus in both June and August, indicating that either they were unable to rid themselves of the fungus through basking or were reinfected. Amphibians can control or rid themselves of chytrid fungus if they are able to bask frequently in the sun and dry off. Cold wet springs with fewer warm sunny days (as in 2011) could limit the efficiency of basking behavior at reducing or eliminating chytrid fungus on individual toads. Finally, we used a different lab to analyze chytrid samples in 2011 than in the past several years (previously we used Pisces Molecular, while in 2011 we switched to the Amphibian Disease Lab at the San Diego Zoo). It is possible that the sensitivity of PCR tests to detect chytrid could differ among laboratories. Thus, if the lab used in 2011 had higher sensitivity, it could cause a perceived increase in the number of infected toads. If the Wyoming Toad Recovery Team deems it important, we can test this possibility by sending a batch of replicate samples to both labs and evaluating differences in the results.

Shoreline Temperature Profiles

We analyzed shoreline depth and temperature profiles separately for flood years (2009, 2010) and non-flood years (2011) because floods substantially altered shoreline characteristics (Estes-Zumpf and Keinath 2011). Furthermore, Crescent Lake and the South Pond are both key toad areas but were engulfed by Porter Lake in flood years. We summarized water temperature and depth for each of 8 shoreline sections around the circumference of the lake (N, NW, W, SW, S, SE, E, NE) and for Crescent Lake and South Pond (Figure 10). In order to assess the amount of potentially suitable shoreline for Wyoming Toads, we examined the distance from dry ground to reach a water depth of 20cm. We also determined the average water temperature for each shoreline section and for the 2 ponds.

Wyoming Toads are typically found along the NW, W, SW, and S shorelines of Porter Lake. They also concentrate at Crescent Lake and South Pond. Shoreline profiles revealed that the NW, W, and SW sections of Porter Lake, as well as South Pond, had gently sloping shorelines with a greater distance of shallow water than other sections of shoreline (Figure 11). These sections, as well as Crescent Lake also had the highest average shoreline water temperatures (Figure 12). Crescent Lake contained the warmest water (78°F) during the breeding season in 2011 and was almost 11°F warmer than the eastern shoreline of Porter Lake (Figure 12).

Flooding at Porter Lake in 2009 and 2010 resulted in more shallow water shoreline habitat for toads (Figure 11). However, shoreline temperatures during flood years were consistently colder than in 2011 (Figure 12). Results suggest that the shoreline of Porter Lake has warmer water temperatures and more shallow water habitat on the NW, W, and SW sections of the lake, as well as at Crescent Lake and South Pond. These areas are also where toads are consistently found across years at this reintroductions site.

Water temperature and depth data also has been collected at locations where adult toads and tadpole aggregations are found. Comparisons between these data and random shoreline

temperature profiles could provide important information on habitat preferences by Wyoming Toads in the wild. This large dataset has been collected by WYNDD during surveys since 2009 and is available for further analyses given interest from the WTRT and LRCD.

Breeding Calls

Male Wyoming Toads began calling on the night of 3 June, 2011. Analysis of recordings from frog loggers is ongoing; however, we have summarized data from the logger on the northwest side of Porter Lake. Calling occurred over 2 intervals, one from 3 June to 10 June, and one beginning on 14 June and continuing through 28 June when the frog logger stopped recording (Figure 13). Daily calling varied in the number of calls/recording throughout the season, but the timing of calls was relatively consistent. Male toads generally began calling in late afternoon/early evening and continued through 4:00 AM (Figure 14). Calling peaked from 10:00 to 11:00 PM. Seasonal weather patterns likely influenced calling behavior. Preliminary analyses suggest that calling began shortly after average daily air temperatures reached over 60°F and maximum daily temperatures reached over 80°F. Although we will further explore relationships between air temperature and other weather patterns with calling behavior, we currently only have data for a single season (n = 1). Seasonal recordings of calling from multiple years (and multiple sites, if possible) and simultaneously collected weather data are necessary to better understand conditions that influence onset of calling by the Wyoming Toad.

Evaluation of Proposed Safe Harbor site

A WYNDD zoologist visited the property surrounding the University of Wyoming's Red Buttes Environmental Biology Laboratory on 16 June, 2011 to conduct a preliminary evaluation of habitat suitability for the Wyoming Toad.

All water on the property is from artesian wells and is very cold all summer long, which could hinder tadpole development. Before introductions take place, it is advisable to conduct temperature profiles of the target wetlands and compare them to suitable temperatures for larval development from the literature and other Wyoming Toad sites. Shallow areas of the two ponds in the South Group, and runoff from the western-most of the two wells in the North Group (Figure 15) are slightly warmer. A beneficial aspect of site hydrology is that since the wells flow continually at a consistent rate, all water bodies are of consistent size throughout the year, even in relatively dry years.

Leazenby Lake is stocked with trout, has fairly deep margins, and receives substantial fishing pressure, making it an unlikely reintroduction location. Outside of Leazenby Lake and the fish runways in the Red Buttes facility, all water bodies on site are trout-free. It should be noted that the large pond in the South Group has previously been stocked with trout for experimental purposes, but is currently thought to be trout-free. Given the potential for trout to prey upon Wyoming Toad tadpoles, the feasibility of keeping this pond free of trout is an important consideration.

Much of the dry upland has subsurface limestone with very little overlaying soil. Moist areas of the property generally have dense grass with ample dead stems from the previous seasons. A low level of cattle grazing occurs on the property, generally in October.

Our initial evaluation suggests that the most suitable reintroduction locations are the shallow margins of the two ponds on the southern end of the property (South Group in Figure 15). We therefore concentrated on developing search blocks for this area. In particular, the eastern edge of the lager pond and all margins of the smaller pond have shallow areas where water is warmed from solar heating. Further, according to facility managers (Bob and Steve) habitat modification, including creating additional shallow areas long pond margins, could be a possibility. The water table for much of the property is quite shallow (seven feet or less), suggesting a high potential for creation of man-made ponds.

RECOMMENDATIONS

1. *Frog loggers*: Very little is known about the calling behavior of the Wyoming Toad. Because calling signifies breeding behavior, information on calling could help the WTRT better understand the breeding biology and requirements of the Wyoming Toad in the wild. WYNDD recorded extensive calling during the spring of 2011 and has provided results from preliminary analyses in this report. However, seasonal and daily weather patterns are known to greatly affect calling behavior in other amphibians. To account for annual variation, we recommend collecting at least two years of calling data for detailed analysis of calling behavior in relation to 1) season, 2) time of day, 3) air temperature, 4) relative abundance of toads, as well as other key variables. Data from both Porter Lake and Mortensen Lake would be ideal, should toads become re-established at Mortensen. Thus, we recommend the WTRT continue using frog loggers to record calling behavior by Wyoming Toads at Porter Lake and Mortensen Lake. If adult toads are repeatedly found at Hardigan Lake, the WTRT also should consider placing a frog logger at that site. We recommend that call data be copied and stored for future detailed analysis.

2. *Egg string searches*: Currently, egg string searches are conducted once during the breeding season. Initial analysis of call data suggests that breeding behavior can continue for several weeks, and may occur in pulses. Thus, a single egg string search is likely insufficient to document breeding in the wild, should it occur. We recommend conducting several egg string searches across the breeding season to improve our ability to detect wild breeding. To this end, WYNDD zoologists are working with the WTRT monitoring committee to improve search protocols for egg strings.

3. *Stable isotope verification of breeding*: Wild breeding may have occurred at Porter Lake in 2011 for the first time since reintroductions began there. However, because tadpoles are regularly released at that site, confirmation that the tadpoles documented in Crescent Lake resulted from wild breeding was not possible. We recommend the WTRT consider the use of stable isotope analyses of tadpoles to determine if larvae come from the wild or from a captive breeding facility. Stable isotope signatures in tadpoles should reflect their food source history, and signatures should be different for captive diets and wild food items. The University of Wyoming has an exceptional stable isotope facility that will run samples from external projects

for a fee. Before implementation of this approach, a pilot study will be necessary to determine the stable isotope signatures of captive and wild toads.

4: *Timing of tadpole releases*: The WTRT is exploring ways to improve the survival and recruitment of captive-bred tadpoles released at reintroduction sites. One suggestion discussed at the WTRT meeting in October, 2011, was to time releases with natural breeding behavior. Because toads theoretically begin breeding calls in spring when conditions are suitable for reproduction, releasing tadpoles after calling has begun may improve tadpole survival. Because the timing of breeding varies with annual weather patterns, the timing of calling behavior signifying suitable breeding conditions may also vary annually. We recommend the use of frog loggers to record calling and weekly download and analysis of recordings to identify when breeding calls have begun in the wild. In that absence of calling on a given year, we recommend timing releases based on past calling patterns and trends with local air temperature.

5. *Synthesis and analysis of temperature data*: In addition to survey data, WYNDD has collected water temperature data at the Buford property for several years. Shoreline temperature profile data are available for each search block and for locations where adult toads were detected. We also have water temperature and depth data for each point location where adult toads and tadpole aggregation were found. Analyses of shoreline water temperatures at Porter Lake provided in this report were done as time and budgets permitted and are thus preliminary. Further analyses of these data, particularly comparisons between random shoreline temperature profiles and profiles where toads were detected could provide insight in to habitat selection by toads at this site. Temperature preferences of adult toads and tadpoles could also be used to inform new release protocols at Buford and other reintroduction sites. If time and budget permit, in-depth analyses of existing temperature data collected by WYNDD at the Buford property over the past several years could yield useful information regarding microhabitat selection of Wyoming toads.

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TABLES AND FIGURES

Table 1. Summary description of Wyoming Toad monitoring searches conducted at the Buford Foundation and Lindzey properties in 2011.

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 15 - 18. Post-Breeding Search: August 15 – 17.
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 15 - 18. Post-Breeding Search: August 15 – 17.
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.
Mortality Search	To document toad mortality due to chytrid fungus (or other causes). To collect specimens of dead toads for necropsy and testing.	All search blocks where toads have been documented in past years.	Detailed, un-timed search.	End of summer

Table 2. Breakdown of release dates, locations, and source for the 10,824 Wyoming Toad tadpoles and toadlets released at the Buford Foundation Property in 2011.

Breeding Facility	Tadpoles	Release Dates	Toadlets	Release Dates
Saratoga	2,859	6/08, 6/22, 6/27	6	8/17
Mississippi River Museum	492	6/24	0	-
Red Buttes	228	6/14, 6/30	19	7/8
Toledo Zoo	0	0	0	-
Detroit Zoo	552	6/15	10	8/11
Como Zoo	1,353	7/6	6	8/11
Cheyenne Mtn. Zoo	1,792	6/21	9	8/26
Omaha Zoo	127	6/21	0	-
Kansas City Zoo	360	6/15	0	-
Toronto Zoo	3,011	6/28	0	-
Total	**10,774**		**50**	

Table 3. Raw counts of individual Wyoming Toads found during monitoring activities at Buford from 2006-2011. 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	
Size Class	**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)
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)
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 (18[c])

[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)	
Size Class	**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)
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)

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

Table 5. Chytrid fungus results and initial capture information for the 34 adult Wyoming Toads captured on the Buford Foundation property in the summer of 2011. Two female toads were captured during both breeding and post-breeding season surveys and results for both captures are presented. Location coordinates are in UTM NAD83.

Adult ID	Recap	Date	Block ID	Sex	Age	Wt (g)	SVL (mm)	GPSE	GPSN	Notes	CHYTRID?
F0090	N	16-Jun	C02	F?	O	10.3	42.0	416556	4571429	Found on dry soil.	**Y**
F0090	Y- from June	16-Aug	H02	F	PB	32.6	60.0	416743	4570813	Found on muddy soil.	**Y**
F0091	N	16-Jun	P02	F	PB	48.4	65.0	416576	4571754	Found in water.	**Y**
F0092	N	16-Jun	P02	F	O	5.4	37.0	416576	4571754	Found on dry soil.	**Y**
F0092	Y- from June	17-Aug	P18	F	PB	27.0	57.0	416877	4571481	Found on muddy soil.	**Y**
F0093	N	17-Jun	P02	F?	O	6.4	35.9	416593	4571746	Found in water.	**Y**
F0094	N	17-Jun	P02	F?	O	7.2	31.5	416583	4571747	Found in water.	**Y**
F0095	N	17-Jun	P02	F?	O	5.5	24.5	416583	4571747	Found in water. Captured at the same location as F0094.	**Y**
F0096	N	17-Jun	P02	F?	O	4.5	32.6	416583	4571747	Found in water. Captured at the same location as F0094.	**Y**
F0097	N	18-Jun	P02	F?	O	7.3	36.1	416597	4571770	Found on muddy soil. Possible recapture.	**Y**
F0098	N	15-Aug	P04	F	O	11.6	46.9	416553	4571764	Found on dry soil.	**Y**
F0099	N	15-Aug	P01	F	O	14.4	47.5	416597	4571739	Found on muddy soil.	**Y**
F0100	N	15-Aug	P04	F	PB	25.6	55.7	416551	4571746	Found on dry soil.	**Y**
F0101	N	15-Aug	P04	F	O	16.5	52.3	416544	4571759	Found on muddy soil. Shedding belly skin	**Y**
F0102	N	16-Aug	H03	F	PB	32.1	65.0	416711	4570835	Found on muddy soil.	**Y**
F0103	N	15-Aug	P04	F	PB	24.4	53.9	416546	4571760	Found on muddy soil with M0028.	**Not sampled**
F0104	N	15-Aug	P18	F	O	7.7	35.9	416835	4571503	Found in water.	**Y**
F0105	N	15-Aug	P18	F	O	20.3	51.1	416832	4571495	Found in water.	**Y**
F0106	N	17-Aug	P01	F	O	5.8	41.3	416593	4571753	Found in water.	**Y**
M0016	Y-2010	15-Aug	P04	M	PB	22.2	50.8	416541	4571776	Found in water.	**Y**
M0017	N	16-Jun	C03	M	PB	16.1	41.2	416506	4571254	Found on dry soil.	**Y**
M0018	N	16-Jun	P02	M	PB	22.9	46.3	416586	4571752	Found in water.	**Y**
M0019	N	15-Aug	P03	M	PB	20.5	53.0	416558	4571765	Found on muddy soil.	**Y**
M0020	N	15-Aug	P04	M	O	15.3	49.1	416543	4571793	Found on dry soil. Hopped into water.	**Y**
M0021	N	15-Aug	P02	M	PB	18.9	52.2	416597	4571742	Found on muddy soil. Molting?	**Y**

M0022	N	15-Aug	P04	M	O	15.1	53.0	416538	4571737	Found on muddy soil.	**Y**
M0023	N	15-Aug	P01	M	O	15.5	50.0	416602	4571749	Found on muddy soil.	**Y**
M0024	N	15-Aug	P04	M	PB	20.2	49.5	416546	4571749	Found on muddy soil.	**Y**
M0025	N	15-Aug	P14	M	PB	21.5	58.0	416706	4571580	captured at 15:23	**Y**
M0026	N	15-Aug	P04	M	PB	21.5	50.9	416544	4571759	Found on dry soil.	**Y**
M0027	N	15-Aug	P16	M	PB	22.0	58.0	416817	4571540	Found on muddy soil.	**Y**
M0028	N	15-Aug	P04	M	O	16.2	46.1	416546	4571760	Found on muddy soil with F0103.	**Y**
M0029	N	17-Aug	H02	M	PB	18.4	52.0	416736	4570818	Found on muddy soil.	**Y**
M0031	N	16-Aug	P19	M	PB	23.5	51.2	416888	4571513	Found on muddy soil.	**Y**
M0032	N	16-Aug	P04	M	PB	20.8	52.7	416552	4571741	Found on moist grass.	**Y**
M0033	N	17-Aug	P01	M	PB	20.0	50.9	416613	4571793	Found on dry soil.	**Y**

Figure 1. Map of the Buford Foundation and Lindzey properties. 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.

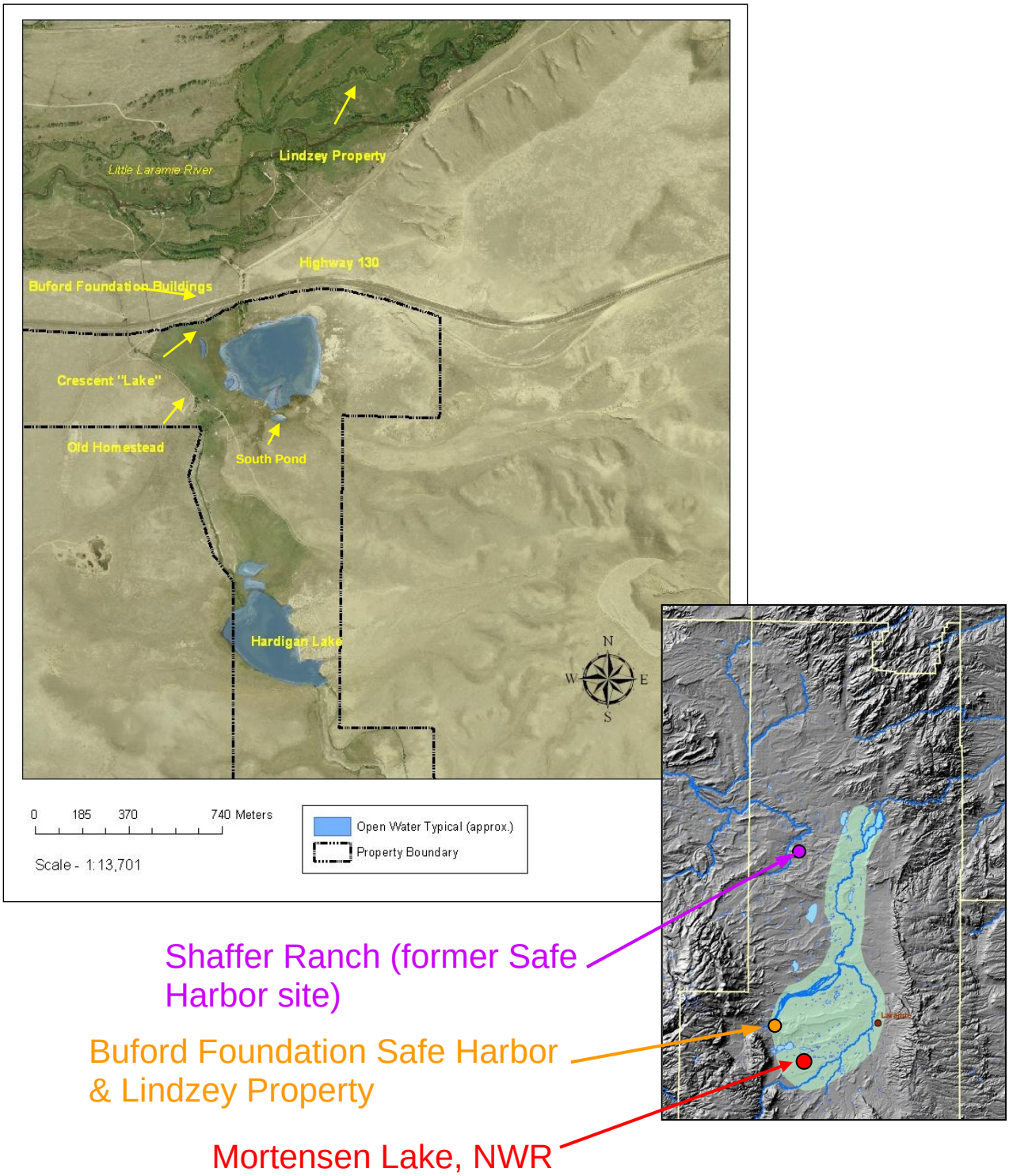

Figure 2. Search blocks used during Wyoming Toad surveys at the Buford Foundation Safe Harbor property in 2006-2008 and in 2011. Inset is a view of the approximate extent of flood waters at Porter and Hardigan Lakes in 2009 and 2010.

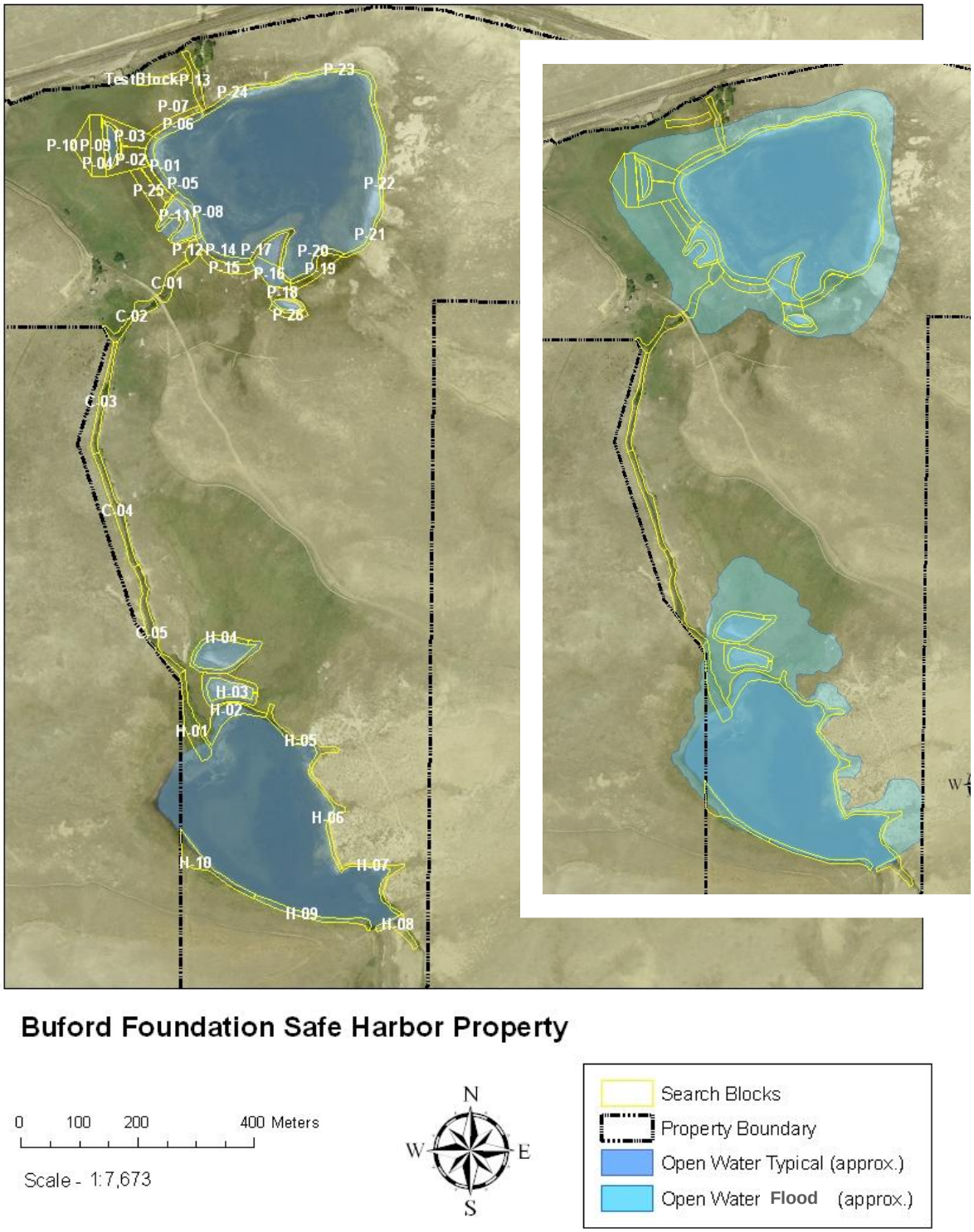

Figure 3. Search blocks used during Wyoming Toad surveys at the Lindzey Property in 2011.

Lindzey Property

0 45 90 180 Meters

Scale - 1:3,200

Search Blocks
Open Water Typical (approx.)
Property Boundary

Figure 4. Size classes defined for Wyoming Toads at reintroduction sites in Albany County, WY.

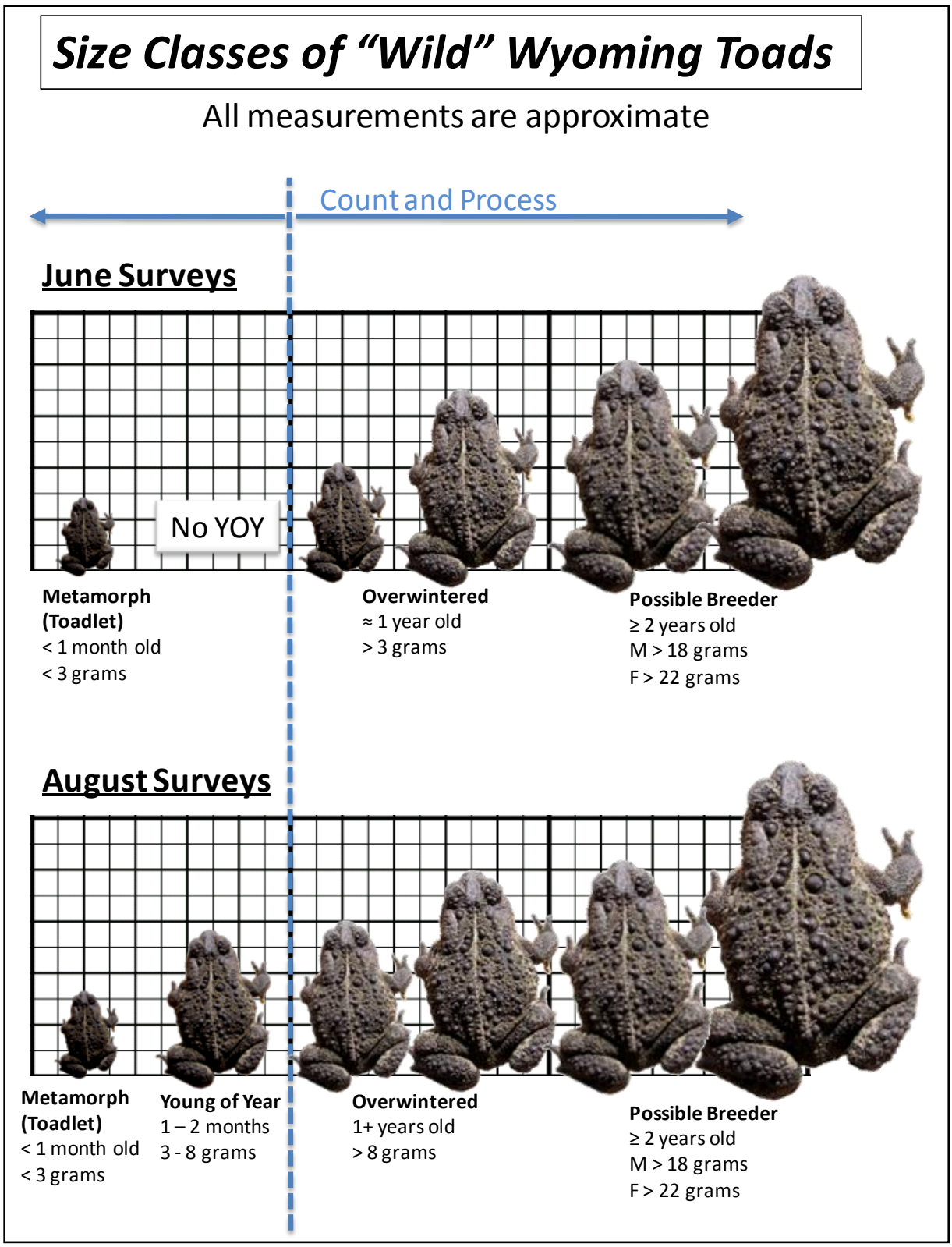

Figure 5. Numbers of captive-reared tadpoles and toadlets released from 2005 to 2011 at Buford

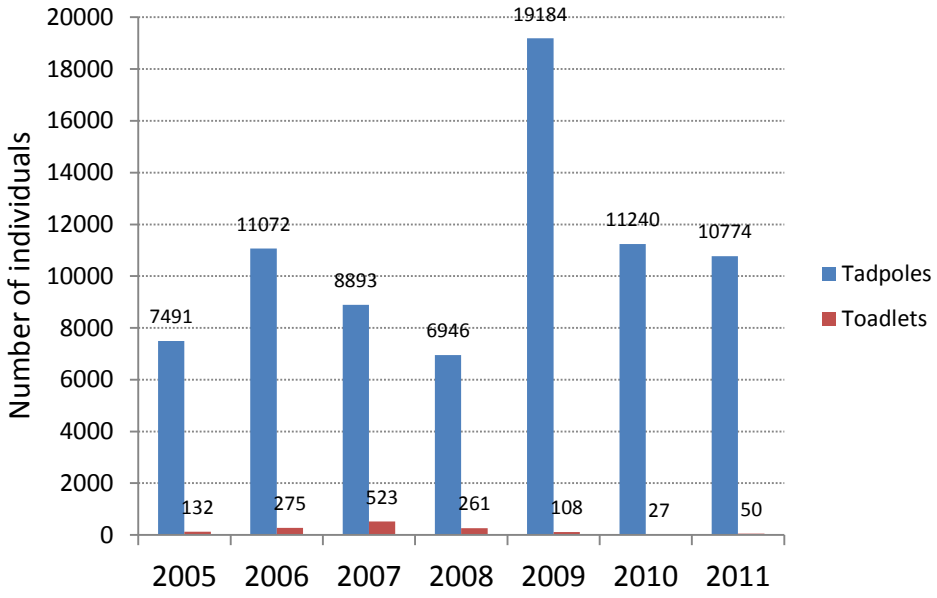

Figure 6. Total number of unique adult toads (including incidentals) detected during breeding and post-breeding season surveys at the Buford Foundation Property from 2006-2011.

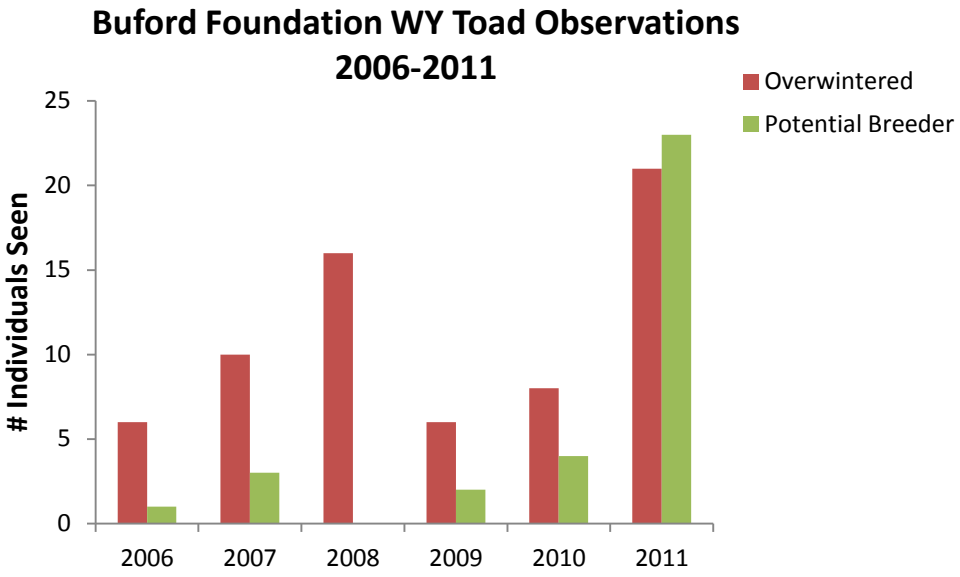

Figure 7. Location of all adult toads (including incidentals) detected during breeding and post-breeding season surveys at the Buford Foundation Property in 2011.

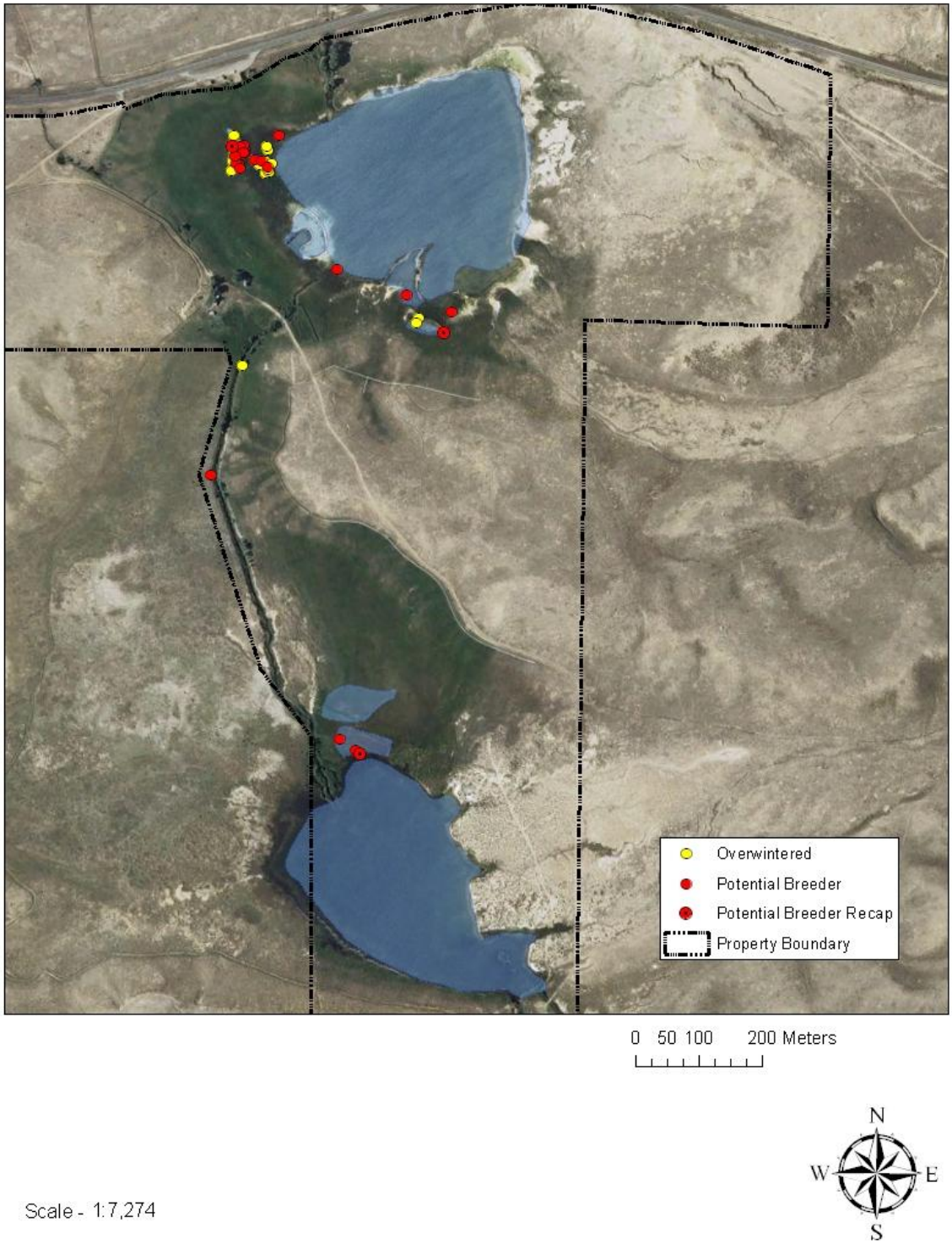

Figure 8. Concentration of adult toads (including incidentals) detected in and near Crescent Lake at the northwest corner of Porter Lake during breeding and post-breeding season surveys at the Buford Foundation Property in 2011.

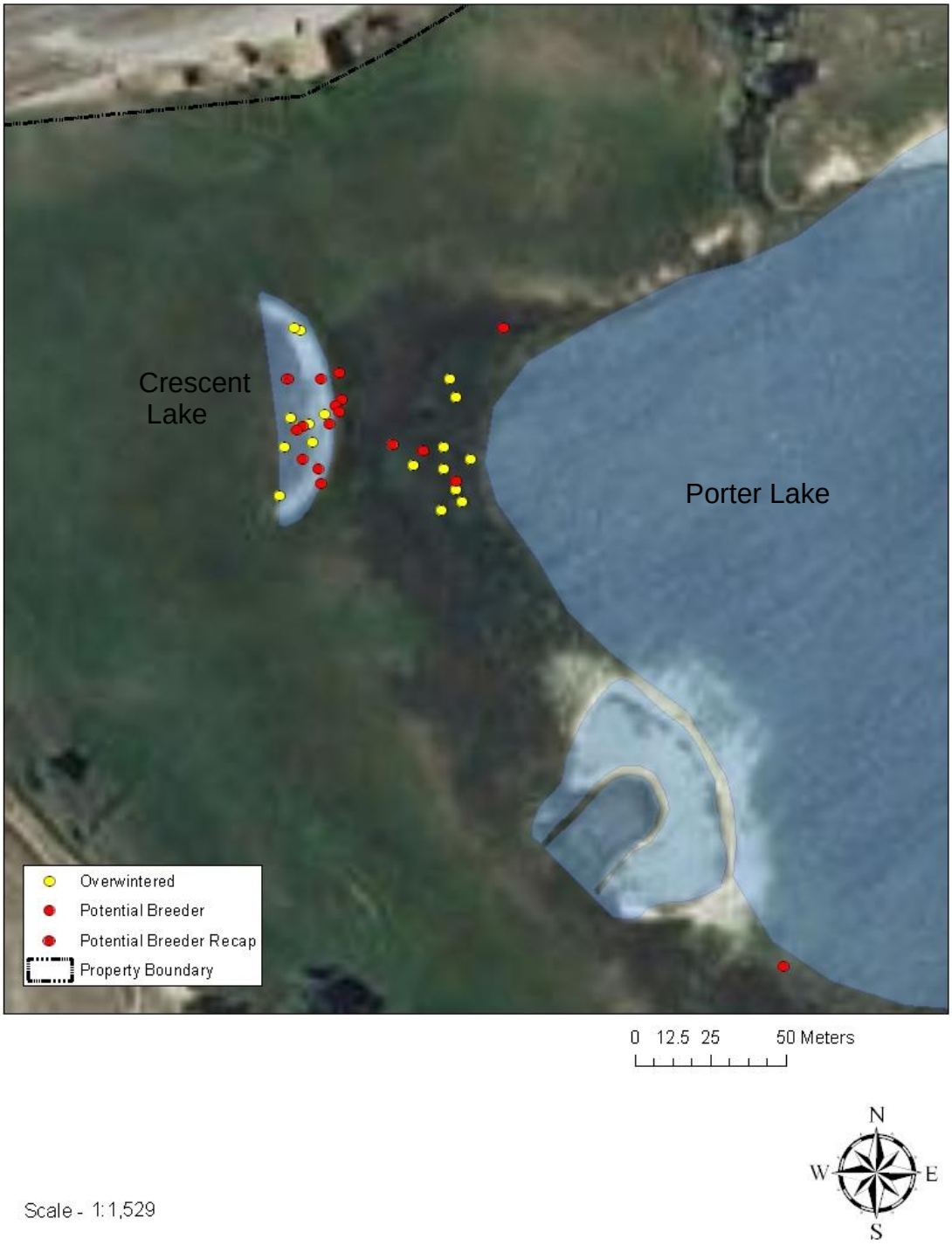

Figure 9. Chytrid fungus infection rates for adult Wyoming Toads at the Buford Foundation property from 2006-2011.

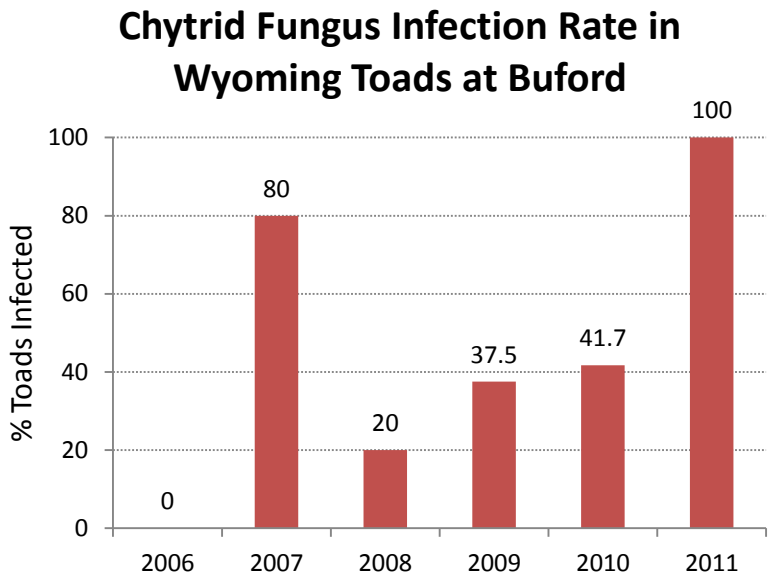

Figure 10. Sketch of Porter Lake shoreline showing section breaks for shoreline temperature and depth profile analyses.

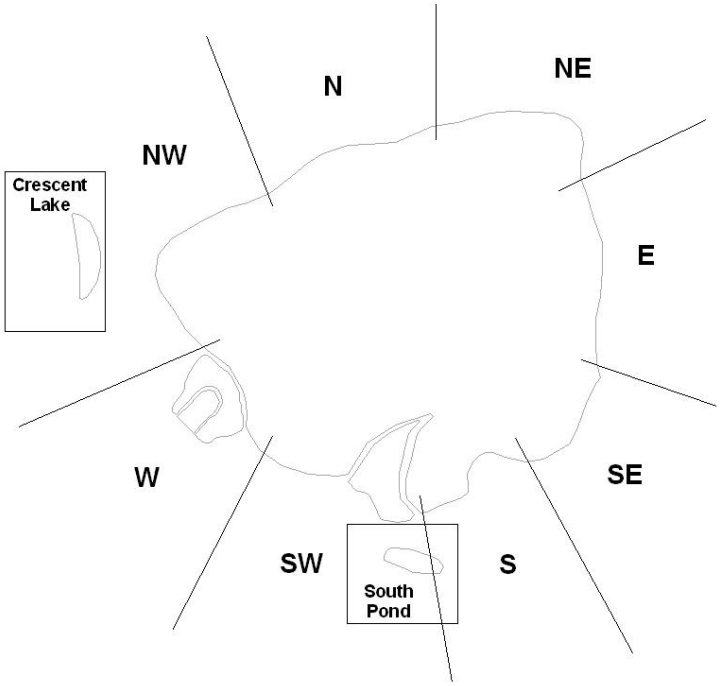

Figure 11. Average distance from dry ground for a transect perpendicular to the shoreline to reach 20cm water depth at Porter Lake. Distance is summarized for 8 sections covering the circumference of the lake, as well as for 2 ponds adjacent to Porter Lake. Results are shown for flood (2009 & 2010) and non-flood years (2011).

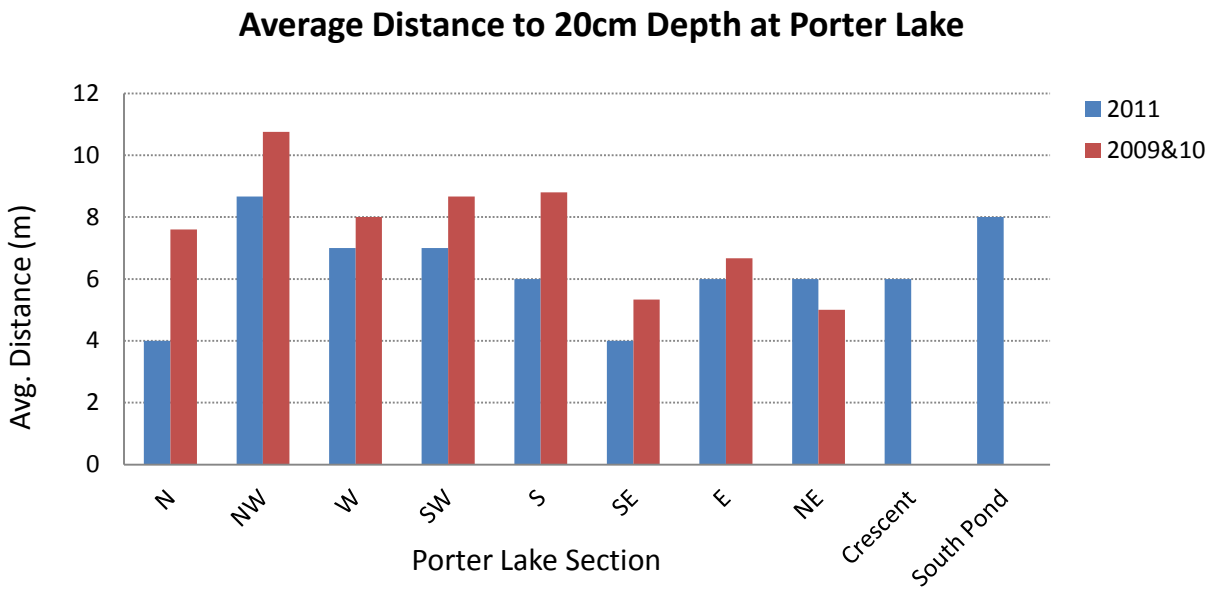

Figure 12. Average shoreline water temperature at Porter Lake. Water temperature is summarized across temperature transects for 8 sections covering the circumference of the lake, as well as for 2 ponds adjacent to Porter Lake. Results are shown for flood (2009 & 2010) and non-flood years (2011).

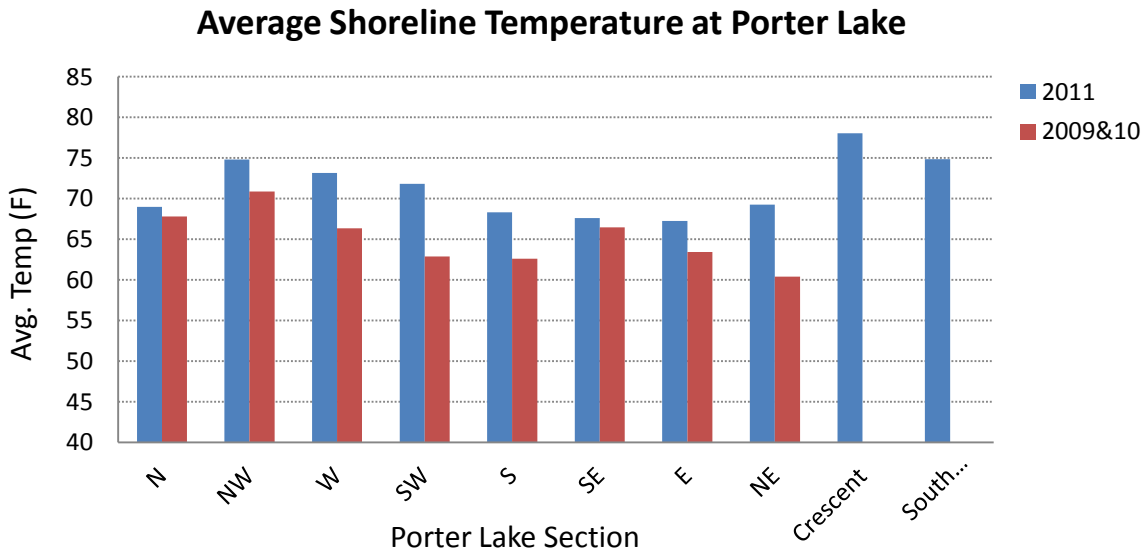

Figure 13. Seasonal patterns of calling by male Wyoming Toads at the Buford Property in 2011. Calling was recorded in two 10-minute sessions every hour by an acoustic recording device. Calls were recorded from 27 May to 28 June, 2011.

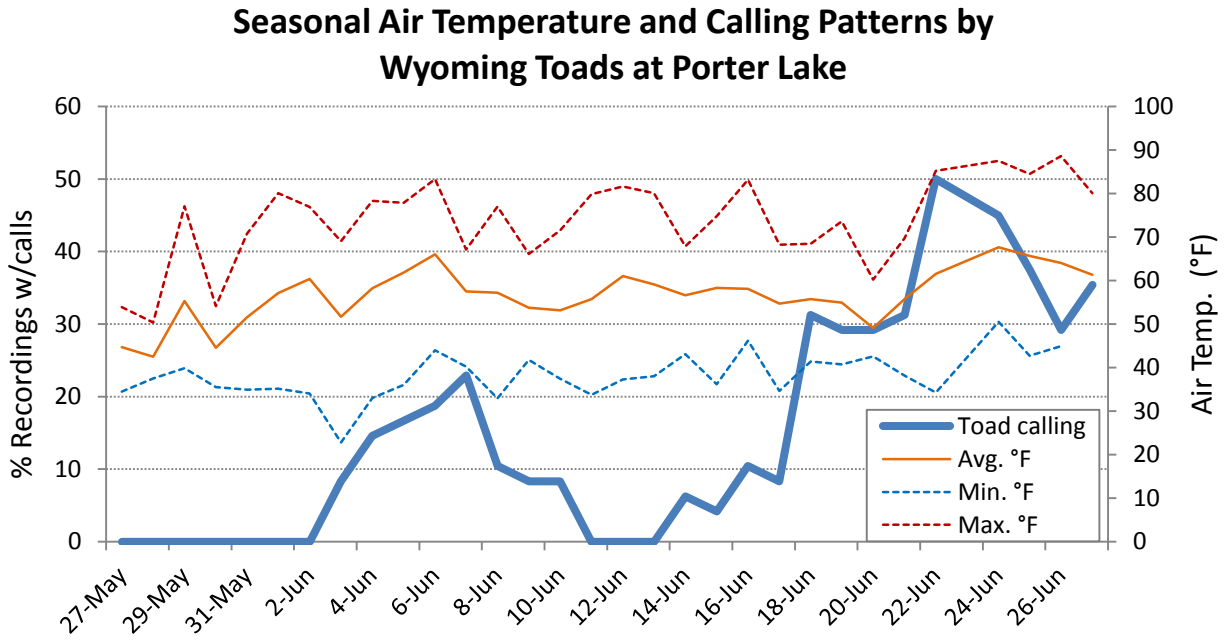

Figure 14. Daily patterns of calling by male Wyoming Toads at the Buford Property in 2011. Calling was recorded in two 10-minute sessions every hour by an acoustic recording device.

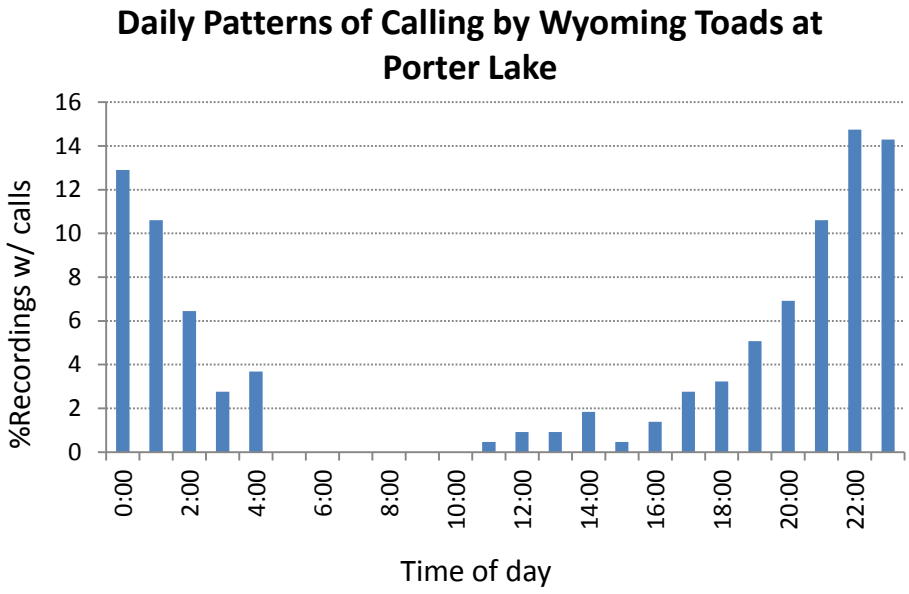

Figure 15. Map of property surrounding the Red Buttes research facility owned and operated by the University of Wyoming and being considered as a Safe Harbor reintroduction site for the Wyoming Toad. Preliminary search blocks (yellow lines) were delineated to facilitate planning, but will necessarily be revised based on reintroduction plans and adjustments to optimize survey efforts.