Wyoming Toad Monitoring on the Buford Foundation Wetland Reserve: 2006

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EXECUTIVE SUMMARY

Introduction

In the spring of 2006, the Wyoming Natural Diversity Database entered into a cooperative agreement with the Laramie Rivers Conservation District (LRCD) to establish a plan and conduct first-year field work to monitor Wyoming toads on property owned by the Buford Foundation and currently enrolled in a Natural Resource Conservation Service Wetland Reserve Program (depicted in Figure 1 and hereafter referred to as BFP). This property is included in a Safe Harbor Agreement between the Foundation and LRCD, and by extension falls under LRCD's Safe Harbor Permit with the U.S. Fish and Wildlife Service (USFWS).

The monitoring plan and its addenda sought to meet the following specific goals identified by the Wyoming Toad Recover Team:

- Population Data
  1. Estimate annual relative abundance of sub-adult life stages
  2. Estimate actual abundance of over-wintered adults
  3. Document wild breeding, if it occurs
  4. Estimate dispersal from the release site
- Habitat Data
  5. Conduct a coarse vegetation site characterization of BFP
  6. Collect habitat data relative to sites used by Wyoming toads
- Threat Data
  7. Collect tissue samples from amphibians found at BFP to determine the presence of chytrid fungus (*Batrachochytrium dendrobatidis*, hereafter referred to as Bd) on the property.
  8. Keep field notes on predators observed during monitoring activities
  9. Determine what potential aquatic invertebrates are present at BFP that might prey on tadpoles of Wyoming toad.

Summary Results

Population Data

Population estimates focused on post-metamorphosis toads. No toadlets were present early in the summer, about 208 toadlets were present (range 203 – 214) in mid-summer, while about 256 toadlets were present (range 189 – 323) in late summer. Over the course of the summer, 20 adult-sized toads were observed at Porter Lake (Table 5, Figure 8, Appendix 2), many of which were young of the year. Only two mature toads were observed (one female and one male), although three males were heard calling. Formal counts (Table 3) combined with detectability estimates (Table 4) suggest that on the order of 16 (range 10-35) adult-sized toads were present at the end of the summer, with 5 (range 3-10) of these being adults that had previously survived a winter in the wild.

No egg masses were seen, which, when combined with the low numbers of potentially breeding adult toads, makes it logical to conclude that successful wild breeding did not occur on BFP in the summer of 2006.

Since reintroduction, toads have dispersed over 500 meters along the shorelines of wetlands surrounding Porter Lake. Average daily movements of between 2 and 152 meters were noted for individual, recaptured toads, with longer movements occurring later in the summer.

Habitat Data

The area around Porter Lake was classified into ten habitat types (Figure 9), with all toads occurring in two (Figure 8): wet flats along lake margins (dominated by Nuttall's alkaligrass and Foxtail barley) and wet meadow footslopes (dominated by Common spikerush, Nebraska sedge, several rushes, and Analogue sedge).

A variety of habitat measurements were recorded at the precise points where toads were found, in a 5 meter radius neighborhood around those points, and at unoccupied locations in the vicinity of toads (see Methods). Of these, only measures of site moisture appeared to distinguish points were toads occurred from points where they did not (Table 10), while no variables distinguished occupied neighborhoods from unoccupied neighborhoods (Table 11). Also, within neighborhoods where toads were found, they appeared to select micro-sites with less plant canopy cover and less litter. From this information we can conclude that toads are selecting consistently wet sites with shallow standing water, and that within wet sites they are selecting open habitats, probably for basking and/or ease of finding a mate.

Threat Data

Tissue samples were collected from live anurans found on BFP (Table 14 and Figure 13) and sent to Dr. Allan Pessier, the Wyoming Toad SSP Pathologist, for analysis via a PCR test to determine if chytrid fungus (*Batrachochytrium dendrobatidis*) was present at the site. Results were not available at the time this report was drafted. Similarly, specimens of aquatic invertebrates were collected twice during the summer and are currently being identified by entomologists at the University of Wyoming. Results of both analyses will be provided when available.

Notes were taken regarding potential predators observed during toad surveys of BFP (Table 15), from which we conclude that the major natural predation threats to adults and metamorphs are Canada geese, gulls, and garter snakes, while the major threats to tadpoles could be tiger salamanders (larval and adult) and predacious diving beetles. An unexpected result of detectability trials using artificial toads was documentation of likely predation events, wherein 5.2% of artificial toads (and 17% of large toads in shoreline habitat) were found to be disturbed after being in the environment for only 24 hours (Table 16). Predation pressure seemed to increase as toad size increased and seemed to be higher in shoreline areas than upland areas.

Summary Recommendations

Based on the results of this pilot project we recommend several modifications to protocol for future monitoring efforts at BFP and other reintroduction sites. These recommendations are detailed at the end if this report. For population surveys, we suggest modifying session dates, adjusting search intensity, re-mapping search blocks, adding and/or eliminating some search blocks, incorporating call surveys, and reducing the extent of egg mass surveys. For habitat studies we suggest changing some of the variables collected, adjusting how paired survey points are selected, and refining the estimation of some variables.

If adopted, several of these recommendations would alter search effort, thus altering project cost. Therefore, future efforts may need to be re-budgeted. However, we believe overall cost will be roughly the same because recommendations increasing cost (e.g., adding more shoreline search blocks and incorporating call surveys) will be roughly balanced by those decreasing cost (e.g., reducing the number of sessions, reducing shoreline egg-mass searches, and eliminating upland and irrigation ditch search blocks).

METHODS

Population Data

Given the multitude of desired goals and constraints on time and budget the entire BFP area could not be surveyed for all purposes. Therefore, we implemented an adaptive sampling (Thompson and Seber 1996) scheme wherein searches were focused on the areas of highest likelihood-of-occurrence. To delineate search effort, BFP was stratified into "search blocks" of known area (Figure 2). These were classified into two habitat categories: wetland and upland. Wetland areas were those areas proximate to fixed bodies of water and deemed to be moist through much of the spring-summer season, as confirmed by their vegetation composition. Upland areas were those areas not proximate to fixed bodies of water that were generally dry except for periods immediately associated with irrigation. Due to the nature of BFP, wetland areas are generally linear features (i.e., shorelines or irrigation channels) while upland features are polygonal (i.e., meadows or hillsides).

We conducted a variety of field searches as part of the toad monitoring effort, which are summarized in Table 1 and detailed in Appendix 1. Unless otherwise noted, searches were standard visual encounter surveys with strict documentation of survey effort (e.g., Heyer et al. 1994). Below we have outlined the search efforts relative to their associated goals.

Estimate annual relative abundance of sub-adult life stages (Goal 1)

This goal was addressed through low- and high-intensity visual encounter searches conducted on a subset of search blocks. Blocks for high-intensity search efforts were selected based on data from associated low-intensity searches. Standardized searches were conducted in three, separate sessions (see Tables 1 and Appendix 1), during which we recorded the number of tadpoles, metamorphs and adults observed in each block. Additional information was recorded for each adult (see Goal 2, below).

Estimate actual abundance of adult toads (Goal 2)

Data from the previously noted low-intensity and high-intensity searches was used in concert with detectability searches (see Table 1 and Appendix 1) to derive estimates of actual abundance for adult toads.

To better track population dynamics and for potential use in future mark/recapture analyses, we attempted individual identification of each adult toad observed. We assigned each adult a unique identification number at the time of its first observation and used these numbers to identify all subsequent captures. Individual identification was achieved in two ways. First, given that toads seem to have unique dorsal wart patterns, we photographed the dorsum of all adults and used these photos as a key to identify future observations. Second, toads greater than 15 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.

For all adult observations we recorded the sex, individual identification number, and exact location via Global Positioning System (GPS) receiver. Additionally, at each adult's first incidence of capture we recorded its snout-vent length (SVL) to the nearest millimeter using analog calipers and its weight to the nearest tenth gram using a digital scale. Also, each observation was followed by documentation of habitat characteristics at the site of observation (see Goal 6 under the section on Habitat Data below).

Document wild breeding, if it occurs (Goal 3)

Since only tadpoles, metamorphs and adults are released at BFP, the presence of Wyoming toad eggs represents evidence of wild breeding. Thus, we conducted three shoreline searches over the course of the breeding season with the specific aim of finding egg masses (see Table 1 and Appendix 1). The second shoreline search coincided with the second session of low and high-intensity searches, but the other two were independent. During these searches, technicians assigned each egg mass an identification number, recorded its exact location, and photographed it. Habitat characteristics at the site of the egg mass were subsequently documented (see Goal 6 under the section on Habitat Data below). Also, observations of adult toads during shoreline searches were documented as per those in high-intensity searches (see Goal 2 above).

Estimate dispersal from release site (Goal 4)

We have coarse information on where toads have been released at BFP (Figure 3). As noted above, the BFP was divided into search blocks and all search blocks were surveyed three times over the course of the summer using the low-intensity search method (Table 1). This resulted in information on which search blocks were occupied by each major life stage (tadpole, metamorph, adult) during each survey session. Distribution of occupied search blocks was then spatially related to the release sites to roughly determine how far each life history stage dispersed from that area.

Habitat Data

The habitat study program had two parts. The first was a general characterization of the composition and structure of the vegetation in and around the wetlands. The second was a detailed study of habitat features in areas used by the toads and areas available to them but unused.

In addition to describing the habitat at the Buford Foundation property, we intended to develop and test a procedure that can be used in other locations for collecting and analyzing data on vegetation structure and a few additional features of potential Wyoming toad habitat. This procedure was required to meet five requirements:

1. It allows one to distinguish habitat used by Wyoming toads from habitat available to them but unused.
2. It allows one to predict whether vegetation in a wetland likely is *unsuitable* for Wyoming toads. By recognizing features of unsuitable habitat, land owners and managers can adjust grazing to avoid creating unsuitable habitat.
3. The procedure is fast and simple enough that field technicians can collect the habitat data at the same time as they conduct toad surveys. Hence collecting and analyzing the habitat data will add little to the cost of toad surveys already being conducted in the same area.
4. Neither the collection nor the analysis of the data requires extensive technical training or sophisticated equipment or software.
5. The procedure is flexible enough to be adapted for use in different situations, such as different types of wetlands, or different densities of toads.

We anticipated the need to revise the habitat sampling procedure after it had been used, and considered the first year to be a pilot project.

Conduct a simple site characterization of BFP (Goal 5)

Information for characterizing the general habitat types around Porter Lake was collected in August, 2006. The distribution of different cover-types was determined from a 2001 color infra-red aerial photograph (ortho-image), and a survey route was selected to include all of the cover-types. The aerial photograph was used in the field to guide the survey. In each general cover-type, information on the species composition and structure of the vegetation were recorded at several survey points, chosen subjectively to illustrate the variation in the vegetation. A global positioning system receiver (Garmin eTrex Legend) was used to document the location of each survey point and also to document the boundaries of general vegetation types in some areas. A map of vegetation types and descriptions of those types were produced from the field survey information.

Collect habitat data relative to sites used by Wyoming toads (Goal 6)

Data Collection

We recorded and analyzed data on the habitat type in which toads were found based on 10 specific habitat features:

1. Habitat type (0 = a point on land and $\geq$ 5 meters from water, 1 = a point on land but within 5 meters of water, 2 = a point in water),
2. Height of plant canopy,

3. Amount of plant canopy cover,
4. Vertical distribution of plant cover,
5. Which major plant types (such as sedges and grasses) are the 3 most common,
6. Amounts of different types of ground cover (bare ground, plant litter, live plants on soil surface, animal droppings, water),
7. Soil wetness,
8. Distance to water,
9. Depth of water,
10. Distance to land.

Data on habitat features were recorded in slightly different ways for two types of sample units:

1. Point Data – A 1 square meter plot centered on each point where a toad was found and a randomly-selected, unoccupied point,
2. Neighborhood Data – A 5-meter radius neighborhood around each toad point and an associated unoccupied point.

-- Point Data

When a toad was found, the location was marked with a pin flag placed into the soil 1 meter due north (true north) of the toad. Within 48 hours of marking each toad point, the habitat sampling crew returned to that point and collected habitat data. For each toad point, an unoccupied point (where no toad was found) was selected 30 meters away by the habitat sampling crew when they returned to the toad point[1].

Data at each point were collected within a 1 m x 1 m plot frame centered on the point, as follows:

1. Habitat type: 0 = a point on land and $\geq$ 5 meters from water, 1 = a point on land but within 5 meters of water, 2 = a point in water.
2. Height of plant canopy. The height above the ground (in centimeters) of the top of the bulk of the plant canopies was measured with a meter stick.
3. Amount of plant canopy cover. The percentage of the plot beneath plant canopies was estimated in 20% intervals (Table 2). (Observers used charts showing various degrees of cover to make estimates more precise.)
4. Horizontal vegetation density (thickness of the vegetation at different heights above the ground or water). A cover pole (a dowel 1 meter long and marked into 10-cm long intervals from one end, with a large nail in the bottom -- see Figure 4) was placed vertically at the center of the plot. The observer looked at the pole from a point 2 meters to the south, with his or her eye 1 meter above the ground or water surface (Figure 5). Starting with the first interval (0-10 cm above the ground), the observer looked at successively higher intervals and recorded the number of the highest interval that was $\geq$

[1] Our intent was to have each unoccupied point (referred to in our proposal as "comparison point") selected in the same habitat type as a toad point and paired with that toad point. Through an error in data collection, though, in many cases the unoccupied point was not located in the same habitat type as the toad point, so the toad points and unoccupied points cannot be analyzed as paired data points.

25% obscured by vegetation. A single measurement, the number of the highest interval ≥ 25% obscured, was recorded for each plot.

5. Three major plant groups present. The observer recorded which three of the major plant groups (grasses, sedges, rushes, thistles, other forbs) contributed the most canopy cover, in decreasing order. This information was recorded as a simple list of the three groups, not an estimate of the amount of canopy cover of each group.
6. Amount of 5 ground-cover categories. The observer recorded the percentage of the ground within the plot covered by *plant litter*, *live plants*, *animal droppings*, *bare ground*, or *water*. For each category, the cover was recorded in one of 6 intervals (Table 2). A single value for each category of cover was recorded for the plot.
7. Soil wetness. When the point was on land (habitat type = 0 or 1), the degree of wetness of the soil was evaluated with the method used by Withers (1992): the observer recorded whether the soil at the center of the plot was *saturated* (water could be expressed from the soil surface with the thumb), *moist* (soil contained moisture but water could not be expressed from the surface), or *dry* (soil apparently contained no moisture). Each wetness category was recorded as a number: saturated = 2, moist = 1, dry = 0.[2]
8. Distance to water. If the point was on land (habitat type = 0 or 1), the observer estimated the distance in meters from standing water to the nearest point of the plot. If standing water was present in the plot (habitat type = 2), the distance was recorded as 0.[3]
9. Depth of water. When the point was in water (habitat type = 2), the observer measured the depth of water in centimeters at the center of the plot.
10. Distance to land. When the point was in water (habitat type = 2), the observer estimated the distance in meters to land.[3]

-- Neighborhood Data

Each toad point or unoccupied point served as the center of a neighborhood of 5 meters radius. After recording data at the point, the observer used a tape to measure the radius of the neighborhood and marked the boundary temporarily with pin flags. Measurements were made in each neighborhood on most of the habitat features that were measured at the toad points and unoccupied points. Measurements differed slightly between neighborhoods in different habitat types.

1. Habitat type: 0 = a point on land and ≥ 5 meters from water, 1 = a point on land but within 5 meters of water, 2 = a point in water.
2. Height of plant canopy. After walking throughout the neighborhood for 3 minutes, the observer recorded the estimated height of the bulk of the plant canopy. A single estimate was made for each neighborhood.

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[2] A fourth category was added for this variable after the field season but before data analysis. The *Flooded* category (value = 3) included those points where ≥ 50% of the soil surface was covered with water, as indicated by the ground cover variable.

[3] The two variables, Distance to water and Distance to land, were combined before data analysis to form the single variable, Distance from shore.

3. Amount of plant canopy cover. After walking for 3 minutes throughout the portion of the neighborhood on land or over emergent vegetation, the observer recorded the percentage of the ground or water surface beneath plant canopies. Any portion of the neighborhood in open water and beyond the edge of the zone of emergent vegetation (Figure 6) was excluded from this estimate. Canopy cover was estimated in 20% intervals (Table 2). A single estimate was recorded when the neighborhood lay entirely over land (habitat type = 0 [Figure 6a]). Two estimates were be made for neighborhoods that lay partly over water (habitat type = 1 [Figure 6b] or 2 [Figure 6c]), one for the terrestrial vegetation and one for the emergent vegetation.

4. Horizontal vegetation density (thickness of the vegetation at different heights above the ground or water). Measurements were made with a density pole, viewed from the south (Figure 6), at points that reflected the range in vegetation thickness throughout the neighborhood. Points were located differently depending on where the neighborhood was located. Where the neighborhood lay entirely over land (habitat type = 0 [Figure 6a]), the observer chose two points to make the measurements. One was the point that the observer thought would give the *maximum* number, and the other was the point that the observer thought would give the *minimum* number. Where the neighborhood included both land and water (habitat type = 1 [Figure 6b] or habitat type = 2 [Figure 6c]), the observer chose four points. Two were on land (one at the point giving the *maximum* number and the other at the point giving the *minimum* number) and two were in the emergent vegetation (again, at points giving the *maximum* and the *minimum* numbers). When taking measurements in water, the observer pushed the base of the density pole into the pond bottom until the lower edge of a 10-cm interval lay at the water surface, and that was the lowest interval counted.

5. Three major plant groups present. After walking through the portion of the neighborhood on land for 3 minutes, the observer recorded which three of the major plant groups (grasses, sedges, rushes, thistles, other forbs) contributed the most canopy cover, in decreasing order. This information was recorded as a simple list of the three groups, not an estimate of the amount of canopy cover of each group.

6. Amount of 5 ground-cover categories. After walking through the portion of the neighborhood on land for 3 minutes, the observer recorded the percent of the ground within the neighborhood covered by *plant litter*, *live plants*, *animal droppings*, *bare ground*, or *water*. For each category, the cover was recorded in one of 6 intervals (Table 2). A single value for each category of cover was recorded for the neighborhood.

7. Soil wetness. Data on this feature were not recorded for the neighborhoods.

8. Distance to water. For neighborhoods entirely on land (habitat type = 0), the observer estimated the distance in meters from standing water to the nearest part of the neighborhood.[3]

9. Percent of neighborhood in water. For neighborhoods around points on land within 5 meters of water (habitat type = 1) or points in water (habitat type = 2), the observer estimated the percentage of the neighborhood beneath water

10. Depth of water. Data on this feature were not recorded for the neighborhoods.

11. Distance to land. Data on this feature were not recorded for the neighborhoods.

Data Analysis

The habitat variables we studied provide several measures of the vegetation and the physical habitat immediately around points used by toads, and also in the larger areas around those points. If those variables are closely related to one another, then measuring all of them may be unnecessary. We used correlation analysis, which shows how much the values of one variable change in concert with changes in a second variable, to examine the strengths of relationships between pairs of variables.

All statistical analyses were run using S-PLUS (2001, Insightful Corporation), SPSS (2004, Apache Software Foundation) or Minitab (1998, Minitab, Inc.) software packages.

-- Occupied versus unoccupied habitat

Differences between areas occupied by toads (points or neighborhoods) and unoccupied areas were examined first with logistic regression, a type of general linear model statistical technique particularly well suited to looking for the influence of a continuous, independent variable (such as percent of the ground covered by water) on a binary, dependent variable (such as presence or absence of toads). Logistic regression calculates the probability that the independent habitat variable correctly identifies a point (or neighborhood) as being occupied or unoccupied.

A second statistical technique, general linear model (GLM) analysis of variance, also was used to examine differences in the habitat variables between occupied and unoccupied areas. In the GLM tests, presence or absence of toads was considered a categorical variable by which points and neighborhoods can be classified. This approach is useful because it allows interactions between variables to be examined. For example, horizontal vegetation density might be greater at toad points than at unoccupied points, but only in soil wetness categories 1 (moist areas) and 2 (saturated areas), and not in category 0 (dry areas). Understanding such interactions is vital to understanding what makes an area suitable for toads, and GLM analysis of variance is the suitable tool for investigating them.

An additional factor made GLM analysis of variance (instead of simple analysis of variance) the method of choice. Initial exploration of the data showed that two categorical variables, presence vs. absence of toads and soil wetness, must be considered, and there are differences between the numbers of toad points (and neighborhoods) versus unoccupied points (and neighborhoods) in the different soil wetness categories -- in statistical parlance, our data set is “unbalanced”. GLM tests are suited for analyzing unbalanced data sets, but simple analysis of variance is not. .

Note that general linear models (like any statistical technique) are imperfect. With the exception of logistic regression, GLM assumes that variables are related in a linear manner, and that the variables are normally distributed. The small sample size in our study made it impossible to test these assumptions, so it is possible that relationships actually do exist among the measured variables that are not reliably detected by these (or any other) statistical analyses.

A third statistical technique, paired t-tests, was used to examine the habitat at each toad point with the habitat in the surrounding neighborhood. In each of these tests, a toad point was paired with its surrounding neighborhood and the two were compared according to each of the habitat variables. The use of this type of analysis is to find out if, for example, toads were found in neighborhoods with dense cover but at relatively open points in those neighborhoods.

Changes were made to several of the variables recorded at the points before the analyses were performed: (i) Two variables, distance to water and distance to land, were combined into a single variable, distance from shore. The distance from shore was positive for sample points on land and negative for points in Porter Lake; points on the shore of Porter Lake were assigned a value of 0. (ii) The variable soil wetness originally had three categories: 0 = dry, 1 = wet, 2 = saturated. A fourth category, flooded (value of 3) was added after initial exploration of the data, to indicate points in Porter Lake and points on land (i.e., not in Porter Lake) for which water covered ≥ 50% of the soil surface in the 1-square-meter plot around the point, as indicated in the data for the ground cover variable.

-- Occupied points versus occupied neighborhoods

Several variables were measured both at the sample point and in the surrounding neighborhood. To see if the habitat immediately around the point differed from larger neighborhood, we used paired t-tests to compare the values measured at the points with the values in their associated neighborhoods. Differences might indicate, for example, that toads were found in areas of sparse canopy cover surrounded by denser canopy cover.

-- Relationships among variables

The habitat variables we studied provide several measures of the vegetation and the physical habitat. If those variables are closely related to one another, then measuring all of them may be unnecessary. We used correlation analysis, which shows how much the values of one variable change relative to changes in a second variable, to examine the strengths of relationships between pairs of variables.

Threat Data

Collect amphibian tissue samples for Bd analysis (Goal 7)

We collected tissue samples from live anurans found on BFP using established procedures approved by the WTRT (Boyle et al. 2004, UCB 2004, Livo 2003). This procedure required that adult amphibians were systematically "swabbed" to collect epidermal DNA. Swabs were air-dried and stored in sterile microcentrifuge tubes labeled with unique specimen numbers. Specimens were placed in a -20°F freezer the same day they were collected. These samples will be sent to Dr. Allan Pessier, the Wyoming Toad SSP Pathologist for analysis via a PCR test to determine if the fungus was present.

Samples were only collected from anurans, since members of the WTRT have reported poor results when using tiger salamanders for this type of Bd assay (Bill Turner, pers. comm.). Further, given the prevalence of Bd in populations of amphibians, it was recommended that 20 such samples be collected from a given site to be certain of the presence or absence of Bd from that site (Bill Turner, pers. comm.). Our goal was therefore to obtain approximately 40 swabs from BFP (20 from the Porter-Crescent Lake complex and 20 from Hardigan Lake and its adjacent wetlands) focusing on adult Wyoming toads and boreal chorus frogs. Initially, we wanted to collect samples during only the first and last sessions of low and high-intensity searches (i.e., spring and late summer), as Bd seems more detectable during those periods.

However, we had difficulty finding and capturing enough anurans during standard surveys, so effort was spent outside these periods to collect additional chorus frogs.

Keep field notes on predators observed during monitoring activities (Goal 8)

Members of the WTRT desired that technicians keep field notes on all potential predators of Wyoming toads that were observed during monitoring activities. Therefore, if potential predators were observed, we recorded the date, time and location of observation as well as an estimate of the number of individuals seen. Since no formal surveys were conducted for these animals and no evidence of actual predation was collected, this data is opportunistic and anecdotal and cannot be formally analyzed. The raw notes are thus reported with minimal synthesis.

Collect samples of aquatic invertebrates (Goal 9)

This effort is meant to collect baseline presence-absence information on aquatic invertebrates that could represent a potential predation threat to Wyoming toads at BFP (most likely by killing tadpoles). Therefore, we did not attempt to sample all aquatic invertebrates, but rather focused on taxonomic groups of large-bodied organisms that potentially contained predatory species.

Technicians sampled aquatic areas of BFP twice during the summer of 2006 (June 7 and July 7). Sampling consisted of sweeping dipnets through ponds and wetland areas in all available substrates, insuring that comparable effort was provided in aquatic vegetation, at the surface of the soil, and in the water column. The resulting samples were strained through a 1mm sieve to remove fine particulate matter and micro-invertebrates and dumped onto a white viewing tray. Invertebrates evident on the viewing tray were sorted into groups that roughly equated to orders or sub-orders (e.g., dragonflies, damselflies, diving beetles, etc.). For each sampling location, representatives of each group were placed into Ziploc bags and preserved with ethanol (one part 95% ethanol was diluted with two parts water from the wetland in which samples were taken). Each bag was labeled with a specimen number which is linked to additional information (sampling location, sampling date, sampling time, taxonomic group). All samples were stored for later taxonomic identification by an invertebrate expert.

RESULTS AND DISCUSSION

Population Data

Estimate annual relative abundance of sub-adult life stages (Goal 1)

Annual relative abundance of Wyoming toads was determined through systematic low- and high-intensity surveys that yielded block-specific counts of toads at several periods during the summer (Table 3 and Figure 7). For metamorphs these counts can then be combined with estimates of detectability from our detectability trials (Table 4), to estimate the number of toadlets on the property during each survey period.

We conclude that no toadlets were present during the first survey session (since none were seen and it would, realistically, have been too early for tadpoles to have metamorphosed). During the second session, about 208 toadlets were present (range 203 – 214), while during the final session about 256 toadlets were present (range 189 – 323).

Estimate actual abundance of over-wintered adults (Goal 2)

A minimum of 20 "adult-sized" toads were documented near Porter Lake (Table 5, Figure 8 and Appendix 2). We define "adult-sized" as post-metamorphosed toads of at least 3 grams, which does not imply breeding condition. Of these 20 toads, 14 or 15 were female and the remainder were male (determination of sex for one toad was not conclusive). Based on their size relative to time of capture, we concluded that 2 of these toads were mature adults (i.e., potential breeders), 10 were observed the summer after their first hibernation (probably not of breeding age), and 7 were young of the year that had not undergone their first hibernation (see Table 5).

To estimate the actual abundance of toads on site, we pooled data from low- and high-intensity searches (Table 3 and 5) and divided them by our estimates of detection rates (Table 4). Given variability in detection from our limited detectability trials, we used the maximum and minimum to obtain a range of abundance estimates for adult-sized toads, as follows:

Session 1: 0 adult-sized toads (None had likely emerged from hibernation)

Session 2: 14 adult-sized toads (range: 8 – 30)

Session 3: 16 adult-sized toads (range: 10 – 35)

Thus, it seems that there were slightly more adult-sized toads present in the third session. However, most adults from session 2 were not found again in session 3. Most of the toads found in session 3 were "young of the year" (i.e., toads that were presumably metamorphs in the earlier session). Thus, we also conducted the above calculations after removing young of the year from our estimates. Thus counting only over-wintered adults, we obtain the following:

Session 2: 14 over-wintered toads (range: 8 – 30)

Session 3: 5 over-wintered toads (range: 3 – 10)

Thus, it seems that the total population of adult-sized toads increased slightly over the summer, while the population of over-wintered adults decreased substantially. There are a couple potential reasons for these numbers. First, they may be a biologically meaningful representation of the population, wherein many adult toads died over the summer from predation. Alternatively, it could be an artificial result caused by seasonal differences in adult detectability. For example, adults that were present in session 2 (breeding season) could have moved out of the survey area in session 3, presumably enroute to hibernation sites, or they could have been less active, and thus less detectable, during session 3. Future seasons of monitoring may help elucidate this issue, as we can see if the adult toads absent from session 3 are found again in future years.

Obviously, population size will vary with time due to births and deaths on site, so the suite of numbers presented above should be considered when evaluating the BFP. However, if a single figure is required, considering all the above data it seems that the most reasonable estimate for the adult population size at BFP is about 14 adult toads, with a margin of error on the order of 6 toads.

Readers should note that there are two basic ways to estimate abundance: basic extrapolation using estimates of detection probability (as shown above) and formal mark/recapture estimators (e.g., White et al 1982, Caughley 1977). Mark-recapture statistics are potentially powerful, but are very sensitive to sample size. Therefore, the low number of toads present at BFP precludes their efficient application in this situation. However, if the suggestions in the section on recommendations are followed, we will have structured our study such that if/when toad numbers become sufficiently large we can apply mark/recapture estimators within the framework of this monitoring effort. These estimates will then supplement the basic extrapolations presented above.

Document wild breeding, if it occurs (Goal 3)

All wet shorelines were surveyed three times during the breeding season for evidence of Wyoming toad egg masses. No egg masses were found. Although it is possible that we did not observe an existing egg mass, low numbers of potentially breeding adult toads (see previous section) make it more logical to conclude that successful breeding did not occur on BFP in the summer of 2006.

Estimate dispersal from release site (Goal 4)

The area noted as "approximate release site" in Figure 3 represents a rough guess as to where most Wyoming toads where released at BFP (no specific records of release location were maintained by USFWS staff). This information was used in combination with location records shown in Figures 7 and 8 to coarsely assess how far toads migrated from the release site since they were introduced. Adult toads were found up to 365 meters from the release area based on GPS locations of toads observed during the summer of 2006. Using the outer boundaries of search blocks in which they were detected, tadpoles and metamorphs were both found up to approximately 370 meters from the release site using a straight line distance.

Based on personal observations, we assume that most dispersal occurred at the tadpole stage and thus along the shoreline where water was shallow and where there was emergent vegetation. Moreover, given their close ties to water during the summer, it is also likely that most metamorph and adult dispersal occurred along shorelines. Using this logic, it may be more appropriate to measure dispersal distances along shorelines, rather than "as the crow flies." Shoreline distance between release areas and capture sites increases the distance dispersed by both tadpoles and adults to just over 500 meters. Further, based on anecdotal reports, it is likely that tadpoles occurred beyond our designated search blocks and therefore had potentially greater dispersal distances. We thus recommend expanding search blocks in subsequent years (see Recommendations, below).

Adult toads were individually identified and several were observed on multiple occasions, so we can draw coarse inference on the magnitude of their movements (Table 6). Since only 7 adult toads were observed multiple times, this is currently a very limited dataset that will be much improved as monitoring continues in future years. From the limited information shown in Table 6, we can see that daily movement varied greatly, from 2 meters to 152 meters per day. Further, there was some evidence that movements after the breeding season (mid to late summer) were substantially larger than movements during the breeding season (over 60 meters compared to less than 3 meters, respectively). However, due to small sample size this comparison is descriptive in nature and therefore allows no firm inference.

Habitat Data

Conduct a simple site characterization of BFP (Goal 5)

The information from the general habitat survey was used to classify the vegetation around Porter Lake into ten types (Table 7). The vegetation in the irrigated meadows was, for the most part, quite distinct from the vegetation in non-irrigated areas (Figure 9). On the irrigated slopes northwest and west of Porter Lake, the most widespread dominant species in the dense vegetation are Timothy, Baltic rush, Analogue sedge, and Creeping bentgrass (redtop). Smooth brome and Kentucky bluegrass dominate in some areas, and Canada thistle forms dense patches in places. A number of other forbs are present but they contribute little canopy cover, except in small patches.

The wetter footslopes support dense meadows of Common spikerush, Nebraska sedge, several rushes, and Analogue sedge. Common threesquare bulrush patches are found along the margins of Porter Lake and Crescent Lake. A number of forbs are present in this wet vegetation, in relatively small amounts. This wet meadow vegetation dominates in the area where some of the adult toads were found in 2006 (Figures 8 and 9).

Flats immediately along the margin of Porter Lake, both in irrigated and non-irrigated areas, support sparse to moderately dense vegetation dominated by two bunchgrasses, Nuttall's alkaligrass and Foxtail barley. The soil in these sites usually was saturated at the time of the survey and often had standing water. This vegetation was also found on and around the berms near Porter Lake, and it seems to indicate areas that have been inundated by the water of Porter Lake or have been recently disturbed. Most of the adult toads found in 2006 were in areas where this is the most common vegetation type (Figures 8 and 9).

Meadows of Inland saltgrass cover most of the non-irrigated lower slopes to the southeast, east, and north of Porter Lake (Figure 9). In a few places, this vegetation grew in wet soil and merged into the Nuttall's alkaligrass - Foxtail barley wet grass vegetation nearer to the lake, but at the time of the survey, the soil beneath most of this saltgrass meadow was dry at the surface. Shrubs are present throughout but are sparse, with the exception of patchy shrub vegetation on dunes east of Porter Lake. Patches of vegetation dominated by Alkali sacaton grow in the Inland saltgrass meadow south of Porter Lake. No adult toads were observed in 2006 in the area where the Inland saltgrass meadow or the Alkali sacaton vegetation predominate.

The Inland saltgrass meadow merges with the Thickspike wheatgrass - Blue grama - Threadleaf sedge steppe that forms the upland vegetation matrix in which Porter Lake lies. Swales in the upland have shrub layers (dominated by Black sagebrush) dense enough that they were considered a separate, shrub-steppe, vegetation type (Table 7). No adult toads were observed in the upland vegetation in 2006.

Collect habitat data relative to sites used by Wyoming toads (Goal 6)

Summary

Adult toads were found in moist areas along much of the west and south shores of Porter Lake, but two areas seemed preferred (see areas highlighted as Group 1 and Group 2 in Figure 8). In general, the two preferred areas seemed to be consistently wet with much shallow water and low emergent vegetation through the summer. During our limited nocturnal call surveys, male toads were only heard calling in Group 1. Nearly all of the toad points were in Porter Lake (habitat type 2) or on land within 5 meters of Porter Lake (habitat type 1). No toads were captured on land farther than 10 m from the shoreline or in Porter Lake farther than 5 m from the shoreline.

There were some notable caveats to our analyses of habitat data. First, data were recorded at 19 toad points and neighborhoods and 19 unoccupied points and neighborhoods (Table 8), which is a small number that limits our ability to draw conclusions about what features make for suitable toad habitat and to recommend changes in the habitat sampling methodology. Second, most of the unoccupied points selected for sampling were on land farther than 5 m from Porter Lake (habitat type 0) and thus not directly comparable to the occupied points, which was the result of an unfortunate misunderstanding by the field crew in how data were to be collected that will be rectified in future years. Finally, data analysis showed that some variables lacked sufficient variability to be useful as predictors of toad presence, so they were not used in the data analysis.

The presence of moisture, as evidenced by amount of water, depth of water and soil wetness (Table 9) appeared to distinguish occupied from unoccupied sites. The main metric distinguishing points occupied by toads from those unoccupied was a higher percentage of the ground covered by water in the area immediately around the toad. Points occupied by toads had twice as much water (according to linear regression analysis) to almost eight times as much water (according to analysis of variance for points on saturated soil) as did the unoccupied points. This difference, though, did not apply to the larger neighborhood around the toads. Similarly, depth of water distinguished occupied points from unoccupied points. The GLM analysis of variance shows that water was only about half as deep at points where toads were found (average depth = 7.1 cm) as it was at unoccupied points (average depth = 13 cm), suggesting that there is a maximum depth of water in suitable toad habitat. We note, though, that this conclusion is based on only three unoccupied points in Porter Lake.

Interestingly, although the data analysis showed no difference between toad points and unoccupied points in the amount of plant canopy cover or litter cover on the ground, the paired t-tests showed that both canopy cover and litter cover were less around the points than in the neighborhoods. Perhaps the toads were selecting open habitats at a very fine scale, for thermoregulation.

<u>Occupied points versus unoccupied points</u>

Table 10 summarizes tests performed on habitat variables and the results in distinguishing occupied points from unoccupied points. We were able to discern little difference between occupied and unoccupied habitat. Logistic regression analysis produced a statistically significant result only for one variable, the percent water around a point (one category of the ground cover variable measured at the points) (Table 11). Water covered, on average, 36.9% of the 1-square-meter sample plot around a toad point, but only 15.0% of the ground around an unoccupied point. Logistic regression showed no statistically significant difference between toad points and unoccupied points for any other habitat variable.

The same variable, percent water, also was shown by GLM analysis of variance to differ between toad points and unoccupied points (Figure 10). In this analysis, where presence or absence of toads was treated as an independent variable used to classify points (rather than as a dependent variable, as it was in the logistic regression analysis), the difference between toad points and unoccupied points is complicated by interaction with the other variable in the analysis, soil wetness. When comparing percent water at all of the toad points against percent water at all of the unoccupied points, GLM analysis found no significant difference. But the interaction between the two variables shows that the comparison between toad points and unoccupied points must be made separately for each soil wetness category. The analysis indicates no difference at the moist points (wetness = 1). (Note, though, the very small sample size of 2 moist toad points). For saturated points (wetness = 2), mean percent water was substantially greater (28.71%) around toad points than around unoccupied points (3.67%), a result that conforms with the result from the logistic regression. For flooded points (wetness = 3), though, mean percent water seems to be less at toad points than at unoccupied points. The overlap in the 95% confidence intervals for the means makes this result inconclusive, but that overlap may come from the small number of flooded unoccupied points (only 3).

A second variable, water depth, also was shown by GLM analysis to differ between toad points and unoccupied points. Water depth was recorded only for the 10 toad points and 3 unoccupied points in Porter Lake, all which are in the flooded soil wetness category (Figure 11). Mean water depth at the 10 toad points was 7.10 cm, and mean depth at the 3 unoccupied points was 13.00 cm. The results of this analysis seem to be complicated by the interaction between soil wetness and type of point (toad or unoccupied). That interaction, though, simply shows that the slope of the line from the soil wetness classes 1 and 2 (where there were no points, hence depth of water = 0) to class 3 is steeper for the unoccupied points than it is for the toad points.

<u>Occupied neighborhoods versus unoccupied neighborhoods</u>

Table 12 summarizes tests performed on habitat variables and the results in distinguishing occupied neighborhoods from unoccupied neighborhoods. At first glance, the GLM analysis of variance suggested that percent plant canopy cover differed between toad neighborhoods and unoccupied neighborhoods (Figure 12). Mean canopy cover did not differ between all toad neighborhoods and all unoccupied neighborhoods, but the interaction between toad presence or absence with wetness has a low probability value (0.092), hinting that canopy cover might differ between toad neighborhoods and unoccupied neighborhoods in some wetness categories. A close look at the results, though, reveals that there was no meaningful difference between toad neighborhoods and unoccupied neighborhoods. Within the wetness = 0 category (dry neighborhoods), there were no toad neighborhoods, making the difference there trivial. Within wetness category = 1 (moist neighborhoods), there appeared to be no difference in canopy cover between the types of neighborhoods, but the very small number of toad neighborhoods robs this comparison of meaning. The contrast within soil wetness = 2 (saturated neighborhoods) is the most robust, because of the relatively large number of both toad and unoccupied neighborhoods, and canopy cover does not differ between the neighborhood types here. Finally, for soil wetness category 3 (flooded points), the small number of unoccupied neighborhoods makes comparison with toad neighborhoods meaningless. Consequently, we are interpreting this test conservatively and concluding that plant canopy cover did not differ between toad neighborhoods and unoccupied neighborhoods.

<u>Occupied points versus occupied neighborhoods</u>

Paired t-tests for all variables measured at both occupied points and their surrounding neighborhoods revealed two statistically significant differences. First, plant canopy cover was significantly less by approximately 22% immediately around points than in the surrounding neighborhoods (t = 4.85, P-value = 0.000). Second, the amount of litter on the ground also was about 15% less at occupied points compared to the associated neighborhoods (t = -2.89 P-value = 0.010). While these differences are small, the may indicate that open spots are important to toads for thermoregulation, by maximizing exposure to the sun during a short activity season in a cold climate.

Relationships among habitat variables

Many of the variables we collected were slightly correlated, which is expected since environmental features at a given location are likely linked to produce the observed habitat. However, only four of the 19 variables were significantly correlated with more than half of the other variables (Table 13), the most evident of which were percent canopy cover and wetness variables. Closer analysis of these variables suggests that we might be able to eliminate some measures of wetness, but shows that no other correlations were sufficiently strong for us to recommend eliminating variables from future data collection and analyses. Moreover, the low sample sizes do not provide a level of confidence wherein we are comfortable eliminating many variables at this time. The details of the percent canopy cover and wetness analyses are noted below.

Percent canopy cover around a point was significantly (although weakly) correlated with 6 of the 7 other variables measured at the points: points with relatively complete canopy cover also had relatively large amounts of water and litter on the soil surface (Pt %Water and Pt % Litter, respectively), lay relatively far from the shore of Porter Lake (Pt Distance from Shore), and had tall plants (Pt Ht Tallest Plant), but they also had low soil wetness values (Wetness) and little bare ground (Pt %Bare Ground). Similarly, percent canopy cover in terrestrial neighborhoods was correlated with the other five variables in terrestrial neighborhoods: the terrestrial neighborhoods with relatively high percent canopy cover also tended to have relatively large amounts of litter on the ground (Neigh %Litter, Terrestrial), relatively dense vegetation (Neigh Max Hor Veg Dens, Terrestrial and Neigh Min Hor Veg Dens, Terrestrial), and tall plants (Neigh Ht Tallest Plant, Terrestrial); but (not surprisingly) they had little bare ground (Neigh %BareGround, Terrestrial). The only strong correlation among these was the negative correlation between percent canopy cover and percent bare ground (r = -0.824).

Soil wetness (Wetness) and percent water at the points (Pt % Water) were strongly correlated with one another[4], and both of these point variables also were strongly correlated with percent water in the surrounding neighborhoods (Neigh % Water). These results show that points at which much of the immediate area was covered by water lay in neighborhoods in which much of the ground also was covered with water. Further, for both points and neighborhoods the percentage of ground covered with water around a point was negatively correlated with the distance between the point and the shore of Porter Lake (Pt Distance from Shore): the farther from shore a point lay in Porter Lake, the greater the coverage of water; and the farther the point lay from Porter Lake on land, the less area covered with water. These correlations between the various measures of wetness around points suggest that data for some of those variables need not be collected in the field, because much of the information contained in those data also is contained in the data for the other variables. Among those wetness variables, the only one that

[4] The positive correlation between soil wetness and percent of the ground around the point covered with water is to be expected because, as explained earlier, the former was partly derived from the latter.

differed significantly between toad points and unoccupied points is percent of the ground covered by water (Table 11). This probably is the most useful variable to retain.

Threat Data

Collect amphibian tissue samples for Bd analysis (Goal 7)

61 tissue samples were collected, as listed in Table 14 and shown geographically in Figure 13. Only 6 samples were collected from the Hardigan Lake complex, despite our pre-season goal of 20 samples and extra effort specifically focused on the area. Although suitable habitat seemed to exist, anurans were scarce around Hardigan Lake and its proximal wetlands. The remaining 55 samples were collected from the Porter Lake complex, 19 of which were from adult-sized Wyoming toads and the remaining 36 from boreal chorus frogs. All samples were sent to Dr. Allan Pessier of the Zoological Society of San Diego for PCR analysis. Results will be provided to the Wyoming Toad Recovery Team directly by Dr. Pessier, independent of this report.

Keep field notes on predators observed during monitoring activities (Goal 8)

While conducting field surveys, personnel recorded observations of animals that could potentially prey on Wyoming toads (Table 15). These observations are *ad hoc* and do not represent a complete list of potential predators on site, nor do they represent a valid estimate of abundance for any animal listed. Further, we did not study, or even witness, predation by any of these taxa, so their impact on Wyoming toads is purely conjecture.

In our opinion, Canada geese, gulls, and garter snakes seem to be the biggest natural threat to adult toads, as they were regularly seen in areas frequented by toads and could easily kill and consume toads of any size. Other predators (e.g., raccoons, coyotes, magpies, cranes) could potentially kill toads, but evidence of their presence was infrequent and often in areas not frequented by toads.

Tiger salamanders (larval and adult) and predacious diving beetles may represent the largest potential predators of tadpoles, although relatively few of the latter were observed. Pelicans could represent an added threat for tadpoles, but they were normally seen feeding off shore, while tadpoles were exclusively near shore. Numerous wading birds (e.g., sandpipers, avocets) and water birds (e.g., phalaropes) frequented areas containing tadpoles, but were not initially deemed to be potential predators since they mostly feed on small invertebrates. In retrospect, it is possible that some of these birds (particularly the avocets) could consume tadpoles, so a more thorough accounting of wading birds should be made in subsequent years.

A potential “predator” not listed in Table 15 is domestic cattle. About 100 head of cattle occupied nearly the same area as the toads during the toad breeding season. Much of the area frequented by toads showed evidence of trampling by cattle, which was often quite extensive. Also, when fake toads were placed during delectability trials, at least one of those toads was stepped on by cattle. Aside from stepping on adults, the biggest danger from cattle could be destruction of egg masses, since even a few cows in wetland areas could completely obliterate an egg mass before it was detected. In the future, it may be beneficial to remove cattle from those wetland areas frequented by toads during the breeding season.

Interesting and unexpected data on predation pressure was collected as part of the adult detectability trials. For these trials, artificial toads were placed in the environment and their observed occurrence was documented as part of standard search efforts. Although the dorsum of these artificial toads was painted to match the cryptic coloration of real toads (see Figure 14), their undersides were white with large red letters indicating their size class. Survey personnel found numerous artificial toads "belly-up" during the surveys (Table 16). The only explanation that seems to account for this phenomenon is that those toads were disturbed by predators, most likely birds or other visually-oriented predators. If this is the case, the number of artificial toads thus overturned provides a coarse estimate of predation pressure on wild toads of similar size. 5.2% of all artificial toads were disturbed after being in the environment for only 24 hours, with the rate on large toads being substantially higher (up to 17% in shoreline habitat). Predation pressure seemed to increase as toad size increased and seemed to be higher in shoreline areas than upland areas (Table 16). We would like to expand our detectability trials in coming seasons and thus expand our predation estimates.

Collect samples of aquatic invertebrates (Goal 9)

We collected numerous aquatic invertebrate organisms from Crescent Land and the west shore of Porter lake, as well as the northwest corner of Hardigan Lake (Table 17). These organisms were binned into coarse groups by field personnel and stored for later identification by invertebrate taxonomists. At the time this report was completed, they were still being identified by Kathleen Meyers, an entomology student at the University of Wyoming, under the supervision of Dr. Scott Shaw, Professor of Renewable Resources and curator of the University of Wyoming entomology collection. Since Ms. Meyers and Dr. Shaw are insect experts, some non-insect organisms will not be identified (e.g., zooplankton, leeches, etc.). However, it is expected that most insects can be identified to family, with some unique taxa identified to genus or even species. Identification should be complete by January 2007, at which time results will be furnished as a memorandum to the Wyoming Toad Recovery Team, independent of this report.

RECOMMENDATIONS

Based on the results discussed above and field notes collected during survey efforts, we recommend the following modifications to future monitoring efforts at BFP and other reintroduction sites. Several of these recommendations suggest altering search effort, which would alter project cost. However, we believe that recommendations that increase cost (e.g., adding more shoreline search blocks and incorporating call surveys) will be roughly balanced by those recommendations that decrease cost (e.g., reducing the number of sessions, reducing shoreline egg-mass searches, and eliminating upland and irrigation ditch search blocks).

Toad Surveys

1. **Sessions:** In this report we conducted three survey sessions: late May, mid June, and late August. No adult toads were found during the first survey session and we now feel that it was too early in the season for toads to have emerged from hibernation. By mid June, however, we found several adult-sized toads and heard males calling, indicating that toads had emerged from hibernation and begun breeding activity. We therefore propose eliminating the first session, resulting in two sessions: one during breeding season and one at the end of the summer (pre-hibernation).

2. **Search Intensity:** The primary goal of high-intensity searches was to see "most of the adults in a block," but high-intensity searches turned up roughly the same number of new toads as low intensity searches while taking much more effort. Further, having differential search intensity makes application of detectability rates difficult. Thus, instead of conducting high intensity searches, we recommend conducting a second (and perhaps third) low-intensity search in those blocks where toads are found. In addition to affording sufficient effort for estimating abundance based on detectability trials, the second and third searches will assist in mark/recapture analysis of data within each session by being considered independent opportunities to mark and/or recapture adult toads. This sampling procedure would better fit assumptions required in the application of standard mark/recapture estimators, such as Schumacher's and/or Bailey's methods (Caughley 1977) to estimate the size of the adult "population" during each session, with minimal likelihood for violation of these methods' assumptions. Good estimates of the size of these seasonal "populations" would allow estimates of both mortality/emmigration during the active season between sessions and overwinter survival. Use of mark/recapture population size estimators would provide an independent check on the population size estimate derived from the detectability studies.

3. **Search Blocks:** We initially proposed uniform search blocks distributed systematically across the study area. These blocks proved difficult to apply in the field, so we altered the size and shape of blocks to relate to geographic features of the BFP (Figure 2). While this made field efforts more efficient, we now believe that the blocks were too large and irregularly shaped to be useful in data analysis. In future years, we recommend sub-dividing the existing blocks into smaller units that are more consistent in size. Further, since we witnessed some tadpoles along the shore of Porter Lake beyond our defined search blocks, we suggest adding additional wetland blocks that encompass most, if not all, of the Porter Lake shoreline.

4. **Uplands:** During this monitoring effort, the following three facts became clear pertaining to upland areas: 1) no toads were found in upland blocks; 2) upland blocks cover a larger area than wetlands (which are primarily shorelines) and therefore require more effort to survey; and 3) detectability of toads in uplands is much lower than shorelines, therefore requiring a greater per-area effort than wetlands to make detection comparable. Considering these facts, it becomes apparent that searching uplands expends much effort for very little gain. We therefore recommend eliminating most upland search blocks from future survey work. A few blocks (i.e., 92, 95 and 112) were initially characterized as upland, but had standing water and emergent vegetation occurring throughout the summer. These blocks should be reclassified as wetlands and monitored in future years.

5. **Ephemeral Irrigation Ditches:** There are several irrigation ditches on the BFP that are dry through much of the summer (see Figure 2; e.g., search blocks 101, 102, 106 and 211). No toads were found on such ditches, nor do we expect that such ditches represent likely toad habitat. Thus, like uplands, we recommend that search effort on transient irrigation ditches be limited or eliminated.

6. **Call Surveys:** Of the males found during this monitoring effort, only one was found during standard visual encounter surveys. In fact, most males were identified during the one *ad hoc* evening of nocturnal call survey conducted during this project. Given the

apparent effectiveness of nocturnal call surveys in documenting adult male toads, we therefore recommend that such surveys be formally incorporated into future monitoring efforts. It will require careful planning to incorporate nocturnal call surveys so that they will fit into our existing analytical framework, but given the potential benefits of such surveys we feel that it is worth the trouble.

7. **Egg Mass Surveys:** Given the effort it takes to conduct shoreline searches for egg masses and the fact that no toads were found outside the Porter Lake complex, we recommend limiting egg mass searches to those areas where toads have recently been observed. For instance, we could limit egg-mass searches to all shoreline within a search block of where toads were found in the previous or current year. With current information, this would limit egg mass searches to the west and south shores of Porter Lake and all adjacent water bodies, including Crescent Lake and the first one-hundred yards of the outlet channel.

Habitat Study

The general habitat description from 2006 should suffice and this effort need not be repeated unless substantial areas are disturbed. For the sampling directed at habitat use by Wyoming toads, we have the following recommendations:

1. **Reduce the number of variables**: Information on the general habitat type and the three most common plant groups present around the point serves no apparent use and this information needn't be collected. Eliminating these two variables from data collection will leave a set of 8 variables for which information will be collected around each point: (1) height of plant canopy, (2) amount of plant canopy cover, (3) horizontal vegetation density, (4) ground cover, (5) soil wetness, (6) distance to water, (7) depth of water, (8) distance to land. We may further decide to limit the metrics that evaluate site moisture, but have not determined the best combination of metrics at this time. Additional data may show that other variables can eventually be dropped from sampling or that we add additional variables to capture information heretofore not recognized as important.
2. **Pair each toad point with an unoccupied point by distance from shore**: Failure to pair each toad point with an unoccupied point was a weakness in the 2006 habitat data collection. Because we recommend eliminating the general habitat type variable, we suggest that distance of a point from the shore of Porter Lake (or Crescent Lake, or other water body) be used as the basis for selecting an unoccupied point to be paired with a toad point. After sampling a toad point, the field technicians will select a comparison point a random distance away but the same distance from the nearest water body.
3. **Refine the estimation of variables:** Values for two habitat variables, amount of plant canopy cover and amount of ground cover (with five categories) were estimated, rather than measured, in 2006. Estimation has disadvantages, chiefly that the values it produces likely have lower accuracy (i.e., are farther from the true cover value) and lower precision (i.e., different people give different estimates). The advantage of estimation over measurement, though, is speed: estimation of cover variables is faster and so a field crew can collect data from more points. We recommend that the cover variables be estimated in the future, with two modifications to the current methods. First, the percentage cover intervals should be narrowed from 20% intervals (as in Table 2) to 10%

intervals. Second, the field crew should be provided with templates showing the area represented by different percentages of 1 square meter, which is the area around a sample point in which habitat variables will be measured. For example, cardboard squares of 0.1 sq m (31.6 cm on a side) and 0.01 sq m (10 cm on a side) would allow the field technicians to estimate values with more accuracy, and also reduce the variability between technicians.

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