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Amphibian Monitoring in the Greater Yellowstone Network - Project Report 2007

Yellowstone and Grand Teton National Parks

Natural Resource Technical Report NPS/GRYN/NRTR—2009/151

ON THE COVER
Amphibian survey, Lewis River, Yellowstone, July 6, 2007
Photograph by: Mark Wiley

Amphibian Monitoring in the Greater Yellowstone Network - Project Report 2007

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Introduction

Concerns about amphibians have escalated since population declines became apparent in diverse areas around the world in the 1980s (Collins and Storfer 2003). Systematic examinations have revealed that in some regions, including North America, rapid declines probably began around the middle of the 20th century, with the rate of decline increasing in the 1990s (Houlahan et al. 2000; Alford et al. 2001). Worldwide, 32% of amphibian species are now threatened with extinction, while 43% exhibit some form of population decrease (Stuart et al. 2004). Amidst the rapid and general decline in global biodiversity, amphibian population extinctions and declines are particularly alarming because they are occurring not only where habitat has been lost, but also in natural, protected areas. The six leading hypotheses for explaining amphibian declines are land use changes, infectious diseases, global change (climate warming and increased ultraviolet radiation), toxic chemicals (e.g., pesticides), invasive species, and over-exploitation of wild amphibians for food or the pet trade (Collins and Storfer 2003). A recent concern is the amphibian disease chytridiomycosis, caused by a species of parasitic fungus first identified in 1998 (Gascon et al. 2007). Many of the rapid amphibian population declines are poorly understood and likely have complex causes involving multiple factors.

Background of the Program

In 1991, the Herpetology Laboratory at Idaho State University (ISU) began a collaborative project with the National Park Service and the U.S. Fish and Wildlife Service in the Greater Yellowstone Ecosystem (GYE) to assess the occurrence and status of amphibians. Work in the 1990s included comparisons of species occurrence at sites where amphibians had been noted in the past, monitoring at 10 selected sites, surveys to provide information about amphibians in areas targeted for park management activities, several graduate level research projects, and a field guide (Koch and Peterson 1995).

From the 1990s work, we learned that 3 amphibian species are widespread and locally common to abundant in Yellowstone (YELL) and Grand Teton National Park (GRTE): tiger salamanders (*Ambystoma tigrinum*), boreal chorus frogs (*Pseudacris maculata*), and Columbia spotted frogs (*Rana luteiventris*). Boreal toads (*Bufo boreas*) are apparently less widespread and less common than in the 1950's. Northern leopard frogs (*Rana pipiens*) have vanished from GRTE. One non-native species, the American bullfrog (*Rana catesbeiana*), thrives in GRTE at Kelly Warm Springs.

Beginning in 2000, survey and monitoring efforts increased considerably due to collaboration among the US Geological Survey (USGS) Amphibian Research Monitoring Initiative (ARMI), the NPS Greater Yellowstone Inventory and Monitoring Network (GRYN), and ISU. As a first step, herpetological (amphibians and reptiles) inventory was completed in YELL and GRTE (Patla and Peterson 2004). In 2004, amphibian occurrence was selected by the NPS as one of 12 Vital Signs for monitoring ecosystem health in GRYN. Concurrently, USGS-ARMI designated the GYE as the central portion of the Great Divide Transect, a system of amphibian monitoring and research projects extending from Glacier National Park to Rocky Mountain National Park (Corn et al. 2005b). Through the collaborative efforts of ISU, GRYN, and ARMI, we developed and tested a sampling design for long term amphibian monitoring in YELL and GRTE. Our

approach involves comprehensive, repeated amphibian surveys of wetlands within small watersheds (referred to as catchments). The goal is to identify trends in amphibian occupancy in the two national parks and to provide information that suggests a possible cause-effect relationship that can then be investigated with a research study. In 2005, we field-tested the new approach with a subset of the selected sampling units (catchments). The 2006 field season was the final year of testing, and it approximated full implementation. By 2007, the main sampling and data collection issues were resolved, and the project was fully implemented with recognition of the need for adaptive modifications and further development of data analysis methodology.

Conceptual Model

The conceptual basis for monitoring amphibians was developed in 2004 during the process of evaluating and selecting GRYN vital signs. Long-term monitoring of amphibians not only provides insights into how well GRYN is maintaining a significant component of biological diversity, but also can help assess the level of overall ecological condition or stress. Amphibians serve as useful indicators because they are sensitive to stressors that are of prominent concern to national park managers and the public, including climate change, diseases, contaminants, habitat alteration, and introduced non-native species (Figure 1).

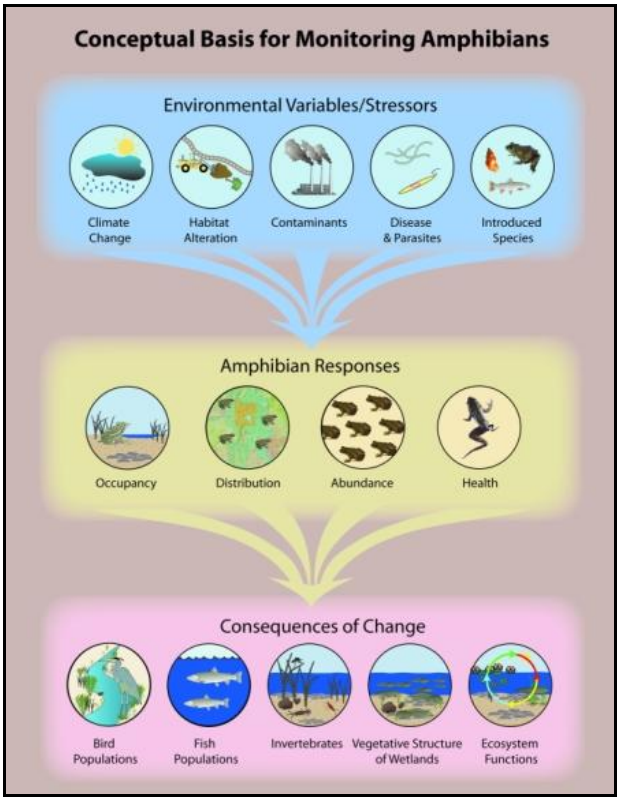

Figure 1. Conceptual basis for monitoring amphibians. Amphibian populations respond in measurable ways to local, regional, or global environmental stressors. Changes in amphibian populations affect the environment through impacts on predator-prey relationships, pond ecosystem structure and interactions, and energy flow between terrestrial and wetland ecosystems.

Amphibian populations can respond in a number of ways to these stressors and variables: occupancy and distribution patterns may change, species may disappear regionally or within administrative units, the abundance of individuals can decline or increase, outbreaks of disease and malformations may occur, and the genetic structure of populations may change. Changes in amphibian populations have consequences for ecosystems. Amphibians often occur in great local abundance, providing prey for many kinds of predators including fish, reptiles, birds, and mammals. Adult amphibians are prodigious predators, consuming insects and other invertebrates. Declines or increases in amphibians thus may alter trophic relationships, and the abundance of other animals. In terms of energy flow in ecosystems, amphibians play a unique role by transporting the high productivity of wetlands to the terrestrial environment, as tadpoles metamorphose and emerge from ponds.

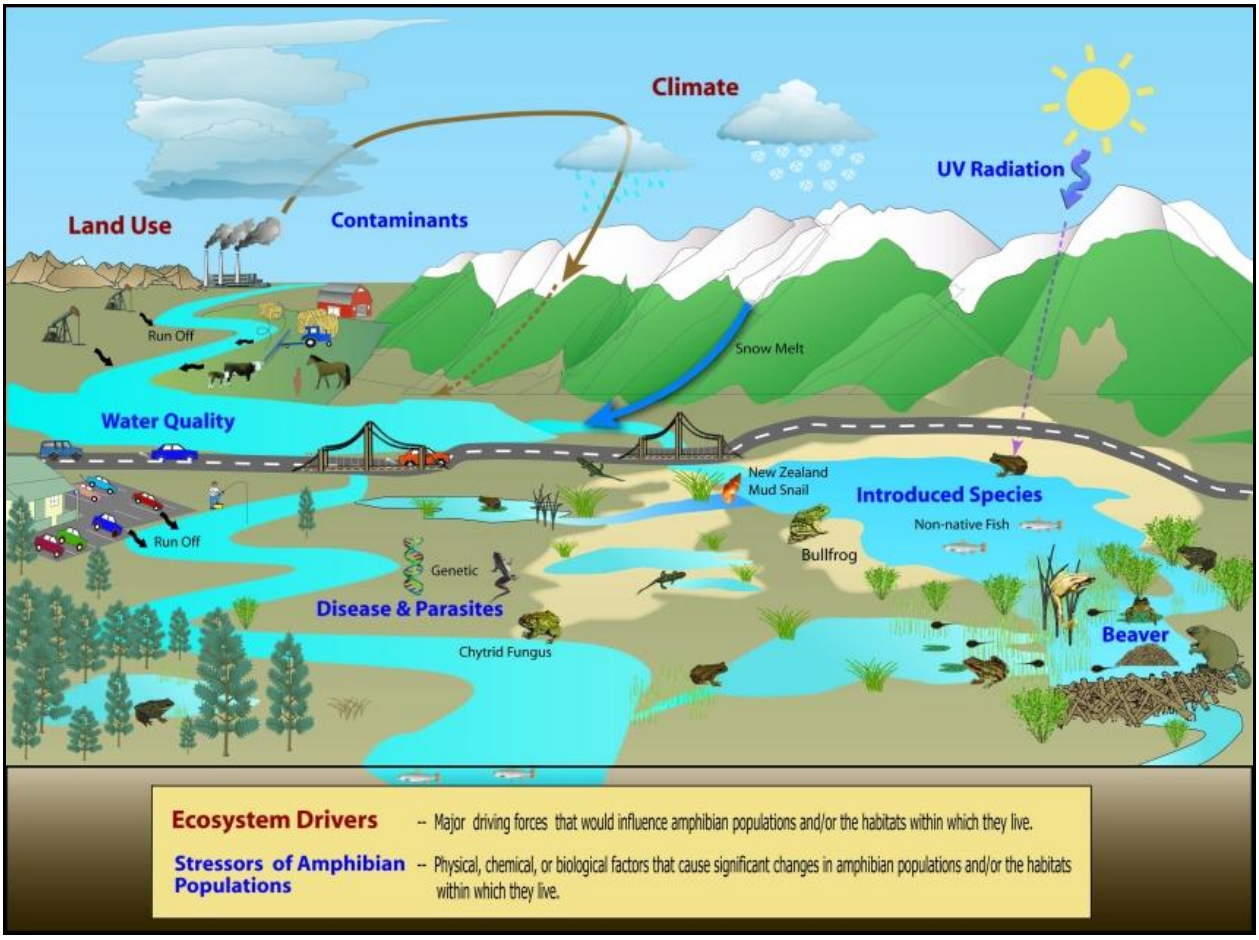

Figure 2. Conceptual model: factors affecting the location, size, and quality of wetlands may affect the distribution and number of amphibian breeding populations.

Figure 2 depicts more specifically how amphibians respond to drivers and potential stressors. All amphibian species of GRYN rely on shallow water bodies for egg deposition and larval development (Koch and Peterson 1995). Thus, factors affecting the location and size of wetlands (drought or climate change, land use, and beavers) are likely to substantially affect the distribution and number of amphibian breeding populations. Some stressors have the potential to directly affect the health, survival, and abundance of amphibians: pathogens, contaminants from

both local and remote sources, UV radiation, and introduced species that compete with or prey on native amphibians (such as bullfrogs, non-native fish, and introduced snails) or plant species that degrade wetland breeding habitat. Some environmental factors may affect amphibians directly as well as indirectly, via their impacts on wetland habitats. For example, roads can cause high mortality rates in frogs attempting to reach their seasonal breeding or wintering habitat, and roads can also cause wetland loss.

The ARMI conceptual approach

The problem of how to monitor amphibians on Department of Interior lands has been the subject of an integrated effort by senior USGS scientists (Corn et al. 2005a). The approach adopted for monitoring amphibians in national parks focuses on the number of populations, as opposed to changes in the size of populations. This is based on Green's (1997) framework for discerning declines:

> *A decline is the condition whereby the local loss of populations across the normal range of a species so exceeds the rate at which populations maybe established, or reestablished, that there is a definite downward trend in population number* (Green 1997).

To assess amphibian status and trends, USGS scientists propose monitoring changes in the proportion of area occupied based on presence-absence data, using estimation techniques that incorporate measures of detection probability and allow for testing how environmental variables affect occupancy dynamics (Corn et al. 2005a). The approach assumes:

> *As populations increase in abundance they should expand into available habitat with a concomitant increase in occupancy. As populations decrease in size, distributions should shrink, with fewer species in the sampling units and a concomitant decline in occupancy. Thus the occupancy estimator can provide indirect information on temporal and spatial variations in species abundance. With simultaneous monitoring at sampling sites of environmental variables and stressors that can affect amphibians, correlation with possible causes of change can be established and studied* (Corn et al. 2005a).

The ARMI conceptual approach was adopted by GRYN for long-term amphibian monitoring in GRTE and YELL. A formal protocol for the program, including the conceptual basis and all the elements of implementation, is under peer review in 2008.

Objectives

The goal of this project is to answer the questions:

- Are native amphibian species declining in YELL and GRTE, exhibiting the global change phenomenon of amphibian population declines? Or, are they remaining stable, or even increasing?
- What are the possible underlying causes of any observed declines?
- What further directed research or management actions are warranted?

Although our specific monitoring objectives are targeted for inferences to GRTE and YELL, they are also intended to complement broader objectives of ARMI that seek to answer similar questions at more regional and/or national scales.

Objective 1

Estimate the proportion of catchments and sites (described below) used for breeding by each native amphibian species annually, and estimate the rate at which their use is changing over time.

Breeding sites are identified by the presence of eggs, larvae, or recently metamorphosed juveniles. The species under investigation in GRTE and YELL are: Tiger salamander (*Ambystoma tigrinum*), boreal toad (*Bufo boreas*), boreal chorus frog (*Pseudacris maculata*), and Columbia spotted frog (*Rana luteiventris*). Work on boreal toads is supplemented by Objective 2, below. If discovered in the future, northern leopard frogs (*Rana pipiens*) and any other rare native species will be included under a new objective.

The proportion of area occupied (PAO) approach we are using provides a measure that: (1) explicitly enables estimation of local extinctions and colonization of sites; (2) account for imperfect detectability of individual species; (3) enables estimation of confidence intervals; (4) is comparable across sites; and (5) has become a widely accepted approach for reliable estimates of occupancy (MacKenzie et al. 2006).

Occupancy will be assessed at two levels: catchments (portions of watersheds containing multiple potential sites, and sites (individual ponds or wetlands). The catchment level approximates the 'breeding population' identified by ARMI as the feasible target for monitoring amphibian population trends in national parks. Occupancy at this level will be the major vehicle for meeting the goal of determining if amphibians are declining, stable, or increasing in GRTE and YELL. Occupancy at the site level is a lower-scale measure that will allow one to investigate the importance of site-specific and survey-specific variables affecting detection probability and occupancy at individual wetlands. The dynamics at the site level can augment the catchment-level results to provide greater understanding of system dynamics.

Objective 2

Determine the number of potential breeding sites within sampled areas that are suitable for amphibian breeding (i.e., have standing water during the breeding season) annually. For reliable comparisons of change in occupancy over time, the sampling frame must be the same from year

to year. However, some wetlands are dry in some years, with no possibility of supporting amphibian breeding. By tracking the number of available breeding sites annually, we can directly observe how amphibian occupancy changes as a function of the proportion of wetlands that are dry, and identify to what extent habitat loss due to drought or climate change is affecting amphibian occurrence.

Objective 3

For boreal toads (*Bufo boreas*, Figure 3), estimate the proportion of previously identified breeding areas that are used annually, and estimate the rate at which their use is changing over time. This objective is intended to supplement Objective 1. Based on pilot studies, occupancy rates provided under Objective 1 would probably be too low to enable reliable estimation of rates of change or to recognize a decline if it is occurring, given the rarity of toads relative to the other amphibian species.

Unfortunately, current funding levels are inadequate to meet this objective, which has a lower priority than Objective 1 and 2. This objective may be partially met through volunteer efforts.

Figure 3. Boreal toads breeding at Indian Pond in Yellowstone NP.

Methods

Study Area

Our primary study area is YELL and GRTE, with inference to all portions of the parks containing shallow wetlands. Sampling units for amphibian monitoring are small portions of watersheds, referred to as catchments. Figure 4 shows the catchments that have been randomly selected for long-term monitoring.

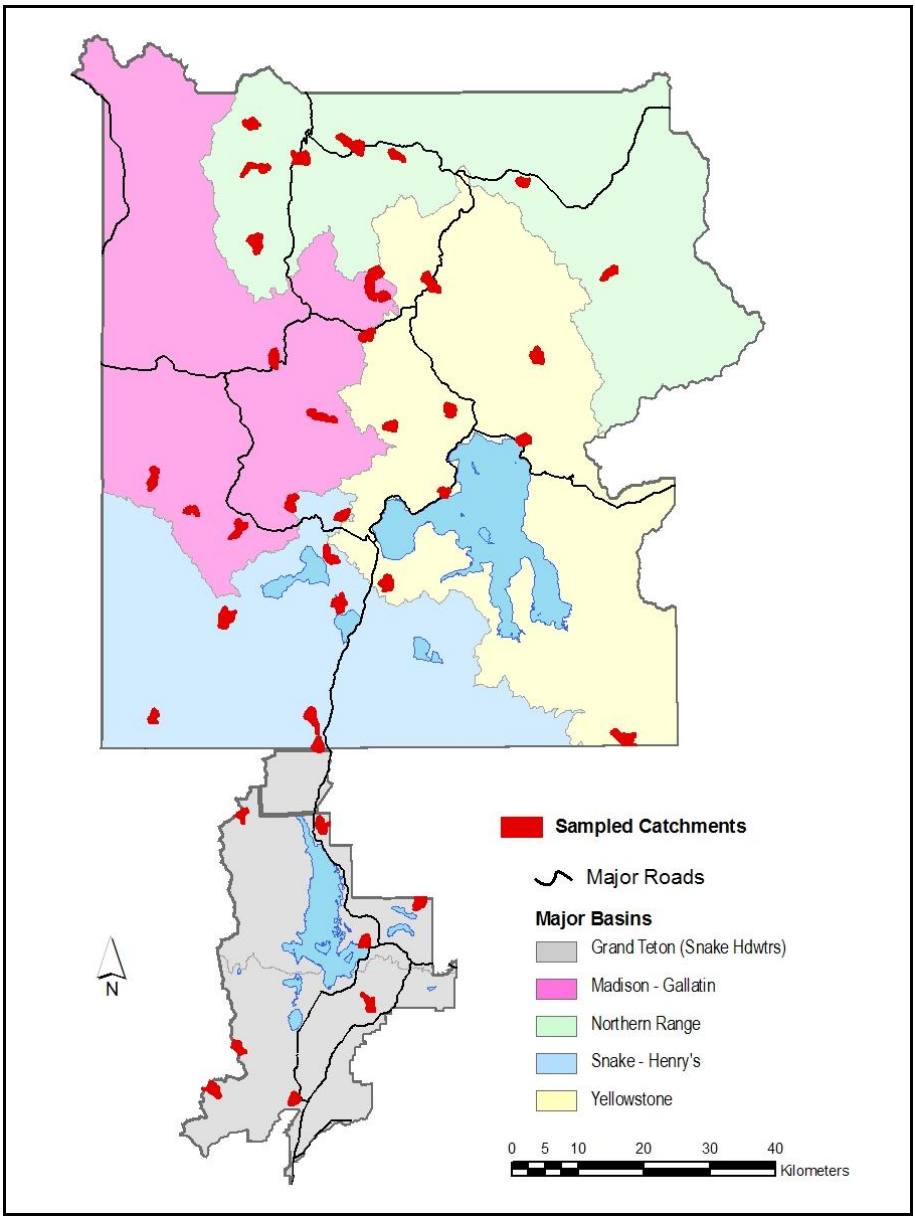

Figure 4. Catchments selected for long-term amphibian monitoring in Grand Teton and Yellowstone national parks. All potential amphibian breeding sites within these 40 catchments are targeted; 32 catchments in YELL, 8 in GRTE.

Sampling scheme

Catchment boundaries are represented in a Geographic Information System (GIS) layer created at USGS Earth Resource Observation Systems (EROS) Data Center. We used a probabilistic sampling scheme to ensure spatial distribution of sampling units among the major drainage basins of GRTE and YELL (depicted by shaded areas in Figure 4). To help ensure that the majority of units could be reached without extraordinary off-trail efforts during the brief field season, we used two accessibility classes ('close' $\leq$ 4 km from roads; and 'remote' > 4 km) in our allocation scheme (Table 1). Since the highest quality habitat within GRTE and YELL is limited to a small amount of the area, we also wanted to ensure sampling this habitat by stratifying on amphibian breeding habitat probability (high, medium, and low), based on National Wetland Inventory (NWI) types and amounts within the catchment (Amphibian Monitoring Working Group 2008). In 2007, we visited all the selected catchments in the high and medium habitat classes but only a subset of the catchments in the low class as per our previous split panel design, which allowed for visiting catchments in this class every 5 years (Patla et al. 2007). Subsequent analysis of the 2007 data has demonstrated that all catchments should be surveyed annually. Table 1 shows the revised sampling scheme that will begin in 2008.

Table 1. Number of catchments per access class, habitat class and basin to be sampled for long-term monitoring beginning in 2008. In 2007 we sampled a subset of catchments in the Low Habitat class.

Access Class[1]	Habitat Class[2]	Basins						Total catchments
		Northern Range	Yellow stone	Madison-Gallatin	Snake-Henrys Fk	GRTE-North[3]	GRTE-South	
Close	High	2	2	2	2	1	1	10
Far	High	1	1	1	1		1	5
Close	Medium	2	2	2	2	1	1	10
Far	Medium	1	1	1	1	1		5
Close	Low	1	1	1	1	1		5
Far	Low	1	1	1	1		1	5
								40

[1]Based on catchment distance $\leq$ or > 4 km from a road.

[2]Based on the amount and type of wetlands in each catchment identified by National Wetland Inventory.

[3]GRTE is in the Snake basin; it was divided into North & South zones to achieve better spatial representation

Amphibian Surveys

Procedures for surveys are detailed in our draft Protocol (Amphibian Monitoring Working Group 2008). Two-person field crews visited all potential amphibian breeding sites within the boundaries of the selected catchments. Surveys were conducted at all sites with suitable water, following standard amphibian visual encounter methodology (Thoms et al 1997). This entailed walking the perimeters of water bodies and transects through shallow ponds and wetlands, and using long-handled dip-nets to sweep the water for amphibian larvae. To determine the presence of breeding populations, we searched for life stages that indicate breeding has occurred: eggs,

larvae, or recent metamorphs. Each field crew member surveyed the site independently (dual observer method), to provide data on species detectability. Catchments were visited once per season.

Data collected in the field included location recorded with a Global Positioning System (GPS) receiver, time spent searching, species observed (life stages and numbers of each), weather, habitat descriptors, water temperature, pH and conductivity (Amphibian Monitoring Working Group 2008). Sites were documented with drawings and photos, and species larvae with photographs. Data were recorded on field sheets and personal digital assistants (PDAs). The PDAs were programmed using forms software (Pendragron Forms), and downloaded directly into a Microsoft Access database provided by USGS-ARMI and slightly modified for use in GRTE and YELL as coordinated by the GRYN Data Manager. The relational database, containing multiple tables, is standardized for use in the Rocky Mountain Region by USGS-ARMI.

In 2007, surveys of catchments began on June 15 and ended on July 26. Two 2-person field crews worked through the season, with a third team working for half the season. Work time to accomplish the surveys included a total of 1350 hours worked by technicians hired through ISU for this project (training, surveys, and related tasks), approximately 700 hours by the field crew supervisor (for hiring/supervising, project planning, and field work), and approximately 60 field hours by a USGS employee sent from Glacier to assist us.

Boreal Toad Monitoring

Based on a comprehensive list of locations compiled from previous amphibian databases and records, 39 toad breeding areas (some containing multiple breeding sites) have been found in YELL and GRTE (Figure 5 and Appendix 1). Four of these areas are in catchments selected for long-term monitoring, and are subject to annual surveys in the surveys described above. Otherwise, toad monitoring is not covered by current funding levels, given that the catchment surveys (Objective 1) have higher priority. In 2007, a subset of the remaining 35 areas were surveyed for toad breeding by an experienced volunteer team who has worked with us on amphibian monitoring in YELL since 1996 (Char and Dave Corkran), and opportunistically by the field coordinator on a time-available basis. Monitoring consisted of conducting visual encounter surveys at the known breeding sites and in the vicinity up to about 500 m, with time limited to 3 hours. Data were collected on paper forms, and then were compiled in a relational Access database by the field coordinator on a time-available basis. The database was adapted from our initial GRTE and YELL database (in use 2002 to 2004), which is more suitable for the toad data because it is not reliant on catchments (as is the ARMI database), allows for convenient manual data entry, and includes fields that are useful for tracking toads at a fine scale (e.g., geospatially referenced coordinates for egg deposition or tadpole congregation sites).

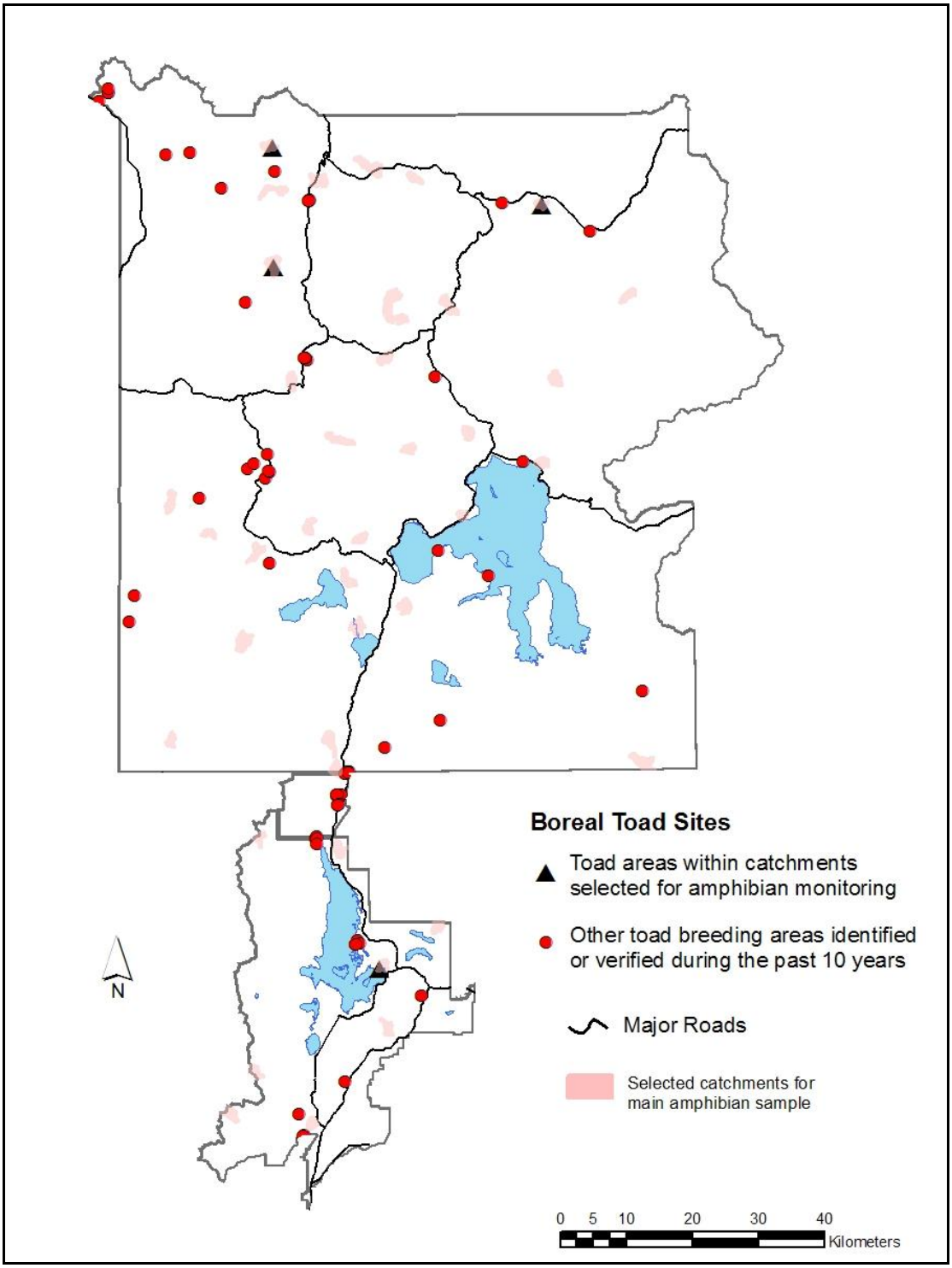

Figure 5. Boreal toad breeding sites in YELL and GRTE. Sites within 500 m of each other have been clustered as 'toad breeding areas' for monitoring. A total of 39 breeding areas have been identified. Dark triangles show the toad breeding areas that occur within catchments that were selected for GRYN long-term monitoring under Objective 1 (4 areas). Red circles indicate other previously identified toad breeding areas (35 areas).

In 2007, we visited 23 of the 35 previously-identified toad breeding areas outside of the long-term monitoring catchments. This included all areas with "easy" access (less than about 2 km from roads), and all but one of the areas that can be reached and surveyed within one day. Four areas were visited multiple times during the season. The 4 additional toad areas within the long-term monitoring catchments were surveyed by ISU field crews, following the normal protocol for catchments surveys. A total of 27 areas, or 69% of all previously-identified toad breeding areas in GRTE and YELL, were surveyed in 2007.

Data analysis

Occupancy modeling provides a statistical framework for assessing changes in species occurrence (MacKenzie et al. 2002; Royle & Nichols 2003; MacKenzie et al. 2006). Information from repeated observations at sample units is used to estimate detectability and adjust occupancy rates for imperfect detection (failure to observe a species that is actually present), providing a metric referred to as Proportion of Area Occupied (PAO). Occupancy models allow for analysis of covariates potentially affecting occupancy (e.g., habitat class), and covariates affecting detectability (e.g., observer). The analysis philosophy is optimization, based on evaluating which model or set of models best explains observed patterns, rather than the elimination of models using hypotheses testing. Our approach uses information theoretic methods, specifically Akaike's information criterion (AIC) differences and weights, to evaluate the models. This is followed by model averaging, which allows one to incorporate model uncertainty into the estimation process as well as providing unconditional variance estimation.

For occupancy modeling, we used the program MARK (White & Burnham 1999). To extract data from the amphibian database for occupancy and detectability analysis, we used an interface tool provided by ARMI, which includes a PAO File Generator.

Modeling of the 2007 catchment data was based on the initial sampling design of the project, with models allowing for stratum-specific estimates of occupancy (Appendix II). Strata were defined by breeding habitat quality (high, medium, and low quality) and access-specific differences (close and remote). Tiger salamanders (AMTI), boreal chorus frogs (PSMA) and Columbia spotted frogs (RALU) were analyzed separately. Boreal toads (BUBO) were excluded from this modeling due to sparse data. By considering multiple models and assessing the relative support for each, it was possible to evaluate stratum–level distinctions, in comparison to treating the data as a simple random sample (Patla et al. 2007). Where there was little evidence for stratum-level distinctions, models without these distinctions were preferred based on the principle of parsimony (include only parameters that contribute meaningfully to describing the process that generated the data). This analysis approach also allowed for a compromise in which one could allow for stratum-specific estimates of occupancy but common estimates of detectability (Gould 2008). To estimate occupancy at the target population level (GRTE and YELL) when stratum-specific models were strongly supported, we combined the stratum-level estimates according to design-based methodology (e.g., Thompson 1992) in a weighted mean (weighted by the number of catchments in each habitat class; Gould 2008).

The weighted average and variance were computed as:

$\overline{\hat{\psi}} = \sum_{h=1}^{L} \frac{N_h}{N} \hat{\psi}_h$ where *h* indexes each stratum, and N_h is the stratum size such that $\sum_{h=1}^{L} N_h = N$.

The estimated variance is $V\hat{a}r(\overline{\hat{\psi}}) = \sum_{h=1}^{L} \left(\frac{N_h}{N} \right)^2 \left(\frac{N_h - n_h}{N_h} \right) V\hat{a}r(\hat{\psi}_h)$ where n_h represents the number of catchments sampled from stratum *h* and $V\hat{a}r(\hat{\psi}_h)$ is the square of the estimated standard error for stratum *h*.

Occupancy and detectability at the site level can also be modeled. At the current time, *a priori* models to apply and evaluate at the site level have not been formulated. To avoid data dredging, formal analysis at the site level should be postponed until *a priori* models have been considered and carefully formulated, with agreement among the collaborators. Some pilot work was attempted with existing data (Gould 2008). Attempts to model detection as a function of the number of observed larvae were unsuccessful (Gould 2008). Modeling the effects of pH and conductivity on tiger salamander, boreal chorus frog, and Columbia spotted frog breeding sites produced variable and inconclusive results (Gould 2008). Sites are secondary units of clusters (the catchments), not a simple random sample, and as such, over dispersion might exist and should be evaluated using appropriate means available in programs MARK and PRESENCE. Gould (2008) also suggests evaluating occupancy at the site level including all sites that are surveyed, even if selected sites are dry in a given year. By doing so, lower overall occupancy at the site level is better reflected (but detectability rates become less meaningful due to the inclusion of sites where detection can only be zero). Based on ARMI methodology and the data extraction tools provided, we have been considering only sites that have surface water and are potentially occupied by amphibian larvae.

In this report, site-level occupancy and detectability treat sites as a simple random sample, assuming constant occupancy and detectability, using ARMI's PAO Generator Interface Tool and Presence 2.0. These data are provided with the recognition that the values will be revised in the future as modeling issues and methodology are updated.

Boreal toad data analysis

To analyze boreal toad data, dual frame methods will be used (Haines and Pollock 1998). This approach allows one to combine an incomplete list frame (e.g., toad breeding sites found opportunistically in the past), and the area frame (catchments randomly selected for long-term amphibian monitoring). Sampling design and analysis methods are still under development for boreal toad monitoring, with insufficient funds for completion at this time. This report summarizes the 2007 efforts and results of surveys at previously identified toad breeding areas, without application of dual frame analysis methods.

Results

Catchments sampled and breeding sites found

In 2007, we sampled a total of 34 catchments, 27 of which were in YELL and 7 in GRTE (Table 2). In terms of sampling design, 4 of these catchments were in the stratum classed as low, 16 in the medium stratum, and 14 in the high stratum. Five catchments were visited for the first time in 2007; 3 of these were replacements for catchments found to be unsuitable in 2006 due to lack of wetlands or administrative area closures, and 2 were in the habitat class surveyed every 5 years rather than annually as per the draft (now revised) sampling design. One of the new catchments sampled in 2007 had no sites suitable for surveys and thus was not used for 2007 occupancy assessment.

A total of 420 wetland sites were visited (331 in YELL, 89 in GRTE), but only 221 sites (156 in YELL, 65 in GRTE) had sufficient water to provide potential amphibian breeding habitat and were subject to surveys. Breeding sites (identified by the presence of eggs, larvae, or recent metamorphs) of four species were found (Table 2). Boreal chorus frog and Columbia spotted frog breeding was detected in all basins and at numerous sites. Tiger salamanders were clustered mostly in the Northern Range basin of YELL and in GRTE. Boreal toad breeding was detected only at 2 catchments in the Northern Range basin and 1 catchment in GRTE. No leopard frogs or bullfrogs were found.

Table 2. Results of amphibian monitoring in 2005 – 2007. Total numbers of catchments and sites surveyed per basin, and the number of catchments and sites containing breeding sites for amphibian species.

	Catch-ments survey-ed	Sites Survey-ed	Tiger Salamander		Boreal Toad		B. Chorus Frog		C. spotted Frog	
			Catch-ments	Sites	Catch-ments	Sites	Catch-ments	Sites	Catch-ments	Sites
2005 [1]										
Northern Range	4	10	2	2	1	1	3	5	1	3
Yellowstone	2	26	0	0	0	0	1	3	2	6
Madison-Gallatin	2	16	0	0	0	0	2	2	1	2
Snake-Henrys Fork	4	31	1	1	0	0	4	9	3	9
Grand Teton	3	38	1	2	1	3	1	3	2	3
Totals	**15**	**121**	**4**	**5**	**2**	**4**	**11**	**22**	**9**	**23**
2006										
Northern Range	6	24	5	10	3	3	4	9	5	7
Yellowstone	5	40	0	0	1	1	2	11	4	6
Madison-Gallatin	6	71	1	1	0	0	5	30	4	13
Snake-Henrys Fork	8	65	0	0	0	0	4	16	5	15
Grand Teton	7	62	3	8	1	4	4	16	4	13
Totals	**32**	**262**	**9**	**19**	**5**	**8**	**19**	**82**	**22**	**54**
2007										
Northern Range	7	22	4	10	2	2	4	8	2	3
Yellowstone	7	46	0	0	0	0	2	5	5	7
Madison-Gallatin	6	46	1	1	0	0	5	25	4	13
Snake-Henrys Fork	7	42	1	1	0	0	5	12	5	12
Grand Teton	7	65	3	5	1	2	3	14	4	4
Totals	**34**	**221**	**9**	**17**	**3**	**4**	**19**	**64**	**20**	**39**

[1]2005 was a pilot year in which only a portion of the selected catchments were monitored.

Occupancy and detectability at the catchment level

Occupancy at the catchment level in 2007 was assessed with a set of 8 candidate models, including the 3 habitat strata (high, medium, and low quality habitat) and the 2 access classes (catchments $\leq$ and > 4 km from roads) that were used in stratifying and selecting catchments for monitoring (Appendix II). Also included were models allowing for variation in detection probability between the primary and secondary surveys. One species, boreal toad, was excluded

from this analysis due to sparse data. Information theoretic methods (AIC differences and weights) were used for evaluating models. Results for tiger salamanders and boreal chorus frogs exhibited substantial model uncertainty and were thus subjected to model averaging. For Columbia spotted frogs, the model stipulating 2 habitat classes (high plus medium versus low) was reasonably well supported and outperformed the other models. Goodness of fit testing for the most general model in each set indicated no evidence of lack of fit (Gould 2008). Occupancy estimates and associated standard errors (SE) based on the averaged models for salamanders and boreal chorus frogs, and the best model for Columbia spotted frogs, are provided in Table 3. To provide inference to entire GRTE and YELL, an unbiased estimator under stratified random sampling (e.g., Thompson 1992:104) was used that considers the relative numbers of catchments within each habitat-access strata.

Table 3. Catchment-level occupancy and detectability from 2007 monitoring data. Occupancy rates were based on model averaging for tiger salamanders and boreal chorus frogs, and the best model for Columbia spotted frogs, as described in the text and Appendix II. Detection rates were based on the top ranked models. Boreal toads, detected in only 3 catchments, provided data that were too sparse for modeling.

Species	Occupancy	Standard Error	Detection Rates	Standard Error
Tiger salamander	0.16	0.08	0.71	0.14
Boreal chorus frog	0.49	0.11	0.97	0.03
Col spotted frog	0.23	0.03	1.00	0.00

Occupancy and detectability at the site level

Site-level estimates of occupancy and detectability for 2007 are provided in Table 4. As described in the Methods section, these occupancy estimates were derived using a simple occupancy model without weighted averages as we did with the catchment-level occupancy. These results do not provide inference to entire GRTE and YELL and should be interpreted with caution until analysis methods are finalized and applied. Detection rates at the site level were high for 3 species (boreal toads, boreal chorus frogs, and Columbia spotted frogs) and relatively low for tiger salamanders. Salamander larvae tend to be more cryptic than the other amphibian species, providing lower detection rates (e.g., Corn et al. 2005b).

Table 4. Site-level occupancy and detectability. Occupancy rates for sites, assuming constant occupancy and detectability across strata. Analysis methods for site-level occupancy are under development.

Species	Occupancy	Standard Error	Detection Rates	Standard Error
Tiger salamander	0.09	0.02	0.64	0.11
Boreal toad	0.02	*	1.00	0.00
Boreal chorus frog	0.29	0.03	0.92	0.03
Col spotted frog	0.21	0.03	0.96	0.02

**Variance not completed successfully for boreal toads due to sparse data*

Boreal Toads

Toad breeding (eggs, tadpoles, or recent metamorphs) was confirmed at 17 of the 23 previously identified toad breeding areas that were surveyed in 2007 (Figure 6). In addition, toad breeding was detected at 3 of the 4 long-term monitoring catchments where toad breeding was previously found.

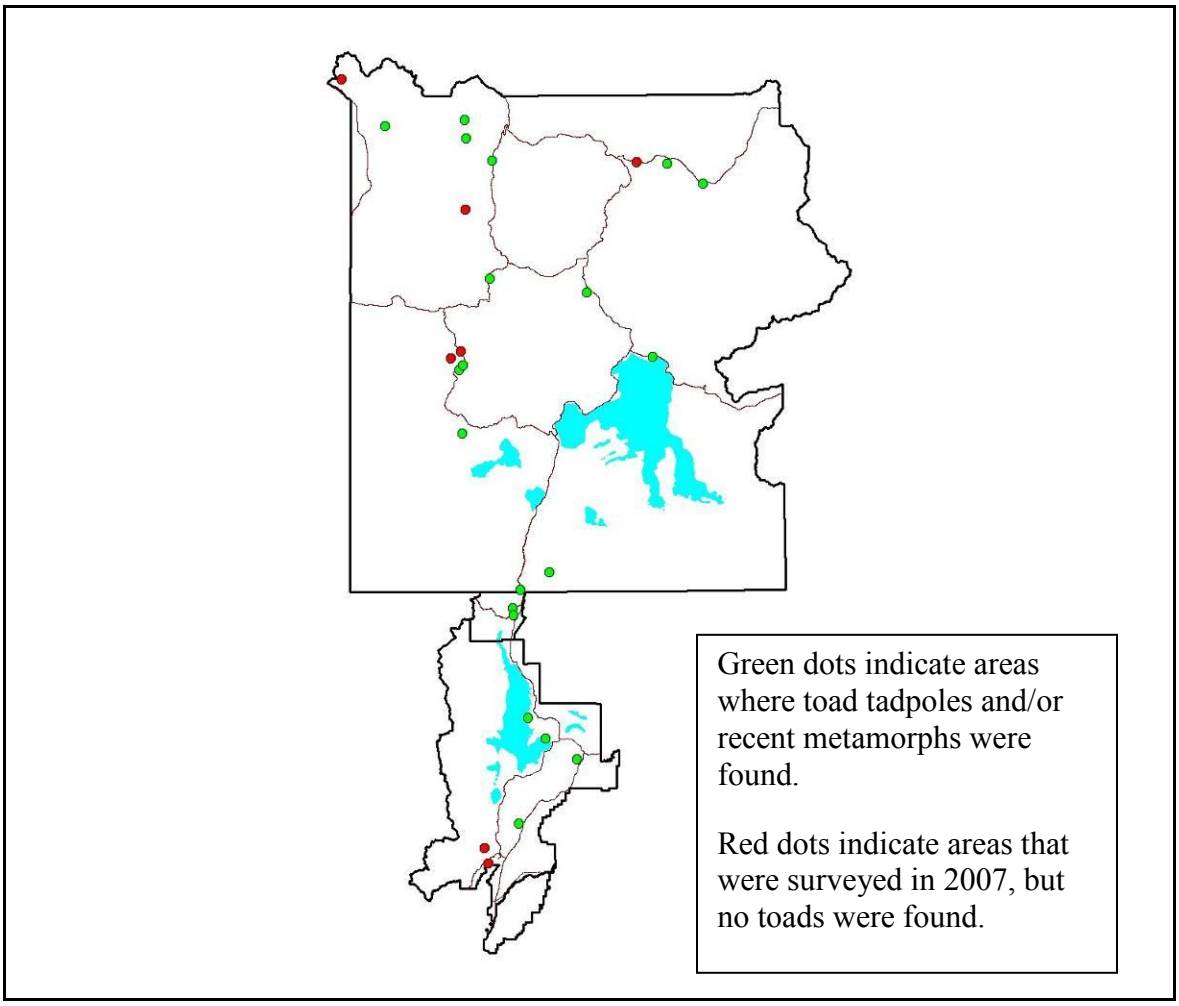

Figure 6. Boreal toad breeding areas surveyed in 2007.

Discussion

In 2007, the number and type of catchments surveyed met the goal set by our draft sampling design, with surveys completed in all the selected catchments within the medium and high quality habitat classes, and a subset of catchments in the low quality habitat class. This represents a slightly higher level of effort than in 2006, when one YELL catchment in the high class was unavailable due to an administrative closure during the field season. Because of intensifying drought conditions, the number of potential amphibian breeding sites surveyed in YELL dropped from 199 sites in 2006 to only 156 sites in 2007. In GRTE, the number of available sites was nearly stable, with 62 sites surveyed in 2006 and 65 sites in 2007.

The raw number of catchments occupied per species remained stable between 2006 and 2007 for tiger salamanders and boreal chorus frogs, but dropped for boreal toads and Columbia spotted frogs (Table 2). The total number of active breeding sites that were detected in 2007 declined for all four amphibian species relative to 2006, with decreases occurring in YELL as well as GRTE (Table 2).

The 2007 adjusted occupancy rates provided by this report were calculated using a modeling approach that was not employed in 2006; reported occupancy for the 2 years should not be compared until the 2006 data are subjected to the same analysis. Our 2006 report (Patla et al. 2007) assumed constant occupancy among the strata that were used in selecting catchments. Gould's (2008) recent assessment of the project revealed that the stratified sampling design must be considered in estimating GRTE and YELL-wide occupancy, and that the occupancy rates reported for 2006 are biased toward high quality habitat.

We will conduct thorough data analysis every 5 years, with basic occupancy and detectability computed annually. The 5-year synthesis period is appropriate to determine if trends are present, with less danger of inflating the importance of annual fluctuations.

Gould's (2008) review of the amphibian monitoring draft protocol resulted in some important changes, which we implement in 2008. In terms of field work, the most important of these is to increase the number of catchments sampled in the low habitat quality stratum, given that they represent the largest portion of catchment types within GRTE and YELL. This should improve confidence in occupancy estimates for entire GRTE and YELL. Determining how to increase sampling with a relatively flat budget for field work will be a challenge.

Recent work has revealed the need for continued development of occupancy modeling, including joint modeling of habitat and species occupancy, analysis of covariates at the catchment and site level and development of methods for trend estimation. Also needed is a pilot study to determine how or if breeding occupancy changes during the survey season (because occupancy modeling assumes occupancy is constant during the survey season). Many aspects of boreal toad monitoring need to be resolved.

Through review and continuing discussion, project objectives have been revised since our 2006 report, including the elimination of Objective 4, amphibian disease (Patla et al. 2007). Disease occurrence, however, will continue to be monitored opportunistically as field personnel collect

and submit specimens encountered. In 2007, we documented mass mortality and abnormalities of juvenile and adult Columbia spotted frogs at Lodge Creek in YELL. Six frog specimens were collected and sent to David E. Green, Staff Diagnostic Pathologist, USGS National Wildlife Health Center, in Madison Wisconsin. The deaths were attributed to ranavirus but the frogs also had mild chytrid infections and parasites. One frog had limb malformation consistent with those caused by metacercariae of *Ribeiroia ondatrae*, a parasite well known for causing deformities in frogs (Blaustein and Johnson 2003) and which has not been previously found in the GYE, to our knowledge. Green's report is provided in Appendix III.

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Appendix I: Boreal toad breeding areas.

Catchments selected for long-term monitoring are indicated in blue.

ID code: Population ID code assigned for easy reference and database use

Access: Easy = <2 km from roads; Day = hike to and survey in one day; Remote = backpacking needed, 2-5 days

Toad Life Stage: E = eggs; L = tadpoles; M = metamorph; J = juvenile (immature, probably 1-2 years old); A= adult.

Single or multiple breeding sites: Toad eggs or tadpoles found in one or multiple distinct sites within the area.

Major or Minor: Minor means <100 tadpoles seen 1 year; Major means hundreds or thousands of tadpoles, usually seen multiple years

ID code	Access	Toad Life Stage found in 2007	Single or multiple breeding sites	Major or minor breeding site	Year of last toad breeding record	Year that area was last checked for toad breeding	General Location
G1	Easy	L, M	multiple	major	2007	2007	Flagg Ranch,
G2	Easy	E,L,M	multiple	major	2007	2007	Snake River pit
G3	Day	--	multiple	major	2006	2007	Snake River above Jackson Lake
G4	Easy	L, M,A	multiple	major	2007	2007	Colter Bay
G5	Easy	L,M	multiple	major	2007	2007	Willow Flats
G6	Day	L,M	multiple	major	2007	2007	Snake River downstream Moran
G7	Easy	L,M,J,A	multiple	major	2007	2007	Schwabacker Landing

ID code	Access	Toad Life Stage found in 2007	Single or multiple breeding sites	Major or minor breeding site	Year of last toad breeding record	Year that area was last checked for toad breeding	General Location
G8	Easy	0	single	minor	2004	2007	Whitegrass Rach
G9	Easy	0	single	minor	2004	2007	Snake River Moose-Wilson Rd
Y1	Day	0	single	minor	2002	2007	Daly Cr ponds
Y2	Day	L,J,M	single	major	2007	2007	Fan Creek
Y3	Remote	--	multiple	major	1999	1999	East Fan Creek
Y4	Remote	--	single	minor	2002	2002	Fawn Pass
Y5	Day	L,M	single	major	2007	2007	Fawn Pass Trail
Y6	Remote	L	single	major	2007	2007	NW Yell
Y7	Easy	L	multiple	major	2007	2007	Swan Lake
Y8	Easy	0	single	minor	1999	2007	Lamar Valley
Y9	Day	L,J/A	single	major	2007	2007	Lamar Canyon
Y10	Easy	L,A	single	major	2007	2007	Soda Butte Creek
Y11	Remote	--		minor	?	?	In NE YELL
Y12	Remote	--	multiple?	minor	2001	2001	Duck Cr
Y13	Easy	L,J	multiple	major	2007	2007	Gibbon Meadow
Y14	Easy	L,J,A	multiple	major	2007	2007	Hayden Valley

ID code	Access	Toad Life Stage found in 2007	Single or multiple breeding sites	Major or minor breeding site	Year of last toad breeding record	Year that area was last checked for toad breeding	General Location
Y15	Remote	--	single	minor	2004	2004	Buffalo Meadow
Y16	Day	0	single	minor	1997	2007	Fairy Creek
Y17	Easy	L,M	multiple	major	2007	2007	Firehole Lake Area
Y18	Easy	0	single	minor	2005?	2007	Mary Mtn trail
Y19	Easy	L,J	multiple	major	2007	2007	Tangled Creek
Y20	Easy	E,L,M,J,A	single	major	2007	2007	Indian Pond
Y21	Remote	--	multiple	major	2002	2002	Boundary Cr watershed
Y22	Remote	--	single	major	2002	2002	Boundary Cr watershed
Y23	Day	L,M	multiple	major	2007	2007	Lone Star
Y24	Remote	--	single	minor	?	2006	Yellowstone Lakeshore
Y25	Remote	--	single	minor	?	2006	Yellowstone Lakeshore
Y26	Easy	L,A	multiple	major	2007	2007	South Entrance
Y27	Day	L	single	major	2007	2007	Snake Hot Springs
Y28	Remote	--	multiple	major	2003	2003	Heart River
Y29	Remote	0	single	minor	2006	2007	Winter Creek
Y30	Remote	--	single	minor	2006	2006	Mountain Creek

Appendix II: Modeling Data at the Catchment Level for 2007.

Analysis was structured to investigate if the stratifications used in random sampling resulted in real distinctions, using a model selection approach in which the strata are maintained or collapsed. The 2007 catchment-level data were analyzed considering the 6 strata-combinations that were used in sample selection: 3 habitat strata (high, medium, low) and 2 access strata (close, far). Based on the likely similarity of high and medium habitat strata (R. Bennetts, pers comm to W. Gould), consideration also was given to models with only 2 habitat categories (high + medium versus low). Also included were models allowing for variation in detection probability between the primary and secondary surveys.

The models based on these distinctions are considered *a priori* models. A set of 8 candidate models was used. The most general model allowed detection probability to vary between survey occasion (the first and second survey within a year) and occupancy to vary among the 6 strata combinations of habitat quality (3 levels) and access (2 levels).

Information theoretic methods (Burnham and Anderson 2002) were used for evaluating models in the set. With this approach, more than one model may be supported, and model uncertainty is considered when making inferences.

The following steps were taken:

1. Akaike's information criterion with a correction for small samples (AICc) was applied (Burnham and Anderson 2002). Differences in ΔAICc of less than 3 were taken as indicating that the model had substantial support, while AICc weights provided the probability or relative likelihood of the model, given the data. Model uncertainty was indicated when the weights were similar for several high-ranked models, and thus none of the models was clearly superior.

2. Modeling was followed by Goodness of Fit testing, performed for the most general model (the one with the most parameters) to see if model fit was adequate. The parametric bootstrap feature within MARK was used to accomplish this task in which the model deviance and estimated chat were compared to generated values under the most general model.

3. Model averaging was applied to occupancy estimates (for model sets exhibiting uncertainty). This provided estimates of occupancy for each of the 6 strata (e.g. high quality habitat - close access; high – far, etc.).

4. A weighted average of the stratum-specific estimates was computed:

$\overline{\hat{\psi}} = \sum_{h=1}^{L} \frac{N_h}{N} \hat{\psi}_h$ where h indexes each stratum and N_h is the stratum size such that $\sum_{h=1}^{L} N_h = N$.

With variance computed as:

$V\hat{a}r(\overline{\hat{\psi}}) = \sum_{h=1}^{L} \left(\frac{N_h}{N} \right)^2 \left(\frac{N_h - n_h}{N_h} \right) V\hat{a}r(\hat{\psi}_h)$ where n_h represents the number of catchments sampled from stratum h.

Stratum size was obtained from the catchment GIS layer used in Sample Design (total number of catchments = 3370):

Habitat	Access	Number of Catchments
High	Close	92
High	Far	43
Medium	Close	565
Medium	Far	425
Low	Close	1009
Low	Far	1236

In the sections below, we provide the results of modeling, model averaging, and occupancy estimation with inference to GRTE and YELL for 2007. The models were applied to the 2007 catchment-level data for tiger salamanders, boreal chorus frogs, and Columbia spotted frogs. Data for boreal toads were considered too sparse for modeling.

Notations used below:
P or p = detection probability.
(.) = constant, does not change during the analysis period or differ among strata.
(t) = varies survey time, first or second survey.
Psi or Ψ = occupancy.
(hab) = the 3 habitat strata (high, medium, low).
(2hab) = habitat strata collapsed into high+medium and low.
(distance) = the 2 access strata (close or ≤ 4 km from roads; far or > 4 km from roads)
(g) = groups, or the 6 combined strata (3 habitat x 2 access)
AICc = Akaike's information criteria, with small-sample correction.
Delta AICc = AICc differences, relative to the smallest AICc value in the set of models.
AICc Weight = the relative likelihood of the model, given the data. These are normalized to sum to 1 and interpreted as probabilities.
#Par = number of parameters.

Modeling results, Goodness of Fit, model averaging, and application of weighted averages to obtain GRTE and YELL occupancy estimates

I. Tiger Salamanders

A. Model rankings for the 2007 tiger salamander data. Parameter estimates from the 4 top ranked models follow the model rankings.

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	#Par	Deviance
{P(.)psi(2hab)}	60.662	0.00	0.26651	1.0000	3.00	53.834
{P(.)psi(hab)}	60.726	0.06	0.25806	0.9683	4.00	51.298
{P(.)psi(.)}	60.983	0.32	0.22694	0.8515	2.00	56.583
{P(t)psi(hab)}	62.474	1.81	0.10773	0.4042	5.00	50.251
{P(.)psi(distance)}	63.013	2.35	0.08225	0.3086	3.00	56.186
{P(t)psi(distance)}	64.568	3.91	0.03781	0.1419	4.00	55.139

{P(.)psi(2hab2dist)}	65.864	5.20	0.01978	0.0742	5.00	53.641
{P(t)psi(g)}	71.981	11.32	0.00093	0.0035	8.00	49.981

Real Function Parameters of {P(.)psi(2hab)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.7142857	0.1369021	0.4016686	0.9030076
2:Psi	0.3379310	0.0978744	0.1780012	0.5460920
3:Psi	0.2700260E-14	0.3834242E-07	-0.7515115E-07	0.7515115E-07

Real Function Parameters of {P(.)psi(hab)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.7142857	0.1369021	0.4016686	0.9030076
2:Psi	0.5025641	0.1565226	0.2284802	0.7751156
3:Psi	0.2041667	0.1076657	0.0654239	0.4845797
4:Psi	0.2517355E-14	0.3702107E-07	-0.7256130E-07	0.7256131E-07

Real Function Parameters of {P(.)psi(.)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.7142857	0.1369021	0.4016686	0.9030076
2:Psi	0.2969697	0.0881276	0.1558934	0.4913924

Real Function Parameters of {P(t)psi(hab)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.8333333	0.1521452	0.3687380	0.9771683
2:p	0.6250000	0.1711633	0.2848446	0.8745944
3:Psi	0.4923077	0.1519781	0.2274858	0.7615179
4:Psi	0.2000000	0.1051454	0.0645055	0.4754540
5:Psi	0.3046066E-15	0.1274586E-07	-0.2498188E-07	0.2498188E-07

B. **Goodness of Fit.**

Goodness of Fit testing for the most general model indicated no evidence of lack of fit (observed deviance= 49.81 at 75th percentile, average deviance = 42.68: chat = 1.17 or observed chat = 3.12 vs. average chat = 3.29).

C. **Model averaged estimates of occupancy**; tiger salamander 2007.

Occupancy (Psi) HighClose Parameter 13

Model	Weight	Estimate	Standard Error
{P(.)psi(2hab)}	0.26651	0.3379310	0.0978744
{P(.)psi(hab)}	0.25806	0.5025641	0.1565226
{P(.)psi(.)}	0.22694	0.2969697	0.0881276

{P(t)psi(hab)}	0.10773	0.4923077	0.1519781
{P(.)psi(distance)}	0.08225	0.3314010	0.1082191
{P(t)psi(distance)}	0.03781	0.3246377	0.1051618
{P(.)psi(2hab2dist)}	0.01978	0.3629630	0.1162016
{P(t)psi(g)}	0.00093	0.5333333	0.1732763
Weighted Average		0.3873879	0.1181845

Unconditional SE 0.1495225
95% CI for Wgt. Ave. Est. (logit trans.) is 0.1553551 to 0.6849451.
Percent of Variation Attributable to Model Variation is 37.52%

Occupancy (Psi) HighFar Parameter 14

Model	Weight	Estimate	Standard Error
{P(.)psi(2hab)}	0.26651	0.3379310	0.0978744
{P(.)psi(hab)}	0.25806	0.5025641	0.1565226
{P(.)psi(.)}	0.22694	0.2969697	0.0881276
{P(t)psi(hab)}	0.10773	0.4923077	0.1519781
{P(.)psi(distance)}	0.08225	0.2177778	0.1389784
{P(t)psi(distance)}	0.03781	0.2133333	0.1358576
{P(.)psi(2hab2dist)}	0.01978	0.2722222	0.1683065
{P(t)psi(g)}	0.00093	0.3555555	0.2915170
Weighted Average		0.3718750	0.1230151

Unconditional SE 0.1634075
95% CI for Wgt. Ave. Est. (logit trans.) is 0.1306378 to 0.6999308.
Percent of Variation Attributable to Model Variation is 43.33%

Occupancy (Psi) MedClose Parameter 15

Model	Weight	Estimate	Standard Error
{P(.)psi(2hab)}	0.26651	0.3379310	0.0978744
{P(.)psi(hab)}	0.25806	0.2041667	0.1076657
{P(.)psi(.)}	0.22694	0.2969697	0.0881276
{P(t)psi(hab)}	0.10773	0.2000000	0.1051454
{P(.)psi(distance)}	0.08225	0.3314010	0.1082191
{P(t)psi(distance)}	0.03781	0.3246377	0.1051618
{P(.)psi(2hab2dist)}	0.01978	0.3629630	0.1162016
{P(t)psi(g)}	0.00093	0.1939394	0.1248835
Weighted Average		0.2785789	0.1004864

Unconditional SE 0.1171652
95% CI for Wgt. Ave. Est. (logit trans.) is 0.1096635 to 0.5476400
Percent of Variation Attributable to Model Variation is 26.44%

Occupancy (Psi) MedFar Parameter 16

Model	Weight	Estimate	Standard Error
{P(.)psi(2hab)}	0.26651	0.3379310	0.0978744
{P(.)psi(hab)}	0.25806	0.2041667	0.1076657
{P(.)psi(.)}	0.22694	0.2969697	0.0881276
{P(t)psi(hab)}	0.10773	0.2000000	0.1051454
{P(.)psi(distance)}	0.08225	0.2177778	0.1389784
{P(t)psi(distance)}	0.03781	0.2133333	0.1358576

{P(.)psi(2hab2dist)}	0.01978	0.2722222	0.1683065
{P(t)psi(g)}	0.00093	0.2133333	0.1914725
Weighted Average		0.2632491	0.1052691

Unconditional SE 0.1213387
95% CI for Wgt. Ave. Est. (logit trans.) is 0.0948875 to 0.5491084
Percent of Variation Attributable to Model Variation is 24.73%

Occupancy (Psi) LowClose Parameter 17

Model	Weight	Estimate	Standard Error
{P(.)psi(2hab)}	0.26651	0.0000000	0.0000000
{P(.)psi(hab)}	0.25806	0.0000000	0.0000000
{P(.)psi(.)}	0.22694	0.2969697	0.0881276
{P(t)psi(hab)}	0.10773	0.0000000	0.0000000
{P(.)psi(distance)}	0.08225	0.3314010	0.1082191
{P(t)psi(distance)}	0.03781	0.3246377	0.1051618
{P(.)psi(2hab2dist)}	0.01978	0.0000000	0.0000000
{P(t)psi(g)}	0.00093	0.0000000	0.0000000
Weighted Average		0.1069241	0.0328761

Unconditional SE 0.1572971
95% CI for Wgt. Ave. Est. (logit trans.) is 0.0047204 to 0.7513893
Percent of Variation Attributable to Model Variation is 95.63%

Occupancy (Psi) LowFar Parameter 18

Model	Weight	Estimate	Standard Error
{P(.)psi(2hab)}	0.26651	0.0000000	0.0000000
{P(.)psi(hab)}	0.25806	0.0000000	0.0000000
{P(.)psi(.)}	0.22694	0.2969697	0.0881276
{P(t)psi(hab)}	0.10773	0.0000000	0.0000000
{P(.)psi(distance)}	0.08225	0.2177778	0.1389784
{P(t)psi(distance)}	0.03781	0.2133333	0.1358576
{P(.)psi(2hab2dist)}	0.01978	0.0000000	0.0000000
{P(t)psi(g)}	0.00093	0.0000000	0.0000000
Weighted Average		0.0933709	0.0365665

Unconditional SE 0.1447968
95% CI for Wgt. Ave. Est. (logit trans.) is 0.0035911 to 0.7463776
Percent of Variation Attributable to Model Variation is 93.62%

D. **Application of weighted averages to compute estimated occupancy for tiger salamanders in GRTE and YELL**.

There are 5 models that have some support (ΔAIC <3). Model averaged estimates of occupancy (see above) for the 6 habitat-access strata arranged from high quality-close access to low quality-far access are 0.3873879 (SE =0.1495225), 0.3718750 (SE=0.1634075), 0.2785789 (SE=0.1171652), 0.2632491 (SE=0.1213387), 0.1069241 (SE=0.1572971), and 0.0933709 (SE=0.1447968).

Estimated occupancy for GRTE and YELL is

$$\bar{\hat{\psi}} = 0.387(92/3370) + 0.372(43/3370) + 0.279(565/3370) + 0.263(425/3370) +$$
$$0.107(1009/3370) + 0.093(1236/3370) = 0.161 \qquad (SE = 0.075)$$

This estimate differs from that assuming the 3rd ranked model is correct, in which occupancy is assumed to be constant (and estimated as 0.297). Model averaging allows one to incorporate the model uncertainty into the estimation process as well as providing unconditional variance estimation.

II. Boreal Chorus Frog

A. Model rankings for the 2007 boreal chorus frog data. Parameter estimates from the 6 top ranked models follow the model rankings.

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	#Par	Deviance
{p(.)psi(.)}	58.609	0.00	0.26467	1.0000	2.00	54.209
{p(.)psi(2habonly)}	59.044	0.44	0.21292	0.8045	3.00	52.216
{p(t)psi(.)}	59.650	1.04	0.15725	0.5941	3.00	52.823
{p(t)psi(2hab)}	60.259	1.65	0.11600	0.4383	4.00	50.830
{p(.)psi(habitat)}	60.968	2.36	0.08134	0.3073	4.00	51.540
{p(.)psi(distance)}	61.002	2.39	0.08000	0.3023	3.00	54.174
{p(t)psi(distance)}	62.217	3.61	0.04358	0.1647	4.00	52.788
{p(t)psi(hab)}	62.376	3.77	0.04024	0.1520	5.00	50.154
{p(.)psi(g)}	67.581	8.97	0.00298	0.0113	7.00	49.101
{p(t)psi(g)}	69.715	11.11	0.00103	0.0039	8.00	47.715

Real Function Parameters of {p(.)psi(.)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.9729730	0.0270172	0.8277566	0.9963056
2:Psi	0.5761785	0.0861010	0.4051702	0.7307002

Real Function Parameters of {p(.)psi(2habonly)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.9729730	0.0270172	0.8277566	0.9963056
2:Psi	0.6211434	0.0901727	0.4361903	0.7765106
3:Psi	0.2501827	0.2166649	0.0335236	0.7624448

Real Function Parameters of {p(t)psi(.)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	0.9473684	0.0512278	0.7061303	0.9926383
2:p	1.0000000	0.3512237E-08	1.0000000	1.0000000
3:Psi	0.5757576	0.0860340	0.4049280	0.7302194

Real Function Parameters of {p(t)psi(hab2g)}

95% Confidence Interval

Parameter	Estimate	Standard Error	Lower	Upper
1:p	0.9473684	0.0512278	0.7061303	0.9926383
2:p	1.0000000	0.0000000	1.0000000	1.0000000
3:Psi	0.6206897	0.0901022	0.4359466	0.7760132
4:Psi	0.2500000	0.2165064	0.0335101	0.7621677

Real Function Parameters of {p(.)psi(habitat)}

			95% Confidence Interval	
Parameter	Estimate	Standard Error	Lower	Upper
1:p	0.9729730	0.0270172	0.8277566	0.9963056
2:Psi	0.5388551	0.1383675	0.2817784	0.7768020
3:Psi	0.6880026	0.1159672	0.4333759	0.8640902
4:Psi	0.2501827	0.2166649	0.0335236	0.7624447

Real Function Parameters of {p(.)psi(distance)}

			95% Confidence Interval	
Parameter	Estimate	Standard Error	Lower	Upper
1:p	0.9729730	0.0270172	0.8277566	0.9963056
2:Psi	0.5656306	0.1034454	0.3632735	0.7482453
3:Psi	0.6004386	0.1550351	0.2974876	0.8420920

B. Goodness of Fit.

Goodness of Fit testing for the most general model indicated no evidence of lack of fit (observed deviance= 47.695 at 80th percentile, average deviance = 40.94: chat = 1.16 or observed chat = 4.77 vs. average chat = 5.89).

C. Model averaged estimates of occupancy; boreal chorus frog 2007.

Occupancy (Psi) HighClose Parameter 13

Model	Weight	Estimate	Standard Error
{p(.)psi(.)}	0.26467	0.5761785	0.0861010
{p(.)psi(hab2g)}	0.21292	0.6211434	0.0901727
{p(t)psi(.)}	0.15725	0.5757576	0.0860340
{p(t)psi(hab2g)}	0.11600	0.6206897	0.0901022
{p(.)psi(habitat)}	0.08134	0.5388551	0.1383675
{p(.)psi(distance)}	0.08000	0.5656306	0.1034454
{p(t)psi(distance)}	0.04358	0.5652174	0.1033665
{p(t)psi(hab)}	0.04024	0.5384615	0.1382642
{p(.)psi(g)}	0.00298	0.5003655	0.1582311
{p(t)psi(g)}	0.00103	0.5000000	0.1581139
Weighted Average		0.5846699	0.0962011

Unconditional SE 0.1017038

95% CI for Wgt. Ave. Est. (logit trans.) is 0.3825062 to 0.7618541

Percent of Variation Attributable to Model Variation is 10.53%

Occupancy (Psi) HighFar Parameter 14

Model	Weight	Estimate	Standard Error
{p(.)psi(.)}	0.26467	0.5761785	0.0861010
{p(.)psi(hab2g)}	0.21292	0.6211434	0.0901727
{p(t)psi(.)}	0.15725	0.5757576	0.0860340
{p(t)psi(hab2g)}	0.11600	0.6206897	0.0901022
{p(.)psi(habitat)}	0.08134	0.5388551	0.1383675
{p(.)psi(distance)}	0.08000	0.6004386	0.1550351
{p(t)psi(distance)}	0.04358	0.6000000	0.1549193
{p(t)psi(hab)}	0.04024	0.5384615	0.1382642
{p(.)psi(g)}	0.00298	0.6671540	0.2723662
{p(t)psi(g)}	0.00103	0.6666667	0.2721655
Weighted Average		0.5896385	0.1030321 Unconditional SE 0.1102096

95% CI for Wgt. Ave. Est. (logit trans.) is 0.3704550 to 0.7782000
Percent of Variation Attributable to Model Variation is 12.60%

Occupancy (Psi) MedClose Parameter 15

Model	Weight	Estimate	Standard Error
{p(.)psi(.)}	0.26467	0.5761785	0.0861010
{p(.)psi(hab2g)}	0.21292	0.6211434	0.0901727
{p(t)psi(.)}	0.15725	0.5757576	0.0860340
{p(t)psi(hab2g)}	0.11600	0.6206897	0.0901022
{p(.)psi(habitat)}	0.08134	0.6880026	0.1159672
{p(.)psi(distance)}	0.08000	0.5656306	0.1034454
{p(t)psi(distance)}	0.04358	0.5652174	0.1033665
{p(t)psi(hab)}	0.04024	0.6875000	0.1158781
{p(.)psi(g)}	0.00298	0.6368288	0.1451497
{p(t)psi(g)}	0.00103	0.6363636	0.1450407
Weighted Average		0.6033466	0.0934257 Unconditional SE 0.1015199

95% CI for Wgt. Ave. Est. (logit trans.) is 0.3984308 to 0.7774497
Percent of Variation Attributable to Model Variation is 15.31%

Occupancy (Psi) MedFar Parameter 16

Model	Weight	Estimate	Standard Error
{p(.)psi(.)}	0.26467	0.5761785	0.0861010
{p(.)psi(hab2g)}	0.21292	0.6211434	0.0901727
{p(t)psi(.)}	0.15725	0.5757576	0.0860340
{p(t)psi(hab2g)}	0.11600	0.6206897	0.0901022
{p(.)psi(habitat)}	0.08134	0.6880026	0.1159672
{p(.)psi(distance)}	0.08000	0.6004386	0.1550351
{p(t)psi(distance)}	0.04358	0.6000000	0.1549193
{p(t)psi(hab)}	0.04024	0.6875000	0.1158781
{p(.)psi(g)}	0.00298	0.8005848	0.1790200
{p(t)psi(g)}	0.00103	0.8000000	0.1788854
Weighted Average		0.6083031	0.0999351 Unconditional SE 0.1093067

95% CI for Wgt. Ave. Est. (logit trans.) is 0.3872311 to 0.7923807
Percent of Variation Attributable to Model Variation is 16.41%

Occupancy (Psi) LowClose Parameter 17

Model	Weight	Estimate	Standard Error
{p(.)psi(.)}	0.26467	0.5761785	0.0861010
{p(.)psi(hab2g)}	0.21292	0.2501827	0.2166649
{p(t)psi(.)}	0.15725	0.5757576	0.0860340
{p(t)psi(hab2g)}	0.11600	0.2500000	0.2165064
{p(.)psi(habitat)}	0.08134	0.2501827	0.2166649
{p(.)psi(distance)}	0.08000	0.5656306	0.1034454
{p(t)psi(distance)}	0.04358	0.5652174	0.1033665
{p(t)psi(hab)}	0.04024	0.2500000	0.2165063
{p(.)psi(g)}	0.00298	0.5003655	0.3538126
{p(t)psi(g)}	0.00103	0.5000000	0.3535534
Weighted Average		0.4275957	0.1480978 Unconditional SE 0.2279085

95% CI for Wgt. Ave. Est. (logit trans.) is 0.1074803 to 0.8225041
Percent of Variation Attributable to Model Variation is 57.77%

Occupancy (Psi) LowFar Parameter 18

Model	Weight	Estimate	Standard Error
{p(.)psi(.)}	0.26467	0.5761785	0.0861010
{p(.)psi(hab2g)}	0.21292	0.2501827	0.2166649
{p(t)psi(.)}	0.15725	0.5757576	0.0860340
{p(t)psi(hab2g)}	0.11600	0.2500000	0.2165064
{p(.)psi(habitat)}	0.08134	0.2501827	0.2166649
{p(.)psi(distance)}	0.08000	0.6004386	0.1550351
{p(t)psi(distance)}	0.04358	0.6000000	0.1549193
{p(t)psi(hab)}	0.04024	0.2500000	0.2165063
{p(.)psi(g)}	0.00298	0.0000000	0.0000000
{p(t)psi(g)}	0.00103	0.0000000	0.0000000
Weighted Average		0.4298911	0.1530538 Unconditional SE 0.2347971

95% CI for Wgt. Ave. Est. (logit trans.) is 0.1033983 to 0.8313790
Percent of Variation Attributable to Model Variation is 57.51%

D. **Application of weighted averages to compute estimated occupancy for chorus frogs in GRTE and YELL**.

The 2007 PSMA analysis resulted in lack of strong support for any particular model. There are 6 models that have some support (ΔAIC <3). Model averaged estimates of occupancy (above) for the 6 habitat-access strata arranged from high quality-close access to low quality-far access are 0.5846699 (SE=0.1017038), 0.5896385 (SE=0.1102096), 0.6033466 (SE=0.1015199), 0.6083031 (SE=0.1093067), 0.4275957 (SE=0.2279085), and 0.4298911 (SE=0.2347971).

Estimated occupancy for GRTE and YELL is

$$\overline{\hat{\psi}} = 0.585(92/3370) + 0.590(43/3370) + 0.603(565/3370) + 0.608(425/3370) + 0.427(1009/3370) + 0.430(1236/3370) = 0.487 \qquad (SE = 0.112)$$

This estimate differs from that assuming the 1st ranked model is correct, in which occupancy is assumed to be constant (and estimated as 0.576). Model averaging allows one to incorporate the model uncertainty into the estimation process as well as providing unconditional variance estimation.

III. Columbia spotted frogs

A. Model rankings for the 2007 Columbia spotted frog data. Parameter estimates from the 2 top ranked models follow the model rankings.

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	#Par	Deviance
{p(.)psi(2hab)}	42.751	0.00	0.63080	1.0000	3.0000	35.924
{p(.)psi(hab)}	44.644	1.89	0.24487	0.3882	4.0000	35.215
{p(t)psi(hab)}	47.438	4.69	0.06058	0.0960	5.0000	35.215
{p(.)psi(.}	48.652	5.90	0.03301	0.0523	2.0000	44.252
{p(.)psi(distance)}	50.411	7.66	0.01370	0.0217	3.0000	43.583
{p(t)psi(.}	51.079	8.33	0.00981	0.0156	3.0000	44.252
{p(t)psi(distance)}	53.012	10.26	0.00373	0.0059	4.0000	43.583
{p(.)psi(g)}	53.458	10.71	0.00299	0.0047	7.0000	34.978
{p(t)psi(g)}	56.978	14.23	0.00051	0.0008	8.0000	34.978

Real Function Parameters of {p(.)psi(2hab)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	1.0000000	0.2356080E-08	1.0000000	1.0000000
2:Psi	0.6896552	0.0859091	0.5029475	0.8299433
3:Psi	0.4088797E-15	0.1429825E-07	-0.2802456E-07	0.2802456E-07

Real Function Parameters of {p(.)psi(hab)}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:p	1.0000000	0.0000000	1.0000000	1.0000000
2:Psi	0.7692308	0.1168545	0.4784491	0.9237344
3:Psi	0.6250000	0.1210307	0.3772319	0.8209747
4:Psi	0.2753602E-15	0.1173372E-07	-0.2299809E-07	0.2299809E-07

P(t) models estimated both P at 1.0 so did not include.

B. Goodness of Fit.
Goodness of Fit testing for the most general model indicated no evidence of lack of fit (observed deviance= 34.97 at 75th percentile, average deviance = 30.24: chat = 1.16 or observed chat = 8.74 vs. average chat = 7.56).

C. Model averaged estimates of occupancy; Columbia spotted frog 2007.
Only 2 models are reasonably well supported. In this case, the top model was selected given its substantial support, and similarity to the second-ranked model. A model averaged estimate of occupancy would have produced an estimate similar to the top model (psi = 0.69)

D. **Application of weighted averages to compute estimated occupancy for Columbia spotted frogs in GRTE and YELL**.

$$\overline{\hat{\psi}} = 0.689(1125/3370) + 0.0(2245/3370) = 0.230$$

With estimated variance = $V\hat{a}r(\overline{\hat{\psi}}) = \left(\frac{1125}{3370}\right)^2\left(\frac{1125-29}{1125}\right)0.086^2 + \left(\frac{2245}{3370}\right)^2\left(\frac{2245-4}{2245}\right)0.00^2 =$ 0.0008030 (SE= 0.028).

Note the difference in this estimate as compared to the estimated occupancy of 0.606 with estimated SE = 0.085 when assuming occupancy is constant among habitat types. In cases where stratum-specific estimates are strongly supported, these differences will occur and are significant. Note the improvement in precision in this case as well with the habitat-specific model. Estimates reported in the 2006 annual report assume no differences among strata. These estimates might be biased toward the high and/or medium quality strata unless the constant occupancy model is strongly supported.

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Appendix III: USGS Pathologist Report

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DIAGNOSTIC SERVICES CASE REPORT

CASE: 20426 | Final Report | 5/5/2008

EPIZOO: 2007-113

Legal ☐ **INV#:** | Declassified ☐

Submitter:
MS. DEBRA PATLA
GYE Amphibian Monitoring Project
PO Box 420
Moran, WY 83013

Date Submitted: 9/5/2007

Specimen description/identification/Location.

AC	SPECIES	SPECIMEN TYPE	BAND NUMBER	SUBMITTER's ID	COUNTY	STATE
001	Frog, Columbia Spotted	CARCASS			TETON	WY
002	Frog, Columbia Spotted	CARCASS			TETON	WY
003	Frog, Columbia Spotted	CARCASS			TETON	WY
004	Frog, Columbia Spotted	CARCASS			TETON	WY
005	Frog, Columbia Spotted	CARCASS			TETON	WY
006	Frog, Columbia Spotted	CARCASS			TETON	WY

DIAGNOSIS

1. Ranavirus infection (culture confirmed) in 4 of 4 Columbia spotted frogs (001-004).
2. Liver: Necrotizing hepatitis with inclusion bodies typical of ranavirus infection in 2 of 6 frogs (002, 005).
3. Spleen: Necrotizing splenitis suggestive of ranavirus infection in 1 of 6 frogs (003).
4. Skin: Necrotizing dermatitis with vasculitis suggestive of ranavirus infection in 1 of 6 frogs (003).
5. Liver: Mild hepatomegaly (enlargement) suggestive of ranavirus infection in 2 of 6 frogs (002, 003).
6. Skin of back: Single or multiple ulcers suggestive of ranavirus infection in 2 of 6 frogs (001, 003).
7. Spleen: Mild splenomegaly (enlargement) suggestive of ranavirus infection in 1 of 6 frogs (002).
8. Body cavity: Mild hydrocoelom (fluid accumulation) consistent with ranavirus infection in 2 of 6 frogs (002, 003).
9. Skin: Chytridiomycosis (infection by Batrachochytrium dendrobatidis) in 3 of 6 frogs (002-004).
10. Limb malformation: Right hindlimb hemimelia suggestive of infection by Ribeiroia metacercariae in 1 of 6 frogs (001).
11. Limb malformation: Right hindlimb skin web at knee joint characteristic of infection by Ribeiroia metacercariae in 1 of 6 frogs (001)
12. Head: Mulitple punctures suggestive of bite wounds (with scavenging of snout) in 1 of 6 frogs (004).
13. Renal parasites: Unidentified encysted metacercariae typical of Echinostoma sp. in 5 of 6 frogs (001-004, 006).
14. Whole body: Mild to advanced autolysis in all frogs with probable postmortem watermold growth on skin in 2 of 6 frogs (001, 005).

PRINTED May 16, 2008

EPIZOO: 2007-113

Legal INV#: Declassified ☐

Comment:

We are pleased to confirm your field diagnosis of ranavirus infection as the cause of death in several of these juvenile Columbia Spotted Frogs. The immediate cause of death in 4 of 6 frogs in this group (001-003 and 005) is attributed to ranavirus infection which was confirmed by isolation of the virus from each of the 4 frozen frogs (001-004). One frozen frog from which ranavirus was isolated in cultures may have been killed by a predator because it had multiple punctures (bite wounds) on its head and much of its snout had been eaten. Ranaviral infection was suspected in one ethanol-fixed frog (005) whose tissues could not be cultured based on characteristic histological abnormalities in its liver (Diagnosis #2, above). Autolysis (decomposition) in one fixed frog (006) was so advanced that characteristic abnormalities of ranavirus infection, if present, could not be detected in histological sections.

Abnormalities in the frozen frogs (001-004) that were seen at necropsy and usually are associated with ranaviral infections included hydrocoelom, skin ulcers, mild enlargement of the liver (hepatomegaly) and mild enlargement of the spleen (splenomegaly) in 2 of 6 frogs (Diagnoses 5 - 8, above).

Histological examinations of all frogs was complicated by varying degrees of decomposition of the organs, but viral hepatitis was detected in 2 frogs (Diagnosis #2, above), and necrosis of the skin and spleen in 1 frog (Diagnoses 3 & 4, above).

Chytridiomycosis (infection by *Batrachochytrium dendrobatidis*, **"BD"**) was detected histologically in skin of the pelvic patch in 3 of 6 frogs (Diagnosis #9, above). Swabs of the toe web skin were collected on frozen frogs 001-004 for PCR tests for "BD." Results of the PCR tests for BD were positive only for frog 002. This group of frogs is one of the few cases in which detection of BD was more efficient by histology than PCR. It is suspected that autolysis (decomposition) and sloughing of the skin from the feet of these frogs had occurred in the field; therefore, the toe web swabs did not capture any BD-infected epidermal cells for the PCR tests. The chytrid infections in all frogs were mild, and it is unlikely the BD infections contributed to their deaths.

Incidental findings in these frogs included limb malformations that were consistent with those caused by metacercariae of *Ribeiroia ondatrae* in one frog (Diagnoses 10 & 11, above) and other species of common encysted metacercaria in the mesonephroi ("kidneys") of 5 of 6 frogs (Diagnosis #13, above).

In summary, the cause of death in many frogs in this group and the likely cause of the die-off at this site was ranavirus infection. This case is unusual (although seen in previous years in amphibians in and around Yellowstone NP) because the infected frogs were juveniles. In the USA, most die-offs are due to ranavirus affected tadpoles and amphibians in active metamorphosis. Infrequent cases of ranavirus infection in adult Tree Frogs, characterized by multiple small skin ulcers, have been seen in southern states, but usually are not associated with the death of the frog. Hence, this die-off and previous die-offs in Yellowstone NP are worthy of publication as a potentially "atypical" form of the infection. However, before these ranaviral die-offs in juvenile frogs can be reported as "atypical", it may be necessary to repeatedly survey tadpoles at the site to determine if "typical" die-offs also affect larvae. While these ranavirus-caused die-offs may be common for the species, they have not yet been seen or reported in other populations of *R. luteiventris* beyond the Greater Yellowstone Ecosystem. Another aspect of these die-offs in Yellowstone that needs to be investigated by our Center is whether the strain of ranavirus is different from isolates from other amphibians nationwide. There is a remote possibility that the ranavirus in these spotted frogs is an introduced strain from Europe, because in Europe, most reports of fatal ranavirus infections occur in adult European frogs rather than larvae. Another likely explanation for "atypical" fatal ranavirus infections in juvenile frogs from Yellowstone is that these populations may be completely naive to all ranaviruses, and therefore, suffer unusually severe fatal infections when exposed. If these populations are naive and unusually susceptible to ranavirus infection, then the ranavirus in these spotted frogs may have come from some other location in the US where it is endemic.

RECOMMENDATIONS: This case and previous amphibian die-offs due to ranavirus infection in Yellowstone are worthy of publication. But before the virus and die-offs can be characterized as "atypical", it would be desirable to perform molecular comparisons to other isolates from the US (limited molecular procedures can be done at our Center) and to determine whether "typical" fatal ranaviral infections also occur in tadpoles of this species. If fatal ranaviral infections also occur in tadpoles in Yellowstone, then it is likely the species or population is naive and unusually susceptible to infection at most life stages. Our Center would welcome submission of any sick or freshly dead tadpoles from this site for further diagnostic examinations. In order to obtain isolates of the virus in cultures, freshly dead tadpoles should be submitted frozen. Also, we welcome submission of additional sick and freshly dead juvenile frogs from any site where mortalities are occurring.

ATTACHMENT: Color Photos

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EPIZOO: 2007-113

Legal ☐ INV#: Declassified ☐

/s/ D. Earl Green

DAVID EARL GREEN DVM, DACVP

Staff Diagnostic Pathologist

If you have questions regarding this case, contact: /s/ Nathan G. Ramsay

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Diagnostic findings may not be used for publication without the pathologist's knowledge and consent.

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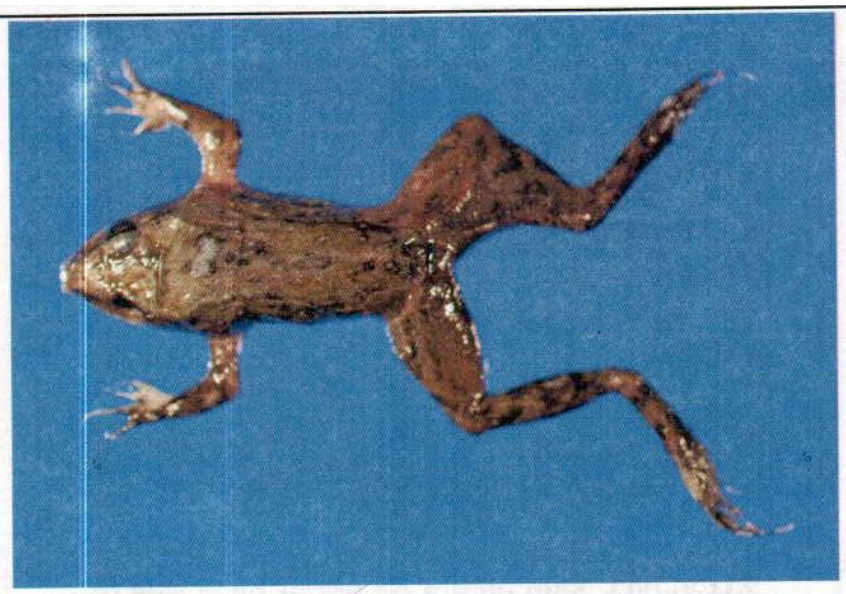
Initial molecular investigations of the virus infecting the 2007 specimens from Lodge Creek indicate that it is closely related to the American Frog Virus-3, which is widespread in North America and probably native to the USA. This eliminates the concern that the ranavirus at the site could be an introduced strain from Europe. (Email communication from David E. Green, 7 August 2008)



20426-001, Hemimelia & skin web on right hindlimb; Skin ulcer (Ranavirus infection) on upper back. Columbia Spotted Frog (*Rana luteiventris*)

20426-001, Hemimelia & skin web on right hindlimb, characteristic of infection by Ribeiroia metacercaria. Columbia Spotted Frog (*Rana luteiventris*)

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20426-003, Skin ulcers on upper back due to Ranavirus infection.
Columbia Spotted Frog (*Rana luteiventris*)

20426-003, Close-up photo of one skin ulcer due to Ranavirus infection (skin culture positive).
Columbia Spotted Frog (*Rana luteiventris*)

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The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

NPS D-1303, January 2009

National Park Service
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