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Final Report

Determining the Effects of Livestock Grazing on Yosemite Toads (*Bufo canorus*) and Their Habitat: An Adaptive Management Study

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1. Executive Summary

This study was designed to provide guidance to managers about the role livestock grazing in montane meadows may play in the decline of Yosemite toad. U.S. Forest Service Region 5 staff formulated two questions for the study to address: 1) Does livestock grazing under Forest/Sierra Nevada Forest Plan Amendment Riparian Standards and Guidelines have a measurable effect on Yosemite toad populations? 2) What are the effects of livestock grazing intensity on key habitat components that affect survival and recruitment of Yosemite toad populations? Key habitat components may include hydrology, water quality, and cover. The overall hypothesis is that Yosemite toad and their montane meadow breeding/rearing habitat are declining due to grazing impacts, and a reduction in grazing intensity will halt/reverse that decline.
In 2005, we initiated two field experiments to address these questions. The first experiment was designed to test for differential toad and breeding pool habitat response to three meadow grazing treatments on the Stanislaus and Sierra National Forests. Data available for this report reflect 4 years of treatment implementation on 14 meadows over 4 years (2006-2009). Treatments were: 1) Grazing in accordance with USFS Region 5 Riparian Standards and Guidelines 120, 121 across the entire meadow (**GRZ** treatment); 2) Exclusion of livestock from breeding (i.e., wet) areas within a meadow (S&G 53; **FBA** treatment); and 3) No grazing within the meadow (**FWM** treatment). Statistical analysis (response ~ year + treatment + year × treatment) was combined with comparison of expected versus observed responses to evaluate treatment effects.

Yosemite toad young of the year density and breeding pool occupancy patterns observed over the first 4 study years do not support the study hypothesis. There was no detectable grazing treatment effect on young of the year density and breeding pool occupancy, nor on breeding pool water quality and cover. In addition to showing no apparent benefit to Yosemite toads, partial meadow fencing increased risk of potential meadow degradation by concentrating grazing in un-fenced portions of meadows; therefore, we do not recommend partial meadow fencing. All nutrient concentration means were low in breeding pools and ranges were narrow regardless of treatment. Lentic (non-flowing) pools were more consistently occupied over the study period by breeding toads than lotic (flowing) pools. Lentic (most occupied) pools were significantly ($P < 0.05$) more nutrient enriched, warmer, and shallower than lotic (flowing) pools.

The second experiment was a longitudinal survey of toad occupancy across 24 meadows, representing a gradient of meadow wetness and ambient cattle grazing intensity on the Sierra National Forest. This

experiment examined the hydrology, cattle utilization, and Yosemite toad occupancy rates of these meadows to simultaneously examine two potential drivers of meadow occupancy by toads: 1) cattle grazing intensity; and 2) relative meadow wetness (i.e., hydrology). The 3 year experiment was initiated in 2006 and continued through 2008. Bayesian structural equation modeling was used to evaluate the relative effects of grazing intensity and meadow wetness on meadow occupancy by toads, as well as the effect of wetness on grazing intensity.

A direct correlation between the intensity of cattle use and toad occupancy of meadows was not found for any portion of the grazing season (early, mid, late). Results strongly indicate that toad presence is driven by meadow wetness (hydrology), rather than cattle utilization. Yosemite toad occupancy rates of meadows increased with meadow wetness, likely driven by toad preference for breeding/rearing habitat associated with wetter meadows. Cattle use of meadows over the grazing season is driven by selection for the nutritious diet associated with plant communities found in drier meadows. Given that meadow wetness was the major determinant of toad occupancy, attention needs to be given to contemporary factors directly impacting meadow wetness (i.e., meadow hydrologic restoration, climate and fire regime changes, conifer encroachment). Properly implemented, USFS Region 5 Riparian Standards and Guidelines 120 and 121 are designed to protect and enhance meadow hydrologic function.

2. Background

The Yosemite toad (*Bufo canorus*) is endemic to the Sierra Nevada mountain range from the Blue Lakes region north of Ebbetts Pass in Alpine County south to Kaiser Pass area in the Evolution Lake/Darwin Canyon region of Fresno Co at elevations from ca. 1950 m ca. 3600m (6400 to 11,800 ft). Yosemite toads are typically associated with high montane and subalpine vegetation in relatively open wet meadows surrounded by forests of lodgepole pine or whitebark pine and are primarily active during the late spring, summer, and early fall (Zeiner et. al. 1988, Jennings and Hayes 1994).

Yosemite toads breed in late spring, usually at snowmelt, laying eggs in shallow areas of lakes and wet meadows; larvae metamorphose by mid-late summer of the same year. Breeding is often 1-2 weeks, but can last up to 5 weeks for males; females often only visit breeding sites for 2-3 days (Kagarise et al. 1993, Sadinsky 2004). Meadow water depth and water temperature appear to be important limiting factors in the survival of eggs and larvae (Kagarise et al. 1993). These characteristics are strongly influenced by winter snow pack, spring temperatures, and meadow topography/hydrology, but direct relationships to toad habitat use and egg/larval survival have not been quantified.

Yosemite toads are a Species of Special Concern in California, a Forest Service Region 5 sensitive species, and a candidate species for federal listing under the Endangered Species Act (USDI Fish and Wildlife Service 2002, 2004). Factors that could be responsible for the decline of Yosemite toads include livestock grazing in montane meadows and riparian zones, airborne chemical toxins, disease, and climatic shifts and variability, though none of these has emerged as a singularly strong candidate (Davidson et al. 2002, USDI Fish and Wildlife Service 2002).

Of the activities occurring on National Forest lands and under the jurisdiction of Forest Service management, livestock and packstock grazing have been identified as activities that may affect Yosemite toads (USDA Forest Service 2001). This is because of the potential overlap of grazing with toad breeding and rearing areas in wet meadows. Though cattle are rarely present during the brief breeding period at snow-melt, they are often present during larval rearing and metamorphosis. While this potential risk factor has been identified in the public record, the supporting data are primarily from anecdotal accounts and unpublished sources (USDI Fish and Wildlife Service 2002).

3. Study Purpose and Approach

This study was designed to provide guidance to managers about the role livestock grazing in montane meadows may play in the decline of Yosemite toad. The overall study hypothesis is that Yosemite toad and meadow breeding/rearing habitat are declining due to direct and indirect impacts of grazing on the toad and its habitat, and that reduction in grazing intensity will halt and/or reverse that decline. At the initiation of this study, USFS Region 5 leadership and staff formulated two guiding questions relative to this hypothesis.

Question 1: Does livestock grazing under Forest/Sierra Nevada Forest Plan Amendment Riparian Standards and Guidelines have a measurable effect on Yosemite toad populations?

Question 2: What are the effects of livestock grazing intensity on the key habitat components that affect survival and recruitment of Yosemite toad populations? Key habitat components may include hydrology, water quality, and cover.

In 2005, we initiated two complementary field experiments to address these questions in the context of the overall study hypothesis. The study was conducted on the Sierra and Stanislaus National Forests. One experiment (Phase II) was a manipulation of meadow grazing management using three different fencing treatments proposed and implemented by USFS staff. This experiment was designed to specifically test for differential toad and breeding pool habitat response to three distinct meadow grazing treatments imposed on study meadows over time. This experiment was initiated in 2005 with treatment implementation and data collection continuing through 2010.

The second experiment (Phase I) was a longitudinal survey of toad occupancy across 24 meadows, representing a gradient of meadow wetness and ambient cattle grazing intensity. This experiment examined the hydrology, cattle utilization, and Yosemite toad occupancy of these meadows to simultaneously examine two potential drivers of meadow occupancy by toads: 1) cattle grazing intensity; and 2) meadow wetness. This experiment was initiated in 2006 and continued through 2008.

This report focuses on analyses and results from these two experiments (Phase I and Phase II). Complete methods and study design descriptions can be found in the attached Study Plan (Appendix A). Data available for analysis at the time of this report covered the period 2006 through 2009 (Year 1 through 4). For Phase II analyses in this report, toad response variables were young of year (YOY) and breeding pool occupancy (# occupied pools). At the time of this report, PSW investigators were continuing to examine these and additional toad population metrics for treatment response over 5 years of treatment implementation (2006-2010).

4. Meadow grazing manipulation experiment (Phase II)

4.1. Phase II Design, Treatments, and Hypothesis

Phase II study design is outlined in the Study Plan (Appendix A). Briefly, we used a randomized complete block (RCBD) experimental design with five allotments (blocks) and three meadow grazing management treatments randomized within each allotment. Treatments were:

1) Grazing in accordance with Riparian S&Gs 120, 121 across the entire meadow (**GRZ** treatment)
2) Exclusion of livestock from breeding (i.e., wet) areas within a meadow (S&G 53; **FBA** treatment)
3) No grazing within the meadow (**FWM** treatment)

Two allotments on the Stanislaus National Forest and three allotments on the Sierra National Forest were included in this study. Originally, seventeen meadows across these allotments were enrolled in the study; however, two were excluded from analysis due to atypical grazing management history (i.e., livestock gathering locations). One additional meadow was excluded from analysis due to incorrect treatment implementation during several years. The dataset available for analysis at the time of this report was thus an unbalanced RCBD with all treatments present in 4 allotments, but only 2 treatments present in one allotment (Herring Creek Allotment, Stanislaus NF lacked the no grazing within meadow treatment (FWM)).

The refined hypothesis tested by analysis reported below is that *over the course of treatment implementation (2006 through 2009), Yosemite toad numbers and habitat quality will increase in fenced meadow treatments (FBA and FWM) relative to grazed meadows (GRZ).* The grazing treatment (GRZ) serves as the control treatment in this experiment, to which the meadow fencing treatments (FBA and FWM) are compared over the course of the experiment (years). Figure 1 conceptualizes the hypothesis, illustrating the **relative** trends in toad population and habitat conditions we expect as years of grazing management treatment implementation progress (2006=year 1, 2009=year4). Over the course of treatment implementation we expect a reduction in Yosemite toad numbers and habitat conditions in grazed (**GRZ**) meadows relative to fenced meadows. In meadows with only breeding areas fenced (**F**ence **B**reeding **A**rea; **FBA**), we expect to see some benefit to toad populations and habitat, while in completely fenced meadows (**F**ence **W**hole **M**eadow; **FWM**) we expect to see maximum benefit to toad populations and habitat.

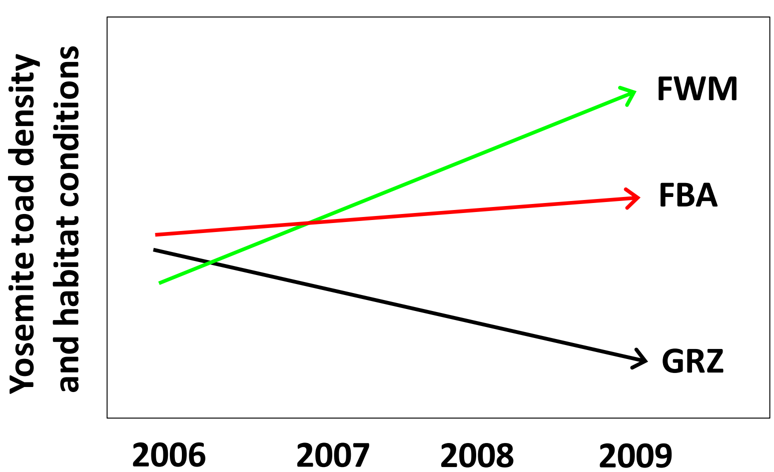

Figure 1. Hypothesized responses of Yosemite toad density or habitat conditions to GRZ, FBA, and FWM treatments.

4.2. Phase II Treatment Implementation

Average annual utilization in grazed meadows (GRZ) ranged from 10-48%; allowable use as outlined in the Sierra Nevada Forest Plan Amendment (2004) is 40%. We consistently found high utilization (36-52%) outside fences in FBA treatment meadows. This treatment reduced the area of meadow available for grazing, concentrated cattle and thus annual utilization in the remaining areas of FBA treatment meadows.

4.3. Phase II Analyses and Results

Phase II analysis objectives for this report are listed below.

1) Examine meadow scale Yosemite toad young of the year (YOY) density and breeding pool occupancy response to grazing management treatments 2006 through 2009.
2) Examine grazing treatment effects on water quality and cover of potential Yosemite toad breeding pools from 2006 through 2008.
3) Examine associations between water quality and cover factors and Yosemite toad occupancy of potential breeding pools from 2006 through 2008.

4.4. Phase II Objective 1: Examine meadow scale Yosemite toad young of the year (YOY) density and breeding pool occupancy response to grazing management treatments.

4.4.1. Analysis

We used generalized linear mixed model (GLMM) regression analysis to test for meadow grazing management treatment effects on: 1) annual YOY density; and 2) annual number of occupied breeding pools per meadow. YOY density and number of occupied breeding pool data were analyzed as count response variables using the log link function (Poisson family) with robust standard errors for over-dispersion (Rabe-Hesketh and Skrondal 2008). Following, is the base model for both analyses:

#YOY or #occupied pools ~ year + trt + meadow wetness + year × trt

In accordance with the experimental design, the specific test of the hypothesis described above is based upon: 1) the significance of the year by treatment interaction (year × trt); and 2) the relative pattern of response among treatment meadows over time (Figure 1). The remaining fixed effect variables are covariates to account for inherent differences in toad densities or number of occupied pools between treatment groups at the outset of the study (Trt), year to year variation (Year), and mean depth to water table (meadow wetness). The area of each meadow surveyed for Yosemite toads (survey area in hectares) was used as an exposure variable to account for unequal meadow area. To account for repeated measures on each meadow, blocking by allotment, and hierarchical nesting of meadows within allotments, meadow ID and allotment ID were specified as serial random effects (i.e., meadow ID nested within allotment ID). A backward-stepping algorithm was used to construct final models based on Wald tests.

4.4.2. Results

Figures 2 and 3 show the relationships between YOY density and occupied breeding pools (respectively) by each treatment from 2006 through 2009. In the occupied pools GLMM analysis, none of the fixed effects were significant. While Figure 3 might suggest some apparent trends in treatment groups over time, there is no statistical support. Basically, number of occupied pools was constant over years and across treatment groups. In the YOY GLMM regression analysis, year, treatment, and the year by treatment interaction were significant ($p < 0.05$). There was no significant year by treatment differences between the GRZ and FWM treatments (p > 0.10). Apparent relative differences in mean annual YOY between these treatments were evident over the course of the study (Figure 1), but relatively large variance in mean annual YOY for these two treatment groups is also evident. Comparing the pattern of treatment response over the study period (Figure 2) to the hypothesized pattern of response (Figure 1) does not indicate an increase in YOY density in FBA treatment relative to GRZ treatment over the first four years of treatment implementation. The significant year by treatment interaction is apparently detecting random annual variation in YOY between treatments, as opposed to a pattern of treatment effect. Random effects in both models (meadow ID nested within allotment ID) were significant for both YOY and occupied pool density, based upon Log likelihood ratio tests to determine if between group (allotment and meadow) variances were significant.

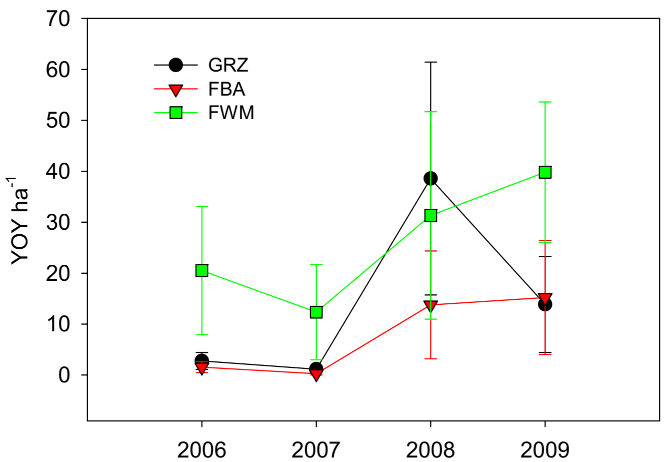

Figure 2. Mean YOY ha^{-1} by treatment across years. Vertical bars represent ± 1 standard error of the mean.

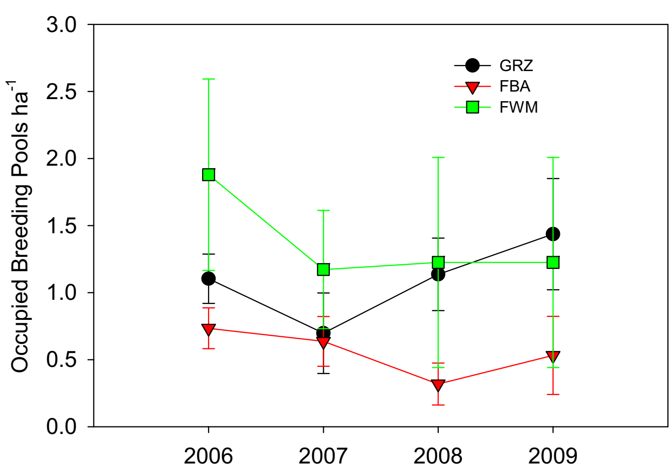

Figure 3. Mean occupied breeding pools ha^{-1} by treatment across years. Vertical bars represent ± 1 standard error of the mean.

4.4.3. Variation in YOY and Occupied Pool Mean Density Estimates

Significant variation exists for annual mean YOY and occupied pool density estimates. This is evident in the magnitude of standard errors reported in Figures 2 and 3, and the spike of YOY counts in GRZ treatment meadows in 2008 (Figure 2). Relatively large variation exists between meadows, between years, and within the same meadow from year to year. This variation could be due to temporal and spatial dynamics with the toad populations associated with these meadows (e.g., naturally variable annual toad reproductive success). It could also be associated with the inherent difficulty in getting accurate counts of Yosemite toad young of the year and pool occupancy (i.e., observation of toads in high herbaceous biomass production years and meadows). It could also be due to sample collection issues such as unequal sample effort between years and meadows (e.g., search time, weather conditions at search time, search area, observer differences). Likely, the variation observed in these estimates is a cumulative result of all these sources. Phase I (reported below) was implemented in anticipation that significant variation in Phase II toad response data could limit our ability to make statistical inference (see Study Plan Appendix A).

4.4.4. Conclusions

1) Observed YOY and occupied breeding site patterns over the first four years of treatment implementation do not support the study hypothesis.
2) There was no detectable pattern of YOY density or number of occupied breeding pools response to grazing management treatments for the study period (2006-2009).
3) Significant variation exists in YOY and occupied pool density estimates between years, meadows, and within meadows over years.
4) Based on the 2006-2009 data, we do not recommend partial meadow fencing (FBA treatment) due to a) potential meadow degradation by concentration of grazing in the un-fenced portion of the meadow, and b) no apparent benefit to Yosemite toads.

4.5. Phase II Study Objective 2: Examine grazing treatment effects on water quality and cover of potential Yosemite toad breeding pools from 2006 through 2008.

4.5.1. Analysis

Nine meadows, three replicates per treatment, were selected for intensive study of grazing treatment effects on water quality and cover of potential toad breeding pools. All meadows were located on the Sierra National Forest. Within each meadow, two occupied and two unoccupied potential breeding pools were enrolled based upon 2005 occupancy surveys. We collected three years (2006-2008) of water quality and cover data from each pool (n=36 pools) during May through June (i.e., toad breading, egg laying, and early tadpole life cycle stages). Pools were categorized as lotic (flowing) or lentic (stagnant) hydrologic pool types. Water depth and vegetative cover were measured for each pool. Water temperature was recorded continuously every 0.5 hrs throughout the breeding and rearing season (approximately late May- mid June each year).

To test grazing management treatment effects on pool water quality and cover response variables, we used linear mixed model (LMM) and generalized linear mixed model (GLMM) regression analyses. A separate model was developed for each response variable. GLMMs were used for count responses (turbidity, EC (electrical conductivity), NO_3-N (nitrate), NH_4-N (ammonium), Total N, Total P, water depth, and vegetative cover) using the log link function (Poisson family) with robust standard errors for over-dispersion (Rabe-Hesketh and Skrondal 2008). LMMs were used for temperature and pH, which were normally distributed with homogeneous variance (Pinheiro and Bates 2000). The base model was:

Response variable ~ Year + Trt + Year*Trt + hydrologic pool type

where hydrologic pool type was a categorical variable indicating lotic or lentic flow conditions in each pool. For water temperature response variables, Julian day and ambient air temperature were also included in the base model. To account for repeated measures, block (allotment management), and non-independence of samples (i.e., hierarchical nesting of pool within meadow within allotment), pool

ID, meadow ID, and allotment ID were specified as serial random effects (i.e., pool ID nested in meadow ID nested in allotment ID). A backward-stepping algorithm was used to construct all final models, with significance for inclusion set at $p \leq 0.05$, based on Wald tests.

In accordance with the experimental design, the specific test of the hypothesis described above (Figure 1) is based upon: 1) the significance of the year by treatment interaction; and 2) the relative pattern of a significant response among treatment meadows over time.

4.5.2. Results

Tables 1 and 2 show statistical analyses results for chemical water quality response variables. There was no significant treatment by year interaction for turbidity, pH, EC, NO_3-N, or Total N. It is important to note that the majority (>70%) of the water samples were below detection limits for NO_3-N and PO_4-P. Most samples were low or at detection level, and there was a narrow range in values for all constituents. This is a nutrient limited system.

	Turbidity (ntu)	pH	EC (dS/dm)
Year	<0.05	<0.05	<0.05
Trt	<0.05	ns	ns
Year*Trt	ns	ns	ns

Table 1: Results of GLMM and LMM tests of grazing treatment effects on general water quality metrics for potential toad breeding pools. We found no significant treatment by year interactions for turbidity, pH, or EC. There were significant differences ($p<0.05$) among years for these three variables.

	NO_3-N (ppm)	NH_4-N (ppm)	Total N (ppm)	PO_4-P (ppm)	Total P (ppm)
%< DL	*77*	*5*	*0*	*74*	*6*
Year	<0.05	<0.05	<0.05	<0.05	<0.05
Trt	<0.05	<0.05	<0.05	<0.05	<0.05
Year*Trt	NS	<0.05	NS	<0.05	<0.05

Table 2: Results of GLMM and LMM tests of grazing treatment effects on nutrient levels of potential toad breeding pools. The majority of samples for NO_3-N and PO_4-P were below laboratory detection levels. We found significant ($p<0.05$) treatment by year interactions for the response variables NH_4-N, PO_4-P, and Total P.

To determine if the significant year by treatment interactions indicate a grazing management treatment effect on NH_4-N, PO_4-P and Total P (Table 2), we examined the pattern of response over the study period. Specifically, we hypothesize that *over the course of treatment implementation (2006 through 2009), nutrient loading will decrease in fenced meadow treatments (FBA and FWM), and increase in the unfenced control (GRZ).* We expect to see elevated nutrient levels due to nutrient deposition and increased nutrient cycling in grazed (GRZ) relative to non-grazed meadows (FBA and FWM). For example, Figure 4 shows observed treatment effects on NH_4-N (similar trends were exhibited for Total P and PO_4-P). Overall, NH_4-N levels were significantly lower for all treatments in 2008. Detection limit for NH_4-N is 0.01 ppm. There is no evidence of the hypothesized patterns for any of the response variables that had significant year by treatment interactions (NH_4-N, PO_4-P, and Total P). There is no clear pattern of decreased nutrient loading in fenced treatments (FBA, FWM) relative to the grazed treatment (GRZ). These significant interactions are detecting apparently random annual variation among treatments as opposed to a pattern of treatment effect.

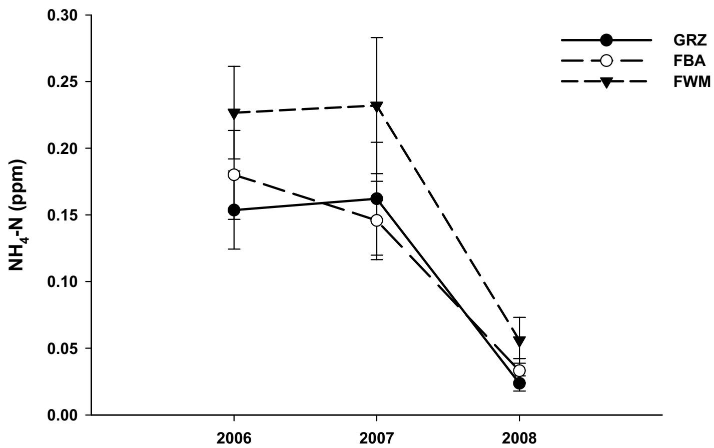

Figure 4: Observed patterns year by treatment interactions for NH_4-N response variable. Vertical bars represent ± 1 SE.

Table 4 shows statistical analyses results for physical response variables. There was no significant treatment by year interaction for water depth or vegetation cover. Water depth and percent vegetation cover were significantly different ($p<0.05$) among treatments, indicating initial, inherent differences among meadows randomly assigned to treatments. There were significant ($p<0.05$) treatment by year interactions for average daily minimum, mean, and maximum temperature variables.

	Water Depth (cm)	Veg Cover (%)	Daily Water Temp (C)		
			Min	Mean	Max
Year	<0.05	ns	<0.05	<0.05	<0.05
Trt	<0.05	<0.05	ns	<0.1	<0.1
Year*Trt	ns	ns	<0.05	<0.05	<0.05

Table 3: Results of GLMM and LMM tests of grazing treatment effects on physical and water quality variables. We found significant ($p<0.05$) treatment by year interactions for average daily minimum, mean, and maximum temperature variables.

Similar to the chemical response variables, we compared expected and observed temperature response to treatments over years to determine if significant year by treatment interactions (Table 3) reflect a pattern of response to grazing management treatments. Figure 5 demonstrates expected temperature responses to grazing treatments over 3 years. With livestock exclusion from breeding pools (FBA and FWM), we expect increased vegetation cover and reduced cattle trampling of pools (i.e., fragmented, widened and shallower pools in grazed meadows). These mechanisms would result in *temperature reductions (i.e., relative cooling over the study period) in fenced treatments relative to the grazed treatment over the course of treatment implementation.*

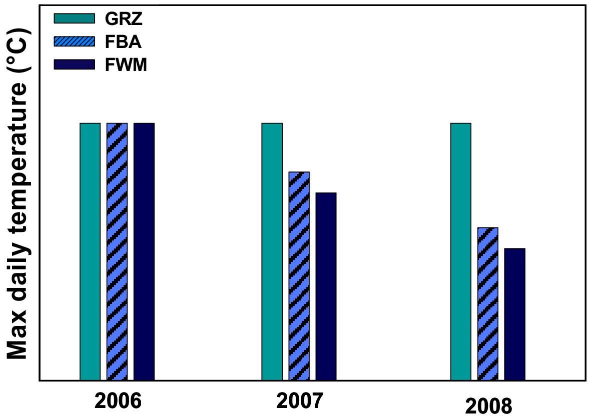

Figure 5: Hypothesized pattern of treatment by year interactions for interpreting potential treatment effects on breeding pool water temperature variables.

Figure 6 shows observed average maximum daily temperature response by treatment over the study period. No clear pattern of relative cooling of pools within fenced treatments was observed. Relative differences among treatments greatly increased in 2007 (fenced treatments were warmer), and then returned to near 2006 levels in 2008. Significant year by treatment interactions are reflecting apparently random annual variations rather than a pattern of grazing management treatment effect. Daily minimum and daily mean temperatures followed a similar pattern to daily maximum temperature (data not shown).

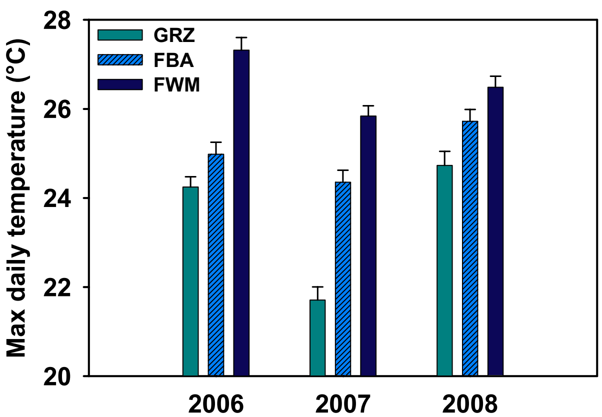

Figure 6: Observed pattern of treatment by year interactions for maximum daily temperature response variable. There is no clear pattern of cooling of fenced treatments relative to grazed treatment. Vertical bars represent 1 SE.

Random effects in all models (pool ID nested within meadow ID nested within allotment ID) were significant based upon Log likelihood ratio tests of GLMMs.

4.5.3. Conclusion

1) There was no detectable change in breeding pool water quality and cover variables due to meadow fencing treatments (FBA and FWM) relative to the grazed treatment (GRZ).

4.6. Phase II Study Objective 3: Examine grazing treatment effects on water quality and cover of potential Yosemite toad breeding pools from 2006 through 2008

4.6.1. Analysis

Data described for Objective 2 above were further analyzed for Objective 3. Objective 3, and associated analysis, is designed to provide guidance on which habitat variables determine Yosemite toad use of potential breeding pools. This information is important for understanding the ecology, biology, and management of Yosemite toad populations.

First, analyses conducted to achieve Objective 2 above determined that potential breeding pool hydrologic type (lotic=flowing, lentic=stagnant) is a significant determinant of several water quality and cover variables. We report these results below, and further examine Yosemite toad occupancy patterns for lotic and lentic potential breeding pools from 2006 through 2008.

Second, we also examined correlations among toad occupancy and all pool water quality and cover variables described under Objective 2 above. We used LMMs and GLMMs for each response variable, with toad occupancy of each pool during the four year period (2005 [initial survey] through 2008 [3rd year of treatment implementation]) as a fixed effect for each analysis. Pools were categorized as occupied 0 years out of 4 (toads never present); 1 to 3 years out of 4 (toads variably present year to year); or toads present 4 years out of 4 (toads always present). In order to account for repeated measures, block (allotment management), and non-independence of samples (i.e. hierarchical nesting of pool within meadow within allotment) pool ID, meadow ID, and allotment ID were specified as serial random effects. Significant coefficients for the toad occupancy model term were directly interpreted as the difference in pool characteristics for pools where toads were never present, variably present, and always present.

4.6.2. Results

We found lentic (non-flowing) pools were more consistently occupied than lotic (flowing) pools throughout the study (Table 4). In 2007, when annual precipitation was lowest (53% of average water year), lotic pool occupancy decreased from 62 to 0%, while lentic pool occupancy rates remained stable.

	Breeding Area Flow Conditions	
	Lotic	**Lentic**
% Occupied 2006	**62**	**30**
% Occupied 2007	**0**	**29**
% Occupied 2008	**33**	**25**

Table 4: Percent toad occupancy each year by hydrologic pool type (lotic vs. lentic).

Lentic habitats, which were more consistently occupied, had significantly higher levels of Total N and Total P (Figure 7), warmer temperatures (Figure 8), and greater turbidity (Figure 9) than lotic pools. Water depth and pH were significantly lower in lentic pools. Maximum and mean daily pool water temperature and pool water depth were significantly correlated with toad occupancy. Occupied pools were significantly warmer and shallower than unoccupied pools (Figure 10). Water temperature and depth were not significantly different between pools occupied all four years and pools occupied at least one year (variably occupied).

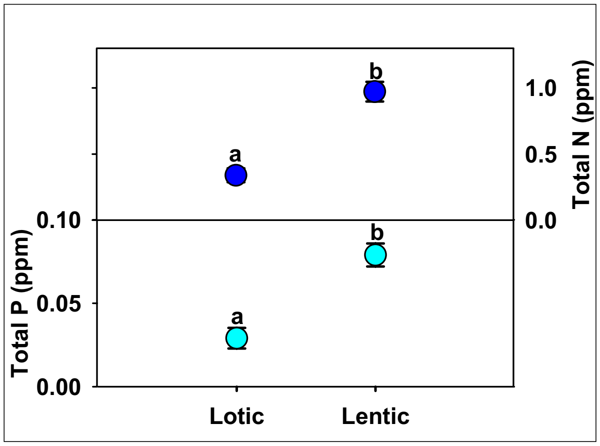

Figure 7: Total P and Total N concentrations by hydrologic pool type (lotic vs. lentic). Nutrient concentrations were significantly ($p<0.05$) higher in lentic pools. For both Total P and Total N, nutrient concentrations were generally low (<1.5 ppm for Total N; <0.10 ppm for Total P). Vertical bars represent ± 1 SE.

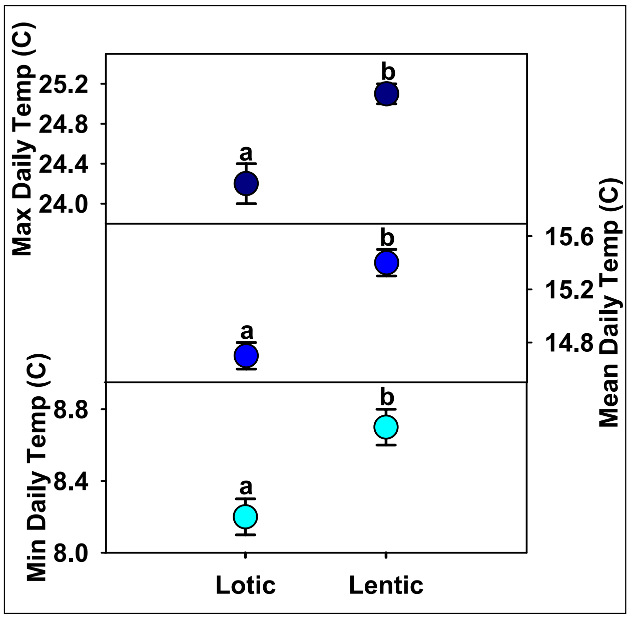

Figure 8: Average daily minimum, mean, and maximum temperatures by hydrologic pool type (lotic vs. lentic). All temperature metrics were significantly ($p<0.05$) higher in lentic pools. Vertical bars represent ± 1 SE.

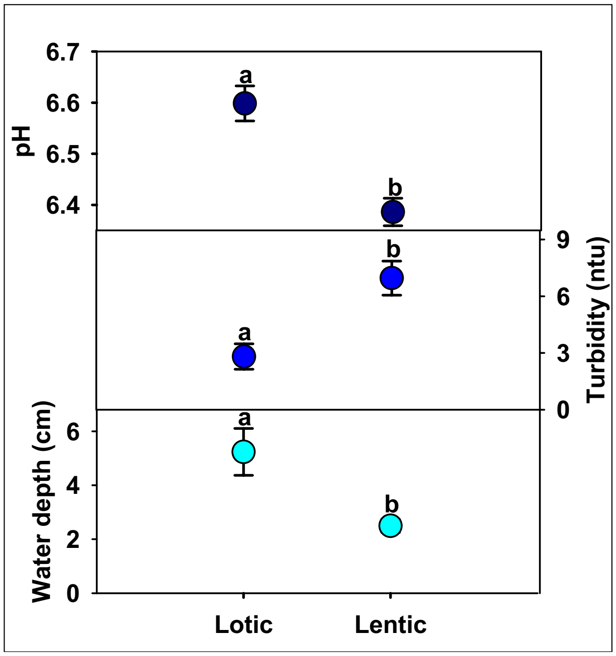

Figure 9: Average water depth, turbidity, and pH levels by hydrologic pool type (lotic vs. lentic). Water depth and pH were significantly ($p<0.05$) lower in lentic pools, while turbidity was significantly ($p<0.05$) higher in lentic pools. Vertical bars represent ± 1 SE.

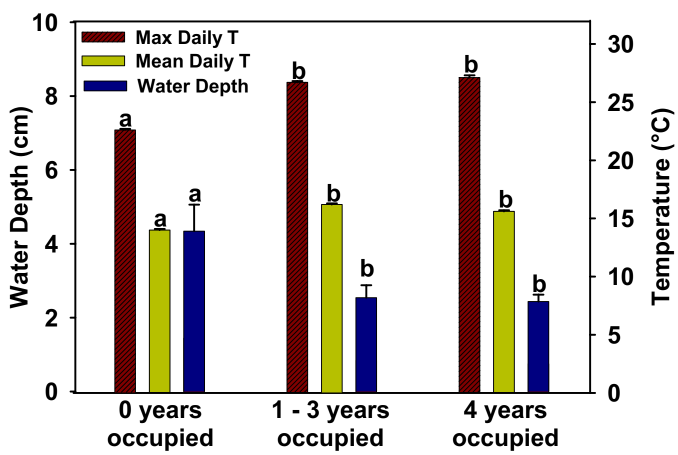

Figure 10: Results of LMMs and GLMMs for pool variables as functions of occupancy rate (0 years, 1-3 years, and 4 years). Average daily maximum and mean water temperature were significantly ($p<0.05$) higher in toad occupied pools. Average water depth was significantly ($p<0.05$) lower in toad occupied pools. Vertical bars represent ± 1 SE.

4.6.3. Conclusions

1) All nutrient levels were low, and the range was narrow for all nutrients and treatment groups. Results indicate this is a nutrient limited system.
2) Lentic (non-flowing) pools were more consistently occupied than lotic (flowing) pools.
3) Lentic pools were significantly more nutrient enriched, warmer, and shallower than lotic pools.
4) Yosemite toad occupied pools were significantly warmer and shallower than unoccupied pools.

5. Grazing intensity, meadow hydrology, and toad occupancy survey (Phase I)

5.1. Phase I Questions and Design

Rational for this experiment can be found in the Study Plan (Appendix A). More Phase I method detail is provided in this report, compared to Phase II, as Phase I study design was fully developed in 2005-06 from the preliminary design described in the Study Plan.

Phase I experiment was designed to quantify relationships among intensity of cattle use, meadow plant and hydrologic characteristics, and toad occupancy. This complements the manipulative grazing experiment (Phase II), and provides allotment scale context for the entire project (e.g., ambient use patterns among meadows under open, equilibrium grazing conditions). Our explicit questions were: 1) How does meadow hydrology influence forage quality and herbaceous biomass productivity, 2) What are the relationships between forage quality, forage productivity, and cattle meadow utilization, and 3) Does Yosemite toad meadow occupancy respond more strongly to cattle utilization or to meadow hydrology? Due to uncertainty about the season of grazing posing the greatest potential impact on this Yosemite toad, we addressed these questions for early, mid, and late season grazing periods.

5.2. Phase I Design and Data Collection

We conducted a cross-sectional, longitudinal survey of Yosemite toad occupancy, cattle use intensity, and meadow hydrology across 24 meadows over three years (2006-2008) on the SNF. SNF Yosemite toad and habitat survey records (conducted in 2002 and 2003; 83% and 94% of average annual precipitation, respectively) were utilized to define an initial set of meadows with potential to support Yosemite toad breeding populations. From this initial set, we randomly selected 24 meadows across three grazing allotments. In 2006, five monitoring sites were selected across each meadow catena (120 total sites), representing the major plant communities and moisture gradient in each meadow. Paired plots (cattle grazed, ungrazed) were permanently marked at each site, and ungrazed caged plots were relocated within the same plant community-water table site each year to avoid introducing a cage effect.

All meadows were open to cattle grazing under ambient allotment scale management. Allotments ranged from 22 000 to 27 000 hectares with 200 to 250 permitted animal units per allotment between 1 June and 15 September (Figure 10). Throughout the study, cattle use intensity and vegetation attributes were recorded at each monitoring site. Cattle use intensity was measured via herbaceous utilization (2006-2008), which was determined via comparative yield-paired plot methods (Interagency 1996) at the end of the early (July), mid (August), and late (September) season grazing periods each year. In the final year of the study (2008), fecal density was measured via multiple belt transects across each meadow to correlate present utilization levels with recent historic use (< 10 yrs). Under the current climate, fecal density in high elevation mountain meadows represents approximately 5-10 years of pat accumulation.

Biomass production data (2006-2008) and herbaceous forage samples (2007-2008) were collected for each site in June, July, and August, representing variation in forage characteristics during early, mid, and late seasons, respectively. Biomass production for each site was determined via the comparative yield method. For forage quality analyses, a minimum of 30 grams dry weight was collected for each sample. Samples were oven-dried at 55-60°C for a minimum of 48 hours, and ground to pass through a 40 mesh screen. Crude protein (CP; protein by nitrogen gas analyzer utilizing induction furnace and thermal conductivity), acid detergent fiber (ADF; using acid detergent, sulfuric acid, and heat), and total phosphorous (TP, microwave acid digestion/dissolution of sample and quantitative determination) were determined for each sample by the University of California Agriculture and Natural Resources Analytical Laboratory, UC Davis, California.

To assess overall meadow hydrology, individual monitoring sites were categorized along a relative wetness scale with scores ranging from zero to six. In 2008, sites were ranked based on dominant plant community, extent and timing of surface flooding and saturation, and soil characteristics (mineral vs. organic dominated soils, depth of peat accumulation in organic soils, presence of redox features in mineral soils). Relatively drier grass/forb dominated sites represented a zero rank, and continuously flooded sites dominated by wetland obligate *Carex* species represented a six rank. Site rankings were averaged within each meadow to provide composite meadow-scale hydrologic rankings. For example, a meadow with a dominant wet *Carex* community and a subdominant drier grass/forb community would have 3 sample sites in the *Carex* community (3 x 6 rank) and 2 sample sites in the grass/forb community (2 x 0 rank), resulting in an average score of 3.6, which is considered a "4" meadow rank assignment on the original scale. Rankings were calibrated at sites within 10 additional meadows (Phase II experiment meadows) in the region, which were instrumented with water table measurement wells. At each site and year, depth to free water was measured approximately every four weeks throughout the grazing season.

Meadow-scale toad occupancy surveys were conducted for all 24 meadows during the early tadpole periods (Figure 10) in 2007 and 2008. Meadows were systematically searched for all toad life stages by three member crews, with search times adjusted for individual meadow size. Searches were conducted during the early season (June – July), when tadpoles (i.e., the most easily detectable stage) were still abundant. Based on pilot study observations, searches were conducted during mid morning hours (0900–1100 hours) on cloudless days, which maximized detection potential.

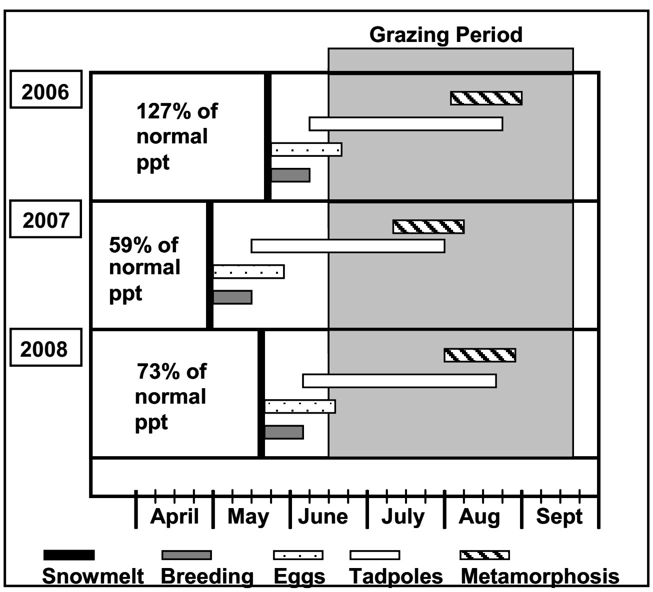

Figure 10. Diagram illustrating timing of Yosemite toad (*Bufo canorus* Camp) life stages and cattle grazing seasons in the High Sierra Ranger District, Sierra National Forest, California, USA. Data were collected for 2006-2008 on cattle grazed meadows in the study area (Phase II experiment meadows). Precipitation (ppt) ranged from 59%-127% of normal for the study period.

5.2. Phase I Data Analysis

Bivariate Relationships. We used linear regression to examine the following bivariate relationships: 1) meadow hydrology and toad meadow occupancy, herbaceous biomass use, and fecal loading; 2) fecal pat density and herbaceous biomass use, and 3) forage quality metrics and meadow hydrology. Meadow hydrology was measured as the composite meadow-scale hydrologic rankings. Meadow occupancy by Yosemite toads was calculated as the proportion of surveys (three total, including the preliminary SNF survey and the project team's surveys in 2007 and 2008) each meadow was occupied. Mean late season herbaceous biomass use (i.e., total use) for each meadow was averaged over 2006-2008, and fecal loading was calculated as fecal pat density in 2008. Means forage quality metrics for each meadow were averaged over 2007 and 2008. All regression analyses were conducted in STATA (StataCorp 2007). For the toad occupancy by meadow hydrology analysis, a logistic regression model was used to fit the response, which was the proportion of surveys each meadow was occupied by toads. The remaining bivariate relationships were fit with linear and quadratic regression models, and AIC and significance tests were used to select final models. Standard diagnostic analyses were utilized to check assumptions of linearity, normality, and constant variance. Box-Cox transformations were used to remedy any violations. The above exploratory analyses were utilized to construct a general conceptual diagram for structural equation modeling (Figure 11).

Bayesian Structural Equation Modeling. SEM is a multivariate analysis technique combining path and factor analyses and permits evaluation of potential causal pathways of intercorrelated variables. The Bayesian approach offers greater flexibility than the classical frequentist approach to SEM. Unlike classical maximum likelihood estimates, Bayesian inferences do not rely on asymptotic normality, and so these estimators are more reliable for smaller samples or cases with other sources of non-normality. We constructed a conceptual model representing our general theoretical scheme for the SEM analyses (Figure 11). The model is based on the results of above bivariate analyses and the following suppositions: 1) plant community characteristics, which may be correlated, may be influenced by environmental wetness; 2) herbaceous biomass use by cattle may be influenced by forage quality and/or productivity; 3) cattle utilization is not likely to influence these perennial plant communities or environmental wetness in the short term; 4) toad meadow occupancy may be directly influenced by environmental wetness, which may determine habitat suitability, or by cattle grazing, which may impact toad habitat physical and/or chemical attributes (e.g., water quality) or lead to direct loss of individuals. To account for repeated measures, year effects, and spatial correlation; meadow and year identifiers were included as crossed random effects, with meadows nested within allotments.

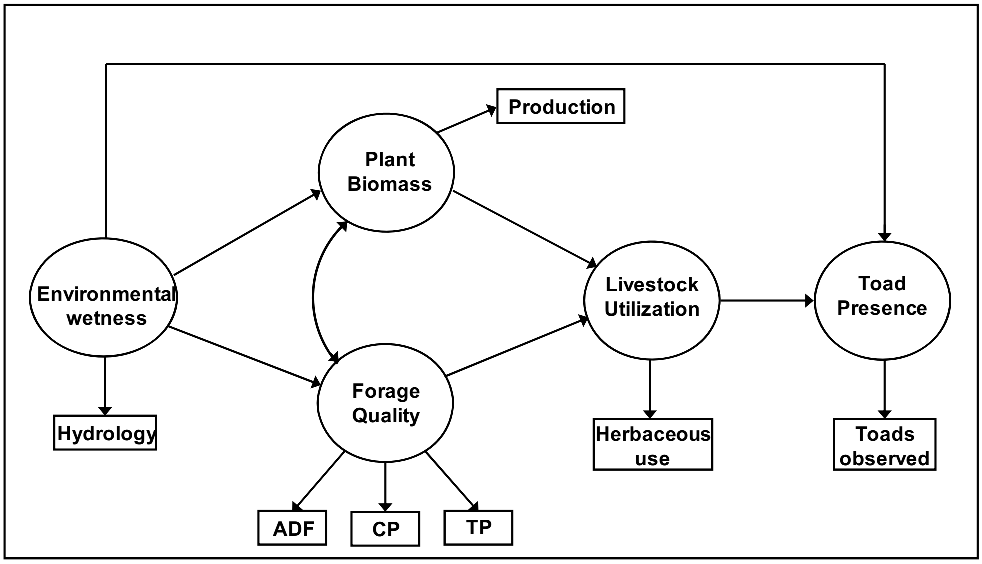

Figure 11. A priori conceptual model of the multiple hypothesized factors influencing toad meadow occupancy in the High Sierra Ranger District, Sierra National Forest, California, USA. Ovals indicate latent variables, which are estimated by observable indicators. Straight arrows represent direct effects of one variable on another and curved arrows represent correlations between variables.

Bayesian SEM analysis was performed with OpenBUGS software, which uses Markov chain Monte Carlo (MCMC) simulation based on Gibbs sampling algorithm. For SEM, we analyzed herbaceous utilization, forage quality, biomass production, and toad occupancy data from 2007 and 2008 collection events, in addition to the one-time meadow hydrology scores. All continuous variables were standardized (mean = 0, standard deviation = 1) to aid model convergence and allow for direct comparisons of model coefficients. Convergence was assessed utilizing trace plots with multiple chain sample values and the bgr diag function, which calculates a modified Gelman-Rubin statistic. Model comparisons and goodness of fit were performed via the Deviance Information Criterion (DIC), a generalization of Akaike's Information Criterion (AIC). Reliability of model coefficients was examined via credible intervals (i.e., Bayesian confidence intervals). To evaluate predictive capacity for toad occupancy and provide an additional measure of model fit, we cross-validated each model. Each data point was predicted by the model developed from the remaining n-1 data points via the R statistical package rjags. Prediction errors for toad occupancy were assessed via receiver operating characteristic (ROC) curves, which have been previously demonstrated for presence-absence models in habitat conservation research. The accuracy of the predictors is measured by the area under the ROC curve (AUC), which ranges from 0.5 to 1. Although no standard classification rules exist, AUC values greater than 0.80 are generally considered good, and values greater than 0.90 are considered excellent.

5.3. Phase I Results

Bivariate Relationships. For the overall study period (2006-2008), enrolled meadows represented a mean annual herbaceous use gradient from 4 to 49%, and an annual biomass production gradient from 1000 to 3200 kg·ha-1 (Table 5). Average forage production for early, mid, and late seasons was 723 kg·ha-1 (+/- 39 SE), 1660 kg·ha-1 (+/- 127 SE), and 1774 kg·ha-1 (+/- 98 SE), respectively. Meadow hydrology (i.e., relative wetness) scores sufficiently reflected the seasonal water table variation between meadow sites with “dry” (score 0), “moderate” (score 3), and “wet” (score 6) hydrologic rankings in additional meadows equipped with ground water wells (Figure 12). Water table depths diverged over a four month period, with hydric sites remaining flooded throughout the season and drier sites experiencing a seasonal drawdown of approximately 55cm.

	Meadow Characteristics			
	Elevation (m)	Area (ha)	Annual biomass (kg/ha)	% Use
Minimum	2118	0.3	1004	4
Mean	2373	1.7	1840	29
Maximum	2670	7.9	3209	49

Table 5. Summary values for elevation, size, annual herbaceous biomass production, and percent herbaceous use by cattle across 24 meadows in the Sierra National Forest, California, USA, during 2006-2008.

Overall toad meadow occupancy was positively correlated with meadow hydrology (pseudo $R^2 = 0.25$, p=0.019), while average late season cattle utilization was negatively correlated with meadow hydrology (herbaceous use: $R^2 = 0.43$, p=0.0005; fecal pat density: $R^2 = 0.22$, p=0.019). There was a strong, significant relationship ($R^2 = 0.80$, p<0.0001) between late season use and fecal loading, indicating that use during the study period was indicative of cattle use during the past 5-10 years for these high elevation meadows (Figure 13). Average peak forage production for 2006-2008 was negatively correlated with meadow hydrology ($R^2 = 0.21$, p=0.026).

Analyses of the 2007-2008 cattle use and forage quality data revealed few differential relationships across the three grazing seasons. There was no significant relationship between herbaceous biomass use and meadow hydrology during the early season; however, there were significant negative relationships between meadow hydrology and herbaceous biomass use for both mid and late seasons (Figure 15; late season data not shown). For all grazing seasons, forage quality metrics (ADF, TP, CP) were negatively correlated with meadow hydrology (Figures 14 and 15).

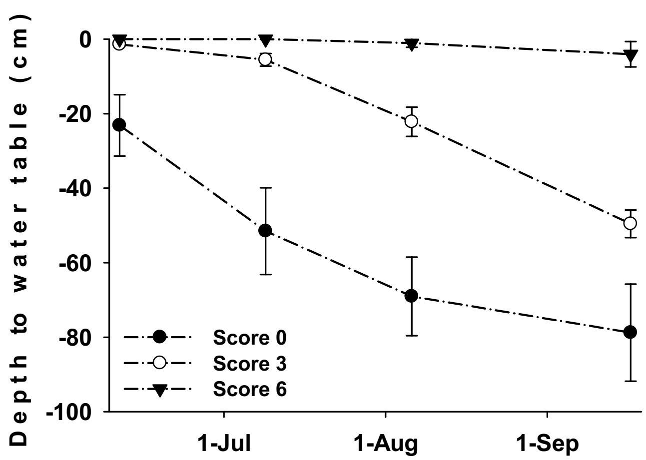

Figure 12. Mean water table by meadow hydrology score for 10 meadows in the High Sierra Ranger District, Sierra National Forest, California, USA, during 2008. Hydrologic scale ranged from 0-6, with 0 representing drier sites and 6 representing the wettest plant community sites. Ground water wells were installed to measure depth to free water approximately every four weeks throughout the grazing season. Water tables diverged over the summer: wet sites (score 6) experienced an average seasonal drawdown of 4cm while drier sites (score 0) experienced an average seasonal drawdown of 79cm. Vertical bars represent ± 1 SE.

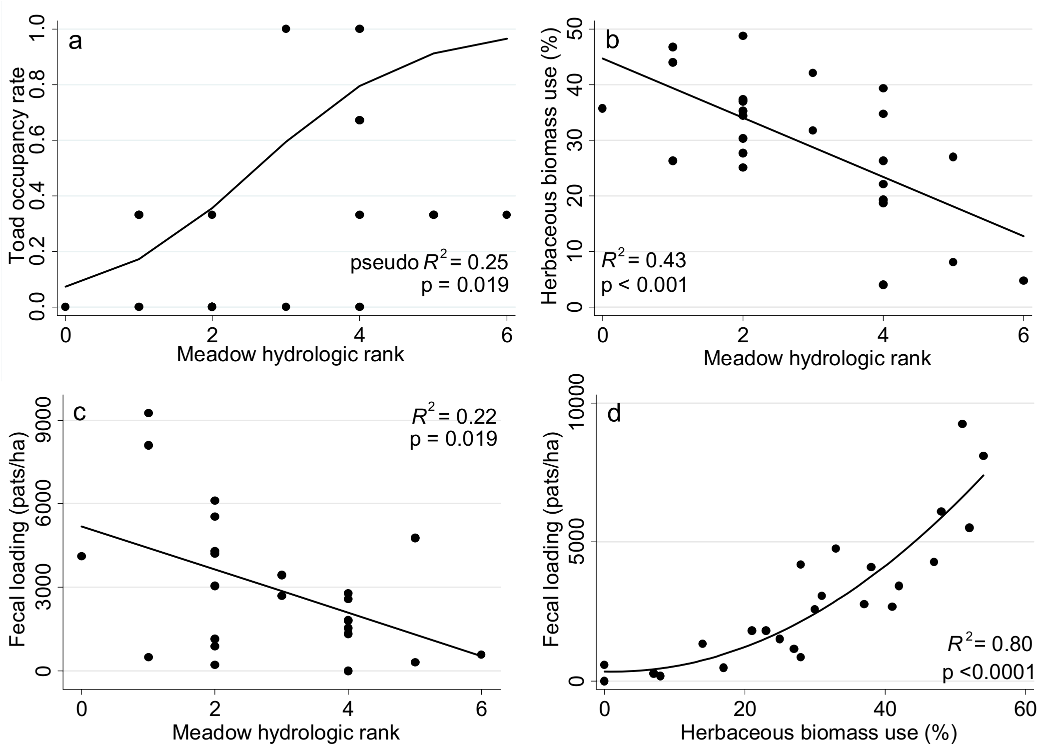

Figure 13. Toad occupancy and annual cattle utilization (percent herbaceous biomass use and fecal pat density) along a hydrologic gradient of meadows (n = 24) in the High Sierra Ranger District, Sierra National Forest, California, USA, during 2006-2008 early grazing seasons (June-July). Toad occupancy rate is calculated as proportion of surveys (three total; 2002/2003, 2007, and 2008) each meadow was occupied. Increases in meadow hydrologic rank (i.e., meadow wetness) correspond with increases in toad occupancy (p=0.019, pseudo R^2 = 0.30), decreases in average total herbaceous biomass use (p=0.0005, R^2 = 0.43), and decreases in cattle fecal accumulation (p=0.019, R^2 = 0.22). For study year 2008, fecal loading and herbaceous biomass use were positively correlated (p<0.0001, R^2 = 0.80).

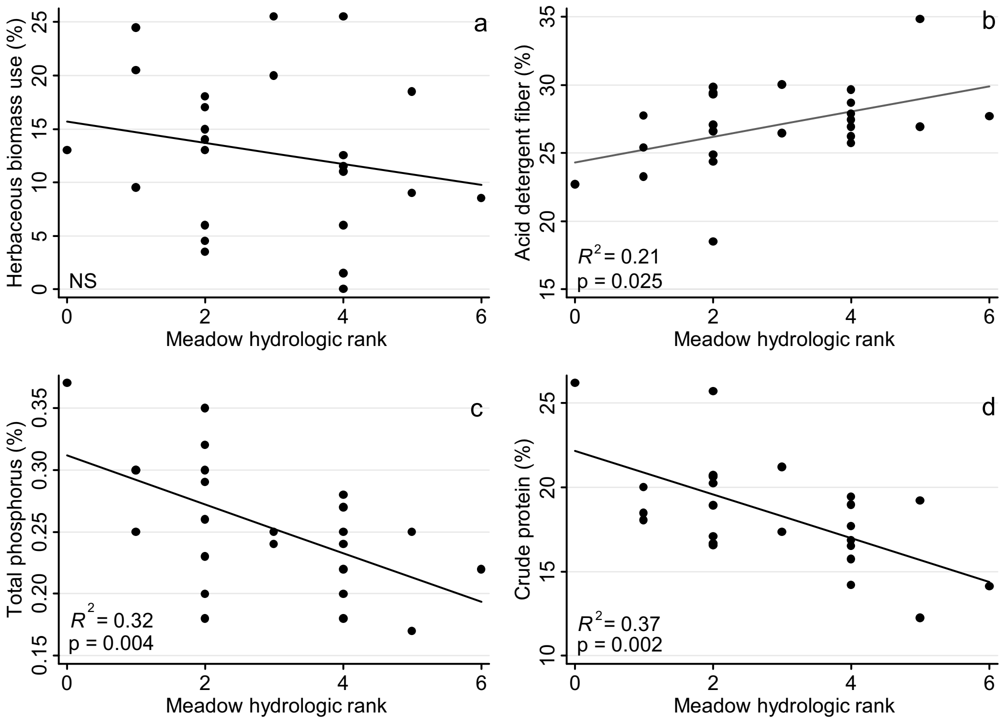

Figure 14. Early season (July) meadow scale cattle use and forage quality along a hydrologic gradient of meadows (n = 24) in the High Sierra Ranger District, Sierra National Forest, California, USA. There was no significant trend in cattle use, as measured by average early season herbaceous biomass use, across the meadow hydrologic gradient. Forage quality (crude protein, total phosphorus [TP], acid detergent fiber [ADF]) significantly declined with increasing meadow hydrologic rank (i.e., meadow wetness).

Bayesian Structural Equation Modeling. Cross validations for toad occupancy predictions produced reasonably good ROC AUC values for all grazing seasons: Early, mid, and late season model ROC AUC values were 0.830, 0.832, and 0.832, respectively. Along with the DIC indicators for general model comparisons, these metrics indicate reasonable model fit.

Bayesian SEM results for all grazing seasons suggest that toad presence strongly responded to variation in environmental wetness, rather than cattle utilization (Figure 16). Potential direct effects of cattle use on toad meadow occupancy were not significant (utilizing 90% Bayesian credible intervals) for any season. Across the grazing seasons, cattle utilization responded differentially to meadow forage quality and productivity. Early season cattle utilization did not significantly respond to any of the measured forage quality or productivity indicators (i.e., plant biomass production, ADF, TP, or CP). During the early season, forage quality fully met the general nutrient requirements of CP and TP (approximately 8 and 0.20 % respectively) for lactating beef cattle (National Research Council 1996), and forage production was limited across meadows early in the herbaceous growing season. As the seasons progressed, productivity exhibited a greater relative effect (0.54 vs. 0.43) on cattle utilization during the mid grazing season, while forage quality had a greater relative effect (0.53 vs. 0.60) during the late grazing season. Comparing the relative importance of CP and TP as indicators of forage quality, CP was relatively more important (1.0 vs. 0.63) during the mid grazing season, while TP became relatively more important (1.10 vs. 1.0) during the late grazing season. Average TP fell far below general nutrient requirements (average = 0.136%, range = 0.076 - 0.174) during the late season. ADF was a significant indicator of forage quality only in the early season analysis. ADF values ranged from 15% to 39% throughout the grazing season. Meadow forage quality and productivity were significantly influenced by meadow hydrology (i.e., environmental wetness) for all grazing seasons (Figure 16). Forage quality and productivity were not significantly correlated for this dataset.

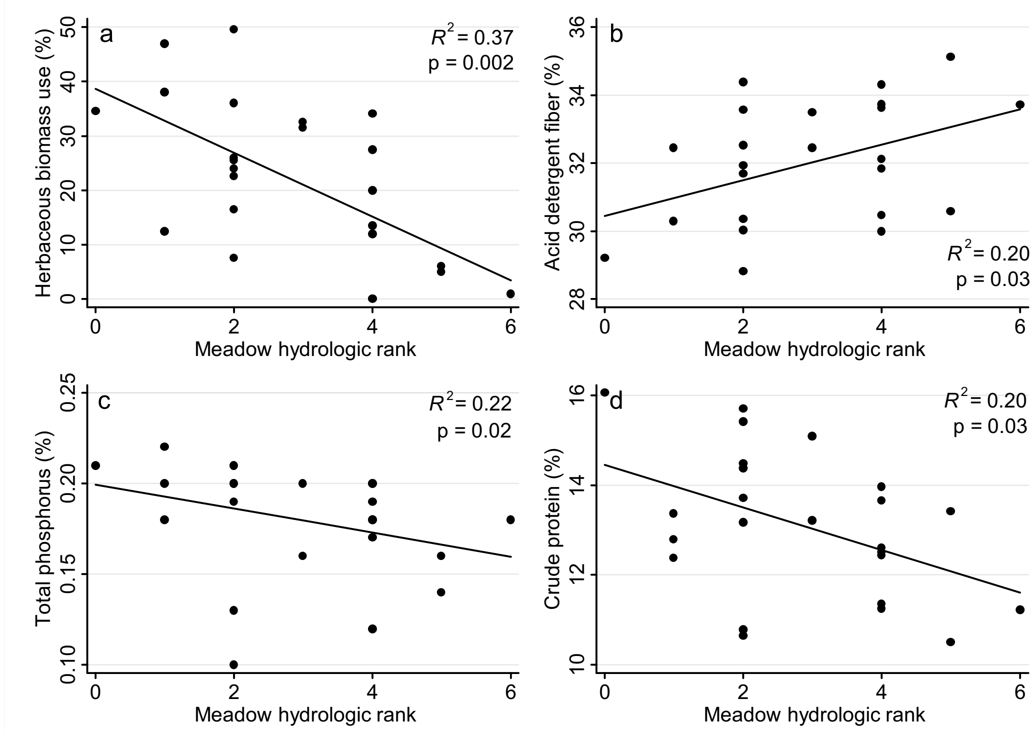

Figure 15. Mid season (August) meadow scale cattle use and forage quality along a hydrologic gradient of meadows (n = 24) in the High Sierra Ranger District, Sierra National Forest, California, USA. Cattle use, as measured by average early season herbaceous biomass use, and average forage quality (crude protein, total phosphorus [TP], acid detergent fiber [ADF; greater ADF values indicate lower digestibility]) significantly declined with increasing meadow hydrologic rank (i.e., meadow wetness). Late season (September) data exhibited similar trends.

5.4. Phase I Conclusions

1) Early season cattle use of meadows was not dependent upon meadow wetness, with use being evenly distributed across all meadows.
2) Mid and late season cattle use targeted drier meadows, which had more forage and higher quality forage than wetter meadows.
3) Cattle use of meadows over the grazing season was driven by selection for the nutritious diet associated with plant communities in drier meadows.
4) Yosemite toad occupancy rates of meadows increased with meadow wetness, likely driven by toad preference for habitat associated with wetter meadows.
5) Bayesian SEM results for all grazing seasons indicate that toad presence was driven by environmental wetness, rather than cattle utilization.
6) A direct correlation between cattle use and on toad meadow occupancy was not found for any season.
7) Given that meadow wetness was the major determinant of toad occupancy and grazing intensity was not directly correlated to toad meadow occupancy, simultaneous management/conservation attention needs to be given to factors likely directly impacting meadow wetness (i.e., meadow hydrologic restoration, climate and fire regime changes, and conifer encroachment in meadows).

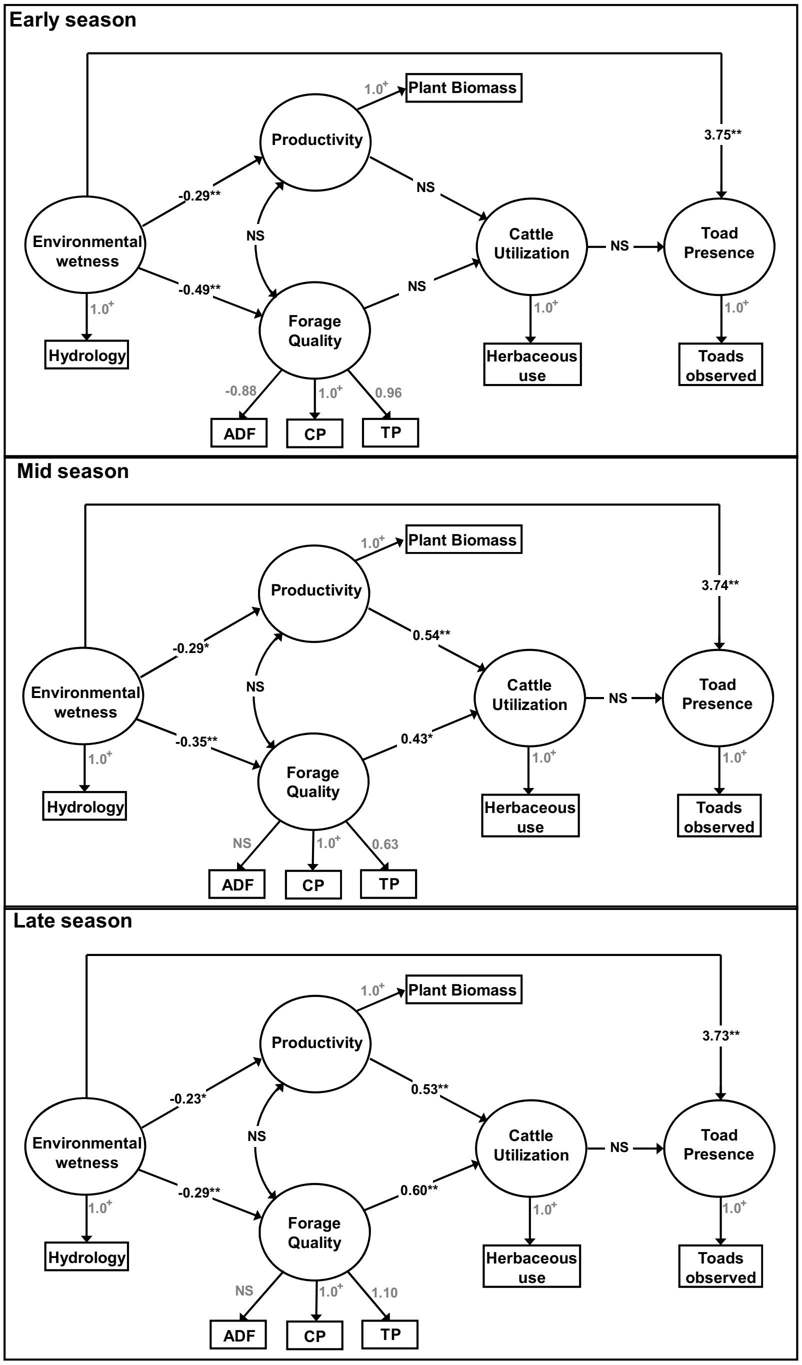

Figure 16. Results of Bayesian structural equation modeling for early, mid, and late season cattle use and forage data for the High Sierra Ranger District, Sierra National Forest, California, USA. All receiver operating characteristic (ROC) area under curve (AUC) values, which measured the accuracy of the predictors for toad occupancy, were ≥0.83. All models suggest toad presence responds to variation in environmental wetness, rather than cattle utilization levels. Meadow and year were included as random effects, with meadows nested within allotments. Ovals indicate latent variables, which are estimated by observable indicators. Straight arrows represent direct effects of one variable on another, and curved arrows represent correlations between variables. + = fixed values, ** = 95% Bayesian credible interval, * = 90% Bayesian credible interval, NS = Not significant.

Final Report

Appendix A – Study Plan

Determining the Effects of Livestock Grazing on Yosemite Toads (*Bufo canorus*) and Their Habitat: An Adaptive Management Study

DETERMINING THE EFFECTS OF LIVESTOCK GRAZING ON YOSEMITE TOADS (*BUFO CANORUS*) AND THEIR HABITAT: AN ADAPTIVE MANAGEMENT STUDY

Final Study Plan – 14 September 2007

Written by: B. Allen-Diaz, A. Lind, S. McIlroy, K. Tate

With technical support from: C. Brown, W. Frost, R. Grasso, N. McDougald, S. Parks, and P. Stine

PROBLEM REFERENCE AND LITERATURE

Introduction

Since the late 1970s, scientists have discovered an alarming decrease in amphibian populations around the world (Stuart et al. 2004, Houlahan et al. 2000). A recent review of amphibian declines identified six potential causes: land use change, introductions of alien species, over-exploitation, pesticides and other toxins, infectious disease, and global change, which includes both increased ultraviolet (UV) radiation and climatic change (Collins and Storfer 2003). Climate change may be particularly detrimental to amphibians because they are sensitive to subtle differences in temperature as well as timing of precipitation and snow events (Beebee 1995). In some environments, trampling of riparian areas, reduction of plant species diversity, and water quality degradation caused by improper livestock grazing are also potential causes of amphibian species declines (Fleischner 1994).

Amphibian species are declining in montane areas, where anthropogenic impact is seemingly low compared to other landscapes (Wake and Morowitz 1991). Recent research has demonstrated that windborne pesticides/toxins and global change are potential factors in these settings (Davidson et al. 2002, Carey and Alexander 2003, Sparling and Cowman 2003). Recent bioregional assessments and land management plans for the Sierra Nevada mountain range have identified declining species and provided recommendations and standards for reducing impacts to these species (Sierra Nevada Ecosystem Project 1996, USDA Forest Service 2001, 2004). Almost half of the 30 or so native amphibian species in the Sierra Nevada are considered to be at risk by State and Federal regulatory agencies (Jennings and Hayes 1994, California Department of Fish and Game 2004, USDI Fish and Wildlife Service 2002, 2004). This mountain range comprises only 20% of the total land area of California, yet 50% of the native plant species in the state occur within it. Over 3,500 plant species, 400 of which are endemic, occur in the Sierra Nevada (D'Antonio et al. 2002). Within this range, montane meadows are areas of high biodiversity, supporting many native and rare species, providing forage and water for grazing animals, and offering a distinct habitat contrast with surrounding areas (Allen-Diaz 1991, Gavin and Brubaker 1999, Ratliff 1985). Meadows comprise less than 10% and riparian areas are less than 1% of the Sierra Nevada, but their ecological importance is disproportionate to their size (Ratliff 1985, Kattleman and Embury 1996).

Yosemite Toads

The Yosemite toad (*Bufo canorus*) is endemic to the Sierra Nevada mountain range from the Blue Lakes region north of Ebbetts Pass in Alpine County south to Kaiser Pass area in the Evolution Lake/Darwin Canyon region of Fresno Co at elevations from ca. 1950 m ca. 3600m (6400 to 11,800 ft). Yosemite toads are typically associated with high montane and subalpine vegetation in relatively open wet meadows surrounded by forests of lodgepole pine or whitebark pines and are primarily active during the late spring, summer, and early fall (Zeiner et. al. 1988, Jennings and Hayes 1994). Adult females are slightly larger (69 versus 66 mm) and heavier (20 versus 17 grams) than adult males. Males and females vary markedly in color; males are olive green while females are gray or brown (Kagarise Sherman and Morton 1984). Cover requirements differ by life history stage. Suitable breeding sites are generally found in shallow, warm water areas such as those found in wet meadows, potholes, the edges of small ponds, shallow, grassy areas adjacent to lakes, slow moving streams, sloughs and backwaters (C. Brown, personal communication). Short emergent sedges or rushes often dominate such sites (Jennings and Hayes 1994). Yosemite toads breed in late spring, usually at snowmelt, laying eggs in shallow areas of lakes and wet meadows; larvae metamorphose by mid-late summer of the same year. Breeding can last up to five weeks for males, while females often only visit breeding sites for two to three days. Breeding is often 1-2 weeks, but can last up to 5 weeks for males; females often only visit breeding sites for 2-3 days (Kagarise Sherman and Morton 1993, Sadinsky 2004, C. Brown, personal communication). Meadow water depth and water temperature appear to be important limiting factors in the survival of eggs and larvae (Kagarise Sherman and Morton 1993). These characteristics are strongly influenced by winter snow pack, spring temperatures, and meadow topography/hydrology, but direct relationships to toad habitat use and egg/larval survival have not been quantified. Species success is heavily dependant on weather, with variations in climate such as changing storm patterns and drought delaying or interrupting breeding and affecting survival of metamorphic toads and adults (Kagarise Sherman and Morton 1993, Jennings and Hayes 1994). Adults utilize aquatic and terrestrial environments for foraging and cover. Springs, upslope from meadows, and rodent burrows are two features that appear to be important for adult dispersal and over wintering habitat (Kagarise Sherman 1980).

Yosemite toads are believed to have declined or disappeared from at least 50% of known localities during the later part of the 20th century (Jennings and Hayes 1994, Drost and Fellers 1996, Jennings 1996) though limited quantitative surveys exist. Long-term monitoring data at Tioga Pass indicate large declines in local populations since the early 1980's (Kagarise Sherman and Morton 1993), although the cause for this decline is unclear. Yosemite toads are a Species of Special Concern in California, a Forest Service Region 5 sensitive species, and a candidate species for federal listing under the Endangered Species Act (USDI Fish and Wildlife Service 2002, 2004). Potential factors that could individually or collectively be responsible for the decline of Yosemite toads include livestock grazing in montane meadows and riparian zones, airborne chemical toxins, disease, and climatic shifts and variability, though none of these has emerged as a singularly strong candidate (Davidson et al. 2002, USDI Fish and Wildlife Service 2002). Research seems to be eliminating possibilities instead of verifying definite causes. For example, an extensive scale study by Knapp (2005) concluded that introduced trout are not a major concern for Yosemite toads. In a predation experiment, Grasso (2005) found that toad larvae and post-metamorphic young of year were unpalatable to common introduced trout species. Another study by Bradford et al. (1994) found no correlation between acid deposition and Yosemite toad decline, and suggested that disease is potentially the greatest cause of decreased numbers. Two diseases that may affect Yosemite toad populations are red-legged disease and chytrid fungus (USDI Fish and Wildlife Service 2002).

Of the activities occurring on National Forest lands and under the jurisdiction of Forest Service management, livestock and packstock grazing have been identified as activities that may affect Yosemite toads (USDA Forest Service 2001). This is because of the overlap of grazing with toad breeding and rearing areas in wet meadows. Though cattle are rarely present during the brief breeding period at snow-melt, they are often present during larval rearing and metamorphosis. While this potential risk factor has been identified in the public record, the supporting data are primarily from anecdotal accounts and unpublished sources (USDI Fish and Wildlife Service 2002). Preliminary evidence suggests that livestock use of wet meadow habitats may affect Yosemite toads indirectly through: (1) changes to meadow stream hydrology and bank stability (increased down-cutting and head-cutting), (2) changes to water quality, and (3) changes in micro-topography of egg deposition and larval rearing areas (USDI Fish and Wildlife Service 2002). The extent of these impacts and their relationship to population level survival and persistence need further study. Due to a reported Yosemite toad population decline and its suspected link to livestock grazing, the USFS placed in non-use a number of previously utilized Sierra Nevada grazing permits in 2001. Many of these allotments had been active for over one hundred and fifty years (USDA Forest Service 2001). A study on this subject is urgently needed both to provide guidance to land managers who are faced with decisions regarding human and livestock use of montane meadows and to better understand the role that livestock grazing may be playing in the decline of the Yosemite toad.

Livestock Grazing

The Spanish introduced livestock to the Sierra Nevada in the mid-1700s, but extensive grazing did not occur until the 1860s (Menke et al. 1996). This was due in part to population increase following the Gold Rush. Drought and flooding also pushed ranchers to higher elevations in the Sierra Nevada during that time. Unregulated and non-sustainable grazing practices were common into the late 1800s (Menke et al. 1996). The establishment of Forest Reserves and subsequently the Forest Service (1905) alleviated the situation by instituting a grazing management policy (Kinney 1996). The Taylor Grazing Act (1934) also led to the implementation of federal land policies and helped end the "open access" era of grazing in the Sierra Nevada (Allen-Diaz et al. 1999). Stocking rates have fluctuated some since then, but have continued to gradually decline (Allen-Diaz et al. 1999, Kosco and Bartolome 1981). The issuance of fewer permits has widespread social (e.g. forcing ranchers to sell their operations) and ecological implications and is a common topic in research and management discussion (Sulak and Huntsinger 2002).

In addition to the subject of permits, other aspects of grazing are often polarized, misunderstood, and/or ambiguous (Brown and McDonald 1995). Researchers and managers frequently identify grazing as either good or bad without consideration of the intensity, timing, frequency, duration, and season of use (Allen-Diaz et al. 1999, Fleischner 1994). Grazing treatments are also often vague, with studies citing "heavy" and "light" levels instead of outlining quantitative intensities (Trimble and Mendel 1995, Tate et al. 1999). To compound the complexity, grazing may also simultaneously negatively and positively impact different aspects of a single system (Hayes and Holl 2003, Allen-Diaz et al. 2004).

Grazing and Ecosystem Interactions

Improper grazing can negatively affect riparian and aquatic systems in a number of ways (Flenniken et al. 2001). Grazing in riparian areas may cause soil compaction, removal of vegetation, physical damage and reduction of vegetation, and alteration of plant growth forms by removal of terminal buds and stimulation of lateral branching (Kauffman and Krueger 1984, Tucker Shulz and Leininger 1990, Szaro 1989). Decreased infiltration, increased erosion and runoff, and increased soil temperature can result from these impacts (Armour et al. 1991). Grazing may change aquatic systems through degrading channel morphology, lowering the groundwater table, decreasing stream flow, altering timing and rate of flood events, and increasing stream temperature (Armour et al. 1994, Kauffman and Krueger 1984). Changes in water quality, such as increased nitrate, phosphate, dissolved solids, and sediment are additional concerns (Kauffman and Krueger 1984).

Grazing is often negatively implicated in other ecosystem changes. A study of pack stock grazing in Yosemite National Park recently found a decrease in plant productivity, a decrease in vegetation cover, and an increase in bare soil cover with increased animal utilization (Cole et al. 2004). Pack stock and cattle can also transport invasive seeds through fur and dung and open habitat for non-native species (Trimble and Mendel 1995). Preliminary assessments indicate that livestock may trample amphibian eggs, larvae, juveniles, and burrows used by adults for hibernation and cover (Jennings 1996, USDI Fish and Wildlife Service 2002).

A number of studies have conversely shown either no effect or a beneficial impact of livestock. For example, appropriate cattle grazing reduces non-native annual grasses around vernal pool margins, benefiting these fragile systems and the organisms that depend upon them (Robins and Vollmar 2002). Hayes and Holl (2003) identified mixed effects of grazing in coastal California grasslands. They found an increase in species richness and cover of native perennial forbs in ungrazed sites, but no difference in native grass cover and species richness between grazed and ungrazed sites. They concluded that a matrix of disturbance regimes is necessary to maintain a suite of native species. A study by Hickman et al. (2004) in tallgrass prairie found higher species diversity and richness in grazed versus ungrazed exclosures. An additional study by Bull and Hayes (2000) concluded that grazing did not negatively impact reproduction and recruitment of the Columbia Spotted Frog (*Rana luteiventris*).

The complexities of species decline, the social and ecological implications of different management decisions, and interactions of livestock with ecosystems are impressive. Ascertaining how to approach these issues is even more challenging. However, we do know that global biodiversity loss is occurring at an accelerated rate, and the complete implications of this decline for society and the environment are unknown (Myers et al. 2000, Forester and Machlis 1996). An array of habitats and species provide cures for human diseases, economically valuable goods and services such as clean air and water, protection from floods and droughts, and recreational opportunities (Allen-Diaz 2000, Spear 2000). As biodiversity declines and species become extinct, we lose knowledge about possible cures for human illnesses, evolutionary connections between organisms, and an understanding of ecosystem processes. Precluding species decline is therefore imperative for human well being in the future. Understanding relationships between species and their habitats and assessing how humans affect these systems in both positive and negative ways will provide the information needed for management and restoration. Our proposed study of Yosemite toads and livestock grazing will provide the knowledge needed for the management and conservation of this species and the unique environments it inhabits.

OBJECTIVES

The overall objective of this study is to understand the effects of varying levels of livestock grazing on Yosemite toad populations and habitats. USDA Forest Service Region 5 staff formulated two key questions to guide this research:

1. Does livestock grazing under Forest/Sierra Nevada Forest Plan Amendment Riparian Standards and Guidelines have a measurable effect on Yosemite toad populations?

Assessment of grazing standard and guideline variables includes monitoring meadows occupied by Yosemite toads under the following treatments:
* Ungrazed meadows (not grazed within recent history, likely outside of an active allotment).
* No grazing within the meadow (i.e. these are meadows that have been grazed recently; they will be fenced to exclude grazing during the study).
* Exclusion of livestock in wet areas within a meadow (S&G 53).
* Grazing in accordance with Riparian S&Gs 103, 120, 121 across the entire meadow.

2. What are the effects of livestock grazing intensity on the key habitat components that affect survival and recruitment of Yosemite toad populations? Key meadow habitat components include hydrology, topography, and cover.

The research proposed here will address these questions and determine whether different treatments result in different population levels and habitat conditions for Yosemite toads. This information can then be used to provide guidance to land managers on relative risk of a set of grazing (or non-grazing) approaches to the long-term survival of Yosemite toads on Forest Service lands. This latter step will require an assessment of the viability of the toad at local and more extensive spatial scales. The data we collect can be used in such an assessment, but additional data on the distribution of Yosemite toads on Forest Service lands will also be needed. Distributional data has been collected at varying degrees of detail over the last few years on the Stanislaus, Sierra, and Inyo National Forests both for range-wide long-term monitoring (C. Brown, pers. comm.) and Forest level survey requirements (S. Holdeman, H. Sanders, pers. comm.). The results of this work will also contribute to an on-going conservation assessment for the Yosemite toad and to any future conservation strategies.

STUDY AREA

The broad study area for this project is the geographic range of the Yosemite toad which includes populations on the El Dorado, Inyo, Stanislaus, Sierra, and Toiyabe National Forests and Yosemite and Sequoia Kings Canyon National Parks. Of these areas, toad populations are most abundant on the Inyo, Stanislaus, and Sierra National Forests and in Yosemite National Park (USDI Fish and Wildlife Service 2002). Because this study specifically deals with livestock grazing and this use is more prevalent in some areas, the focal areas for meadow selection include the Stanislaus and Sierra National Forests (livestock treatment meadows and possible reference meadows) and potentially Yosemite National Park (reference meadows).

METHODS AND ANALYSIS

Study Design

Overview

The project will be based upon two complementary study designs (described here as Phases I and II) both focused on achieving the established study objectives. The experimental unit for each phase is an individual meadow. The population of interest is meadows in the central Sierra Nevada between 1829 and 3048m (6000 and 10,000 feet) in elevation which currently support a Yosemite toad population and/or have the potential to support a toad population. Inherent meadow to meadow variability (hydrology, geomorphology, disturbance regime, etc.) combined with the complexities of conducting research at the management scale make strict application of experimental methods (treatment, control, replication, complete randomization) difficult. For instance, no two meadows have replicate biology, ecology, hydrology, geology, and disturbance regimes. A combination of experimental and observational methods will be applied with heavy reliance upon multivariate statistical procedures to: 1) identify and quantify general associations between cattle grazing management, critical toad habitat factors, and toad population dynamics with quantitative evaluation of if and how these relationships are conditioned by meadow specific factors/covariates (Phase I) and 2) specifically test the effects of 3 grazing treatments on toad populations and habitat (Phase II). The design for each phase is described in detail in the following sections, as are the strengths and weaknesses of each design.

Phase I

Approach

Phase I will be an observational, cross-sectional survey of a larger set of meadows (n>50) which will be sub-sampled from the population of interest. Cattle grazing management will not be manipulated on-the-ground; rather we will take opportunistic advantage of the diversity of grazing management which we know exists across the population of meadows of interest (defined above). Previous grazing management surveys in the region (Ward et al., 2001) confirm that significant gradients exists for management factors such as: 1) grazing intensity, frequency, and season; 2) grazing distribution practices (*e.g.*, none, herding effort, drift fencing); 3) proximity to cattle attractants (*e.g.*, significant forage resources, drinking water), and 4) exclusion (*e.g.*, 1, 10, 20 years of exclusion). The assumption inherent in this approach is that toad population and habitat metrics at a point in time integrate recent grazing management (last 5 to 10 years), and are sensitive to variation in grazing impact levels. Similar assumptions have been accepted and widely applied in development and application of bioassessment protocols such as those based on aquatic macroinvertebrate community composition (Wohl et al., 1996; Hawkins et al., 2000; Weigel et al., 2000).

This design is based upon the quantification (directly measure) and/or classification (assign to a category such as “low” or “high”) of toad population, habitat, vegetation, and cattle grazing variables. Multivariate analysis of these variables in conjunction with covariates (*e.g.*, elevation, meadow size) is then used to identify and quantify associations between toad population, habitat, and specific grazing management factors dependent upon site specific conditions. Significant variation can be expected across the population of meadows of interest for all factors of interest. It can also be expected that factors will potentially interact to condition toad response to grazing. It is crucial that the sub-sample of meadows selected for this survey: 1) are proportionately representative of the range of conditions (elevation, disturbance regime, etc.) found in the population of interest; 2) represent the complete gradient of variables of specific interest (toad population, habitat, grazing management) found across the population; 3) have sufficient overlap in levels of variables of interest to allow examination of potentially important interactions between these variables (*e.g.*, grazing frequency by meadow size interaction might describe the effect of grazing frequency upon toad presence or absence). We will not be able to determine adequate sample size for this phase until we have compiled a sufficient sub-sample for preliminary analysis. We were able to identify and quantify relationships between grazing and riparian health metrics in mountain meadows via a cross-sectional survey that enrolled ~60 Sierra Nevada meadows (Ward et al., 2001).

As a first step, existing data on toad population, habitat, vegetation, grazing management, and meadow covariates will be capitalized upon. It is crucial that the data collected across meadows be comparable in terms of methodology, collection date/season, units, etc. This will certainly be an issue in utilizing existing datasets. However, it is reasonable to expect that there will be reliable metrics common among datasets for toads, habitat, and vegetation, respectively. For instance, the simple metric of toad presence (yes, no) can almost certainly be derived from existing datasets with relative certainty of accuracy and comparability across meadows. However, number of adult toads per meadow is a metric that will most likely not be consistently available or credible for all datasets.

We will limit our use of existing datasets to those which were collected as part of a wide-spread, consistent survey or data collection effort with clear quality control measures in place. Such data are known to exist in the region for both toads and vegetation (Personal Communications: C. Brown, H. Sanders, and S. Holdeman, USDA Forest Service Stanislaus and Sierra National Forests, C. Miliron, California Department of Fish and Game). Overlap of these datasets across a sufficiently large and diverse sub-set of meadows will be an important factor in determining the feasibility of using only existing data. Strategic field data collection will occur during year 1 if augmentation and/or confirmation of existing datasets is required. Grazing data will certainly need to be collected via interviews with grazing managers, permit holders, and district staff. We have a cattle management survey available for this purpose which was developed and used extensively in a similar previous project.

Statistical Analysis

Phase I will generate data on several dependent or response variables, requiring multiple analyses (development of statistical models for each response variable). Dependent variables available in this phase will be toad population (*e.g.*, presence or absence, density of breeding areas per meadow) and toad habitat/vegetation (e.g., number and condition of breeding areas, meadow hydrology measures, emergent vegetation) metrics which are both categorical and continuous. Independent variables will include grazing management metrics (*e.g.*, season of grazing, stocking rate, herding effort as days/year). Covariate data from each meadow will be available for a suite of factors (*e.g.*, elevation, pack-stock use levels, and mean annual snowfall).

We have had success in modeling similar survey datasets using linear models, linear mixed effects analysis, and logistic regression models (Ward et al., 2001; Tate et al., 2003; Tate et al., 2004*b*). Potential co-dependence among meadows in the same management unit (allotment) will be investigated and modeled as random effects if required. Spatial correlation among meadows in close proximity will also be evaluated and modeled if required (Pinheiro and Bates 2000). Compliance with assumptions of normality and constant variance will be checked and variance and correlation structures for handling heteroscedasticity within the data as well as transformation of response variables applied if required. Analysis approaches will be refined in consultation with statisticians as the project progresses and the complete data structure is evident.

Strengths and Weaknesses

Strengths of this study design include: 1) examines a broad suite of grazing management factors; 2) does not depend upon implementation of grazing treatments over multiple years with associated logistical difficulties and risks; 3) can potentially yield management recommendations within 1 to 2 years; and 4) capitalizes upon the variability found across the population of interest, potentially increasing the scope on inference of the results. Weaknesses of this design include: 1) identifies and quantifies associations, cannot absolutely determine cause and effect; 2) requires significant data compilation and is vulnerable to unknown faults in existing data; and 3) variability may be so great that sample size requirements become infeasible with current resources.

<u>Phase II</u>

Treatment Definitions

The treatment in this phase of the study is cattle grazing. Three levels of cattle grazing will be examined in detail, and an additional 2-3 reference (long-term ungrazed) will also be monitored. Grazing treatment levels were derived from specific guidance provided by the Steering Committee and statistical requirements for a reference condition identified by the Design Team. Cattle grazing treatment levels are: 1) compliance with annual grazing standards and guidelines as defined in the most recent Sierra Nevada Forest Plan Amendment (USDA Forest Service 2004); 2) exclusion of cattle from wet areas of the meadow; 3) exclusion of cattle from the entire meadow; and 4) ungrazed by cattle for >15 years (reference condition). Baseline data will be collected in year 1 and grazing treatment levels 1 through 3 will be implemented and maintained during years 2 through 5 of this study by the study team. Grazing treatment level 4 is a reference condition which is already in existence and will be maintained throughout the course of the study. The use of the term "grazing treatments" in following sections refers to grazing management treatment levels 1 through 3, and the term "reference condition" refers to grazing management treatment level 4. The statistical importance of a reference condition treatment level is discussed below.

Approach

Phase II will be a randomized complete block design based upon collection of data before and after implementation of grazing treatments across 15 meadows (5 replicates of 3 grazing treatment levels), with the inclusion of 2-3 reference condition meadows to serve as a baseline across the study period (17-18 meadows total). This phase will focus on meadows known to contain toads and which have low pack-stock use. Blocks are spatial clusters of 3 meadows. The primary purpose for clustering is to simplify formidable data collection logistics by having clusters of meadows in close (~10-20 km) proximity to each other. For statistical purposes, each treatment level will be represented in each cluster (1 replicate). Thus each cluster will have 3 meadows either currently or recently grazed under ambient grazing conditions. Grazing management prior to this phase will be quantified for each meadow for use as a covariate in analysis. Due to potential constrains imposed by location of ungrazed meadows, cluster location selection may not be completely random. Every effort will be made to introduce randomization in cluster site selection and to insure cluster site selection does not introduce bias towards one or more treatment levels. Depending upon project goals, cluster locations could be randomly selected in a stratified manner (*e.g.*, northern, central, southern range of the population; Stanislaus NF, Sierra NF). *After substantial reconnaissance efforts in 2005 and 2006, reference meadows (long-term ungrazed) of appropriate elevation and vegetation type could not be found in proximity to the study areas on the Sierra and Stanislaus National Forests. In 2007, two meadows in Yosemite National Park were added to fill the role of reference meadows and these will be monitored for the remainder of the study.*

Following a minimum of one year of baseline data collection on all 3 meadows in each cluster, grazing treatments (comply with annual grazing standards and guidelines, exclude cattle from wet portions of meadow, exclude cattle from entire meadow) will be randomly allocated to the 3 currently/recently grazed meadows within each cluster. Grazing treatments will be implemented annually during the remaining 4 years of the study, as will data collection across all 17-18 meadows.

Rationale for Baseline Data and Reference Condition

Grazing treatment level effect on toad populations, habitat, vegetation, etc. will be determined by comparison of relative changes in treated meadows to changes in ungrazed meadows over the study period (statistically significance/insignificant treatment level by year interaction). Collection of baseline data allows us to quantify initial differences between meadows scheduled for treatment implementation and reference condition meadows. Without baseline data, we cannot determine if any differences between treatment and reference meadows at the end of the study are due to grazing treatments or if these differences simply existed prior to grazing treatment implementation. For statistical purposes, the reference condition is the treatment level which is consistently applied across the entire study period allowing a reference or baseline condition against which to compare changes in treated meadows. Implementation of consistent management on a sub-set of experimental units across the study period reduces our vulnerability to confounding factors such as long or short term annual weather patterns. From a statistical perspective, a consistently applied heavy grazing treatment level could just as easily serve as a reference or baseline condition. From a biological and ecological standpoint, the ungrazed reference condition is of specific interest due to its potential to provide context for understanding the relative effects of cattle grazing.

Statistical Analysis

This phase of the study will generate data on a large number of dependent or response variables, requiring multiple analyses (development of statistical models for each response variable). Dependent variables available for analysis will be metrics of toad population (*e.g.*, # of toads by life stage, ratios of life stage abundances, density of breeding areas per meadow), habitat (*e.g.*, water temperature, aquatic plant cover), and vegetation (*e.g.*, species cover, diversity, structure). Independent variables will be grazing treatment which has 3 levels and year of which there will be five. Covariate data from each meadow will be available for a suite of static and dynamic factors (*e.g.*, lotic v. lentic, elevation, past grazing pressure, annual pack-stock use, annual snowfall). Reference meadow data will provide context, specifically related to background seasonal and annual variation in toad population sizes in ungrazed meadows. Several factors complicate the interpretation of this dataset and must be addressed directly within the analysis process. There will be co-dependence within the data due to repeated measures on each experimental unit (meadow) across a season and across years. Data from meadows within the same cluster will potentially be subject to spatial correlation. Based upon previous experience, we suspect that these data will not comply with the normal distribution nor will they have a constant variance. Finally, inherent variability between meadows due to non-cattle grazing related variable such as elevation, annual snowfall, geomorphology, etc. will almost certainly reduce or confound our ability to ascribe treatment effect. This will be addressed as much as possible by inclusion and modeling of these variables as covariates during statistical analysis.

We have had success in modeling similarly complex datasets using both linear mixed effects analysis and count-based regression models (Atwill et al., 2002; Tate et al., 2004*a*; Jackson et al., 2006). Linear mixed effects analysis is one feasible approach that will be explored in the analysis of this data (Pinheiro and Bates, 2000). Meadow identity and cluster will be treated as group effects to account for co-dependence introduced by repeated measures and spatial proximity, respectively. The model allows, if warranted, application of variance and correlation structures for handling heteroscedasticity within the data. Transformation of response variables will also be explored. We will also explore the application of negative binomial regression analysis and other count-based regression techniques, which we have found valuable in the analysis of data similar to that available in this study (*e.g.*, counts of toads). Software which allows for cluster or group variables (meadow identity and cluster) will be used (as explained in volume 2 of *Stata Statistical Software: Release 7.0*, *Reference H-P*, p. 530-534 [Stata Corporation, College Station, Tex.], 2001). Analysis approaches will be refined in consultation with statisticians as the project progresses and the complete data structure is evident. Preliminary analysis will be conducted on an annual basis to provide guidance for final analysis strategies, and as a standard quality control and assurance measure.

Strengths and Weaknesses

Strengths of Phase II study design include: 1) explicitly targets the three grazing treatment levels identified by the Steering Committee; 2) has potential to establish cause and effect, rather than identify associations/correlations; 3) is longitudinal, allowing examination of cumulative effects of treatment application over multiple years; 4) targets data collection efforts on a reasonable number of meadows allowing quantification of seasonal dynamics toad population, habitat, hydrologic, vegetation, etc. variables which may be important for interpreting study results. Weaknesses of the study design include: 1) treatment effects may take longer than 4 years of treatment implementation to become significant; 2) one year of baseline may be inadequate to accurately characterize initial conditions and differences between treatment and reference condition meadows; 3) the study is vulnerable to unforeseen confounding factors, and/or is under sampled to account for a large number of potential confounding factors; 4) the study is vulnerable to unforeseen logistical problems with grazing treatment implementation, and/or events such as prolonged drought, wild fire, etc.; 5) study results will likely not be available for incorporation into conservation strategies until 5 to 6 years after the study commences.

Study Meadow Selection

Phase I

As described above, this phase of the project primarily involves the use of existing data sources. Once a preliminary assessment and analysis of these sources and their applicability to our main questions has been completed, we will strategically select additional field sites for rapid assessment and/or return to field sites from existing data to gather data on missing variables of interest. Our goal will be to ensure that we have data from a gradient of livestock grazing levels and a gradient of Yosemite toad occupancy levels.

Phase II

Random Selection of Meadows/Allotments

All available distributional data on Yosemite toad was collected. Appendix A provides a list of the data sources considered for use in the analysis to determine sample sites. Each occurrence data point is associated with geographic coordinates (utm northing and easting) and/or a meadow. Because meadows are the sampling unit and not all Yosemite toad occurrences are associated with a meadow, all occurrences were overlaid in a Geographical Information System (GIS) environment with a meadows layer. Meadows that overlapped with the point occurrences are thought to have Yosemite Toad present. Only meadows with an elevation between 6000 and 10,000 feet and meadows in Stanislaus NF, Sierra NF and Yosemite NP are considered.

A randomized block design was utilized so that each block would contain four meadows which could later be assigned to each of the four grazing treatment types. The primary purpose for clustering the sample meadows into blocks is to simplify data collection logistics by having clusters of meadows in close proximity to each other (maximum of about 12 km apart). A secondary benefit from blocking is that all meadows within the block should have similar environmental conditions, such as temperature and precipitation.

Each block, which was actually a hexagon, had an area of 100 km^2 (Fig. 1). The hexagons were overlaid with the distribution of the Yosemite toad. Hexagons containing at least five meadows occupied by Yosemite toad (n = 27) were assigned random numbers and arranged in order from lowest number to highest number (Fig. 2). At least one meadow in the block must not have been grazed by cattle for at least the previous 15 years. Meadows adjacent (within 2km) to the block were included in the list of potential sample meadows to provide options for this ungrazed category.

For initial meadow selection, we reviewed the blocks in their assigned random order and assessed whether they met minimal qualitative criteria for final selection. These criteria included: presence of relatively robust toad populations (e.g. multiple life stages, multiple breeding sites, relatively population sizes) and accessibility (distances to drivable roads, etc). If a block did not meet these criteria, then the next block was assessed for inclusion into the study. In addition, we imposed a criterion to ensure representation of study meadows on both National Forests. Because more blocks were initially present on the Sierra than the Stanislaus National Forest, we required that minimum of two blocks be chosen from the Stanislaus National Forest. The selected blocks were associated with 8 livestock grazing allotments; they are presented here in the random order in which they were chosen: Dinkey, Collins, Patterson Mountain, Highland

Lakes, Blasingame, Kaiser, Mt. Tom, and Herring Creek. These allotments thus became our focal set. Even though we ultimately need only 5 allotments to conduct the study (3 meadows x 5 allotments = 15 study meadows + 2-3 reference meadows), we will evaluate all 8 in the next step of site selection to allow some flexibility. In addition, we plan to collect baseline data on more than 15 meadows, and assess their general condition. Meadows that are extreme outliers may be eliminated during the later years of the study which should increase our ability to provide more reliable and precise results to managers.

Final Meadow Selection

To aid in final study meadow selection, we sent a questionnaire to Forest staff to gather the following information on all meadows in these 8 allotments: proximity to ungrazed meadows, detailed population data on Yosemite toads (number of breeding sites, multiple years, abundance by life stage, etc.), potential logistical difficulties, road access and condition, current packstock and recreational (hiking, camping) use, types of livestock controls employed (e.g. fencing, herding), availability of records on history of livestock use, and availability of quantitative information on meeting standards and meadow/stream conditions, occurrence of meadow restoration activities, and rodent densities/control measures (Appendix B).

Based on information provided to the design team via the questionnaire, logistical constraints (i.e., accessibility and the need for clusters of 3 meadows for sampling efficiency), and field visits in spring 2005, we reduced the set of 8 allotments to 5: Dinkey, Patterson Mountain, and Blasingame on the Sierra National Forest and Highland Lakes and Herring Creek on the Stanislaus National Forest.

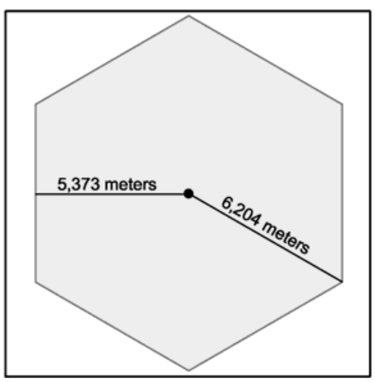

Figure 1. Depiction of the 100 km^2 hexagon.

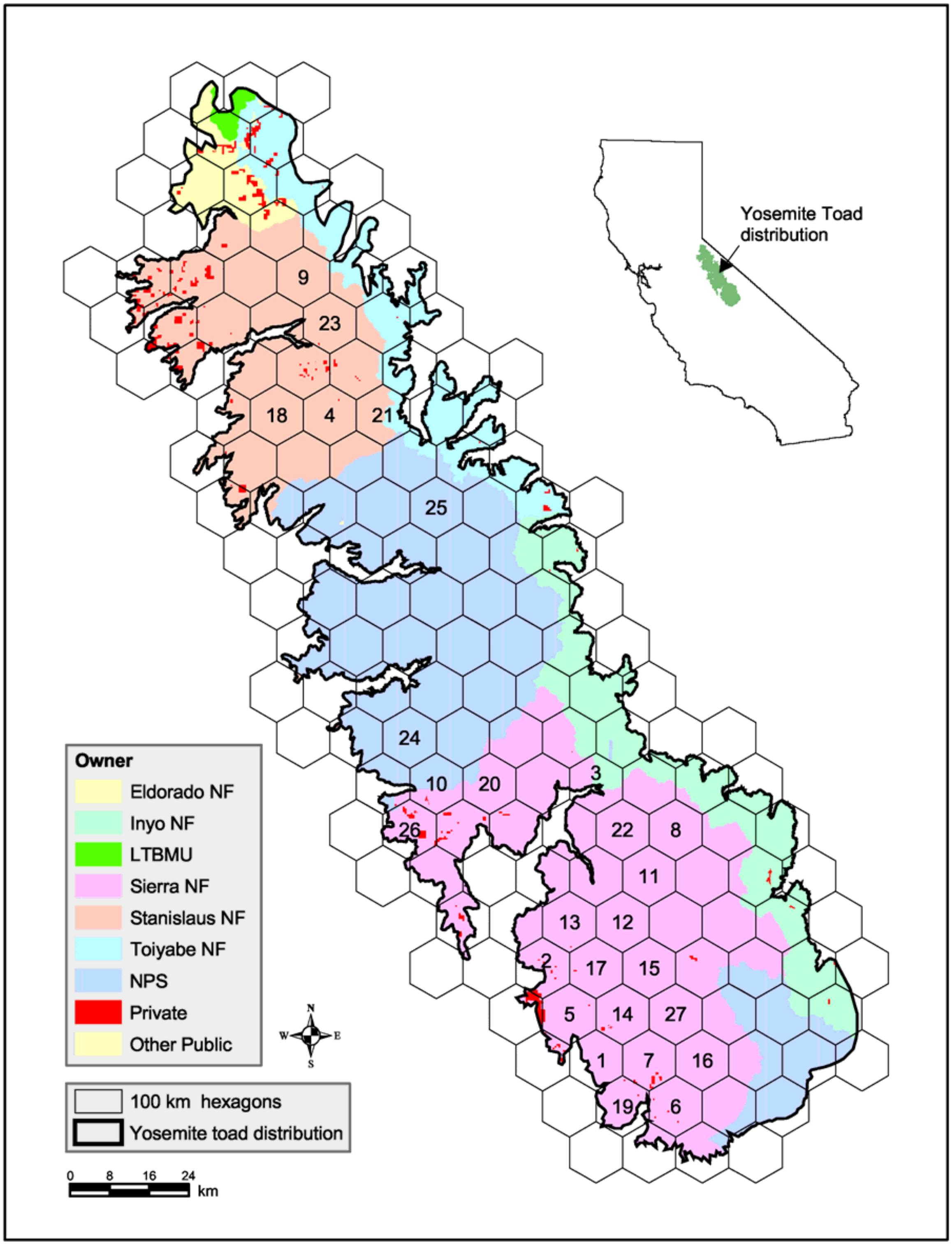

Figure 2. Yosemite toad distribution with randomized block order.

Data Collection Methods

Yosemite Toad Populations and Microhabitat

Determination of Population Attributes/Parameters

To develop reliable estimates of the effects of livestock grazing on Yosemite toad populations, we will need to use repeatable sampling methods that will ideally provide information on relative abundances or densities of all life stages. Yosemite toads have a relatively short breeding season; up to 5 weeks, but at some locations as few as 1-2 weeks. Research and anecdotal accounts indicate that adults are not easy to detect outside of this season as they may be active primarily at night and take refuge in rodent burrows (USDI Fish and Wildlife Service 2002, Sadinsky 2004). Thus the timing and intensity of our sampling will dictate the potential methods we will use.

Phase I – For analysis of existing data, we will rely on toad occupancy, breeding status, and potentially indices of abundance as response variables. Because these data will be from a variety of sources, we are developing metrics that are consistent among the sources and performing a "validation" of data by comparing multiple sources for the same meadow locations. For example, extensive surveys for Yosemite toads have been conducted on the Sierra National Forest. Prior to using these data for analyses of occupancy status, we will compare ("validate") the occupancy status to data collected by the Sierra Nevada Amphibian Monitoring Team (C. Brown, pers. comm.) and other researchers (R. Grasso, pers. comm.) at the same sites.

Similarly for any additional meadows where new field data are collected, we will use metrics that can be rapidly recorded and converted to the same indices used for the existing data analysis. These metrics also need to be somewhat consistent over the timeframe of the field work since different life stages will occur at different relative abundances depending on the timing of sampling (e.g., early vs. late summer). However, because occupancy is the key toad population parameter here, the timing of this work is less critical. For example, we could use a metric that represented the number of breeding/rearing areas which could be determined through observation of eggs, larvae, or early metamorphic toads.

Phase II – The overall study design for this phase includes only five replicate meadows per grazing treatment, so identifying population attributes that will provide appropriate sensitivity in the face of the expected high levels of variability is critical. We will use a combination of abundance data, capture-recapture population estimates, and indices of breeding/rearing areas to characterize toad populations. Detailed information on relative abundance by sex and life stage (eggs, larvae, metamorphic toads [young of the year], subadults, adults) collected during the baseline year will provide insights into ambient levels of variability in toad populations and guidance on selection of meadows. In subsequent years of the study, we will collect more detailed information on abundance, including estimates of population size by life stage using capture-recapture techniques. We will make an effort to collect information on all life stages, though adult and subadult (1+ year olds) toads present challenges because of their limited activity periods at meadows. Following assessment of data from the baseline year, we will adjust the timing of sampling as needed to increase likelihood of encountering adults at least a subset of our sites (e.g. at all meadows within 2 blocks/allotments).

In addition, we will record information on body size/weight and evidence of disease for a subset of individual larvae and toads to develop a description of the health of each population. We are currently working with researchers in the Briggs Lab at U.C. Berkeley to evaluate levels of chytrid fungus (*Batrachochytrium*) presence (and prevalence) in our study populations (C.

Briggs, pers. comm.). To determine if we will have the power to detect differences in grazing treatments at biologically appropriate levels if they exist, we will conduct sample size analyses and analyze sources of variability from existing data. We will use data on intra and inter-annual toad abundance gathered by the Sierra Nevada Monitoring Team (C. Brown, pers. comm.). Baseline data collected in the first year of our study will provide additional information so that sample sizes or sub-sampling approaches can be modified to improve estimates of and confidence in effect size detection.

Microhabitat associations for individual toads and for groups of eggs or larvae will also be measured and will include attributes that characterize surrounding immediate (< 1m) aquatic environments, such as water depth, vegetation, etc. This information, when analyzed in combination with the meadow and pool habitat data, will give insights on the specific features of the environment that are utilized by and important to different life stages of toads. The potential effects of cattle grazing on these features can then be assessed.

Field Methods

The observational component of Phase I is dependent on the inclusion of a large number of meadows along a gradient of conditions, so we will use metrics of toad populations that can be collected rapidly in the field. Presence/absence of toads and counts of breeding/rearing areas are two such metrics (Thompson et al. 1998, Knapp et al. 2003). Phase I meadows will be visited only once during the study so we will use techniques that have a high likelihood of detecting toads if they are there. Visual encounter surveys and dip-netting (Heyer et al. 1994) will be the primary methods used to detect toads and these surveys will focus on areas of the meadow where toads are most likely to occur (e.g. pooled water) to increase efficiency. Depending on the timing of sampling, we should encounter either or both larval and metamorphic life stages so we will also be able to quantify the density of breeding/rearing areas. Counts of all life stages will be made so that it will be possible to develop abundance indices, though differential timing of sampling may ultimately confound our ability to generate reliable estimates of abundances by life stage.

For Phase II, our sampling approaches need to generate precise estimates of toad population sizes. The following are potential metrics: 1) abundance of toads by life stage (eggs, larvae, metamorphic toads, sub-adults, adults), 2) ratios of life stage abundances, especially as an index of survival from larval to metamorphic stages, 3) population size and survival rates of toads can be calculated (if capture-recapture techniques can be used successfully), and 4) density of breeding/rearing areas per meadow. Because of life history of the toad and limited access to meadows under snow, we will likely encounter primarily larvae and metamorphic toads in the study meadows. These life stages are notoriously difficult for estimates of population size because their numbers can vary dramatically within meadows, among meadows, and among years (e.g. Jung et al. 2002). We will deal with this in several ways: 1) by choosing sub-sites within meadows and doing multiple counts of all life stages within sub-sites over the course of a day or two (counts of the same area by different observers may area also being tested to increase precision of estimates), 2) using a temporary mark on larvae for sub-sites within meadows and returning within a day to count marked and unmarked larvae, and 3) visiting each meadow in two different time periods so that counts of larvae are made during the first time period and counts of metamorphic toads are made during the second time period, which should provide a rough estimate of survival from larval stage to metamorphosis. We will also attempt to survey a subset of meadows early in the season so that counts and population estimates of subadult and adult toads (using capture-recapture techniques) can be made. Finally, we will also utilize information on the density and occupancy of breeding/rearing areas as an index of toad population size. Draft protocols for toad population data collection can be found in Appendices C1-C3.

Microhabitat data will be collected on groups of eggs or larvae. Variables representing immediate habitat conditions in the wet areas of the meadow will be the main focus including: water depth, substrate, presence of emergent vegetation, etc. (Figure 3). In addition, habitat characteristics that are believed to represent features important to terrestrial life stages of toads (subadults and adults) will be quantified, especially burrow abundance within and immediately adjacent to meadows and connectivity to upland springs/streams. Draft protocols for microhabitat data collection can be found in Appendices C1-C3.

Current and Past Cattle Grazing Management

Cattle grazing management (current and past) will be quantified from: 1) review of allotment management plans; 2) standardized survey of USFS range management staff about grazing management practices and observed grazing intensity for each allotment and meadow enrolled in the survey; and 3) standardized survey of on-the-ground cattle managers for each permitted allotment and meadow enrolled in study 1 and 2. We will use a standardized grazing management survey-questionnaire which has been specifically designed and extensively used to quantify both current and past grazing management on meadows (Ward et al. 2001 – over 150 interviews with grazing managers on both public and private rangelands). The survey allows us to quantify standard grazing management practices such as number of cattle, duration of grazing, season of grazing, frequency of grazing, and implementation of cattle distribution practices such as herding and placement of off-site supplemental feeds for each meadow enrolled in the survey. Questions designed to quantify meadow use by packstock will be incorporated into the questionnaire for this project. Our model for obtaining detailed management information from individual managers is to work closely with county-based UC Cooperative Extension advisors, the California Cattlemen's Association, and the California Farm Bureau Federation to make individual managers aware of objectives of the project, it's potential to aid in science-based resolution of critical resource management issues, and the importance of their participation and assistance in collecting objective and accurate data (Lewis et al., 2001; Ward et al., 2001; Lewis et al., 2005).

Hydrologic and Water Quality Data

Hydrologic and water quality related habitat variables to be monitored at each study site include: water temperature, pool habitat volume (area and depth of pools), water table dynamics and connectivity of pools to meadow water table dynamics, flow rate and volume for lotic systems, dissolved oxygen, pH, conductivity, and turbidity in pools (Figure 3). Data on these parameters will be collected bi-weekly during May/June through August/September snow and weather permitting. Water temperature in pools in each meadow will be collected via placement of automatic temperature loggers set to record temperature (Onset Computer Corp. Optic StowAway) on a 0.5 hour time step, allowing for characterization and analysis of a suite of temperature metrics (Tate et al., 2005*b*). Air temperature will be collected in each meadow as a covariate (Tate et al., 2005*c*). Pool surface area and depth will be hand measured relative to bench marks for consistency from sample period to sample period. Two inch wells will be dug to bedrock or clay-stone layer and lined with PVC pipe to allow monitoring of water table depth.

Wells will be set out in a grid of transects perpendicular to and transecting pools to facilitate correlation of pool volume to water table dynamics (*e.g.*, connectivity). Naturally conservative tracer (*e.g.*, Cl, Br) injection and recovery techniques may be employed to further characterize hydrologic connectivity in lentic systems and hydrologic residence times in lotic systems (stream associated). Flow rate and volume in and out of lotic meadows will be measured by hand using the area-velocity method at the top and bottom of each meadow (instream). Dissolved oxygen levels, conductivity (multi-parameter YSI meter) and turbidity (Orbeco-Hellige portable turbidity meter) will be determined in the field. All equipment (water quality meters, flow meters, temperature loggers, etc.) will be calibrated and used according to manufacture's instructions. Initial and follow-up training of field crew in operation of equipment and collection of field data will be conducted and documented.

Vegetation

Vegetation and related grazing intensity variables to be collected in this study include: plant species composition and cover, residual dry matter, stubble height, and above ground biomass (Figure 3). Vegetation parameters will be collected in conjunction with piezometer wells as described in the Hydrologic and Water Quality section above since species composition and productivity are related to water table hydrology (Allen-Diaz 1991). The wells will be laid out in transects perpendicular to and transecting meadow pools. Within a 3 m radius of each well, we will randomly place a livestock exclusion cage. The number of cages will be determined in conjunction with the number of piezometer wells, and cages will not be placed in pools. A paired, uncaged plot, will be located and marked with a wooden redwood stake at the same time that the cage is installed. A modified 10-pt frame (collecting a total of 100 points) will be used to determine species composition within the cage. Then the biomass inside the cage will be clipped (using a 25x25 cm square quadrat) as close to peak standing crop as possible in order to determine above-ground biomass (grams per $1/16^{th}$ m^2). At the uncaged plot, stubble height will be recorded from 5 points inside the $1/16^{th}$ m^2 quadrat using a centimeter stick, and biomass will be clipped at the same time in order to determine utilization. Periodic random sampling of grazed meadow biomass will be conducting during the season to track stubble height S&Gs. Cages will be built of woven wire, with a 1 m^2 base, narrowing to 50 cm^2 at the top and be approximately 5 ft tall. Residual dry matter and end-of-season stubble height will be measured at a random location within the 3 meter radius of each piezometer well in September when cages will be collected for the season. The cages will be moved to a new random location near the piezometer well the following spring to remove any cage effects.

Again using the random piezometer transects as the focal point for vegetation data collection, we will collect vegetation species information by functional group at 10 cm intervals along a line point transect placed adjacent to the piezometer transect in order to examine meadow vegetation gradients. At this time we propose to use grass, sedge, rush, forb, shrub and semi-shrub as functional groups. Water, rock, hoof print, small mammal burrows, litter, and manure will also be recorded. Vegetation data will be collected and recorded in a spatially explicit fashion so that relationships between and among hydrologic and vegetation variables can be examined using gradient analysis and spatial statistics.

One time environmental variables include: slope, aspect, and elevation will be recorded using standard field instruments at each meadow. At each piezometer well within the meadow, slope, aspect and soil profile information will be recorded. Soil samples will be collected from each 10 cm interval along the soil core when the piezometer well holes are dug. Soil samples will be placed in sealed plastic bags, placed in a cooler, brought back to the lab and frozen for later analysis.

Integration of Toad Population, Habitat and Livestock Grazing Data

The success of this study will ultimately depend on the integration and analysis of a data covering a diverse set of variables collected at several spatial scales. Table 1 provides a conceptual approach to that integration and Figure 3 shows a schematic of field data collection. The most extensive scale represents the context for each study meadow and will be based on a hydrologically defined basin and/or whole grazing allotments. Data at this scale will be derived primarily through GIS and include information such as elevation, number of occupied meadows surrounding study meadow, etc. Some of these data have already been gathered to aid in study meadow selection and we will continue to refine and add to this as needed. The next less extensive scale is that of the whole meadow which reflects general vegetative and hydrologic conditions, the distribution of occupied and potential breeding sites, and overall livestock use information. Within a given meadow we expect to find multiple discreet or definable wet areas that could be used for breeding/rearing of toads and toad larvae. At this scale, we will characterize the environmental context, identify the habitats available to toads, and document finer scale effects of livestock on these environments. Finally, we will also collect data on larval groups and individual toads, including estimates of abundance of larvae and metamorphic toads, descriptions of local microhabitats, and evidence for direct effects of livestock on these life stages. Habitat preferences of toads will then be assessed through comparison with data gathered on available habitats/environmental context.

Table 1. Scales of interest and potential field methods for Yosemite toads, their habitat, and livestock use. Scales (underlined text, e.g. "whole meadow") are ordered from most extensive to most local. Field methods should be considered preliminary; more details are provided in sampling protocols (Appendices C1-C3).

TOADS	TOAD HABITAT	LIVESTOCK USE	FIELD METHODS
Allotment/Basin/Multi-Meadow (Context)			
number of populations, proportion of meadows/area occupied	GIS generated environmental variables such as: elevational range, climate regime / history, topographic features	allotment-wide use, distribution over time	- Forest GIS data on toad populations and meadows - Information from Forest range conservationists and permittees (livestock)
distribution of occupied and unoccupied meadows	spatial distribution of suitable meadows, connectivity among meadows		
Whole Meadow			
number of breeding/rearing areas	spatial distribution of breeding/rearing areas and other wet areas of meadow - e.g. area of aquatic habitats by type	cattle use levels /disturbance of all breeding/rearing areas and other wet areas of meadow	cross meadow transects or grids and point intercept data ("permanent")
movement/dispersal corridors – e.g. hydrologic connectivity to other meadows/upland springs, overland distances to upland springs	number/size/length? of streams or moist riparian areas	cattle use of riparian corridors	
	area of meadow	pattern of cattle use of whole meadow	
	meadow hydrologic patterns vegetation composition/structure in wet and dry areas		
	slope/aspect of meadow		
	predators/competitors?		
	other disturbance – e.g. recreation, packstock		
Suitable Breeding/Rearing Areas (occupied and unoccupied for comparison)			
population size (and survival rates?) by life stage per breeding area	characteristics of breeding/rearing areas - e.g. water depth, temperature, vegetation, substrate, water quality	cattle effects on breeding/rearing areas – e.g. chiseling?, hoof punches, water quality (cow pies?)	collection of more intensive data – via stations, short transects, or area based estimates, capture-recapture of terrestrial toad life stages
	predators/competitors – snakes, birds, other amphibians		
Individual Aquatic Toads, Egg Masses, Larval Groups			
health – e.g. disease, length/weight ratios.	microhabitat associations of individual toads, metamorphic toads, egg masses, larvae groups - e.g. associated substrate, water temps, water quality	evidence for direct effects on toads - e.g. trampling, stranding of eggs, larvae in hoof prints.	visual inspection / local scale measurements, capture-recapture and/or repeated counts of aquatic toad life stages

Table 1., continued			
Individual Terrestrial Toads			
relative use/importance of burrows and other cover (logs, vegetation) by life stage	characteristics of burrows and other cover in meadow, at edge, in uplands, toad microchabitat	rodent control efforts by permittees? collapsing of burrows by cattle	cross meadow or meadow-edge focused transects, individual toad-focused microhabitat measurements
use of upland springs by life stage	characteristics of upland springs/riparian areas – e.g. distance from meadow, type; toad microhabitat	use of upland areas by cattle	visual encounter survey of upland cover and springs, individual toad-focused microhabitat measurements

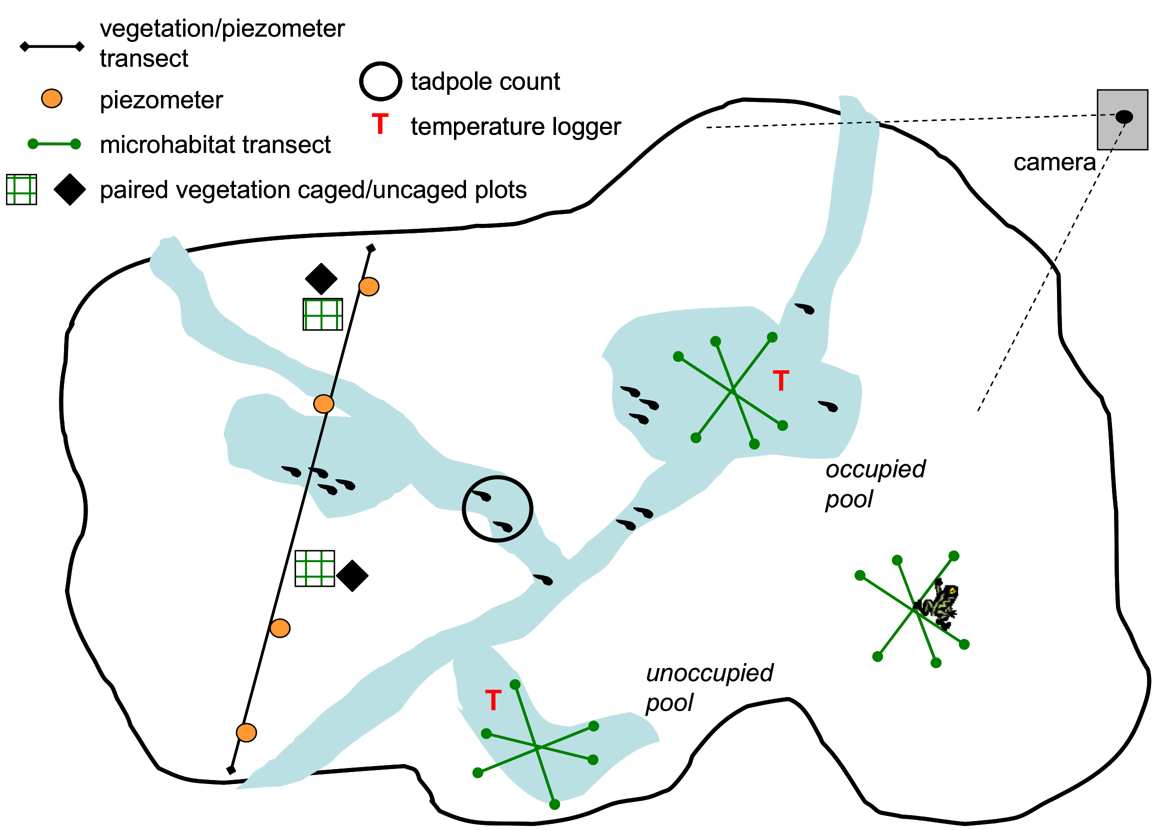

Figure 3. Schematic of integrated field data collection methods.

Quality Assurance and Control

The principle investigators have significant experience in design, management and analysis of studies evaluating the implementation of grazing, restoration, and water quality management practices on environmental response variables (*e.g.*, Allen-Diaz et al., 2004; Bedard-Haughn et al., 2004; Jackson et al., 2006; Jones et al., 2005). They have similar experience with observational studies relating grazing, agricultural, and restoration management practices to environmental responses on private and public range and forest lands (*e.g.*, Lewis et al., 2001; Ward et al., 2001; Welsh and Lind, 2002; Lennox et al., 2004; Tate et al., 2004*b*; Lewis et al., 2005; Tate et al., 2005*a*). The multi-disciplinary nature of the team significantly increases our capacity to appropriately design and implement these study designs as well as to insure a holistic analysis and appropriately cautious interpretation of results given the potential study strengths and weaknesses identified above. Consultation with statisticians and applied researchers outside the core study team will occur throughout the study design and analysis process. All results will be subjected to peer-review in significant scientific journals.

LOGISTICAL CONSIDERATIONS AND IMPLEMENTATION

Animal Care and Use Certification and Permits

The Sierra Nevada Research Center (SNRC) of the Pacific Southwest Research Station is in the process of working out an agreement with the Institutional Animal Care and Use Committee (IACUC) at the University of California, Davis. Once finalized, this arrangement will allow SNRC researchers to submit animal care and use protocols to the IACUC for review and approval. If this agreement is not in place at the start of our study, we will follow the guidelines by the American Society of Ichthyologists and Herpetologists (Herpetological Care and Use Committee 2004) for handling of live amphibians. All necessary scientific collecting permits will obtained from the California Department of Fish and Game.

Radio Telemetry and Communications Considerations

This study does not currently contain a radio telemetry component. If that changes in the future, we will revise this section of the study plan.

Data Management and Archiving

We will establish relational databases (e.g. Microsoft ACCESS) to house data and we will follow standard approaches for quality control (e.g. entry and checking by different individuals). More details will be incorporated here as the data collection procedures are developed. All data collection will be done using Forest Service (FS) corporate standards and protocols, including GIS data dictionaries and NRIS (Natural Resource Information System) databases. Data will be provided to the NRIS database once quality control, including data checking, has occurred.

Expected Products

Results of the study will be presented to Forest Service Regional office and National Forest staff in several formats: presentations at annual Regional meetings, brief verbal or email updates, written quarterly and annual reports, and contributions of data to NRIS databases. Once particular phases of the study are complete, we will provide an analysis of effects of grazing treatments and standards and guidelines on Yosemite toad populations and habitat and recommendations for future livestock grazing management.

Results pertaining to Yosemite toad ecology and responses to livestock grazing will be presented at conferences and scientific meetings such as herpetological societies, wildlife and conservation organizations, and potentially agricultural or range ecology organizations. Results pertaining solely to meadow ecology and livestock utilization will be presented at ecology and rangeland conferences and meetings. Publications will be collaborative and integrative including data analysis on Yosemite toad population and habitat relative to various livestock grazing utilization levels, seasons of use, etc. and targeted publications will included both layman and applied science journals.

Opportunities for Partnerships and Collaboration

This study is unique at the outset because it has been designed and will be implemented through collaboration between USFS biologists, managers, and researchers and University of California (U.C.) faculty, cooperative extension program leaders, and graduate students. A benefit of the study's adaptive management framework is the inclusion of multiple stakeholders in the project design and implementation. We have the unique opportunity to engage a diversity of researchers, managers, and land-users and we will work to facilitate a collaborative process throughout the study's implementation. The opportunities for public outreach and education are endless and we will continue to identify them as the study progresses. Hopefully our work will lead to the continuation of an adaptive framework for long-term monitoring and management of Yosemite toad populations and habitat.

Oversight Team

A small team of interested resource/land managers, other interested parties, and other researchers (the "Steering Team") has been established to provide for guidance and communication. This team will be engaged at all phases of the project, from the development of the study plan through execution of the field work and ultimately to data analysis and reporting.

Statistical Review

PSW has a process in place for statistical review of study plans. Once a final draft plan has been written, we will submit it for review by PSW statisticians. In addition, we will seek peer review from appropriate individuals for various components or the entire study plan as needed.

Health and Safety

The primary risks of this research involve those typically associated with field work in high elevation areas – e.g. inclement weather conditions, remote locations, etc. Other studies under the auspices of the Sierra Nevada Research Center have developed Job Hazard Analyses for the type of work we will be conducting (e.g. "Aquatic Ecology" and "Ski and Snowshoe in Forests"). We will draw from these and add any specific safety issues as necessary.

Site Specific Plan to Conform to 2004 ROD (S&G 54)

Selection of livestock grazing allotments for this study is not yet complete. As soon as we have a proposed set of allotments and/or meadows within allotments for this study, we will forward them to appropriate Forest personnel so that they can integrate that information into and allotment and site specific plans. For baseline data collection in 2005 and all years of the experimental phase of this study, we will be installing instruments to record meadow water temperatures, soil moisture, etc. and potentially fencing some areas. We will work with Forest Range Conservationists to communicate with permittees and minimize disruption of their activities.

Expectations of Design Team Relative to Livestock Grazing During the Study

In March 2005, a subset of the Design Team (K. Tate, P. Stine, A. Lind, B. Frost, and S. McIlroy) developed a statement to clarify our expectations regarding the management of livestock grazing in the meadows and allotments included in the study. That statement is included below as well with one addition on livestock grazing in the dry areas of the meadows where only wet areas are exclusion zones. We consider these to be the conditions necessary for a successful study.

The following statement applies only to the five allotments tentatively selected for baseline data collection in the livestock grazing study: Dinkey, Patterson Mountain, and Blasingame on the Sierra National Forest, and Herring Creek and Highland Lakes on the Stanislaus National Forest.

1. The Design Team is only concerned about how the individual meadows selected for the experimental study are managed, not the entire allotment. This applies to these meadows during both the baseline year (2005) and experimental grazing treatment years (2006+).

2. It is essential that baseline grazing represent grazing levels in terms of current utilization and streambank condition standards without the specific season of use or exclusion restrictions that have recently been implemented for Yosemite toads. This will enable testing response of toads and their habitat to a grazing approach that represents grazing as it is currently proposed to be done, balancing reasonable and effective meadow conservation measures with efficient grazing practices. If this study is executed with these premises we anticipate that all concerned parties will find the foundation of the study to be credible and acceptable.

3. We assume that baseline grazing to current utilization and streambank condition standards will result in relatively consistent activity and forage consumption across all study meadows, certainly within each block of treatment meadows (allotment=block).

4. Allotment scale grazing management logistics do matter from a scientific perspective if the risk of not achieving desired grazing on study meadows is greatly increased due to allotment scale problems associated with one standard for part of the allotment, another for the rest. The Design Team presumes that this risk needs to be assessed allotment by allotment and in concert with each permittee. The reason we care about this risk is that we could end up with baseline data that do not represent grazing to current utilization and streambank condition standards (too light, to heavy, no grazing at all), thus confounding the design of our study.

5. One of the treatments we are evaluating is exclusion of livestock from the wet areas of meadows. For that treatment, we expect that the rest of the meadow (i.e. the dry portions) will be grazed to meet current utilization and streambank condition standards.

6. Beyond these factors we believe it to be the responsibility of the Forest and District biologists and range conservationists to determine the administratively and legally appropriate grazing practices to apply to the meadows that are not part of the study. This would include both meadows not selected for the study within allotments that contain study meadows as well as meadows in allotments that do not contain any study meadows. We presume that existing Forest Standards and Guidelines will direct these decisions.

Implementation Plan and Potential Impacts to Yosemite Toads

In general, U.C. researchers will provide oversight of and collect field data on relevant meadow habitat and environmental variables, as well as characterizing livestock use. PSW-SNRC researchers will provide oversight of and collect field data on Yosemite toad populations and associated microhabiat features. Field work will utilize a single large crew (4-6 individuals) or potentially two smaller crews. The crews will combine U.C. and SNRC employees to efficiently and simultaneously collect data on both Yosemite toad populations and meadow environments. Field work will take place as early in each spring as possible. This will depend on when meadows are accessible (i.e. can be reached by vehicle or by a reasonable hike/snowshoe) and could be as early as late May and as late as early July. Field work will continue through late summer or for as long as Yosemite toads are active within the study meadows. Field work for this study began in the spring of 2005, including Phase I data collection and baseline data for Phase II. Phase II experiments will begin in spring of 2006 and continue for 4 years (2006-2009).

Meadows selected for grazing treatments and examples of potential fence lines are provided in Appendix D. Note that two allotments have meadows in addition to the 3 grazing treatments. These two meadows were initially chosen to be part of the study and instrumentation was installed there in 2005. However, after discussions with range conservationists and permittees it was apparent that these meadows had historically been used as holding pastures and that use would necessarily continue through the study. We thus selected one additional meadow in each allotment to receive the necessary treatment, but kept the holding pastures in the study to gain additional information (Appendix D).

This study is designed to gain an understanding of the effects of livestock grazing on Yosemite toads, thus at this point we cannot fully know if the grazing treatments implemented in the study will negatively impact the toad. However, we do not anticipate that the level of grazing in the study (i.e. 10 meadows distributed over 2 National Forests with some amount of grazing over 5 years) will have an extensive scale or long term negative impact on the Yosemite toads. In addition, we do not expect the field methods we are using (e.g. counting, mark/recapture, measuring habitat) to negatively affect the relatively robust populations of toads we will be working with in the study meadows. Any methods that could have detrimental effects are being tested on a surrogate species of toad (western toad, *Bufo boreas*) prior to use on Yosemite toads (Appendix C-4).

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APPENDIX A. LIST OF YOSEMITE TOAD DATA SOURCES CONSIDERED FOR INCLUSION IN THE SAMPLE SITE SELECTION.

1) Yosemite toad GIS file from Carlos Davidson - EXCLUDED
→ Many of the records appear to have location information that is questionable. S. Parks does not think that the locations are accurate enough for this analysis.

2) "TOI99bbcalb" GIS file obtained from C. Brown – INCLUDED
→ 6 records from 1999

3) "CDF_buca_all.shp" GIS file obtained from C. Brown. – INCLUDED
→ 45 records from 2001

4) "cas_sierra_buca.shp" GIS file obtained from C. Brown – EXCLUDED
→ Most records are historic (before 1990) and are thought by S. Parks to be museum data, which usually does not have very accurate location information.

5) "sfrog_buca.shp" GIS file obtained from C. Brown – EXCLUDED
→ Many of the locations placed from PLS coordinates (township, range and section). Not enough spatial accuracy.

6) "seibcalb" GIS file obtained from C. Brown. – INCLUDED
→ 21 records form 1999

7) "SCK_buca" GIS file obtained from C. Brown - EXCLUDED
→ Records too old for this analysis (1980's and older)

8) "ENF_03_buca" GIS file obtained from C. Brown – EXCLUDED
→ Only four records and the species appeared questionable (w. toad possibly)

9) "amp_mon_strm_fy03" GIS file obtained from C. Brown – INCLUDED
→ Six records. Placed on each stream where BUCA is present.

10) "amp_mon_mdw_fy03" GIS file obtained from C. Brown – INCLUDED
→ 46 records

11) "seki_np_buca" GIS file obtained from C. Brown – INCLUDED
→ Only records from 1993 and later were used in analysis (18 records)

12) "amp_mon_lakes" GIS file obtained from C. Brown – INCLUDED
→ 16 records form lakes.

13) "Buca_recs" GIS file from Eldorado NF. – INCLUDED
→ Only four records from 2001

14) "Yotoad_shightings.shp" GIS file from Inyo NF – INCLUDED
→ Records from 1998 and later used in analysis (204 records)

15) "SNF_draft_survey_sites_2002_2003_2004.shp" GIS file obtained from Sierra NF – INCLUDED
→ 313 BUCA sites on Sierra NF

16) "stf_aquasurv" GIS file obtained from Stanislaus NF – INCLUDED
→ 118 BUCA records, but some are same location in different years. 84 locations used in analysis.

17) "Rolandk_buca" MS Access file from R. Knapp – INLCUDED
→ 113 records mostly from Yosemite NP

18) "knapp" GIS file. – INCLUDED
→ Included the ten records that did not overlap with the above dataset

19) "fellers" GIS file presumably obtained from G. Fellers – INLCUDED
→ 191 records from Yosemite NP

20) "stfsur_buca" GIS file presumably obtained from Stanislaus NF – EXCLUDED
→ Basically the same file as "stf_aquasurv" (# 15).

APPENDIX B. **YOSEMITE TOAD OCCUPIED MEADOW QUESTIONNAIRE (MARCH 2005)**

Background Information/Instructions

This year (2005) a study is being initiated on the effects of livestock grazing on Yosemite toads. The first year of the study will involve baseline data collection for at least 20 meadows on the Stanislaus and Sierra National Forests known to be occupied by Yosemite toads. Subsequent years will include continuing data collection on these meadows under different livestock grazing scenarios. The study is primarily funded by Region 5 of the U.S. Forest Service with some matching funds (= salary of principal investigators and statisticians from PSW and U.C. Berkeley and Davis).

As a starting point, we have selected 8 grazing allotments (Dinkey, Collins, Patterson Mtn, Blasingame, Kaiser, and Mt.Tom on the Sierra NF and Herring Creek and Highland Lakes on the Stanislaus NF) that contain potential study meadows. However, it is likely that only 5 of these will be included in the final set of study allotments/meadows. To facilitate development of a logistically feasible set of meadows that can be field checked for final selection, we need some additional information. Included with this email is an excel spreadsheet and GIS point coverage of the meadows that we have determined already meet some basic criteria for Yosemite toad occupancy and elevational limits. We have developed a unique naming/numbering system (FNL_ID) for the meadows, but have included other known names to be sure we are all talking about the same meadow (the GIS coverage should help with that too).

The spreadsheet contains two worksheets; one sheet ('8 allotments - whole allotment') with a list of allotments and one additional column to answer the allotment level question and a second sheet ('8 allotments-meadows') with a list of the meadows in each allotment and columns for the meadow level questions. This second sheet also contains other descriptive information for each meadow compiled by Sean Parks. Feel free to rearrange/delete columns in the spreadsheet for ease of answering the questions but please retain the "FNL_ID" column at a minimum.

Please answer the questions and fill in the spreadsheet in order. If initial questions (1 and 2) are answered in the negative, you may stop filling out the rest of the information, but please document these situations clearly. **Provide any information you feel is relevant to the question.** All of this information will help us select meadows for a successful study. The first question on the questionnaire below refers to the whole allotment and the rest refer to individual meadows.

A. Contacts for more information:

Questionnaire questions:

Amy Lind *or* *Ken Tate*
alind@fs.fed.us, 530-759-1702
kwtate@ucdavis.edu
530-754-8988

Meadow ID or GIS questions:

Sean Parks
sean_parks@fs.fed.us, 530-759-1717

B. Who should fill out this questionnaire?
An individual (or team of individuals) from each Forest (Stanislaus and Sierra) preferably biologists and range conservationists who are familiar with the grazing allotments and meadows listed in the attached file.

The Questionnaire

Whole Allotment: Is there an historically ungrazed (control/reference) meadow (not grazed in the past 10-15 years at a minimum) that meets the criteria in questions (1) and (2) below within the allotment or within close proximity to (10 miles) the allotment? If so, please identify the meadow from the enclosed point coverage or provide UTM coordinates and indicate the date it was last grazed.

Individual Meadows (need 4 per allotment):

1. Does the meadow contain a healthy population of Yosemite toads? Specifically, has breeding been documented in the meadow in multiple years? If so, which years? Do the toads use/breed in more than one area of the meadow? [We have included the toad data, that we received from you previously, in the excel spreadsheet. For this question, we would like you to consider that data along with any additional field knowledge or other data you have.]

2. a. We will be applying 4 livestock grazing treatments (4 meadows) in each of the allotments that contain ungrazed meadow or have an ungrazed meadow nearby, and have toads in several meadows:
(1) no grazing in the meadow/upland grazing allowed;
(2) no grazing in wet areas of the meadow/grazing in dry meadow areas and upland areas allowed;
(3) grazing to utilization standards and guidelines throughout the meadow (no exclusions);
(4) no grazing in recent history (control/reference, 10-15 years).
Treatments (1) and (2) will require electric fencing which we will provide and maintain. Do you foresee any difficulties (logistics, permitee cooperation, etc.) in applying treatments (1) through (3) to three meadows in the allotment?

b. Is there road access to the meadow? 2WD or 4WD? Trails? Distance from nearest road? Any other access considerations for the meadow?

3. What are the current packstock and recreational (trail, hiking, camping, ohv) uses of the meadow and immediate surrounding area? Any other uses or activities in/near the meadow that could affect the study?

4. a. What type of livestock controls does the permitee typically employ to control cattle distribution and meadow use levels – e.g. herding, drift fences, supplements?
b. Are there records available on the history of livestock use of the meadow – e.g. season of use, frequency, stocking rate?

5 a. Is there quantitative data for annual use (meeting/not meeting standards) for the meadow?
b. Are there any meadow/stream condition trend data available – e.g. vegetation, SCI, PFC? For what time period?

6. What is travel time from state highway or main county road? Road quality? Snow melt timing?

7. Is there any information on rodent densities in the meadow? Are any rodent control measures used there?

8. Have any restoration activities taken place in the meadow? (E.g. any controls on head-cutting, stream back stabilization.)

APPENDIX C. YOSEMITE TOAD POPULATION AND HABITAT DETAILED FIELD METHODS.

Appendix C-1. Overview of Phase II Yosemite toad population and microhabitat field sampling.

The following is a draft field protocol for the Yosemite toad population and microhabitat component of a study of the effects of livestock grazing on this species. The relevant scales of interest and a broad outline for the whole study are provided in Table 1 of the main study plan document.

Study Meadows

The toad population and microhabitat sampling components of the study will be conducted to meet the broad goal of detecting effects among three grazing treatments (no grazing in meadow, exclusion from breeding areas, and meadow-wide grazing to utilization and streambank standards and guidelines). Each treatment will occur on 3 grazing allotments on the Sierra National Forest and 2 allotments on the Stanislaus National Forest, resulting in 5 replicates of each treatment. Allotments may be treated as blocks in data analysis. A smaller number of reference meadows will be studied to provide context for overall toad population size and trends over time. The selection and monitoring of these meadows is part of Phase I of the study. Because they will necessarily occur outside of active allotments, these meadows are not part of the blocked design.

Timing of Sampling

Our goal is to sample in three time periods with a different toad lifestage focus in each period:
* Late spring – adult toads, egg masses
* Early-middle summer – tadpoles (larvae)
* Late summer – young of the year (metamorphs).

Within each period, we will sample with an appropriate intensity to derive reliable count data (tadpoles) or population estimates via capture-recapture (adults, young of the year). These metrics can also be used to develop ratios among lifestages within a year; for example, to derive an index of survival from the tadpole to young of the year stage. Over a period of at least three years, we expect to derive survival estimates for at least the adult life stage.

Timing of sampling is primarily based on block location, logistics, and developmental phases of toads. Lower elevation meadows will be accessible first and breeding should occur at these locations first. To the extent feasible, we will time our sampling of tadpoles so that they are at roughly the same developmental stage each year, thus reducing variability for estimates of change over time.

Appendix C-2. Yosemite toad population and microhabitat Reconaissance and Baseline Data Collection (2005).

In 2005, the following full protocol was used only on the set of meadows receiving one of three grazing treatments. Only reconnaissance surveys (quick toad/tadpole counts, no habitat data) were conducted at potential reference (ungrazed for at least 15 years) meadows.

Early to Mid-Summer Surveys

Primary Survey

Each study meadow was visited during the tadpole period and a complete survey (continuous parallel transects, after Sadinsky 2004) of the meadow was conducted to identify and permanently mark (with stakes) breeding areas.

During these surveys the following information was recorded: meadow, date, start and end time, start and end air and water temperatures, count by life stage of all amphibian and reptile species seen, delineation of area (length x width) of tadpole groups, and GPS location of each tadpole group.

Tadpole Abundance

Tadpole groups were defined as continuous collections of tadpoles not connected by aquatic habitat to other tadpoles. That is, each group had to be surrounded by relatively dry soil such that tadpoles could not swim from group to group.

Unoccupied wet areas were also randomly selected to represent "available", unused areas of each meadow (see microhabitat below).

In each tadpole group, tadpoles were counted using ¼ sq. m hoops with the number of hoops based on the area of the group. Each hoop was counted by 2 observers to increase the precision of density estimates. Water depth and temperature, detritus depth, dominant cover and substrate, flow condition were recorded for each hoop. A subset of 5 tadpoles were staged (tadpole stages 1-4) and measured (total length) for each of the first 5 hoops.

Juvenile and Adult Toads

Any juvenile (1+ years) or adult toads that were found during these surveys were captured and marked appropriately – passive integrative transponder (PIT) tags for ≥50mm snout-vent length or visible implant elastomer (VIE) injection for < 50mm snout to vent length. PIT tags provide a unique mark for each individual. A two color system was used for VIE– one color represented the year of sampling (e.g. 2005) and the second color represented the meadow the toad was captured in. The following data were also recorded for each individual: GPS coordinates, mark type/number, sex, snout-vent length, substrate, cover, distance to water, or if in water, temperature, detritus depth, and flow conditions.

Micro-Habitat

To quantify habitat characteristics important to toads at local spatial scales, especially those characteristics that may be affected by livestock grazing, we conducted line transect sampling at occupied and an equal number of unoccupied sites in each meadow.

We determined the approximate center of each tadpole group, and delineated a 5m diameter circle by laying out a tape measure. Three, 5m transects were then evaluated. The first transect was randomly placed, the second one was placed perpendicular to the first, and the third was placed on either diagonal.

The following data were recorded for the whole 5m diameter circle: date and time of data collection, signs of livestock use, deer sign, a count of mammal burrow openings, fish presence, and an indication of whether the meadow has been grazed in the current year.

At each meter point (0, 1, 2, 3, 4, and 5), along each of the 3, 5m transects, the following data were recorded: water depth, detritus depth, water temperature, flow/water conditions, and live vegetation/stubble height.

Along the whole length of each 5m transect, we recorded the amount of the transect covered by: rocky substrates, logs/downed wood, live herbaceous vegetation total and by type (grasses, forbes, sedges, rushes), dead herbaceous vegetation, moss, detritus/silt, shrubs, and trees/branches.

For each transect overall, we recorded: water condition - estimate of % of transect that has surface water, saturated soil, or is dry, slope/gradient (in degrees) with clinometer, and an estimate of % canopy closure (0=open, 100=closed).

Late Summer Surveys

Young of the Year (Metamorphs)

Surveys for metamorphs were conducted by starting at each previously identified tadpole group site and working in concentric circles out from the center, until reaching the edge of the meadow or until no more metamorphs were found. Spot surveys of the dryer parts of each meadow and of microhabitats where clusters of metamorphs were found (e.g., open dry, sandy patches) were also conducted. All metamorphs (with the exception of a few that were too small) were marked using the two color VIE system described above for juveniles.

Two meadows were revisited (approx. 3 weeks after their first visit) to get a count of marked and unmarked young of the year for a quick population estimate and potential recapture rates.

Juvenile and Adult Toads

Any juvenile (1+ years) or adult toads that were found during these surveys were captured, marked appropriately (PIT tag or VIE injection) and the following data was recorded: GPS coordinates, mark type/number, sex, snout-vent length, substrate, cover, distance to water, or if in water, temperature, detritus depth, flow conditions.

Micro-Habitat

To examine change in habitat through the summer, we used line transect sampling as described above for a subset of 3 (maximum) previously occupied tadpole group sites and 3 (maximum) previously identified unoccupied sites.

Appendix C-3. Yosemite toad population and microhabitat during treatment implementation (2006-2009).

Late Spring Surveys

Primary Survey

A cursory whole meadow survey (e.g., broad zigzag pattern) is done on the first visit to each meadow, with more intensive searching in the vicinity of 2005 breeding areas. If no breeding activity is found in 2005 breeding areas, a more intensive survey will be done of the entire meadow. If no breeding activity is found in the entire meadow, a revisit will be scheduled for several days later.

During these surveys the following information is recorded: meadow, date, start and end time, start and end air and water temperatures, and count by life stage of all amphibian and reptile species seen.

Juvenile and Adult Toads

Any juvenile (1+ years) or adult toads that are found during primary or revisit surveys are captured and marked appropriately – passive integrative transponder (PIT) tags for ≥50mm snout-vent length or visible implant elastomer (VIE) injection for < 50mm snout to vent length. PIT tags provide a unique mark for each individual. A two color system is used for VIE– one color represents the year of sampling (e.g., 2006) and the second color represents the meadow the toad is captured in. The following data are recorded for each individual: GPS coordinates, mark type/number, sex, snout-vent length, substrate, cover, distance to water, or if in water, temperature, detritus depth, and flow conditions.

Up to 20 juvenile or adult toads per meadow are swabbed to document presence/prevalence of Chytrid fungus in our study populations.

A second visit is made to a subset of meadows (e.g., all meadows in 1 allotment on the Sierra NF and all meadows in 1 allotment on the Stanislaus NF) so that within season population estimates can be derived for those meadows.

Egg Strings/Clusters

Locations of egg clusters are identified based on permanent tadpole group numbers (established in 2005). If clusters are found in new areas during the primary survey, they are given a new/unique group number and GPS'd. These new breeding areas are added to the permanent set of tadpole groups for each meadow and all subsequent data (tadpole counts, microhabitat conditions) area collected there in the current and future years of the study. Habitat data including substrate, water temperature, detritus depth, and flow conditions are collected for each discreet cluster.

Early to Mid-Summer Surveys

Primary Survey

During this time period, a cursory whole meadow survey (e.g., broad zigzag pattern) is done for each meadow, with more intensive searching in the vicinity of 2005 breeding areas and any new areas found during late spring surveys.

During these surveys the following information is recorded: meadow, date, start and end time, start and end air and water temperatures, count of all amphibian and reptile species seen, delineation of area (length x width) of tadpole groups associated with 2005 breeding areas. If new groups of tadpoles are found they are given a new number and the GPS location is recorded.

Tadpole Abundance

Tadpole groups were defined as continuous collections of tadpoles not connected by aquatic habitat to other tadpoles. That is, each group had to be surrounded by relatively dry soil such that tadpoles can not swim from group to group.

In each tadpole group, two strata of tadpole abundance are delineated by visual assessment – high density and low density. Within each strata, tadpoles are counted using ¼ sq. m hoops with the number of hoops based on the area of the group and the strata. Water depth and temperature, detritus depth, dominant cover and substrate, flow condition were recorded for each hoop. A subset of 5 tadpoles were staged (tadpole stages 1-4) and measured (total length) for each of the first 5 hoops.

Juvenile and Adult Toads

Any juvenile (1+ years) or adult toads that are found during these surveys are captured, marked appropriately (PIT tag or VIE injection) and the following data was recorded: GPS coordinates, mark type/number, sex, snout-vent length, substrate, cover, distance to water, or if in water, temperature, detritus depth, flow conditions.

Micro-Habitat

For these mid-summer surveys, we collect a subset of the full microhabitat data at the permanently marked occupied and unoccupied breeding areas/tadpole groups (3 of each maximum). We use the previously staked (2005) centers of tadpole group areas and do two 5m transects and one 10m transect at each point. The 10m transect is placed randomly along the long-axis of tadpole group and the second one is placed perpendicular to the first, and the third is placed on either diagonal.

The following data are recorded for the whole 5m diameter circle: date and time of data collection, signs of livestock use, deer sign, a count of mammal burrow openings, fish presence, and an indication of whether the meadow has been grazed in the current year.

At each meter point along each of the transects, the following data are recorded: water depth, detritus depth, water temperature, flow/water conditions, and live vegetation/stubble height.

(Vegetation/substrate cover is not recorded during this time period.)

For each transect overall, we record: water condition – estimate of % of transect that has surface water, saturated soil, or is dry, slope/gradient (in degrees) with clinometer, and an estimate of % canopy closure (0=open, 100=closed).

Late Summer Surveys

Young of the Year (Metamorphs)

Surveys for metamorphs are conducted by starting at each previously identified tadpole group site and working in concentric circles out from the center, until reaching the edge of the meadow or until no more metamorphs were found. Spot surveys of the dryer parts of each meadow and of microhabitats where clusters of metamorphs are found (e.g., open dry, sandy patches) are also conducted. All metamorphs (with the exception of a few that were too small) are marked using the two color VIE system described above for juveniles. A second visit is made to all meadows so that within season population estimates can be derived.

Juvenile and Adult Toads

Any juvenile (1+ years) or adult toads that are found during these surveys are captured, marked appropriately (PIT tag or VIE injection) and the following data was recorded: GPS coordinates, mark type/number, sex, snout-vent length, substrate, cover, distance to water, or if in water, temperature, detritus depth, flow conditions.

Micro-Habitat

For these late summer surveys, we collect full microhabitat data at the permanently marked occupied and unoccupied breeding areas/tadpole groups (3 of each maximum). We use the previously staked (2005) centers of tadpole group areas and do two 5m transects and one 10m transect at each point. The 10m transect is placed randomly along the long-axis of tadpole group and the second one is placed perpendicular to the first, and the third is placed on either diagonal.

The following data are recorded for the whole 5m diameter circle: date and time of data collection, signs of livestock use, deer sign, a count of mammal burrow openings, fish presence, and an indication of whether the meadow has been grazed in the current year.

At each meter point along each of the transects, the following data are recorded: water depth, detritus depth, water temperature, flow/water conditions, and live vegetation/stubble height.

Along each 5m transect, at each 20cm point (from 0.0-5.0m) we use point intercept methods and record: rocky substrates, logs/downed wood, live herbaceous vegetation total and by type (grasses, forbs, sedges, rushes, legumes), dead herbaceous vegetation, moss, detritus/silt, shrubs, and trees/branches.

For each transect overall, we record: water condition - estimate % of transect that has surface water, saturated soil, or is dry, slope/gradient (in degrees) with clinometer, and an estimate of % canopy closure (0=open, 100=closed).

Appendix C-4. Yosemite toad population and microhabitat testing and validation of methods.

Marking Techniques
We tested both temporary and permanent marking techniques on a related species of toad (the western toad, *Bufo boreas*) prior to using the techniques on Yosemite toads.

Neutral Red Dye
In April of 2005, we tested neutral red dye on a surrogate species (western toad) in a laboratory setting. We exposed 60 tadpoles (6 groups of 10) to a single recommended nonlethal concentration of neutral red dye. Each group was exposed for a different length of time (1, 2, 3, 4 or 5 hours, control) to test the duration of the dye visibility (to the naked eye) and to document any effects on larval survival and growth. Tadpoles were monitored intensively for the first twenty-four hours and then less often after that, though feeding and water changes were done daily. Dye was clearly visible on tadpoles for all exposure times during the first 24 hours. However, at 48 hours dye visibility was reduced for all exposure times, but especially for the 1 and 2 hour exposures. There also appeared to be negative effects on growth and survival for the longer exposure times.

We tested neutral red dye in the field on Yosemite toad tadpoles at two of our study meadows (Swainson's Thrush and Hash, Patterson Allotment). At Swainson's Thrush meadow, we dyed 25 tadpoles with subsets of tadpoles evaluated after 1 hour, 1 ½ hours, and 2 hours. At Hash Meadow we dyed 5 tadpoles for 1 hour and 5 for 2 hours. At both meadows, we then put a known number of dyed tadpoles with a known number of undyed tadpoles in a white bucket and each crew member attempted to identify how many were dyed and undyed. Results were inconsistent and inaccurate among crew members. We did not pursue this marking method again for Yosemite toads in 2005.

Passive Integrative Transponder (PIT) Tags
PIT tags are electronic microchips housed in biocompatible glass, and are typically shaped like a grain of rice. Each tag has a unique number/letter combination which can be read with a hand held reader (similar to a price scanner in a grocery store). We implanted 12mm long PIT tags in each of 3 adult western toads on 11 May 2005 using a v-shaped incision on their dorsum. These toads had been and continue to be held in captivity. One toad initially lost its tag because the incision was too large. The tag was reinserted and pushed away from the incision under the skin. One day later all toads retained their tags, the incisions were beginning to heal, and the toads consumed live food when offered. As of the writing of this appendix, two+ years post-tagging (August 2007), all three toads have readable PIT tags, are healthy, eat regularly, and the incisions are no longer visible.

Visible Implant Elastomer (VIE)
VIE is a medical grade biologically compatible material. In general, it is injected into a study animal as a liquid and becomes a pliable solid as it cures. There are 6 color options which are initially visible with the naked eye and eventually may be visible only with violet colored LED light. We have tested VIE marking on both western toads and Yosemite toads. In the field, we are using a 2 color mark combination in the field to represent each meadow/study year

combination. In the lab, we have tested multiple colors, single colors, variety of mark positions (e.g., dorsum, ventrum, foot). In July 2005, we marked 3 of 5 young of the year western toads by injecting a small amount of orange VIE into their ventrum. These toads had been used as controls in the red-dye experiment described, were thus raised from tadpoles, and had been in captivity for 3 months. Four months later (November 2005) one of the unmarked toads died following period of lethargy. Approximately 10 months post-marking (May 2006), the remaining 4 toads are healthy and growing. Of the three marked toads, 2 marks are still visible with the naked eye and one is visible only with a violet LED light.

We have also marked two groups of Yosemite toad young of the year (metamorphs) that were collected for a fish palatability experiment in summer 2005. The first group (n=20) were marked in October 2005 with yellow and blue VIE primarily in their ventrum. Seventeen toads from the same group were left unmarked. We tracked these 37 toads over the course of the next 5 months. They experienced substantial mortality and tag loss, such that by March 2006, only 5 toads were alive and retained their marks, 27 were alive and appeared unmarked and 5 had died. In addition, some of the marks had mixed and changed color (yellow+blue=green). A second group of 20 toads were marked in March 2006. Marks were placed on ventrum only or ventrum and left rear foot using red VIE. Approximately 2 months post marking (May 2006), 6 had died due to unknown causes and marks were visible on the remaining toads, though the positions of the marks had sometimes shifted.

<u>Tadpole Counts – Precision and Validation</u>

We used double observer counts at all meadows and we "oversampled" (i.e. high proportion of hoops to area of tadpoles) at one meadow. In general, adding a second observer did not improve the precision of estimates substantially (e.g., at one meadow, it was improved by approximately 15%). The spatial variability in tadpole abundance was a more significant factor in precision (e.g., individual hoop counts ranged from 0 to 35 tadpoles in one tadpole group). Starting in 2006, we stratify the tadpole area by tadpole density (e.g. 2 strata - sparse and dense) and then allocate hoop counts based on the area of each strata, to improve precision of estimates.

We validated the hoop count technique by conducting a removal/depletion study on two groups of tadpoles in a non-study meadow (tadpole group areas were 26 m^2 and 89 m^2). For this validation, observers identified a tadpole group as was typically done in a study meadow and determined the area and the proportional number of hoops. The area was blocked off to immigration and emigration using synthetic garden border material. Once hoop counts were complete, all tadpoles were removed from the area by dip netting, placed in buckets, and counted by two different observers. A second dip netting pass was made to ensure that all tadpoles have been removed from the area. The total removal sample was then compared to the estimated density of tadpoles from the hoop counts. For both the large and small groups, we found that the hoop counting substantially over-estimated the actual number of tadpoles present. This is likely due to the fact the even though the area of the defined tadpole group is continuous, tadpoles are not continuously distributed in the area. Stratification and more careful definition of the area of the group should reduce this bias. However, as long as our validation continues to indicate a consistent bias across different sizes and types of tadpole habitats (e.g., consistent % overestimate) then the resulting error is not problematic for the overall results of the grazing study. In 2005, we conducted the validation at two meadows. We intend to continue to conduct depletion count validations each year so that over the course of the study we will have evaluated a variety of different types and sizes of tadpole habitats.

Capture-Recapture Field Methods

Coverboards

We tested coverboards in the three study meadows to determine if they offered an efficient method for capturing young of the year toads. Coverboards were made from pine shelving and were ~300x600mm in size with legs on each end so that they could be raised off the ground by ~20mm. While we did find young of year toads using coverboards, capture rates were not as high as by the visual encounter surveys we conducted in, and adjacent to, known breeding areas. It is possible that the coverboard method could be utilized to capture toads early in the morning before temperatures warm up and toads become more active.

Preliminary Abundance Estimates for Young of the Year

We conducted an initial VIE marking visit at all of our study meadows in late August and early September 2005. We revisited two meadows, three weeks later to determine if we could still find marked toads and if marks were clearly visible. The following table summaries these results and provides a simple Lincoln-Peterson estimate for the population size (N). Here n1 = the number of toads captured and marked on the first visit, n2 = total number captured on the second visit, and m2 = the number (out of n2) that had marks on the second visit. Recapture rates (% marked toads seen on the second visit) while relatively high, would likely be improved by reducing the time between visits (e.g. several days).

MEADOW	n1	n2	m2	N	% marked
Exchequer	113	59	8	833	13.6
Bear Paw	59	24	5	283	20.8

APPENDIX D. FENCING ESTIMATES FOR STUDY OF LIVESTOCK GRAZING EFFECTS ON YOSEMITE TOADS

(*based on design team meetings and Forest Service staff input as of 5/5/2006*)

Following the rules below, and using randomly generated treatment assignments, we drew proposed fence lines and measured linear distance of fencing needed using GIS tools (summarized in the table below with two examples on the following pages).

FENCING RULES

Both fence treatments:

* Use straight lines and minimize the number of corners.

Fence the whole meadow:

* Put fence line in forested habitat to provide options for fence posts, using a minimum 5m / 16ft buffer from the meadow edge based on a digital air photo.

Fence the wet (breeding) areas:

* Fence both breeding areas and randomly selected, suitable, unused areas (all of these areas were identified, GPS'd, and marked in the field during the 2005 season). Note that in actual practice (post-study) you may be able to modify this to fence only breeding areas, but for the study we need the unoccupied sites and occupied breeding areas to receive the same treatments.
* Use water flow patterns through the meadow and proximity of breeding/random areas to each other to create fenced areas that are continuous, rather than fragmented. The rationale for this approach was that tadpoles typically spread out, often moving downstream, during the spring and summer and we wanted to err on the side of providing more protection through the rearing season.
* If a particular breeding/random areas was isolated and fenced individually, a 60m / 200ft perimeter square (= 15m x 15m / 50ft x 50ft) buffer was placed around it.

ESTIMATES OF FENCING NEEDS BY MEADOW AND ALLOTMENT

Allotment	Meadow*	Meadow Area (acres)	Treatment	Fencing (linear distance)	Area Fenced (acres)
Sierra NF					
Patterson Mnt.	Swainson's Thrush	2.6	fence whole meadow	531m / 1743ft.	4.1
Patterson Mnt.	Continental (Guitar)	9.7	fence wet areas	454 m / 1489 ft.	2.8
Patterson Mnt.	Hash	10.3	standard grazing	0	0
			Patterson Total	**985 m / 3232 ft.**	**6.9**
Dinkey	Bear Paw	4.5	fence whole meadow	671 m / 2202 ft.	7.3
Dinkey	Exchequer	15	fence wet areas	856 m / 2808 ft.	7.9
Dinkey	Cabin	5.9	standard grazing	0	0
			Dinkey Total	**1527 m / 5010 ft.**	**15.2**

Allotment	Meadow*	Meadow Area (acres)	Treatment	Fencing (linear distance)	Area Fenced (acres)
Blasingame	Mono (Paradise)	8.4	fence whole meadow	847 m / 2779 ft.	12.1
Blasingame	Back Badger Flat (Shotgun)	4.7	fence wet areas	322 m / 1056 ft.	1.3
Blasingame	"New meadow" (SNF-516M301)	23.3	standard grazing	0	0
Blasingame	Weldon Camp (Sponge)	6.7	standard grazing + holding pasture	0	0
			Blasingame Total	**1169 m / 3835 ft.**	**13.4**
Sierra NF Reference	***"Near Helms Meadow" (SNF-521M311)***	***25.4***			
<u>Stanislaus NF</u>					
Highland Lakes	Snag	1.8	fence whole meadow	460 m / 1509 ft.	2.6
Highland Lakes	Rock Top	4.0	fence wet areas	208 m / 682 ft.	0.5
Highland Lakes	Bear Tree	5.9	standard grazing	0	0
			Highland Lakes Total	**668 m / 2192 ft.**	**3.1**
Herring Creek	Middle Three	17.1	fence whole meadow	1439 m / 4722 ft.	26.4
Herring Creek	Groundhog	11.6	fence wet areas	329 m / 1079 ft.	0.8
Herring Creek	Castle	43.0	standard grazing	0	0
Herring Creek	Lower Three	10.9	standard grazing + holding pasture	0	0
			Herring Creek Total	**1768 m / 5801 ft.**	**27.2**
Stanislaus NF Reference	***Red Peak 1***	***6.9***			

* Alternate names for meadows are in parentheses – we are still working on finalizing study meadow names and rectifying Forest and permittee names for meadows with the names we used in the field last summer.

Total linear fencing estimate for all treatments = 6117 m / 20,070 ft = 3.8 miles.

Example Fencing Maps for Dinkey Allotment, Sierra National Forest
Meadow name and treatment are given in yellow text. Yosemite toad tadpole groups are indicated by yellow circles and random (unoccupied) sites are blue circles.