Wyoming Toad Monitoring on Safe Harbor Reintroduction Sites: 2010

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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 3 reintroduction sites (Buford, Lindzey, and Shaffer) in the Laramie Plains area of Wyoming in 2010. 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 were released at the Buford site; however, no toads were reintroduced at the Shaffer site in 2010 since the Wyoming Toad Reintroduction Team (WTRT) is considering removing that site from the reintroduction program.

As in 2009, flooding at the Buford site during breeding season surveys necessitated the use of modified search blocks at Porter Lake. Although no adult males were heard calling during breeding season surveys, we detected 4 potential breeders and 8 overwintered adults across the two survey periods. We also found 65 young of the year, presumably metamorphosed from captive-bred tadpoles, suggesting that tadpoles were able to survive and develop despite the flooding and the cold, wet spring. Overwintered adults detected in June, however, had substantially lower body masses than those in previous years. This could indicate that the toads were in poor condition when they entered hibernation, or that conditions in winter/early spring negatively impacted hibernating toads.

The rate of chytrid fungus infection in toads at Porter Lake increased slightly from 37.5% in 2009 to 41.7% in 2010. Bullfrogs, another potential threat to reintroduction efforts, also might have been detected at Porter Lake during August surveys, however, we were unable to catch and confirm that the large tadpoles seen were those of bullfrogs. WYNDD and WGFD will continue to monitor Porter Lake for presence of bullfrogs in 2011.

WYNDD conducted a pilot study to modify protocols for photographing toads and to test the performance of photographic identification software. Modified protocols resulted in less handling of toads while yielding photos of consistently higher quality than past efforts. The photo identification software used dorsal wart patterns on toads to identify recaptured toads quickly and with a high level of certainty. If wart patterns do not change sufficiently over time, then photo recognition software could prove a useful mark-recapture tool both within and among years. Recommendations for reintroduction efforts and field logistics also are provided.

INTRODUCTION

The Wyoming Toad (*Anaxyrus baxteri*) is 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 is at Mortensen Lake National Wildlife Refuge.

In 2010, the Wyoming Natural Diversity Database (WYNDD) entered into a cooperative agreement with the Laramie Rivers Conservation District (LRCD) to continue monitoring Wyoming Toads at 3 locations in the Laramie Plains. Two of the sites are covered by U.S. Fish and Wildlife Service (USFWS) Safe Harbor Agreements with the LRCD and include ponds and waterways owned by the Buford Foundation and Carl and Alice Shaffer. The third site is located along the Little Laramie River and is owned by Fred Lindzey. Wyoming Toad tadpoles and toadlets have been reintroduced at all 3 sites in the past, and reintroductions are continuing at the Buford Foundation Property. 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 3 sites. This report summarizes reintroduction efforts in 2010 and presents survey results for the Buford, Lindzey, and Shaffer sites.

Project Goals for 2010:

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. Optimize protocols for photographing wart patterns used to identify toads.
6. Evaluate the influence of current survey protocols on toad behavior (i.e. movement between replicate searches).

METHODS

Study Areas

Surveys were conducted with the help of WGFD at the Safe Harbor reintroduction sites and the Lindzey property in 2010 (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 (dry) 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.

The Shaffer property is located 3 miles north of Rock River, WY, and lies just outside the perceived historic range of the Wyoming Toad. The Shaffer site includes a main pond and neighboring marsh areas (Figure 4). Grasses and sedges tend to be taller and denser at this site than at Buford, and the Shaffer property has a larger relative proportion of emergent vegetation than Buford. Reintroductions have occurred at Shaffer since 2006. In spring of 2010, however, the Wyoming Toad Recovery Team (WTRT) decided to stop reintroductions at this site due to the apparent lack of overwinter survival of toadlets at this site. The WTRT is considering dropping this site from the Safe Harbor reintroduction program following 2010 survey results. WYNDD has been monitoring toads at the site since 2007.

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, Lindzey and Shaffer 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 on several occasions throughout spring and early summer 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.

At the onset of surveys on each property, search areas were stratified into “search blocks” of known size (e.g. Figures 2-4), within which we conducted the field searches summarized in Table 1 (for detailed description of search types see Griscom et al. 2009, Appendix 1). In 2009 and 2010, however, flooding at Buford precluded use of previously identified search blocks (Figure 2 inset). Thus, new search blocks of approximately 0.3 acres were established (Figure 5). As before, 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).

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. Within 2 days of these searches we conducted 2 replicate searches (using the same search intensity) in blocks where at least one adult toad was found. 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 as in previous years except at the Buford site, where extensive flooding required use of search blocks established in 2009, another flood year.
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 6). 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).

Data collected during surveys in 2010 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 in two ways. First, given that toads appear to have unique dorsal wart patterns, we photographed the dorsum of all adults and compared them to previously photographed toads. Second, toads greater than 18 grams were implanted with Passive Integrated Transponder (PIT) tags. During all searches, toads of this size were checked for PIT tags using a reader and 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 also were recorded.

We also recorded more specific information when we found tadpole aggregations and egg strings. 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.

2010 Changes to Protocols

1. In 2009, we found a consistent trend for decreasing numbers of toads found during replicates searches at Safe Harbor sites and at Mortensen Lake, where the USFWS and volunteers conduct surveys for toads using the same protocols. We hypothesized that 1) surveyors were not conducting replicate searches with the same level of effort, 2) replicate searches tended to be conducted later in the day and time-of-day could influence toad behavior, 3) toads were responding to being processed during surveys by moving out of search blocks, or 4) toads processed during searches had decreased survival rates due to predation or stress. To begin to assess the cause of the pattern, we conducted replicate surveys approximately 24 hours apart at about the same time of day as the initial search to remove variability due to timing of searches. We also emphasized that replicates must be done with same level of effort.

2. We increased the minimum weight required for wild toads to be PIT tagged from 15g to 18g in 2010 to decrease health risks and increase PIT tag retention. This change was based on conversations with members of the captive breeding program and more closely follows new PIT-tag protocols being developed as part of the Species Survival Plan (SSP) for captive breeding programs.

3. We standardized protocols for photographing dorsal wart patterns on toads for identification using pattern recognition software. We constructed tubes of flexible translucent plastic (12cm long; 10cm diameter) and placed tubes over toads in holding containers (Figure 7). By using these tubes we hoped to improve quality and consistency of photos by:

a. Decreasing glare by filtering sunlight through the translucent tube walls

b. Decreasing blur by allowing the photographer to stabilize the camera on the top of the tube

c. Restricting toad movements to within the photo frame and allowing the photographer to rapidly obtain photos of the entire dorsum as the toad remained motionless.

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 either the Lindzey or Shaffer sites 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 Safe Harbor sites (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 Dr. John Wood at Pisces Molecular LLC in Boulder, Colorado, 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. No other amphibians were captured or swabbed.

Influence of Surveys on Toads

We used location data from individuals recaptured in 2006-2010 at Buford and from 2009 at Mortensen Lake to investigate the influence of repeated searches on toad movement. We calculated the percentage of individuals that moved to different search blocks between recaptures. Because we had coordinates for recaptured toads at Buford, we also calculated the average distance moved between searches within survey sessions (breeding and post-breeding) and between sessions. These analyses, however, underestimate the frequency of movement and the distance moved because replicate searches are only conducted in search blocks where toads were found during initial searches. Thus, toads that moved to blocks where replicate searches did not occur, or that moved outside of the search area, were not recaptured and are not included in this analysis.

RESULTS AND DISCUSSION

Surveys

Breeding season and post-breeding season surveys were conducted from 15-18 June and 10-13 August, respectively. Because water was being diverted from the Little Laramie River to Lake Hattie through the supply canal during the breeding season survey period, Porter Lake and surrounding ponds and canals were flooded to approximately the same level as in 2009. Thus, we used the "ad hoc" search blocks of about 0.3 acres each established in 2009 (Figure 5; Estes-Zumpf and Keinath 2010) at Porter Lake to adjust for the drastic shift in shoreline and wetlands. Unlike in 2009, however, we were able to get across the supply canal to survey the south shore of Hardigan Lake in June despite the flooding. Flood waters receded sufficiently by August to allow post-breeding surveys to be conducted using the standard search blocks, though blocks were shifted slightly to encompass swollen shorelines and extended marsh areas. Although results for the modified June search blocks are not directly comparable to individual search block results from previous non-flooded years, overall results remain directly comparable since search effort was the same (i.e., 30 minutes/acre across all available wetland habitats).

Reintroductions

A total of 11,240 tadpoles and 27 toadlets were released from 6 different captive breeding facilities in 2010, all at the Buford site (Table 2). No Wyoming Toads were released at the Shaffer site in 2010 in preparation for eliminating that site from the reintroduction program. Although the number of tadpoles and toadlets released in 2010 was significantly less than the 20,200 in 2009, it was still the second highest number released at Safe Harbor sites since the

program began (Figure 8). As in 2009, flooding at the Buford site combined with an unusually cold, wet spring might have impacted success of reintroduced tadpoles by washing tadpoles out of Porter Lake and down the supply canal, or by lowering average water temperatures and, thus, slowing tadpole development and delaying metamorphosis (Smith-Gill and Berven 1979, Hayes et al. 1993, Olsson and Uller 2002). However, impacts remain purely speculative at this point.

Counts:

Buford Foundation and Lindzey Properties

We documented no Wyoming Toads of any age class at Hardigan Lake, along the outflow canal, or at the Lindzey property in 2010. We did, however, find toads from each age class at Porter Lake. We documented 6 adult toads (5 overwintered and 1 potential breeder) including 1 incidental overwintered toad during the breeding season (Table 3). All adults documented during the breeding season were found along the northwest shoreline in 2 search blocks, WG01 and WY03 (see Figure 5 for search block location). During post-breeding surveys, we documented 7 adult toads (4 overwintered toads and 3 potential breeders). Adults during the post-breeding season were found along the west and southwest shorelines. We found a total of 4 potential breeders in 2010, matching the number found (including those heard) in 2009. Thus, 2009 and 2010 mark the highest number of potential breeders documented at Porter Lake since reintroductions began at that site. Abundance estimates for 2010 at Porter Lake range from 5-27 overwintered toads and 4-8 potential breeders (Table 4). Unlike in 2009, no adult males were heard calling during breeding season surveys despite flooding. However, data from an acoustic recording device (frog logger) deployed during the breeding season is ready to be analyzed by the USFWS and may reveal calling toads.

Overwintered adult toads found during breeding season surveys in June had substantially lower body masses than overwintered adults found in previous breeding season surveys (Table 5). Typically, overwintered toads are >3 grams in June. In 2010, however, the average weight of overwintered toads was 2.2g (range was 1.6 to 3.0g). The low body masses could indicate that toads entered hibernation in poor condition, or that environmental conditions during the winter and/or spring of 2009/2010 were not favorable for hibernating toads. Despite the low body masses of overwintered toads, however, the number of adult toads found in 2010 (13 including incidental toads) was greater than the number of adults found in 2009 and comparable to the number found from 2006 to 2008 (Table 3).

Despite flooding and approximately 8,000 fewer tadpoles being released at Porter Lake than were released in 2009, we counted over 2.7 times more young of the year (YOY) toads than in 2009 (Table 3). All YOY toads were recorded during post-breeding surveys and were concentrated along the west and southwest shorelines which remained moist and slightly flooded even in August. Because no egg strings were found during breeding season surveys and tadpoles were released into Porter Lake prior to surveys, all YOY are assumed to have metamorphosed from tadpoles released from breeding facilities.

Shaffer Property

No tadpoles were released at the Shaffer Property in 2010 and we found no Wyoming Toads of any age class at that site during breeding and post-breeding season surveys (Table 6). As in

2009, detectability at Shaffer was likely low due to a wet spring resulting in dense tall grasses, high water levels, and large expanses of emergent vegetation. However, the lack of adult-sized toads is consistent with previous years, and we hypothesize that conditions at the Shaffer Property do not promote overwinter survival of reintroduced Wyoming Toads. Because no tadpoles or toadlets were released at Shaffer in 2010, the lack of toads of any age class further supports our assertion that the current Safe Harbor site on the Shaffer Property is not habitat for the Wyoming Toad and that annual occupancy of this site by toads is entirely dependent upon spring reintroductions.

Flooding and Water Temperatures

Cold spring runoff water was diverted from the Little Laramie River through the supply canal and into the Buford site off and on from 26 April to 3 July (T. Hiegel, *personal communication*, 2010). This cold water influenced water temperatures on both the north shore (closest to the canal) and the south shore (Figure 9) and likely dropped the water temperature below average temperatures for that site in June, when most tadpoles were released. Comparisons of temperature loggers from 2008, when the supply canal was not opened, and from 2009 and 2010, when the canal was opened in the spring, reveal a trend for consistently lower water temperatures along the north and south shores during flood years when the supply canal was opened (Figure 10). However, trends between years are not directly comparable because temperature loggers were placed in slightly different locations, and water depth varied within and between years. As noted in 2009, the influence of cold flood waters on Wyoming Toad tadpole development at Porter Lake in 2009 and 2010 is unknown at this point. Optimal temperatures for tadpole development vary among species (Denver 1997), and optimal temperatures for Wyoming Toad tadpole development may be lower for this cold-adapted species than for many other species of toads.

Currently, we are unsure if flooding Porter Lake in the spring by opening the supply canal from the Little Laramie River benefits or harms Wyoming Toads. Although we hypothesized that flooding might mimic more historic floodplain-like conditions and, thus, could increase breeding behavior in adult toads (Estes-Zumpf and Keinath 2010), we did not hear calling males in 2010. We are also uncertain whether the cold flood waters influence tadpole development, or if the increase in moist marsh habitat associated with retreating flood waters is beneficial to toadlets and YOY. However, we do feel that drastic shoreline shifts associated with opening and closing the supply canal potentially could influence hatching success of egg strings should breeding in the wild occur. We noted that the shoreline at Porter Lake receded approximately 20m in less than 6 hours after the supply canal was closed, and well over 20m in 24 hours. This rapid drastic retreat of the shoreline could leave egg strings stranded on drying ground. Because it is likely that opening the supply canal causes as similarly rapid advance in the shoreline, eggs laid in pre-flood waters could be submerged at depths that result in suboptimal egg development due to colder water temperatures. In 2010, the supply canal was opened and closed 3 times between 26 April and 3 July. It is possible that the resulting unnatural rapid advance and retreat of the shoreline at Porter Lake could decrease hatching success of future egg strings should reproduction occur. Managing the opening and closing of the supply canal to mimic a more natural flood regime or in a way that minimizes the rapidness of the change in shoreline, if possible, could reduce the risk to egg strings in the future.

Chytrid Analyses

We collected tissue swabs from 12 adult toads at Buford (Table 7). Chytrid fungus was detected in 5 toads (41.7%), a slight increase from the 37.5 % infection rate in 2009 but much lower than the 80% detected in 2007 (Table 8) when the fungus was first detected in toads at Porter Lake. Although the prevalence of chytrid has increased in the past 2 years from the 20% infection rate in 2008, the increase is not surprising given the cold wet springs of 2009 and 2010. 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 could limit the efficiency of basking behavior at reducing or eliminating chytrid fungus on individual toads.

Optimization of Photo Protocols

The new protocols for photographing Wyoming Toads in the field substantially increased the number of high quality photos for use with the photographic identification software. We found that automatic focusing performed better than manual focusing because strong mid-day sunlight appeared to affect a photographer's ability to accurately focus on a toad. Using the translucent tube and automatic focusing, we observed a reduction in both blurred photos and photos with glare, problems that are difficult to correct sufficiently with photo manipulation software. Furthermore, because toads were contained in a small circular area, most photos contained pictures of entire toads, in contrast to previous years where a large number of photos contained only portions of toads as they hopped out of the photo frame. Because toads could not hop out of the photo frame, this also reduced the amount of handling required to obtain quality photos.

The software used to identify toads based on photos of dorsal wart patterns performed extremely well with the photos obtained using the new protocols. We took multiple pictures of each adult toad found, several of which were recaptured during repeat surveys and photographed on different days with different lighting conditions. In all cases, the photo identification software ranked the matching toad first, with the highest level of certainty. Thus, all toads recaptured during the 2010 season were quickly and reliably confirmed using the photo identification software and the new photo protocols. The last step in evaluating the utility of the photo identification software is to test whether wart patterns on individual toads remain similar enough across years for the pattern recognition software to correctly identify individual toads across years and age classes.

Influence of Surveys on Toads

Although the trend was not as strong as in past years, the number of toads found during replicate searches tended to be less than the number found during initial searches. Because all surveyors were made aware of the problem and were specifically instructed to conduct searches with the same level of effort, surveyor effort is likely not the cause of the pattern. Time of day was also held relatively constant for repeat searches at individual search blocks and, thus, likely does not account for the decrease in toads detected.

Analyses of toad movement between searches, however, did reveal a tendency for some toads to move between recaptures. At Buford and Mortensen, 30% and 50%, respectively, of

toads were recaptured in different search blocks during the same survey season. In most of these cases, however, toads moved to immediately adjacent search blocks. At Buford, recaptured toads moved an average of 15m between searches. These movements can be made immediately after processing as evidenced by one overwintered adult captured in one search block and found approximately 15m away in an adjacent search block 20 minutes later. Although the number of recaptures between survey sessions within a summer was low, 100% of recaptured toads moved to different search blocks later in the season at both Buford and Mortensen. The average distance moved between breeding and post-breeding season surveys at Buford was 90m. These trends seem to indicate that while seasonal movement at a site is common, surveys (or the handling process) could cause small-scale movements in Wyoming Toads, thus resulting in decreased detections during replicate searches.

Predators

Although this report does not include a detailed documentation of potential predators found at reintroduction sites, we would like to report two potential sightings of bullfrog larvae along the west shore of Porter Lake. Wyoming Game and Fish Department Herpetologist, Zack Walker, and his technician, Chris Moan, both independently documented several very large tadpoles with silver-dollar sized heads while conducting a formal survey of search block P-01 on 10 August during post-breeding season surveys. Several return attempts to locate and catch the tadpoles with dip nets were unsuccessful, and we were unable to identify the species of tadpole.

With the permission of the USFWS and the Buford Foundation ranch manager, WGFD and WYNDD returned to Porter Lake in the fall and set out approximately 20 minnow traps for 3 nights from 5-8 October, 2010. Because bullfrogs often overwinter as tadpoles before metamorphosing into frogs the following spring, we hoped to catch the tadpoles using minnow traps. We were unable to catch any tadpoles, however, and, thus, cannot confirm at this time that the tadpoles seen in August were those of bullfrogs. The description of the tadpoles, however, most closely resembles that of bullfrog tadpoles. Although the bullfrog, a prolific invasive species and a potentially significant predator of Wyoming Toads, is not known to occur in the upper Little Laramie River drainage, it is possible that an undocumented population exists and could have been introduced into Porter Lake when the supply canal was opened in the spring. WYNDD and WGFD will closely monitor Porter Lake for evidence of bullfrogs in 2011. Should bullfrogs be found at Porter Lake in 2011, we recommend that the WTRT consult with the Buford Foundation manager and the WGFD to explore immediate options for eradication of this invasive species.

RECOMMENDATIONS

1. *The Shaffer Safe Harbor property*: Reintroductions of Wyoming Toad tadpoles and toadlets at the Shaffer property occurred from 2006-2009. Because, neither overwintered toads nor potential breeders have ever been documented at Shaffer, and no tadpoles or toadlets were found in 2010 after reintroductions had been stopped, we believe that the current Safe Harbor site at the Shaffer Property does not provide habitat for Wyoming Toads. We recommend that the current Shaffer site be eliminated from the Wyoming Toad reintroduction program.

This site could be replaced by one or both of two other ponds located on the Shaffer Property, or by another site elsewhere in the toad's historic range.

2. *Frog-loggers*: Calling is an essential breeding behavior for all anurans. Increased calling activity by adult male Wyoming Toads at Porter Lake in 2009 coincided with the flooding of Porter Lake via the supply canal connecting the lake with the Little Laramie River. This led to the hypothesis that flooding could trigger breeding behavior in Wyoming Toads. We attempted to test this hypothesis in 2010 by setting up an acoustic recording device (frog-logger), on loan from the USFWS, at Porter Lake during the 2010 breeding season. We also obtained water and air temperature data, and the dates and times that the supply canal was opened or closed. Once recordings are analyzed by the USFWS, we will look for correlations between calling intensity, flooding, and weather patterns to try to better understand conditions that promote breeding behavior in the wild. Due to annual variability in weather patterns, flood patterns, and the overall abundance of adult toads large enough to breed, we recommend continuing use of frog-loggers at Porter Lake during the breeding season to investigate breeding behavior.

3. *Use of automated photo-recognition software for adult mark-recapture analysis*: The photo-recognition software developed by Tom Morrison and colleagues at Dartmouth College, in consort with WYNDD, was able to successfully match photos from recaptured toads with a high degree of certainty during the 2010 pilot study. Improvement to protocols for photographing toads implemented in 2010 were inexpensive and resulted in photos of sufficiently quality to be used by the software. More importantly, the revised protocols also resulted in less handling of toads during the photographing process. Because the 2010 pilot study showed the software to be a quick and reliable way to identify recaptured toads, we recommend conducting an expanded trial with captive toads to confirm it's applicability to long-term monitoring of Wyoming Toads. Unlike PIT tags, which can be used only with toads large enough to tag (>18g), the photo identification software can be used with toads of all age classes, as long as wart patterns on individuals don't change substantially between recapture events. Collection of dorsal photographs is also less time consuming and requires less training than PIT tag implantation, making in more practical for continued long-term monitoring efforts. Finally, collecting photographs is far less invasive to toads that PIT tagging and lowers overall handling time.

4. *Influence of surveys on toads:* Surveyor effort and time of day do not appear to be responsible for the consistent decrease in number of toads found during replicate search within a survey season. Movement patterns of recaptured toads, however, suggest that surveys (or the handling process) could lead to small-scale displacement of Wyoming Toads, thus resulting in decreased detections during replicate searches. The movement of toads out of the search blocks in which they were first observed, though likely temporary, violates the closed population assumption of many mark-recapture models if toads move outside the search area or into search blocks in which replicate searches are not conducted. Because the toad population at Buford is currently too small for formal mark-recapture analyses, this violation is not yet an issue at Buford. The toad population at Mortensen, however, could

soon be large enough for formal mark-recapture analyses, at which point the violation of the closed population assumption would have to be dealt with either by using "open population" models in analyses, or by modifying search protocols. Open population models, while useful in situations like these, require more data for accurate estimation of parameters than closed population models. Given the low number of toad recaptures and low populations sizes, this could lead to much less accurate abundance estimates. Thus, modifying search protocols might be preferable to using open population models. Because most recaptured toads were found in adjacent search blocks, this could mean that rather than just searching the block where a toad was initially found, searches should be conducted in that block and all immediately adjacent blocks. Anecdotal evidence suggests that movements often occur perpendicular to the shoreline, with toads moving from upland blocks to shoreline blocks, or vice versa. Thus, conducting replicate searches in both upland and shoreline blocks proximate to toad observations from initial searches might solve the analytical problems associated with displaced toads. We recommend that the WTRT discuss these concerns in the near future (i.e., before any toad population attains a size where mark-recapture methods become feasible) and decide on a suitable course of action.

REFERENCES

Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan, and A. D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60:141-148.

Denver, R. J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. American Zoologist 37:172-184.

Estes-Zumpf, W. A., and D. A. Keinath. 2010. Wyoming Toad Monitoring on Safe Harbor Reintroduction Sites: 2009. Prepared by the Wyoming Natural Diversity Database, Laramie, Wyoming, for the Laramie Rivers Conservation District and the United States Fish and Wildlife Service. January 29, 2010

Griscom, H., D. Keinath, and A. Redder. 2009. Wyoming Toad monitoring on Safe Harbor reintroduction sites: 2008. Prepared by the Wyoming Natural Diversity Database, Laramie, Wyoming for the Laramie Rivers Conservation District and the United States Fish and Wildlife Service. January 7, 2009.

Hayes, T. B., R. Chan, and P. Licht. 1993. Interactions of temperature and steroids in larval growth, development, and metamorphosis in a toad (*Bufo boreas*). Journal of Experimental Zoology 266:206–215.

Heyer, W.R. M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster. 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press, Washington, D.C.

Keinath, D.A., H. Griscom, and A.J. Redder. 2007. Wyoming Toad monitoring on safe harbor reintroduction sites: 2007. Prepared by the Wyoming Natural Diversity Database, Laramie, Wyoming. December 31, 2007.

Livo, L.J. 2003. Methods for obtaining *Batrachochytrium dendrobatidis* (Bd) samples for PCR testing. Department of Integrative Physiology, University of Colorado, Boulder, Colorado.

Olsson, M., and T. Uller. 2002. Developmental stability and genetic architecture: a comparison within and across thermal regimes in tadpoles. Journal of Evolutionary Biology 15:625–633.

Smith-Gill, S. J., and K. A. Berven. 1979. Predicting amphibian metamorphosis. American Naturalist 113:563–585.

Thompson, S.K., and G.A.F. Seber. 1996. Adaptive Sampling. J. Wiley and Sons, New York, New York.

UCB (University of California Berkeley). 2004. UC Berkeley Briggs NIH Group: Swab Protocol (2004-2007). Unpublished sampling protocol drafted by members of the Department of Integrative Biology, University of California, Berkeley, California.

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TABLES AND FIGURES

Table 1. Summary description of Wyoming Toad monitoring searches conducted at Safe Harbor properties in 2010.

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 10 – 13.
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 10 – 13.
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 cohorts for the 11,267 Wyoming Toad tadpoles and toadlets released at Safe Harbor reintroduction sites in 2010.

Breeding Facility	Release Date	Tadpoles	Toadlets	Cohort	Location of release	Notes
Cheyenne Mtn. Zoo	7/12/2010	15	0	CMZA10	Buford	
Cheyenne Mtn. Zoo	7/12/2010	650	0	CMZB10	Buford	
Cheyenne Mtn. Zoo	7/12/2010	50	0	CMZD10	Buford	
Cheyenne Mtn. Zoo	7/12/2010	285	0	CMZF10	Buford	
Kansas City Zoo	6/21/2010	44	0	Kansas	Buford	
Mississippi River Museum	6/10/2010	1700	0	MRMA10	Buford	
Mississippi River Museum	6/10/2010	800	0	MRMB10	Buford	

Mississippi River Museum	6/10/2010	500	0	MRME10	Buford	
Mississippi River Museum	6/31/2010	269	0	MRMA10	Buford	
Mississippi River Museum	6/31/2010	834	0	MRMB10	Buford	
Mississippi River Museum	6/31/2010	269	0	MRMD10	Buford	
Mississippi River Museum	6/31/2010	154	0	MRME10	Buford	
Mississippi River Museum	6/31/2010	246	0	MRMG10	Buford	
Mississippi River Museum	8/19/2010	0	1	MRME10	Buford	Released by Saratoga
Mississippi River Museum	8/19/2010	0	5	MRMG10	Buford	Released by Saratoga
Red Buttes	6/25/2010	62	0	RBE10	Buford	
Red Buttes	6/25/2010	82	0	RBG10	Buford	
Red Buttes	6/25/2010	209	0	RBI10	Buford	
Red Buttes	8/19/2010	0	6	RBG10	Buford	Released by Saratoga
Saratoga	6/14/2010	55	0	SARA10	Buford	
Saratoga	6/14/2010	2112	0	SARC10	Buford	
Saratoga	6/17/2010	669	0	SARC10	Buford	
Saratoga	6/17/2010	2214	0	SARB10	Buford	
Saratoga	8/19/2010	0	4	SARB10	Buford	
Saratoga	8/19/2010	0	11	SARC10	Buford	
Toledo Zoo	6/22/2010	10	0	TOLB10	Buford	
Toledo Zoo	6/22/2010	11	0	TOLD10	Buford	
Total		**11,240**	**27**			

Table 3. Raw counts of individual Wyoming Toads found during monitoring activities at Buford from 2006-2010. 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 documented at Buford, all young of the year are the result of reintroduced tadpoles and toadlets.

	2006		2007		2008		2009		2010	
Size Class	**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)
Overwintered	5 (9)	1 (1)	8 (8)	2 (4)	9 (9)	7 (7)	5 (5)	1 (1)	4 (5)	4 (4)
Potential Breeder	1 (2[a])	0 (1)	0 (0)	3 (3)	0 (0)	0 (0)	2 (4[b])	0 (0)	1 (1)	3 (3)

[a] 1 male toad was heard calling but was never found.
[b] 2 male toads were heard calling but were never found.

Table 4. Abundance estimates for overwintered and potential breeder Wyoming Toads at Buford during breeding and post-breeding season surveys from 2006-2010. 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)	
Size Class	**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)
Potential Breeder	2* (1-3)	0*	0*	5 (4-8)	0*	0*	3* (3-5)	0*	2* (1-3)	5 (4-8)

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

Table 5. Average body mass (in grams) of overwintered adult toads found during June breeding season surveys at Buford. Because overwintered toads < 3g could be mistaken for reintroduced toadlets, we excluded all toads < 3g found after the first captive-bred toadlets were released each year. This resulted in the removal of one 2.4g toad found in 2007.

	2006	2007	2008	2009	2010
Average	7.7	4.5	4.4	8.5	2.2
Range	3.2 - 15.2	1.7 - 7.1	2.1 - 6.4	4.0 - 16.5	1.6 - 3.0
n	9	9	10	5	5

Table 6. Raw counts of individual Wyoming Toads found during monitoring activities at Shaffer from 2007-2010.

	2007		**2008**		**2009**		**2010**	
Size Class	**Breeding**	**Post Breeding**	**Breeding**	**Post Breeding**	**Breeding**	**Post Breeding**	**Breeding**	**Post Breeding**
Young of the Year	0	6	0	29	0	0	0	0
Overwintered	0	0	0	0	0	0	0	0
Potential Breeder	0	0	0	0	0	0	0	0

Table 7. Chytrid fungus results and initial capture information for the 13 adult Wyoming Toads captured on the Buford Foundation property in the summer of 2010 (no adults were found at Shaffer or Lindzey). Location coordinates are in UTM NAD83.

Adult ID	Recap	Date	Block ID	Sex	Age[1]	Wt (g)	SVL (mm)	GPSE	GPSN	Notes	Chytrid
F0078	N	15-Jun-10	WY03	F	PB	38.0	65.0	416487	4571795	Found on dry soil.	N
F0079	N	15-Jun-10	WG01	F	O	2.2	29.0	416527	4571837	Found on muddy soil.	N
F0080	N	15-Jun-10	WY03	F?	O	1.9	24.0	416496	4571808	Gave female ID #. Found on dry soil.	**Y**
F0081	N	16-Jun-10	WY03	F	O	2.1	28.0	416519	4571837	Found on muddy soil.	**Y**
F0082	N	15-Jun-10	WY03	F?	O	1.6	–	416508	4571826	Gave female ID #. Originally assumed YOY based on small size/weight.	Not tested
F0083	N	16-Jun-10	–	F	O	3.0	28.0	416477	4571794	Found on dry soil.	**Y**
F0084	N	10-Aug-10	P19	F	O	12.1	44.2	416880	4571505	Found in water.	N
F0085	N	10-Aug-10	P02	F	O	10.3	49.3	416565	4571734	Found in water.	**Y**
F0086	N	11-Aug-10	P02	F	O	9.4	45.6	416572	4571759	Found in water.	**Y**
F0087	N	10-Aug-10	P25	F	PB	21.0	50.0	416628	4571664	Found on muddy soil.	N
F0088	N	10-Aug-10	P26	F	PB	23.0	60.1	416844	4571480	Found in water.	N
F0089	N	11-Aug-10	P18	F	O	10.3	43.0	416845	4571480	Found on muddy soil.	N
M0016	N	10-Aug-10	P18	M	PB	19.1	55.9	416823	4571507	Found on muddy soil.	N

[1] Age classification estimated at time of capture based on weight.
O = Overwintered toads captured after one or more hibernation events.
PB = Potential Breeders; female toads ≥ 22g or male toads ≥ 18g.

Table 8. Chytrid fungus infection rates for Wyoming Toads at the Buford Foundation property from 2006-2010.

Size class	2006 rate of infection	2007 rate of infection	2008 rate of infection	2009 rate of infection	2010 rate of infection
Metamorphs	0%	69%	not swabbed*	not swabbed*	not swabbed*
Young of the Year	0%	25%	not swabbed*	not swabbed*	not swabbed*
Overwintered Adults	0%	80%	20%	37.5%	41.7%

* WYNDD no longer processes these size classes.

Figure 1. Map of (a) the Buford Foundation and Lindzey properties and (b) the Shaffer Ranch Property. Both the Buford and Shaffer properties are under Safe Harbor Agreements for the Wyoming Toad. Properties are shown approximately to scale, with key landscape features identified. Locator map is shown below, with approximate historic range of Wyoming Toad highlighted in green.

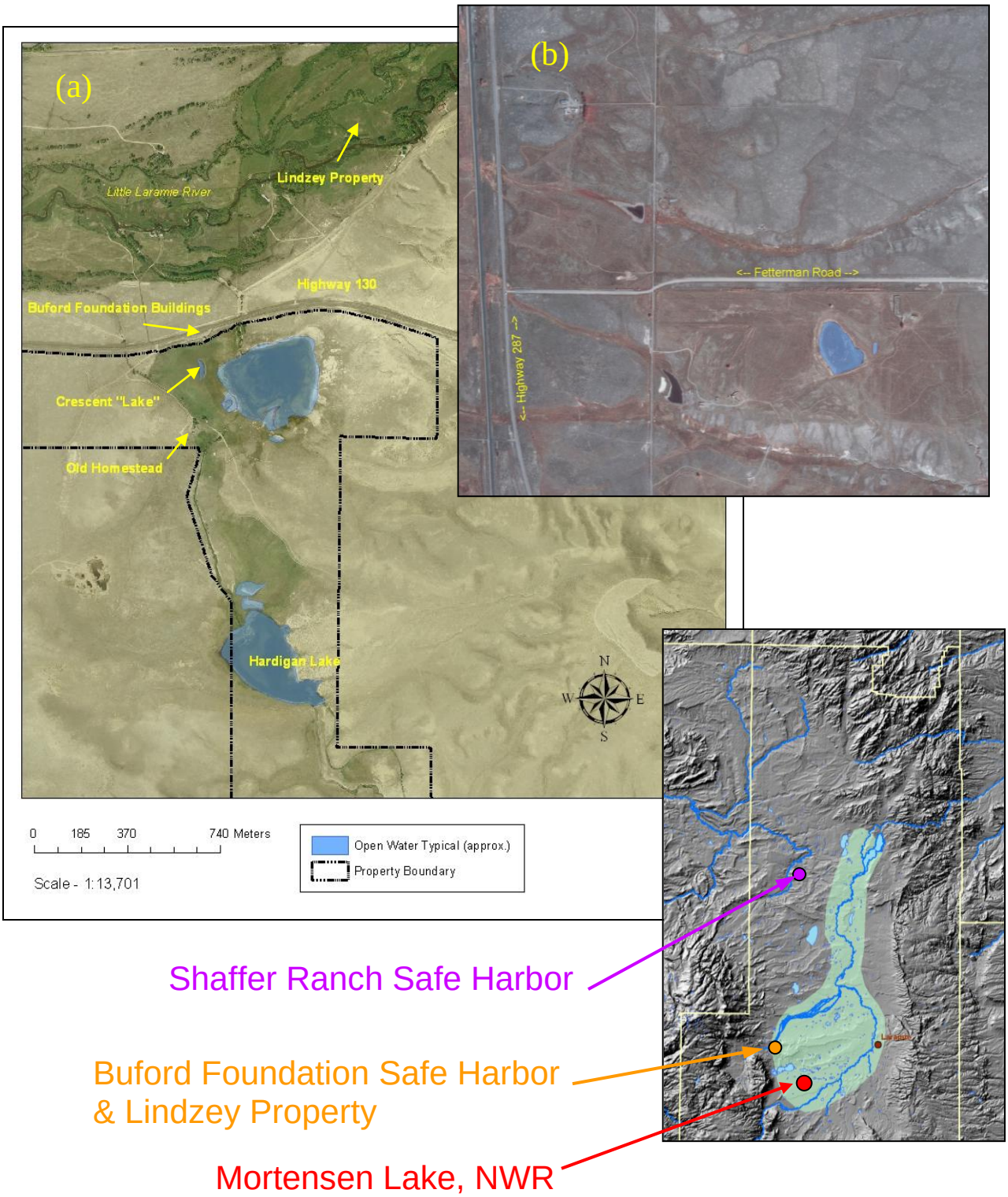
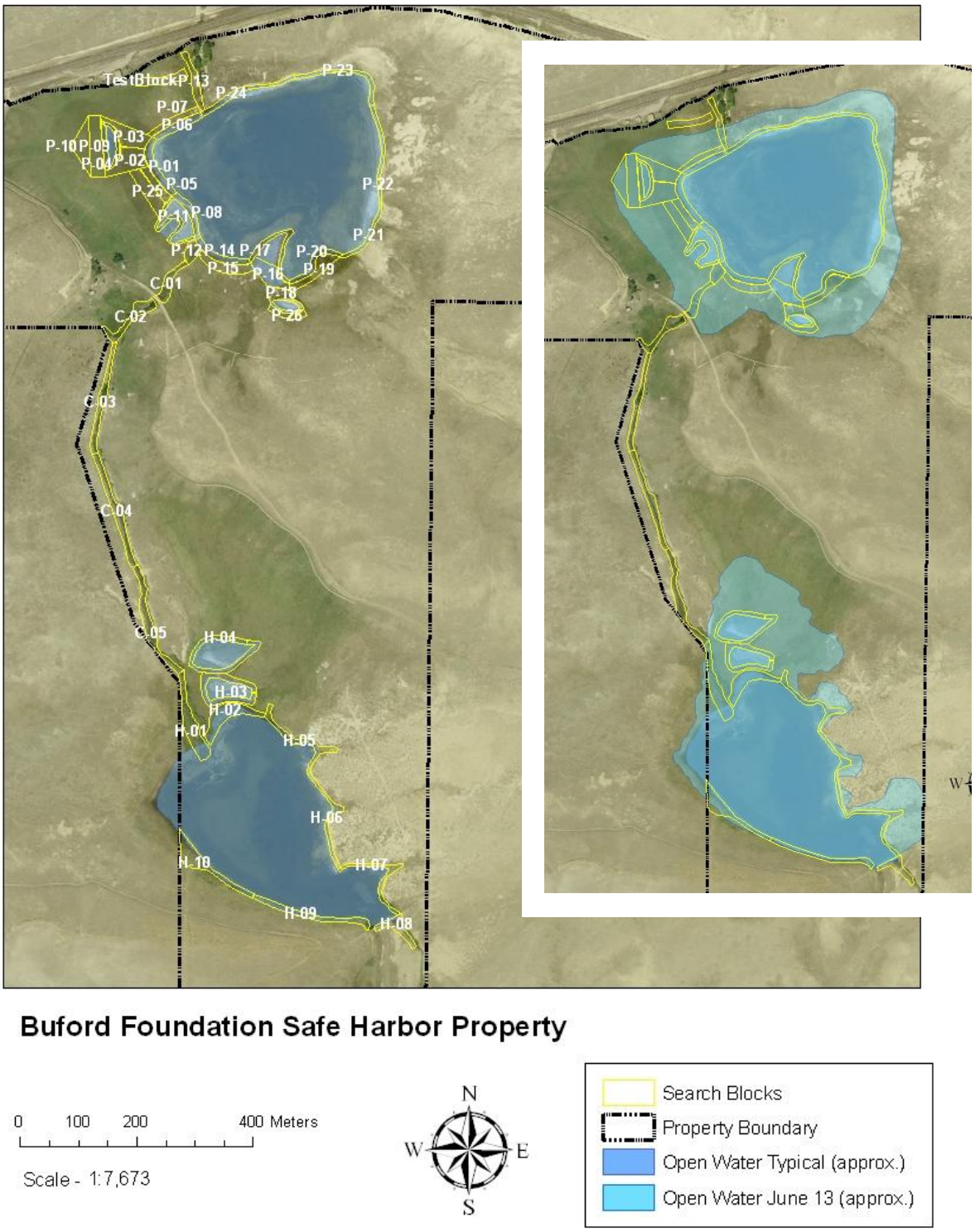


Figure 2. Search blocks previously used during Wyoming Toad surveys at the Buford Foundation Safe Harbor property. Inset is a view of the approximate extent of flood waters at Porter and Hardigan Lakes in 2009 and 2010.



Buford Foundation Safe Harbor Property

0 100 200 400 Meters

Scale - 1:7,673

N W E S

Search Blocks
Property Boundary
Open Water Typical (approx.)
Open Water June 13 (approx.)

Figure 3. Search blocks used during Wyoming Toad surveys at the Lindzey Property in 2010.

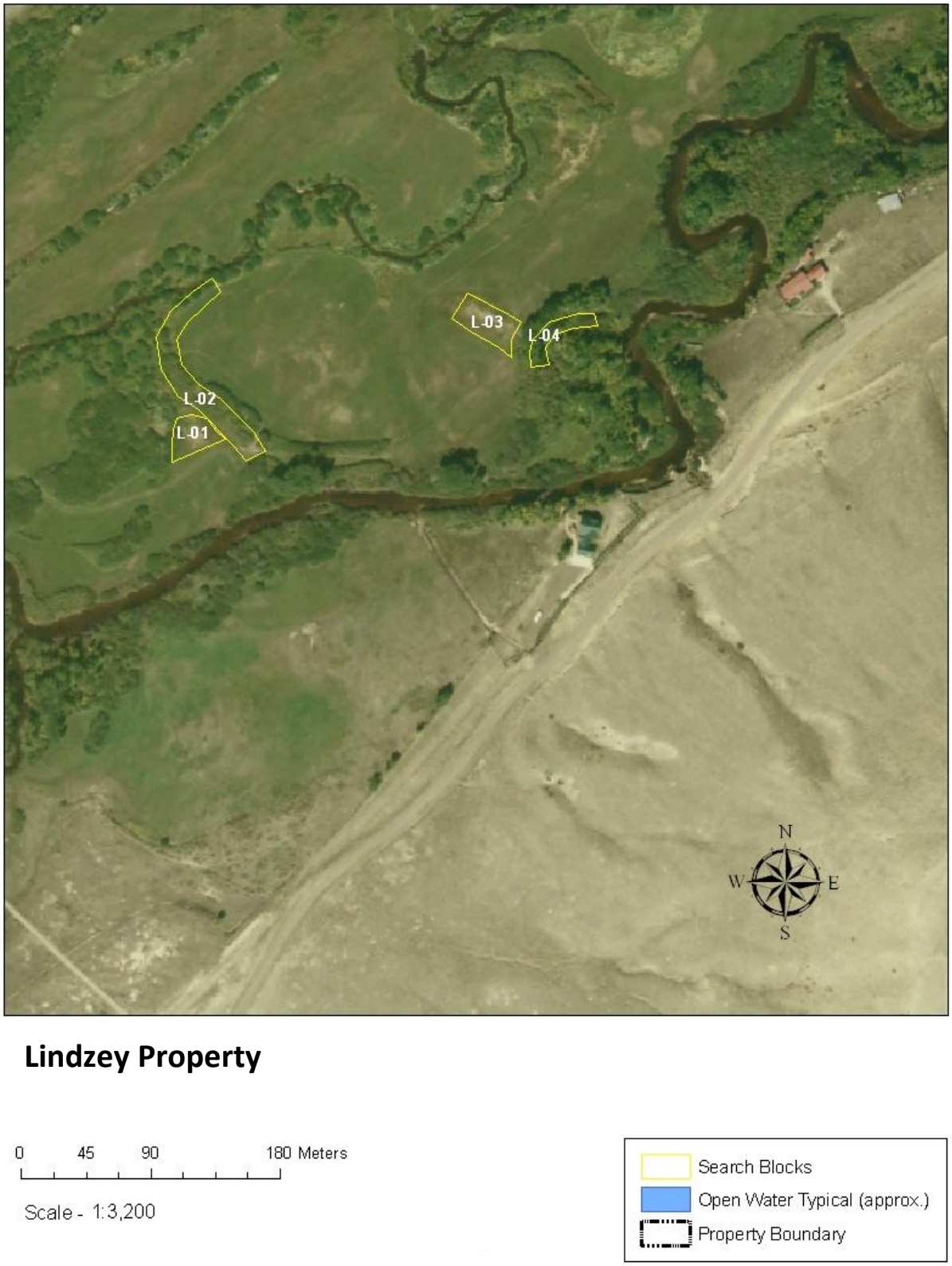


Figure 4. Search blocks used during Wyoming Toad surveys at the Shaffer Safe Harbor property in the summer of 2010.

Figure 5. Modified search blocks developed in 2009 and used during breeding season surveys for the Wyoming Toad at the Buford Foundation Safe Harbor property in 2010. Search blocks were modified from established blocks to accommodate shifted shorelines (light blue area) due to excessive flooding in 2009 and 2010.

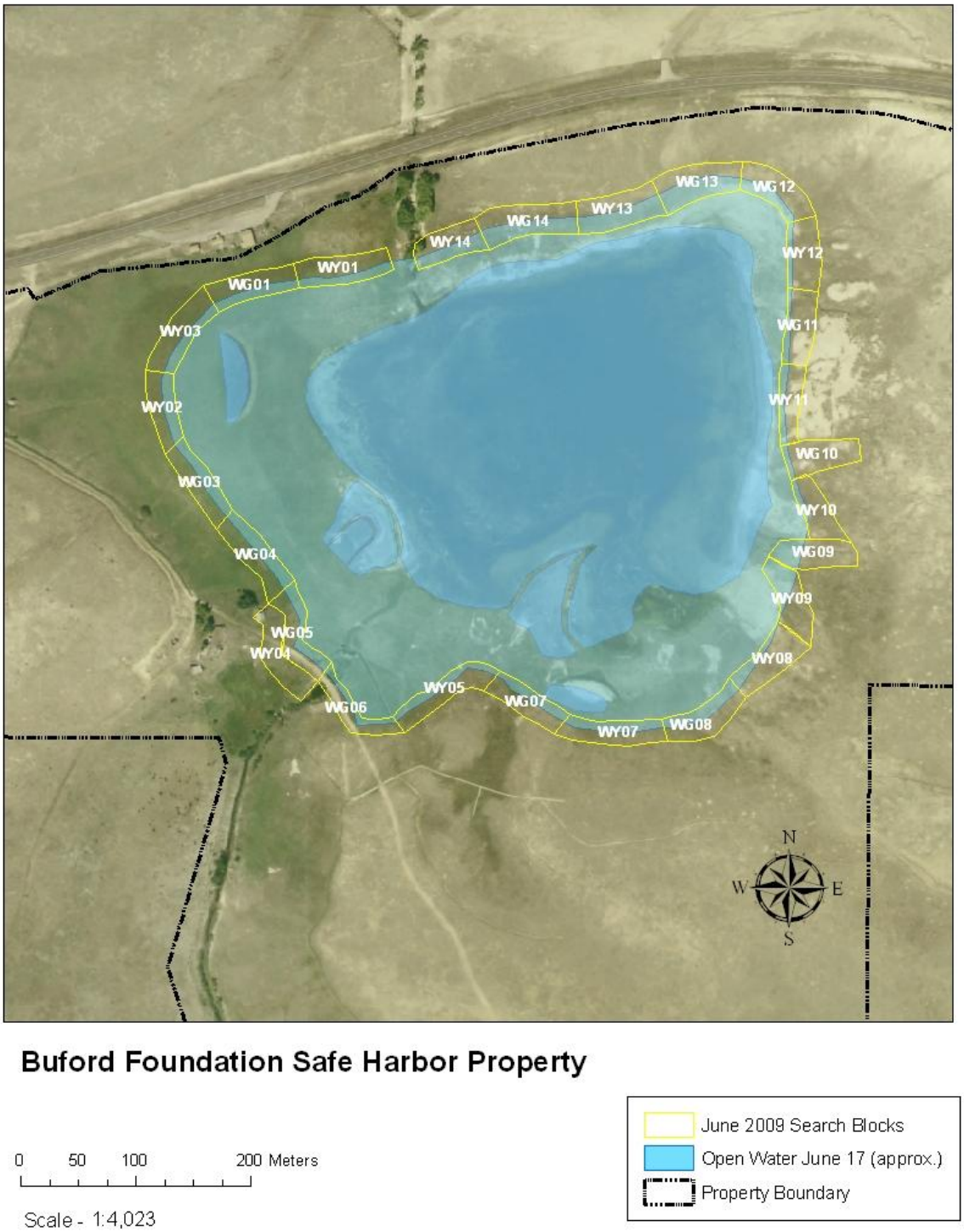


Figure 6. Size classes defined for Wyoming Toads at reintroduction sites in Albany County, WY.

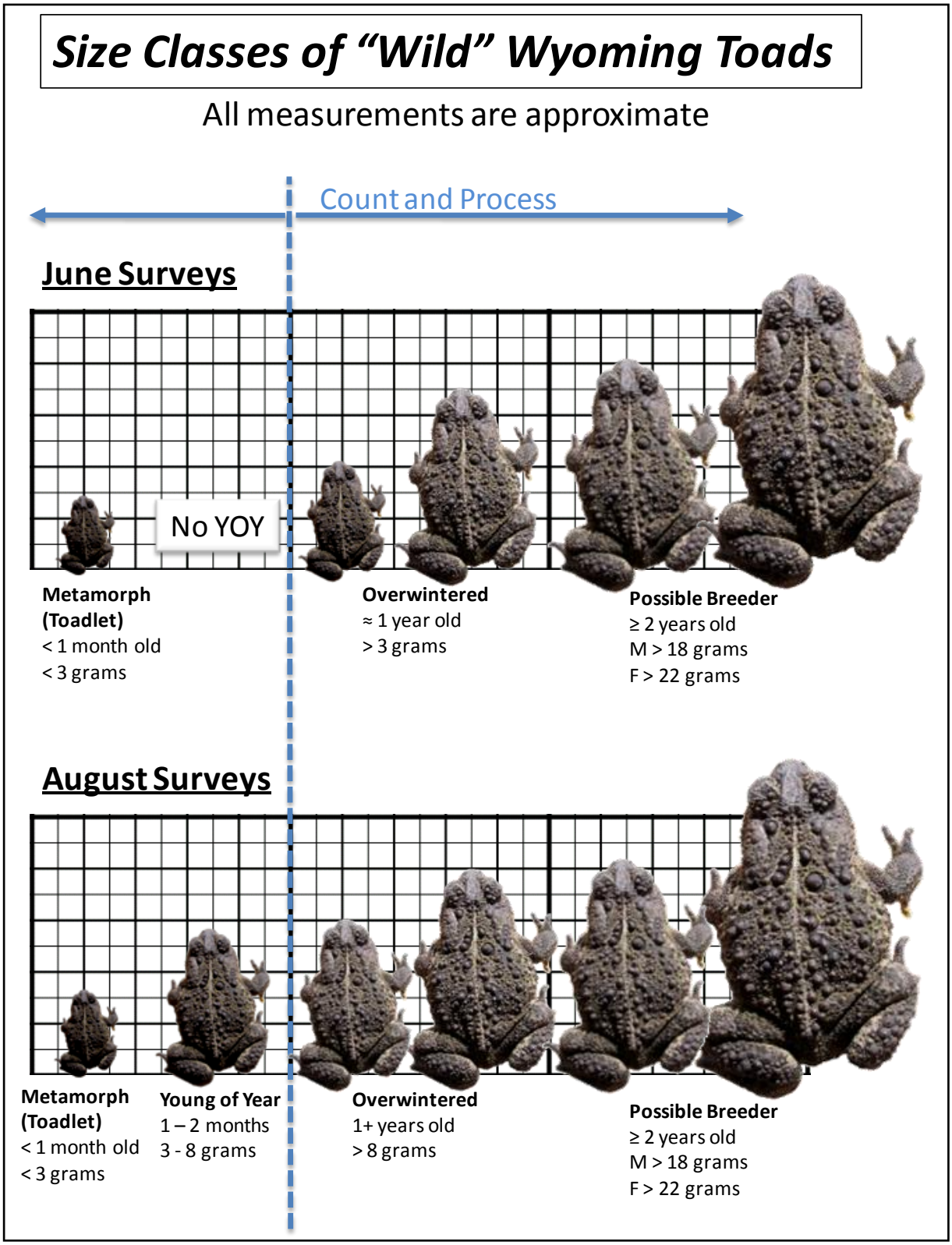
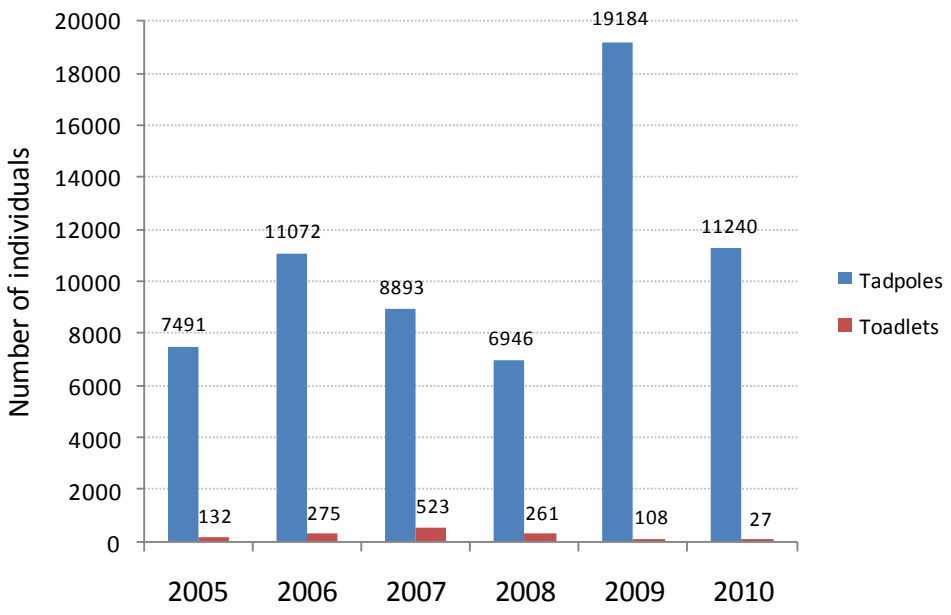


Figure 7. Picture tube set-up and example of a resulting photo. Picture tube was made of flexible translucent plastic and could be easily disinfected between toads.

Figure 8. Numbers of captive-reared tadpoles and toadlets released from 2005 to 2010 at a) Buford and b) Shaffer.

a)



b)

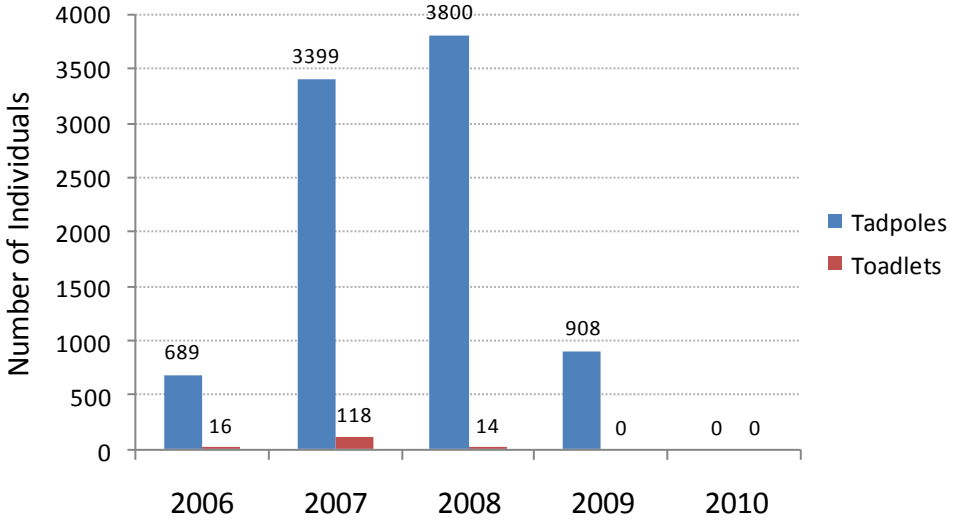


Figure 9. Air and water temperature readings from mid-June to mid-July at Porter Lake in 2010. Water temperature data were recorded with HOBO temperature loggers deployed in the Lake Hattie Supply Canal #2, on the north shore, and on the south shore. Air temperature was recorded via a temperature logger attached to a post on the southwest shore.

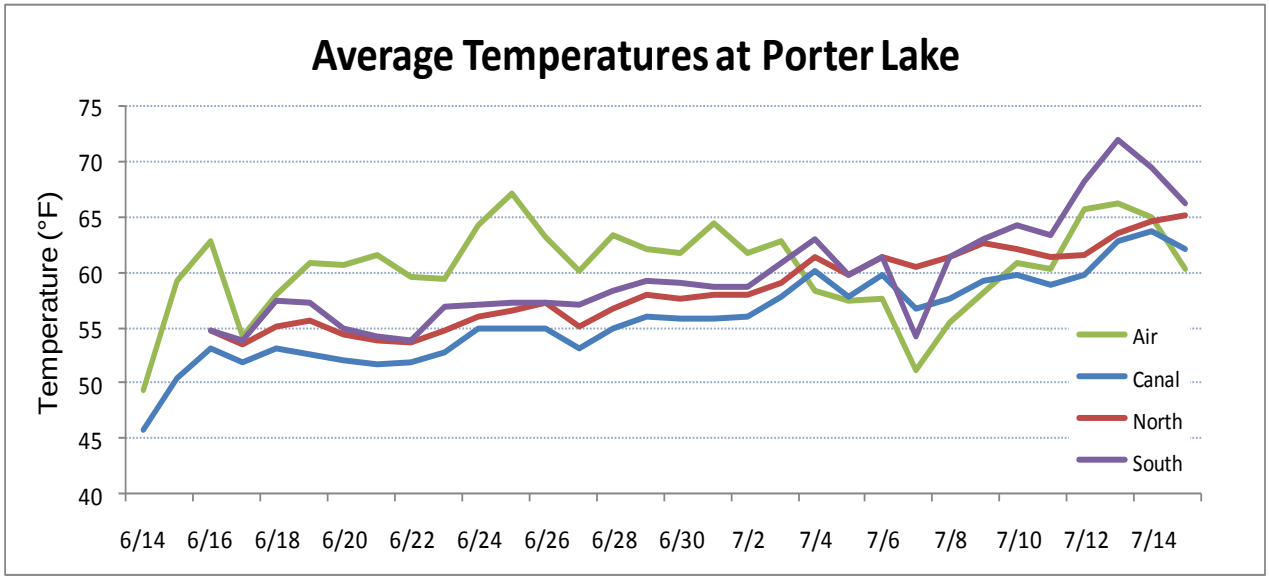
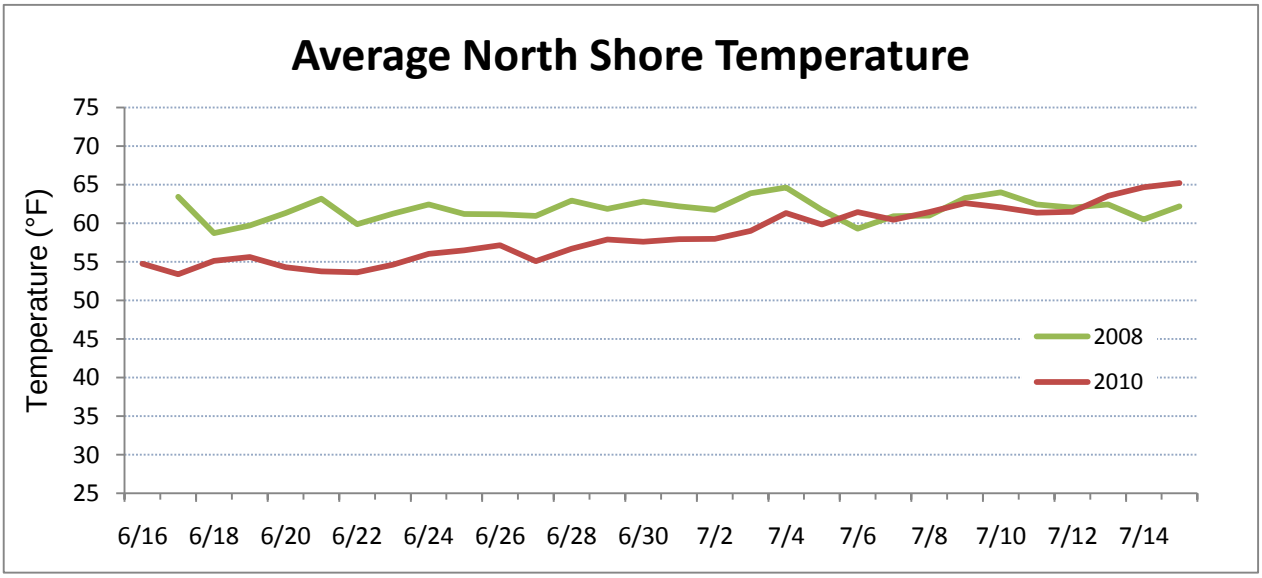


Figure 10. Water temperature readings from mid-June to mid-July at Porter Lake along a) the north shore (2008 & 2010) and b) the south shore (2008, 2009, and 2010). Water temperature data were recorded with HOBO temperature loggers.

a)



b)