

DISSERTATION

MONITORING AMPHIBIAN POPULATIONS AND THE STATUS OF WOOD  
FROGS AND BOREAL CHORUS FROGS IN THE KAWUNEECHE VALLEY OF  
ROCKY MOUNTAIN NATIONAL PARK

Submitted by

Richard D. Scherer

Department of Fish, Wildlife and Conservation Biology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2010

COLORADO STATE UNIVERSITY

June 10, 2010

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY RICHARD DEAN SCHERER ENTITLED MONITORING AMPHIBIAN POPULATIONS AND THE STATUS OF WOOD FROGS AND BOREAL CHORUS FROGS IN THE KAWUNEECHE VALLEY OF ROCKY MOUNTAIN NATIONAL PARK BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

---

Kevin Crooks

---

Denis Dean

---

Paul Doherty

---

Erin Muths

---

Advisor: Barry Noon

---

Department Head: Kenneth Wilson

ABSTRACT OF DISSERTATION

MONITORING AMPHIBIAN POPULATIONS AND THE STATUS OF WOOD  
FROGS AND BOREAL CHORUS FROGS IN THE KAWUNEECHE VALLEY OF  
ROCKY MOUNTAIN NATIONAL PARK

The selection of a state variable is an important component of any program for monitoring a wildlife population. Annual counts of egg masses have been promoted as an appropriate state variable for monitoring populations of some amphibian species. In Chapters 1 and 2, I evaluate different aspects of the use of counts of egg masses as a state variable for monitoring wood frogs and other pond-breeding amphibians. Specifically, I assessed patterns of detectability in wood frog egg masses and evaluated the statistical power of using counts of egg masses and linear regression to detect trends. In Chapter 1, I used closed capture-recapture models to estimate detection probability and evaluate potential sources of variation in the detectability of wood frog (*Rana sylvatica*) egg masses. Model selection results and model-averaged estimates provided evidence that detection probability varied between years which emphasize the importance of accounting for detection probability when using egg mass counts to infer population trends in amphibian populations. In Chapter 2, I used computer simulation to evaluate the statistical power of detecting trends in counts of egg masses. A previous study identified important benefits of using counts of egg masses as the state variable in a monitoring

programs for wood frogs (e.g., low cost) but did not evaluate the statistical power of this approach. The results of the simulations indicate that a minimum of nine years of monitoring is necessary to achieve high statistical power ( $\geq 0.80$ ). For populations experiencing low rates of annual decline ( $\leq 4\%$  annually), two decades or more of monitoring data were required to achieve high levels of power. Unless populations are changing at high rates, counts of wood frog egg masses will be a poor state variable for monitoring programs interested in detecting trends or evaluating the effects of management action or other perturbations. Jeff Tracey designed the computer simulations for this chapter and co-authored the chapter. In Chapter 3, I estimated the proportion of wetlands that were occupied by breeding wood frogs (*Rana sylvatica*) and boreal chorus frogs (*Pseudacris maculata*), as well as extinction and colonization probabilities, from 2004 to 2006 in a valley in Rocky Mountain National Park. I also evaluated hypothesized relationships between occupancy and attributes measured at multiple spatial scales. Erin Muths and Barry Noon were co-authors on this chapter. Breeding wood frogs occupied approximately 12% of the wetlands across the three years of the study, while occupancy by boreal chorus frogs increased from approximately 20% in 2004 to 37% in 2006. Wood frog occupancy was positively associated with the amount of streamside habitat adjacent to a wetland and negatively associated with the cost-based distance to the nearest occupied wetland. Boreal chorus frog occupancy was positively associated with the number of neighboring, occupied wetlands. For wood frogs, model-averaged estimates of colonization probability were  $< 10\%$  and estimates of extinction probabilities were  $> 30\%$ . Model-averaged estimates of colonization for boreal chorus frogs, on the other hand, were higher ( $> 10\%$ ) than for wood frogs, while estimates of extinction

probabilities were much lower (< 10%). I discuss the management implications of these results. Finally, in Chapter 4, I used eight microsatellite markers to assess the genetic population structure, estimate genetic variability and test for evidence of recent bottlenecks in a population of wood frogs (*Rana sylvatica*) in Rocky Mountain National Park, Colorado. The results from a genetic clustering algorithm indicate the population is partitioned into two genetic clusters, and an estimate of  $F_{ST}$  provides strong evidence of differentiation between the clusters. Though I found no evidence of recent population bottlenecks, genetic variability in the population was relatively low. Sara Oyler-McCance completed many of the genetic analyses in this chapter and was a co-author, as were Erin Muths and Barry Noon.

Richard D. Scherer  
Department of Fish, Wildlife and Conservation Biology  
Colorado State University  
Fort Collins, CO 80523  
Fall 2010

## ACKNOWLEDGMENTS

Many people made valuable contributions to this project. I thank my advisors, Barry Noon and Erin Muths for their support, advice, and encouragement and Kevin Crooks, Denis Dean, and Paul Doherty for serving on my committee. Several members of the staff at Rocky Mountain National Park provided logistical support for the fieldwork. In particular, I thank Judy Visty, Cheri Yost, and the staff at the Kawuneeche Valley Visitor Center. Many people at the Rocky Mountain Center for Conservation Genetics and Systematics processed genetic samples for the project, and Sara Oyler-McCance was an outstanding collaborator on the subsequent analyses. Jeff Tracey contributed his outstanding programming skills to the project, and Tammy Fancher, Terry Giles and Bob Waltermire developed maps for each field season. The following technicians and volunteers collected field data or contributed in other important ways: Mary Beth Albrechtsen, Greg Albrechtsen, Mandy Cluck, Angie Hansleben, Susan Harris, Jason Helvey, Scott Ratchford, Kara Rowan, Vid Rowan, Christine Sednek, Ginny Sednek, Chris Symmes, and Jessie Tigner. Clif Knopf was a crew leader for three years of field work, and I thank him for his commitment to the project and leadership in the field. The fieldwork for this project started early in the spring, and in the first field season, Clif and I nearly froze in our tents. I thank Kevin, Patty, Michael and Kristina Schwindt for providing us with housing over the remaining field seasons. Dennis Bellinghausen and

Bob Smithwick put in many hours as volunteers and made the field seasons much more enjoyable.

Funding for this project was provided by the U.S.G.S. Amphibian Research and Monitoring Initiative (ARMI), Denver University's Partners in Scholarship, the Douglas L. Gilbert Memorial Scholarship, and the Jay Hokenstrom Memorial Scholarship. In addition, Jerry Chaney provided timely funding for field equipment.

Finally, I thank my wife, Katie, and daughter, Callae, for their unwavering support in spite of the long hours and months away from home, and my sister, Missy, for her many contributions to our family. I am lucky to have all of you.

## TABLE OF CONTENTS

ABSTRACT OF DISSERTATION.....	iii
ACKNOWLEDGMENTS.....	vi

### Chapter 1. DETECTION OF WOOD FROG EGG MASSES AND IMPLICATIONS FOR MONITORING AMPHIBIAN POPULATIONS

Abstract .....	1
Introduction .....	2
Methods .....	4
Study site .....	4
Field methods .....	4
Hypotheses and statistical analysis .....	6
Results .....	7
Discussion .....	9
Literature cited .....	12
Figures .....	14
Tables .....	15



Chapter 2. A POWER ANALYSIS FOR THE USE OF COUNTS OF EGG MASSES TO  
MONITOR WOOD FROG POPULATIONS

Abstract .....	16
Introduction .....	17
Methods .....	19
Abundance model .....	20
Sampling model .....	22
Decision model .....	23
Assessing the temporal variability in simulated data .....	24
Results .....	25
Discussion .....	27
Literature cited .....	29
Figures .....	34

Chapter 3. OCCUPANCY PATTERNS OF WOOD FROGS AND BOREAL CHORUS  
FROGS IN A MONTANE LANDSCAPE

Abstract .....	38
Introduction .....	39
Methods .....	42
Study area .....	42
Amphibian surveys .....	43
Occupancy model .....	44
Hypotheses of detection probability – single season .....	48

Hypotheses of occupancy probability – single season .....	50
Individual wetlands .....	52
Seasonal migration .....	56
Dispersal .....	58
Estimating the amount of accessible seasonal habitat .....	59
Developing cost surfaces .....	59
Delineating buffers and estimating the amount of seasonal habitat ....	64
Estimating distance to and number of neighboring occupied wetlands ....	65
Hypotheses – multiple seasons .....	66
Modeling procedure, model selection and goodness-of-fit .....	69
Results .....	71
Evaluation of models and parameter estimates – single season .....	71
Wood frogs .....	71
Boreal chorus frogs .....	73
Evaluation of models and parameter estimates – multiple seasons .....	74
Wood frogs .....	74
Boreal chorus frogs .....	75
Discussion .....	75
Management implications .....	80
Literature cited .....	82
Figures .....	93
Tables .....	97
Appendix 1 .....	103

Appendix 2 .....	109
Appendix 3 .....	112

#### Chapter 4. THE GENETIC STRUCTURE OF A RELICT POPULATION OF WOOD

##### FROGS

Abstract .....	114
Introduction .....	114
Methods .....	117
Study area .....	117
Field methods .....	118
Genetic processing of samples .....	119
Genetic analyses .....	120
Results .....	121
Discussion .....	123
Literature cited .....	129
Figures .....	138
Tables .....	140

**CHAPTER 1**

**DETECTION OF WOOD FROG EGG MASSES AND IMPLICATIONS FOR  
MONITORING AMPHIBIAN POPULATIONS**

**Abstract**

Annual counts of egg masses have been promoted as an appropriate state variable for monitoring populations of some amphibian species. However, if some egg masses are not detected and detectability changes over time, the use of egg mass counts is unreliable. Variation in counts of egg masses may be indicative of variation in actual abundance or variation in detectability. I used closed capture-recapture models to estimate detection probability and evaluate potential sources of variation in the detectability of Wood Frog (*Rana sylvatica*) egg masses in a pond in Rocky Mountain National Park in 2003 and 2004. Model selection results and model-averaged estimates provided evidence that detection probability varied between years. However, I found no evidence of variation between observers within each year. The results of this study provide additional evidence that detection probability needs to be accounted for if egg mass counts are to be used to infer abundance and population trends in amphibian populations.

## **Introduction**

Concern over declines in amphibian populations has led to the implementation of many monitoring programs (e.g., the Amphibian Research and Monitoring Initiative [ARMI]; Muths et al. 2005). Historically, a variety of natural resource management and conservation activities have been characterized as monitoring (Thompson et al. 1998). In this paper, I define population monitoring as the repeated measurement over time of a variable that is informative of a population's status (i.e., a state variable; Thompson et al. 1998, MacKenzie et al. 2006). The purpose of repeated measurements is to detect change in the status of the population so that one may implement management or conservation action.

Abundance is often the most desirable state variable for monitoring programs. However, the data required to estimate and monitor the number of individuals in a population can be costly to obtain, particularly over large areas (Thompson et al. 1998, Pollock et al. 2002). Consequently, monitoring programs often use other metrics as indices of abundance. An index of abundance is any measure that is assumed to be correlated with the true abundance of the population (Thompson et al. 1998, Williams et al. 2002). For example, a commonly used index of abundance is a count of the number of individuals that are detected in an area. Counts are used as indices, because they can be collected relatively inexpensively and analyzed using statistical methods with which researchers and managers are often more familiar.

Unfortunately, counts nearly always represent a fraction of the total number of individuals in the population. At reasonable levels of sampling intensity, some proportion of individuals in the population are not detected and, therefore, not included in the count.

Temporal variation in the proportion of individuals that are detected can cause temporal variation in counts even when abundance remains the same, or it can cause counts to be similar from year-to-year even when abundance varies. Without knowledge of the relationship between a count and actual abundance, it is not possible to know if differences in counts over time are the result of change in true abundance (the information being sought by the monitoring program) or change in the detectability of individuals in the population.

Crouch and Paton (2000) argued that counts of egg masses are a valid state variable for monitoring some amphibian species (e.g., Wood Frogs [*Rana sylvatica*]) because they are accurate, precise, and relatively inexpensive to obtain. Yet, an accurate estimator is unbiased (Williams et al. 2002). Unless all egg masses in a pond are detected (i.e., the detection probability for egg masses is one), a count is necessarily biased low. This bias would not preclude detection of trends in abundance, if the same proportion of the total population was detected each year (Thompson et al. 1998). The results of a recent field study suggest the proportion of egg masses that are detected at a breeding pond is variable across years. Grant et al. (2005) reported evidence that detection probability varied across observers and other factors. Perhaps most importantly, they found the factors that explained variation in detection probability differed between years and between ponds. Based on the results of their study, Grant et al. (2005) argued that the use of simple counts for monitoring Wood Frog populations was insufficient. They concluded that detection probability must also be estimated if the goal was to make inference regarding change in the status of Wood Frog populations.

To further evaluate the utility of counts of Wood Frog egg masses for population monitoring, I conducted a capture-recapture study at a pond in Rocky Mountain National Park in 2003 and 2004. The aims of this study were to evaluate a proposed field protocol for applying closed capture-recapture methods to the estimation of detection probability and abundance of Wood Frog egg masses, to determine if the detection probability of Wood Frog egg masses was less than one, and to evaluate hypotheses regarding sources of variation in detection probability. In particular, I was interested in evaluating the hypotheses that detection probability varied between years and field workers.

## **Methods**

### ***Study site***

Gaskil Pond is a temporary pond located in the Kawuneeche Valley of Rocky Mountain National Park. During visits to the pond in the spring of 2002, I observed over 50 wood frog egg masses at the pond. The water in the pond comes primarily from snowmelt, and the pond has dried by the end of summer each year from 2003 – 2006. It is elliptically shaped (approximately 90 m long and 20 m wide) and has a maximum depth of approximately 0.65 m. Emergent vegetation is dense along the outer 0.5 to 5 meters of the pond and in the northernmost portion of the pond. The pond substrate is mud.

### ***Field methods***

Closed capture-recapture models are a common method of estimating abundance in animal populations (Williams et al. 2002). During multiple surveys of a population, records are kept of the individuals that are captured, marked and recaptured, and these data are used to estimate detection probability and abundance. In 2003 and 2004, three

field workers conducted capture-recapture surveys for Wood Frog egg masses at Gaskil Pond. Though all field workers were trained, only one field worker had previous experience searching for egg masses. Wood Frogs in the Southern Rocky Mountains are explosive breeders and deposit egg masses over a period of five to ten days in early spring (Corn and Livo 1989). Their egg masses are globular and approximately the size of a baseball (Hammerson 1999). Because they are large and are deposited at or just below the surface of the water, they are relatively conspicuous. Egg masses were marked using two colors of 8.75 cm deck screws (colors one and two). While two workers waited out of sight, the first worker searched the pond for egg masses. Each detected egg mass was marked by sinking a screw of color one right-side-up into the substrate immediately below the egg mass. Screws were pushed into the substrate so that approximately 2.5 cm of the screw remained above the substrate and they were not readily visible to subsequent workers. The first worker also used a drawing of the pond to mark the locations of each detected egg mass. Thirty minutes after the first worker started, the second worker began searching for egg masses. For each egg mass the second worker detected, he searched for a screw by moving his hand across the substrate below the egg mass. If no screw was located, the worker pushed a screw of color two right-side-up into the substrate immediately below the egg mass. If a screw was detected, the worker removed the screw and pushed it back into the substrate upside-down. The second worker used a drawing of the pond to mark the location of any egg masses that were not previously detected. Finally, the third worker searched the pond 30 minutes after the start of the second worker and used the same protocol as the second worker. After the third worker finished searching the pond, the maps of detected egg masses made by the first and second



workers were used to locate egg masses missed by the third worker. Capture histories for each egg mass were based on the presence, color and orientation of screws. For example, if the third worker found a screw of color one that was right-side-up below an egg mass, the egg mass was given the detection history 101 (the egg mass was detected by the first worker, not detected by the second worker, and detected by the third worker).

### ***Hypotheses and statistical analysis***

I used the closed capture-recapture model in Program MARK (White and Burnham 1999) for analysis of the data. In these models, detection probability,  $p$ , is defined as the probability an individual is detected during a sampling session. Alternatively, it can be defined as the proportion of individuals in the population that are detected during a sampling session. I evaluated four models of detection probability,  $p$ . The model denoted  $p(\cdot)$  represents the hypothesis that detection probability,  $p$ , does not vary between years, workers or egg masses. Because Wood Frog egg masses are highly conspicuous and Gaskil Pond is relatively small and easily searched, I anticipated that  $p$  would be high and of low variability. Model  $p(y)$  represents the hypothesis that  $p$  varies between 2003 and 2004. This model was of particular interest, because variation in  $p$  across years compromises the utility of counts in monitoring programs. Model  $p(y*w)$  represents the hypothesis that  $p$  differs between the first, second, and third workers within each year and between years. This model generates six estimates of  $p$ ; one for each of the three workers that surveyed over 2003 and 2004. Finally, I used the same person as the first field worker in 2003 and 2004. Therefore, I evaluated a fourth model,  $p(\text{first equal})$ . This model made  $p$  equal for the first field worker in 2003 and 2004. However,  $p$  varied between the second and third workers and between years in this model.

I used  $\Delta\text{AIC}_c$  values, represented as  $\Delta_i$  (where  $i$  indexes model), and Akaike weights,  $w_i$ , to determine which model had the most support in the data. Both measures quantify the strength of evidence in support of a particular model as the best model in the set of competing models (Burnham & Anderson 2002). The  $\Delta_i$  of any model  $i$  is computed as  $\text{AIC}_{c(i)} - \text{AIC}_{c(\text{best model})}$ . As  $\Delta_i$  increases, the strength of evidence for model  $i$  decreases. The Akaike weight of a particular model can be interpreted as the probability that the model is the best model of those models in the set of competing models given the sampling situation (Burnham & Anderson 2002). Finally, I used a goodness-of-fit test to evaluate the fit of the capture-recapture data to the most highly parameterized model,  $p(y*w)$ .

## Results

Eighteen egg masses were detected each year. In 2003, the first and second observers detected 15 egg masses, while the third observer detected 17 egg masses. In 2004, the first observer detected 12 egg masses, while the second and third observers detected 13 and 15 egg masses, respectively. The results of the goodness-of-fit test suggest good fit of the data to the  $p(y*w)$  model ( $\chi^2 = 10.14$ , d.f. = 13,  $P$ -value = 0.68).

Model  $p(y)$  had the most support in the data ( $w_{p(y)} = 0.56$ ; Table 1), though support for model  $p(\cdot)$  was also high ( $\Delta\text{AIC}_c = 0.78$ ,  $w_{p(\cdot)} = 0.38$ ; Table 1). Relative to the top two models, models  $p(\text{first equal})$  and  $p(y*w)$  had considerably less support in the data. Thus, there is weak evidence that  $p$  varied between years, and  $p$  showed little variation between workers within each year.

Given the uncertainty between the top two models, I derived model-averaged estimates of the detection probabilities for each worker in each year ( $\hat{p}_{j,k}$ , where  $j$  indexes worker [ $j = 1$  to 3] and  $k$  indexes year [ $k = 2003$  to 2004]), using the following equation from Burnham and Anderson (2002):

$$\hat{p}_{j,k} = \sum_{i=1}^4 w_i \hat{p}_{i,j,k},$$

where  $i$  indexes model. The  $\hat{p}_{j,2003}$  were less than 1 and varied little between field workers (from 0.84 to 0.85 with 95% CIs from approximately 0.69 to 0.93; Fig. 1). The point estimates for the  $\hat{p}_j$  in 2004 were smaller than those in 2003 ( $\hat{p}_{j,2004}$  ranged from 0.76 to 0.77 with 95% CIs from approximately 0.61 to 0.87), but the 95% confidence intervals for the estimates from each year overlapped one another (Fig. 1).

Because of the high estimates of  $p$  for each worker, the probability that an egg mass was missed by all three workers in a year was very small. In 2004, for example, the detection probabilities for each worker were 0.76, 0.76, and 0.77 (Fig. 1). Therefore, the probability that an egg mass was missed by all three workers is  $(1 - 0.76) * (1 - 0.76) * (1 - 0.77) = 0.013$ . Estimates of  $p$  for each worker in 2003 were higher, so the probability an egg mass went undetected was even smaller. Thus, Program MARK provided the numbers of egg masses counted each year as estimates of actual abundance.

## **Discussion**

Many state, regional, and national programs for monitoring animal populations make use of various counts as indices of abundance. If detection probability is not accounted for, however, changes in counts over time may be a consequence of actual change in abundance (the information being sought by the monitoring program) or change in the proportion of individuals in the study area that are detected. Grant et al. (2005) showed that the detection probability of Wood Frog egg masses has many possible sources of variation and, consequently, argued that detection probability needs to be accounted for if egg mass counts are to be used to infer abundance and population trends. Though the small sample precludes strong inference, this study used different field methods and a different capture-recapture model to provide additional evidence of variation in the detectability of Wood Frog egg masses between years.

In this study, three field workers conducted a three-sample closed capture-recapture study. The field protocols appear to have resulted in data that met the assumptions of the model. The assumptions of these models are: egg masses are not added to or lost from the population during sampling each year, the screws used to mark egg masses are not lost or overlooked during sampling, and variation in detection probability is modeled appropriately (Williams et al. 2002). Since sampling took place over approximately 2 hours, egg masses were probably not added or lost during sampling. The second assumption is more difficult to evaluate without double-tagging egg masses. However, the high estimates of detection probability and the fact that the third worker did not find any unmarked egg masses suggest screws were not lost or overlooked. Finally, goodness-of-fit tests suggested that the most highly parameterized

model provided an adequate fit to the data. However, it is important to note that the small number of egg masses each year likely caused the goodness-of-fit test to have low power to detect a lack of fit.

Though the field protocols were appropriate for this study, ecological and logistical constraints limit the applicability of this approach to other sampling situations. For example, female Wood Frogs in Colorado tend to deposit all their egg masses over a few days (Corn and Livo 1989) and egg masses remain recognizable for more than a week. Sampling can be conducted after all egg masses are present and before they are overly degraded. Thus, annual abundance of egg masses can be estimated. However, other species (e.g., Western Chorus Frogs [*Pseudacris maculata*]) have a longer period of deposition. Egg masses that are deposited early can mature and decompose before other egg masses are deposited. For these species, it will not be possible to identify a time for sampling when an estimate of the total number of egg masses can be derived. Issues that compromise reliable marking of individual egg masses will also limit the applicability of this study's methods to other populations. For example, females in some populations of Wood Frogs are known to deposit their egg masses in aggregations of > 100 egg masses (Grant et al. 2005). The largest aggregation at Gaskil Pond contained seven egg masses. Smaller aggregations were also present in both years, but they were loosely aggregated and individual egg masses were able to be reliably delineated and marked. At sites where aggregations are much larger, the method described in this paper will not be a reliable way to mark egg masses such that the capture history of individual egg masses can be determined.

While estimates of detection probability in this study were high, they were less than 1. Model-averaged estimates of detection probability for each observer ranged from 0.84 to 0.85 in 2003 and 0.76 to 0.77 in 2004 and the 95% confidence intervals did not include one (Fig. 1). Based on these estimates, a count of the number of egg masses at a pond would produce a negatively biased estimate of the actual number of egg masses. In addition, the results of this analysis suggest that detection probability of Wood Frog egg masses varied between 2003 and 2004 but not between observers within each year. The small number of egg masses at the pond in each year limited the power of the analysis and precluded strong inference regarding the effect of year and observer on detection probability. In spite of the small sample size, however, the top model included the year effect, and model-averaged estimates of detection probabilities in 2003 were greater than estimates in 2004. This difference in detection probability was apparent in spite of the fact that the first worker was the same in 2003 and 2004, which suggests an environmental cause for the difference. Though differences between observers within years were not apparent in this study, Grant et al. (2005) reported strong evidence of variation in detection probability between observers.

Variation in detection probability can lead to inappropriate inferences from monitoring programs that use counts of individuals as the state variable. The results from Grant et al. (2005) provided strong evidence of variation in detection probability of Wood Frog and Spotted Salamander (*Ambystoma maculatum*) egg masses due to a variety of factors. Similarly, the results of the present study provide evidence of variation in detection probability in Wood Frog egg masses between years. Consequently, inferences regarding population status or trends in amphibian populations based on counts of egg

masses should be considered unreliable. Temporal variation in detection probability is not restricted to egg masses. Capture-recapture studies of post-metamorphic amphibians have consistently found evidence of variation in detection probability (Anholt et al. 2003, Scherer et al. 2005). In spite of the added expense and the additional statistical expertise, monitoring programs for amphibians must use methods that allow detection probability to be estimated (e.g., distance sampling, double-observer approaches, capture-recapture methods).

### **Literature cited**

- Anholt, B.R., H. Hotz, G.-D. Guex, and R.D. Semlitsch. 2003. Overwinter survival of *Rana lessonae* and its hemiclonal associate *Rana esculenta*. *Ecology* 84:391–397.
- Burnham, K.P., and D.R. Anderson. 2002. Model selection and inference: a practical information–theoretic approach. 2<sup>nd</sup> edition. Springer–Verlag. New York, New York, USA.
- Corn, P.S., and L.J. Livo. 1989. Leopard frog and wood frog reproduction in Colorado and Wyoming. *Northwestern Naturalist* 70:1-9.
- Crouch, W.B., and P.W.C. Paton. 2000. Using egg-mass counts to monitor wood frog populations. *Wildlife Society Bulletin* 28:895-901.
- Grant, E.H.C., R.E. Jung, J.D. Nichols, and J.E. Hines. 2005. Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. *Wetlands Ecology and Management* 13:305-320.
- Hammerson, G.A. 1999. *Amphibians and Reptiles in Colorado*. University Press of Colorado, Boulder, CO and Colorado Division of Wildlife.

- MacKenzie, D.I., J.D. Nichols, J.A. Royle, K.H. Pollock, L.L. Bailey, and J.E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press. Burlington, Massachusetts, U.S.A.
- Muths, E., R.E. Jung, L.L. Bailey, M.J. Adams, P.S. Corn, C.K. Dodd, Jr., G.M. Fellers, W.J. Sadinski, C.R. Schwalbe, S.C. Walls, R.N. Fisher, A.L. Gallant, W.A. Battaglin, and D.E. Green. 2005. Amphibian Research and Monitoring Initiative (ARMI): a successful start to a national program in the United States. *Applied Herpetology* 2:355-372.
- Pollock, K.H., J.D. Nichols, T.R. Simons, G.L. Farnsworth, L.L. Bailey, and J.R. Sauer. 2002. Large scale wildlife monitoring studies: statistical methods for design and analysis. *Environmetrics* 13: 105-119.
- Scherer, R.D., E. Muths, B.R. Noon, and P.S. Corn. 2005. An evaluation of weather and disease as causes of decline in two populations of boreal toads. *Ecological Applications* 15:2150-2160.
- Thompson, W.L., G.C. White, and C. Gowan. 1998. Monitoring vertebrate populations. Academic Press. San Diego, California, U.S.A.
- White, G.C., and K.P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study* 46 Supplement:120–130.
- Williams, B.K., J.D. Nichols, and M.J. Conroy. 2002. Analysis and management of animal populations. Academic Press. San Diego, CA, U.S.A.



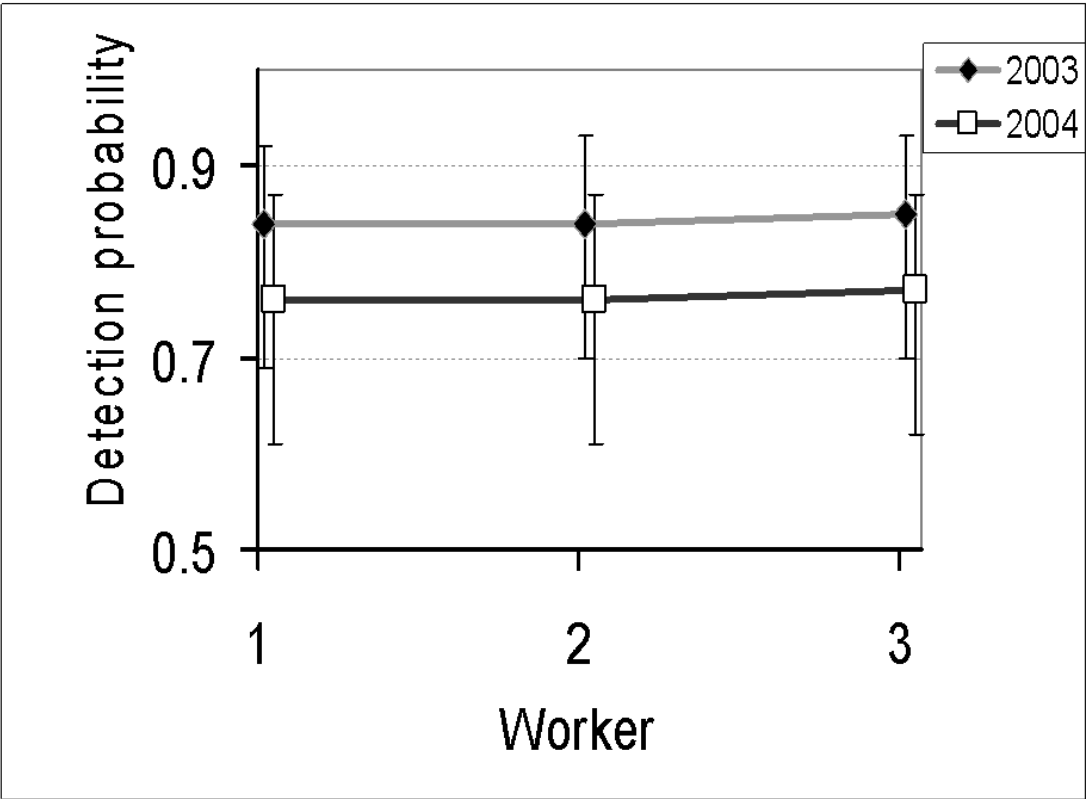


Fig. 1: Model-averaged estimates of detection probability. Error bars represent 95% confidence intervals using unconditional standard errors.

Table 1. Model selection results showing the relative support for each model. The number of parameters in each model is shown in the column labeled  $k$ .

Model	$\text{AIC}_c$	$\Delta \text{AIC}_c$	$w_i$	$k$
$p(y)$	-33.73	0	0.56	4
$p(\cdot)$	-32.95	0.78	0.38	3
$p(\text{first equal})$	-28.54	5.19	0.04	7
$p(y*w)$	-27.46	6.18	0.03	8

## CHAPTER 2

### A POWER ANALYSIS FOR THE USE OF COUNTS OF EGG MASSES TO MONITOR WOOD FROG POPULATIONS

#### Abstract

Counts of egg masses have been proposed as a state variable for monitoring wood frog (*Rana sylvatica*) populations, and some important benefits of this approach have been identified (e.g., low cost). If the power to detect trends in time series of egg mass counts is low, however, this approach may not be useful. I used computer simulation to evaluate the statistical power of detecting trends in counts of egg masses. The results of the simulations indicate that a minimum of nine years of monitoring is necessary to achieve high statistical power ( $\geq 0.80$ ). For populations experiencing low rates of annual decline ( $\leq 4\%$  annually), two decades or more of monitoring data were required to achieve high levels of power. Unless populations are changing at high rates, counts of wood frog egg masses will be a poor state variable for monitoring programs interested in detecting trends and/or evaluating the effects of management action or other perturbations.

## **Introduction**

Over the last 20 years, increasing evidence of declines in amphibian populations has led to the development of many monitoring programs (e.g., the North American Amphibian Monitoring Program, NAAMP; Weir and Mossman 2005). Population monitoring is the repeated measurement over time of some attribute (i.e., state variable) that is informative of a population's status (Thompson et al. 1998, Noon 2003). For example, the Amphibian Research and Monitoring Initiative (ARMI) is using the proportion of sample units (e.g., ponds, stream stretches) occupied as an indicator of the status of amphibian populations in some national parks in the U.S. (Muths et al. 2005). In most cases, a primary objective of monitoring programs is to detect a declining trend in the state variable across time, so that action can be taken to prevent unacceptable losses or extinction (Thompson et al. 1998, Maxwell and Jennings 2005, Marsh and Trenham 2008).

In developing a population monitoring program, several important decisions are necessary. These decisions relate to a variety of issues from determining the goals of the program to selecting the analytical methods that will be used. Of all these decisions, however, selection of an appropriate state variable is among the most critical (Noon and McKelvey 2006). Given that a primary goal of most monitoring programs is to detect declines in the status of a population, the state variable used in these programs should meet the following criteria at a minimum: i) change in the variable should be responsive to declines in the population's status and ii) change in the state variable should have a high probability of being detected (Gibbs 2000, Noon 2003, Maxwell and Jennings 2005, Noon and McKelvey 2006).

Crouch and Paton (2000) argue that counts of egg masses are a valid state variable for monitoring wood frog (*Rana sylvatica*) populations, and egg mass counts have been used to monitor wood frogs and other species of amphibians (Vasconcelos and Calhoun 2006). In assessing the appropriateness of egg mass counts for monitoring programs, Crouch and Paton (2000) consider some important issues (e.g., the cost of collecting counts of egg masses relative to collecting other kinds of data). However, time series of counts of many species of pond-breeding amphibians have other attributes that suggest they may be a poor state variable for monitoring programs. First, amphibian time series tend to be highly variable. Across 10 time series of counts of wood frog adults from Berven (1990, 1995) and Vasconcelos and Calhoun (2006), coefficients of variation (CV) ranged from 0.29 to 1.29 (average = 0.71; CV was computed as the standard deviation of a set of counts divided by the mean of the set of counts as in Lande et al. [2003] and Green [2003]). Time series of counts of ranid egg masses have also shown high levels of variability (CV = 0.90; Richter et al. 2003). Therefore, tests for a trend in these time series will have low power (i.e., a low probability of detecting a trend given a trend is present), unless the time series are of considerable length, the rate of decline is high, or covariates are used to control for some of the variation (Reed and Blaustein 1995). Hayes and Steidl (1997) calculated the power of detecting an annual decline of 5% in abundance for a population of ornate chorus frogs (*Pseudacris ornata*) and found that a time series of 32 years would be required to achieve power of 0.80. Second, the use of counts in monitoring programs compromises inference regarding trends in a population. Change in counts over time may represent change in actual abundance (the change of interest in

monitoring programs) or change in the proportion of individuals in the population that are detected and counted (Williams et al. 2002, Grant et al. 2005).

The objective of this project was to assess the statistical power of using counts of egg masses in monitoring programs for wood frogs. Assessment of statistical power for monitoring programs is uncommon (Gibbs et al. 1998, Marsh and Trenham 2008). I addressed the following questions: How many years of count data will be required to have high power (power  $\geq 0.80$ ) to detect declines in the abundance of wood frog egg masses of various magnitudes? How does variation in detection probability of wood frog egg masses and the number of workers conducting surveys affect power? I chose to base the simulations on wood frog egg masses, because sufficient data were available to parameterize a simulation model. Several time series of counts for wood frogs have been published (Berven 1990, 1995, Vasconcelos and Calhoun 2006), and detection probabilities of egg masses have been estimated (Grant et al. 2005, Scherer 2008). Because time series from populations of other pond-breeding amphibians show similar levels of temporal variability (Green 2003), however, I suspect that the results of this analysis will provide useful guidance for monitoring other species as well.

## **Methods**

I used computer simulation to generate time series of counts of wood frog egg masses similar to the kind of data that come from annual surveys at a breeding pond. The simulation model has three components: the abundance model, sampling model, and decision model. I used the abundance model to generate time series of egg mass abundance with an underlying decline rate, and the sampling model to mimic the process

of field workers counting the egg masses at a pond. The sampling model assumed detection probability was less than 1 and, consequently, generated a time series of counts that were often smaller than the number egg masses present at the pond. Finally, I used the decision model to conduct a trend analysis on each simulated time series of egg mass counts and determine if the results of the analysis provided the correct inference regarding the underlying negative trend in egg mass abundance.

Since I was interested in the effects of study duration (i.e., the number of years of monitoring), decline rate, detection probability and number of workers on the power to detect trends, I generated 20,000 replicate time series each under a particular combination of model parameters (Table 1). For example, one set of 20,000 replicate time series was generated assuming a monitoring program of 10 years, a decline rate of 5% per year, a detection probability of 0.75, and 2 field workers.

### *Abundance model*

The purpose of the abundance model was to project egg mass abundance across a variable number of years using different rates of decline (Table 1). To test the effect of monitoring duration on statistical power, I generated time series of egg mass abundance from 4 years to 30 years in length. The abundance model is a stochastic exponential model of the following general form

$$N_{t+1} = \lambda_t N_t,$$

where  $N_t$  is the abundance of egg masses during the breeding season in year  $t$  and  $N_{t+1}$  is the abundance of egg masses the following breeding season. The finite rate of increase,  $\lambda_t$ , defines the proportional decline or increase in abundance between years  $t$  and  $t + 1$ .

Values of  $\lambda_t$  less than 1 indicate a decreasing population, while values equal to or greater than 1 indicate a stable (no change in abundance) or increasing population (Gotelli 2001). For example, the abundance of egg masses will decline by 5% between the breeding seasons in years  $t$  and  $t + 1$  if  $\lambda_t = 0.95$ . In a deterministic exponential model, the same value of  $\lambda_t$  would apply across every interval  $t$  to  $t + 1$ , and a plot of abundance across years would appear as a smooth curve. Time series of amphibian counts, however, are not smooth curves. Rather, they often show high levels of variability around a trend line (Pechmann et al. 1991, Richter et al. 2003, Loman and Andersson 2007), which indicates a deterministic model is inappropriate. Therefore, I incorporated environmental and demographic stochasticity into the abundance model (Fig. 1). Environmental stochasticity is change in  $\lambda_t$  across intervals due to changes in environmental conditions (Gotelli 2001). During years of favorable environmental conditions, individuals have higher probabilities of survival and produce more offspring, and abundance of egg masses increases between  $t$  and  $t + 1$  ( $\lambda_t > 1$ ). In years of poor conditions, reduced survival and reproductive rates cause a decrease in abundance of egg masses between  $t$  and  $t + 1$  ( $\lambda_t < 1$ ). I incorporated environmental stochasticity into the abundance model by randomly selected  $\lambda_t$  from a gamma distribution at every interval  $t$  to  $t + 1$ . I chose a gamma distribution because it is flexible and appropriate for modeling non-negative random variables ( $\lambda_t$  is always  $\geq 0$ ; Rice 1995). The expected value of the gamma distribution was the decline rate of interest for the current set of replicate time series. For example, the expected value of the gamma distribution from which each  $\lambda_t$  was drawn was 0.95 for all simulated time series with a decline rate of 5% per year. To implement demographic stochasticity into the abundance model, I multiplied the abundance of the previous year,



$N_t$ , by the selected value of  $\lambda_t$ , and the resulting product became the expected value of a Poisson distribution. I drew the abundance value for  $N_{t+1}$  from this Poisson distribution (Allen 2003; Fig. 1).

For each simulation, the initial population size,  $N_0$ , was 500 egg masses which was in the range of counts of adult female wood frogs (Berven 1995) and wood frog egg masses (Egan and Paton 2004) from field studies. To examine the effect of decline rate on the power to detect a trend, I projected egg mass abundance with an expected value of  $\lambda_t$  as low as 0.9 (i.e., a 10% annual decline in abundance) to as high as 1 (i.e., no change in abundance across years) with 18 increments in between. Reported rates of decline in published analyses of amphibian time series ranged 0.004% to 8% per year (Hayes and Steidl 1997, Meyer et al. 1998).

### *Sampling model*

The abundance model projected the number of egg masses for every breeding season,  $t$ , in each simulated time series. I developed the sampling model to simulate the process of one to three workers searching a pond and counting egg masses. Because some proportion of wood frog egg masses are not detected during many surveys, a count will represent a fraction of the total number of egg masses in the pond (Grant et al. 2005, Scherer 2008).

I used the binomial model to simulate the sampling process. The binomial model can be used to simulate the number of successful outcomes given a particular number of trials and a probability of success and is often illustrated using the example of flipping a coin. Each flip of a coin can be considered an independent trial, and the binomial model

is used to simulate the number of times ‘heads’ will be observed given  $n$  flips of the coin and some probability of ‘heads.’ In this project, I used the binomial model to randomly select the number of egg masses that were detected and counted during a breeding season,  $C_t$ , given the total number of egg masses at the pond,  $N_t$ , and a probability of detection by a worker,  $p$ . Previous studies suggest the probability of detection of wood frog egg masses varies between years and workers (Grant et al. 2005, Scherer 2008). Therefore, I randomly selected the probability of detection for each breeding season and worker from a beta distribution. The beta distribution is often used to model probabilities because, like a probability, it is defined on the interval from 0 to 1. To evaluate the effect of detection probability on power, I created three beta distributions with expected values that represented scenarios of high ( $p = 0.94$ ), medium ( $p = 0.75$ ) and low ( $p = 0.50$ ) detection probability. The detection probability of wood frog egg masses is generally high, because they are large (approximately the size of a baseball) and tend to be deposited at or just below the surface of the water (Hammerson 1999, Grant et al. 2005). For the high detectability scenario, I estimated the beta distribution by calculating the mean and variance of 37 estimates of  $p$  from 2 field studies of wood frog egg masses (Grant et al. 2005, Scherer 2008) and using a method-of-moments approach (Rice 1995). The expected value of  $p$  was 0.94 (variance,  $\sigma_p^2 = 0.0064$ ). For the medium and low detectability scenario, I used the same variance as in the high detectability scenario but changed the expected values of  $p$  to 0.75 and 0.50.

In simulations where 2 or 3 workers survey a pond, the probability of detection had a slightly different interpretation than for a single worker. In those simulations,  $p$  represented the probability that an egg mass was detected and counted by at least one of

the workers. The probability that 2 workers fail to detect and count an egg mass can be represented as:  $(1 - p_1) * (1 - p_2)$ , where  $p_1$  and  $p_2$  represent the detection probabilities for each worker. Therefore, the probability that an egg mass was detected and counted by at least one of the workers is  $[1 - ((1 - p_1) * (1 - p_2))]$ . I denoted this quantity as  $p^{*2}$  and used it as the probability of detection in the binomial model. Similarly, in simulations with three workers, the probability that an egg mass was detected and counted by at least one of the workers is  $[1 - ((1 - p_1) * (1 - p_2) * (1 - p_3))]$  and was denoted as  $p^{*3}$ .

### ***Decision model***

I used the decision model to determine if a trend in the time series of egg mass counts was present. For each time series, I log-transformed the counts and used linear regression (LR) with  $\alpha = 0.05$  to evaluate the null hypothesis of no trend (as in Meyer et al. 1998). I used the following rule to determine if the sampling methods and LR resulted in the correct inference regarding the underlying decline in egg mass abundance: if the  $P$ -value  $< \alpha$  and the estimated slope ( $\hat{\beta}_1$ ) was significantly  $< 0$ , the results of the LR gave the correct inference (i.e., a significant decline was detected in the time series of counts and reflects the underlying decline in egg mass abundance across time). Otherwise, I concluded that the inference was incorrect. I conducted simulations in the R statistical software package (R Core Development Team 2007) and used the proportion of time series that resulted in a correct inference as a measure of statistical power.

### *Assessing the temporal variability in simulated data*

A key factor in the power of detecting a trend is the amount of temporal variability in a time series (Gibbs 2000). Therefore, it is important that the variability in the simulated time series was comparable to the variability reported from field studies of wood frog populations. Berven (1995) provided time series of counts of wood frog females from 10 populations in Michigan, Maryland, and Virginia. The duration of the time series ranged from 6 to 14 years, and I computed the CV for each time series as an estimate of the variability in numbers of wood frog egg masses across years. I compared the variability in the simulated time series of egg mass counts to the variability in the time series of females from Berven (1995) both graphically and numerically.

For the graphical analysis, I extracted and graphed the counts that represented the 5<sup>th</sup> and 95<sup>th</sup> percentile and the 25<sup>th</sup> and 75<sup>th</sup> percentile for each year from a simulated set of 20,000, 14-year time series. I compared this graph to the 14-year time series from Berven (1995; Fig. 2). To make this comparison meaningful, I estimated the trend in the time series of wood frog females from Berven (1995) by fitting an exponential model to the data and used the estimate as the decline rate in the simulated time series. In addition to the graphical comparison, I compared the CVs from the simulated time series with the CVs of the 10 time series from Berven (1995).

### **Results**

The graphical comparison indicated that the variability in the simulated time series of egg mass counts is a reasonable approximation of the variation in the time series of counts of female wood frogs from the field study (Fig. 2). Of the 13 counts from

Berven (1995), 11 (85%) are within the middle 90% of counts from the simulation model. Seven of the 13 (54%) are within the middle 50% of counts from the simulation model. The numerical comparison suggested that the simulated time series are less variable than the time series from field studies. The mean CV across the 20,000 simulated time series was 0.59, and the middle 50% of CVs ranged from 0.4 to 0.75. The CV of the 14-year time series of counts of wood frog females from Berven (1995) was 0.81 (Fig. 3). The average CV from the other 9 wood frog populations in Berven (1995) was 0.85 (range = 0.29 to 1.59) larger than the average CV across the simulated time series.

The poorest conditions for detecting a negative trend in the simulated time series of egg mass counts included the smallest rate of decline (1% loss per year), a detection probability of 0.50 and 1 field worker. Under these conditions, the simulations indicate that more than 30 years of counts would be required to attain power of 0.80 (Fig. 4a). Even at the highest rate of decline (10% loss per year), 10 years of counts would be required to achieve power of 0.80 with a detection probability of 0.50 and 1 field worker (Fig. 4a). After 10 years of declines of 10% per year, the number of egg masses in the population would have declined by more than 60% from the starting value of 500 egg masses.

Previous research suggests the detection probability of wood frog egg masses is much higher than 0.50 (Grant et al. 2005, Scherer 2008). Improved detection probability, whether by improving the ability of workers to find egg masses or adding workers to field crews, resulted in only slight improvements in power (Fig. 4b and 4c). Increasing detection probability and the number of workers only decreased the amount of time required to achieve power of 0.80 for a 10% annual decline by 1 year (Fig. 4b and 4c).

Time series of 2 decades or more were still required to achieve power of 0.80 for declines of smaller magnitude (4% loss per year or less, Fig. 4c).

## **Discussion**

A common reason for the failure of monitoring programs is insufficient duration of financial and institutional support for collection of data (Elzinga et al. 2001, Field et al. 2007). A power analysis should be a central component in the evaluation of a proposed monitoring program's survey design and state variable (Gibbs 2000, Maxwell and Jennings 2005, Legg and Nagy 2006, Field et al. 2007) and can provide valuable insights regarding the duration of the project. The purpose of this study was to assess the power of detecting trends in annual abundance of wood frog egg masses from time series of annual counts of egg masses. In addition, I evaluated the effects of monitoring duration, decline rate, detection probability and number of workers conducting surveys on the power to detect trends.

The results of the power analysis indicate that a minimum of nine years of monitoring will be required to achieve high power to detect trends in counts of wood frog egg masses unless rates of decline are greater than 10% per year (Fig. 4). At lower rates of decline, two decades or more of count data may be necessary to achieve high power (Fig. 4). Without a long-term commitment, therefore, initiation of a monitoring program for a wood frog population with egg mass counts as the state variable will likely represent a poor use of resources. In addition, the results suggest that improving the ability of workers to find egg masses (e.g., via better training) or increasing search effort would only marginally increase power for a given duration and rate of decline. Reductions in

power due to the use of counts were small compared to the reductions caused by the high levels of variability observed in time series from wood frog populations (Berven 1990, 1995, Vasconcelos and Calhoun 2006).

Noting that the simulations used in this study may underestimate the number of years required to achieve power of 0.80 is important. The simulated time series of egg mass counts were less variable than time series of female counts from 10 populations of wood frogs in Maryland, Michigan and Virginia (Berven 1995). If time series of abundance from populations of wood frogs tend to be more variable than the simulated time series, more years of data than suggested by this study will be required to achieve high power. A second reason that the results of this study may underestimate the number of years required to achieve high power is that the model used to generate the count data was known. When beginning a time series analysis from field data, the structure of the process that generated the counts of egg masses is not known. A mismatch between the structure of the biological and observational processes that generated the data and the model used to analyze the data will reduce power.

On the other hand, the simulations may overestimate the number of years required to achieve high power. Unless a complete count of the number of individuals in a population each year is achieved, the variability in a time series of abundance is comprised of two sources: variation in actual abundance over the time series (i.e., process variation) and variation due to the fact that abundance is estimated from a sample (i.e., sampling variation) (Link and Nichols 1994). Therefore, sampling variation increases the total variation in a time series and, consequently, reduces the power to detect a trend. Though this step is rarely completed in monitoring studies, sampling variation should be

removed from a time series prior to conducting a trend analysis (Barker and Sauer 1992, Link and Nichols 1994). I did not remove sampling variation from the simulated time series in this study; therefore, estimates of power may be biased low. However, I simulated the sampling of egg masses under low and high detectability scenarios. Under the highest detectability scenario (3 observers and probability of detection of 0.94), a complete census of egg masses would be achieved in most years and sampling variation would be low. Estimates of power from these simulations are nearly identical to estimates from the low detectability scenarios (Fig. 4) which suggests the presence of sampling variation in the simulated time series did not cause the number of years required to achieve high power to be overestimated.

While the conclusions in this study are based on simulations using counts of wood frog egg masses as the state variable, the temporal variability in time series across many populations suggest the results are applicable to other amphibian species and counts of other life history stages (e.g., juveniles and adults). Green (2003) calculated CVs for over 500 time series from populations of pond-breeding amphibians and reported a mean CV of 0.75 (SE = 0.037). This value is similar to the mean CV from the 10 populations of wood frogs studied by Berven (1995) and to the levels of variability used in the simulations for this study. Monitoring populations of amphibians with lower levels of temporal variability (e.g., stream-breeding species and terrestrial direct-developing species; Green 2003), however, may require fewer numbers of years to achieve high power.

Although the use of linear regression on log-transformed counts with  $\alpha = 0.05$  has been used to detect significant population trends in previous analyses of amphibian time



series (Meyer et al. 1998), the indiscriminant use of  $\alpha = 0.05$  does not take costs of a Type II error (concluding no trend when a trend is actually present) into consideration. For management of wildlife populations, a Type II error may be more costly than a Type I error (Field et al. 2007). For example, failing to take action early in the decline of a population may result in more costly management actions (e.g., captive rearing programs) or irreversible losses in populations. Many authors have argued that increasing  $\alpha$  is appropriate in order to increase power in monitoring programs (Gibbs et al. 1998, Field et al. 2007). Further, the use of traditional hypothesis testing in monitoring programs constrains the range of hypotheses that can be examined with a data set. An information-theoretic framework that utilizes an information criterion (e.g.,  $AIC_c$ ) will likely produce more informative results (Burnham and Anderson 2002). Under this framework, several models of different structure could be evaluated using a single data set. For example, a model of no trend (the null model in linear regression) could be compared to a variety of linear and nonlinear models of change in abundance over time, and inference could be made from the model with the most support from the data or by model-averaging across the set of models.

### **Literature Cited**

- Allen, L.J.S. 2003. An introduction to stochastic processes with biology applications. Prentice-Hall. Upper Saddle River, New Jersey, USA.
- Barker, R.J., and J.R. Sauer. 1992. Modeling population change from time series data. Pages 182-194 *In* D.R. McCullough and R.H. Barrett (eds.). *Wildlife 2001: populations*. Elsevier Applied Science. London, U.K.

- Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71:1599-1608.
- Berven, K.A. 1995. Population regulation in the wood frog, *Rana sylvatica*, from three diverse geographic localities. *Australian Journal of Ecology* 20:385-392.
- Burnham, K.P., and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2<sup>nd</sup> edition. Springer-Verlag. New York, New York, U.S.A.
- Crouch, W.B., and P.W.C. Paton. 2000. Using egg mass counts to monitor wood frog populations. *Wildlife Society Bulletin* 28:895-901.
- Egan, R.S., and P.W.C. Paton. 2004. Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. *Wetlands* 24:1-13.
- Elzinga, C.L., D.W. Salzer, J.W. Willoughby, and J.P. Gibbs. 2001. Monitoring plant and animal populations. Blackwell Science. Malden, Massachusetts, U.S.A.
- Field, S.A., P.J. O'Connor, A.J. Tyre, and H.P. Possingham. 2007. Making monitoring meaningful. *Austral Ecology* 32:485-491.
- Gibbs, J.P. 2000. Monitoring populations. Pages 213-252 *In* L. Boitani and T.K. Fuller (eds.). *Research techniques in animal ecology: controversies and consequences*. Columbia University Press. New York, New York, U.S.A.
- Gibbs, J.P., S. Droege, and P. Eagle. 1998. Monitoring populations of plants and animals. *Bioscience* 48:935-940.
- Gotelli, N.J. 2001. A primer of ecology. 3<sup>rd</sup> edition. Sinauer Associates. Sunderland, Massachusetts, U.S.A.

- Grant, E.H.C., R.E. Jung, J.D. Nichols, and J.E. Hines. 2005. Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. *Wetlands Ecology and Management* 13:305-320.
- Green, D.M. 2003. The ecology of extinction: population fluctuation and decline in amphibians. *Biological Conservation* 111:331-343.
- Hammerson, G.A. 1999. *Amphibians and reptiles in Colorado*. University Press of Colorado. Boulder, Colorado, U.S.A.
- Hayes, J.P., and R.J. Steidl. 1997. Statistical power analysis and amphibian population trends. *Conservation Biology* 11:273-275.
- Lande, R., S. Engen, and B. Saether. 2003. *Stochastic population dynamics in ecology and conservation*. Oxford University Press. Oxford, U.K.
- Legg, C.J., and L. Nagy. 2006. Why most conservation monitoring is, but need not be, a waste of time. *Journal of Environmental Management* 78:194-199.
- Link, W.A., and J.D. Nichols. 1994. On the importance of sampling variance to investigations of temporal variation in animal population size. *Oikos* 69:539-544.
- Loman, J., and G. Andersson. 2007. Monitoring brown frogs *Rana arvalis* and *Rana temporaria* in 120 south Swedish ponds 1989-2005: mixed trends in different habitats. *Biological Conservation* 135:46-56.
- Marsh, D.M., and P.C. Trenham. 2008. Current trends in plant and animal population monitoring. *Conservation Biology* 22:647-655.
- Maxwell, D., and S. Jennings. 2005. Power of monitoring programmes to detect decline and recovery of rare and vulnerable fish. *Journal of Applied Ecology* 42:25-37.

- Meyer, A.H., B.R. Schmidt, and K. Grossenbacher. 1998. Analysis of three amphibian populations with quarter-century long time-series. *Proceedings of the Royal Society of London B* 265:523-528.
- Muths, E., R.E. Jung, L.L. Bailey, M.J. Adams, P.S. Corn, C.K. Dodd, G.M. Fellers, W.J. Sadinski, C.R. Schwalbe, S.C. Walls, R.N. Fisher, A.L. Gallant, W.A. Battaglin, D.E. Green. 2005. Amphibian Research and Monitoring Initiative (ARMI): a successful start to a national program in the United States. *Applied Herpetology* 2:355-371.
- Noon, B.R. 2003. Conceptual issues in monitoring ecological resources. Pages 27-71 *In* D.E. Busch and J.C. Trexler (eds.). *Monitoring ecosystems: interdisciplinary approaches for evaluating ecoregional initiatives*. Island Press. Washington, D.C., U.S.A.
- Noon, B.R., and K.S. McKelvey. 2006. The process of indicator selection. Pps. 944-951 *In* C. Aguirre-Bravo, P.J. Pellicane, D.P. Burns, and S. Draggan (eds.). *Monitoring science and technology symposium: unifying knowledge for sustainability in the western hemisphere*. September 20-24, 2004. Denver, Colorado. Proceedings RMRS-P-42CD. Fort Collins, Colorado. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- Pechmann, J.H.K, D.E. Scott, R.D. Semlitsch, J.P. Caldwell, L.J. Vitt, and J.W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* 253:892-895.

- R Development Core Team. 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.  
<http://www.r-project.org>.
- Reed, J.M., and A.R. Blaustein. 1995. Assessment of “nondeclining” amphibian populations using power analysis. *Conservation Biology* 9:1299-1300.
- Rice, J.A. 1995. *Mathematical statistics and data analysis*. 2<sup>nd</sup> edition. Duxbury Press. Belmont, California, U.S.A.
- Richter, S.C., J.E. Young, G.N. Johnson, and R.A. Seigel. 2003. Stochastic variation in reproductive success of a rare frog, *Rana sevosia*: implications for conservation and for monitoring amphibian populations. *Biological Conservation* 111:171-177.
- Scherer, R.D. 2008. Detection of wood frog egg masses and implications for monitoring amphibian populations. *Copeia* 2008:669-672.
- Thompson, W.L., G.C. White, and C. Gowan. 1998. *Monitoring vertebrate populations*. Academic Press. San Diego, California, U.S.A.
- Vasconcelos, D., and A.J.K. Calhoun. 2006. Monitoring created seasonal pools for functional success: a six-year case study of amphibian responses, Sears Island, Maine, USA. *Wetlands* 26:992-1003.
- Weir, L.A., and M.J. Mossman. 2005. North American Amphibian Monitoring Program (NAAMP). Pps. 307-313 *In* M.J. Lannoo (ed.). *Amphibian declines: conservation status of United States species*. University of California Press. Berkeley, California, U.S.A.
- Williams, B.K., J.D. Nichols, and M.J. Conroy. 2002. *Analysis and management of animal populations*. Academic Press. San Diego, California, U.S.A.

Table 1: A list of the parameters in the abundance and sampling models, as well as the number of levels and range of possible values for each parameter. The range of possible values for study duration and decline rate are abbreviated in the table to minimize the length of the table. Study duration ranged from 4 to 30 years (in intervals of 1 year) and decline rate ranged from 0% to 10% decline in egg mass abundance per year (in intervals of approximately 0.5%).

		Parameters			
		Study Duration	Decline Rate in	Detection	Number of
		in number of	% loss/year	Probability	Workers
		years	(20 levels)	Per Worker	(3 levels)
		(27 levels)		(3 levels)	
Possible Values	4	0	0.50	1	
	5	1	0.75	2	
	6	2	0.94	3	
	...	...			
	30	10			

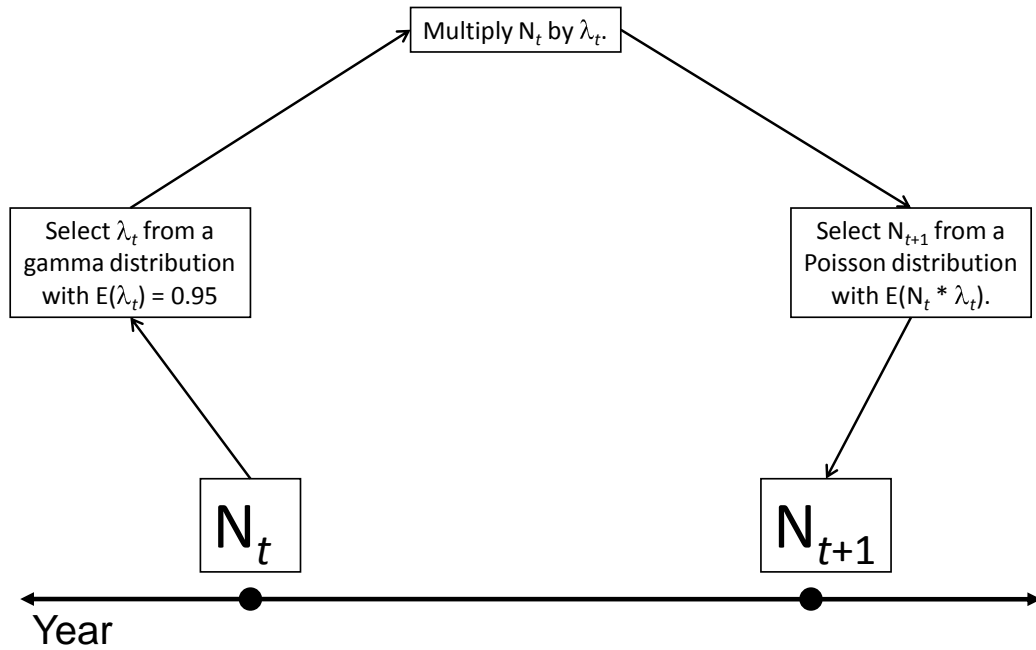


Figure 1: Schematic of the approach used to incorporate stochasticity into the time series of egg mass abundance.  $N_t$  and  $N_{t+1}$  represent egg mass abundance at years  $t$  and  $t + 1$ .  $\lambda_t$  represents the rate of decline between years  $t$  and  $t + 1$  and, for the purposes of this figure, is set at 0.95 (5% decline per year).  $E(\lambda_t)$  represents the expected value of the gamma distribution (i.e., the expected rate of decline).  $E(N_t * \lambda_t)$  represents the expected value of the Poisson distribution.

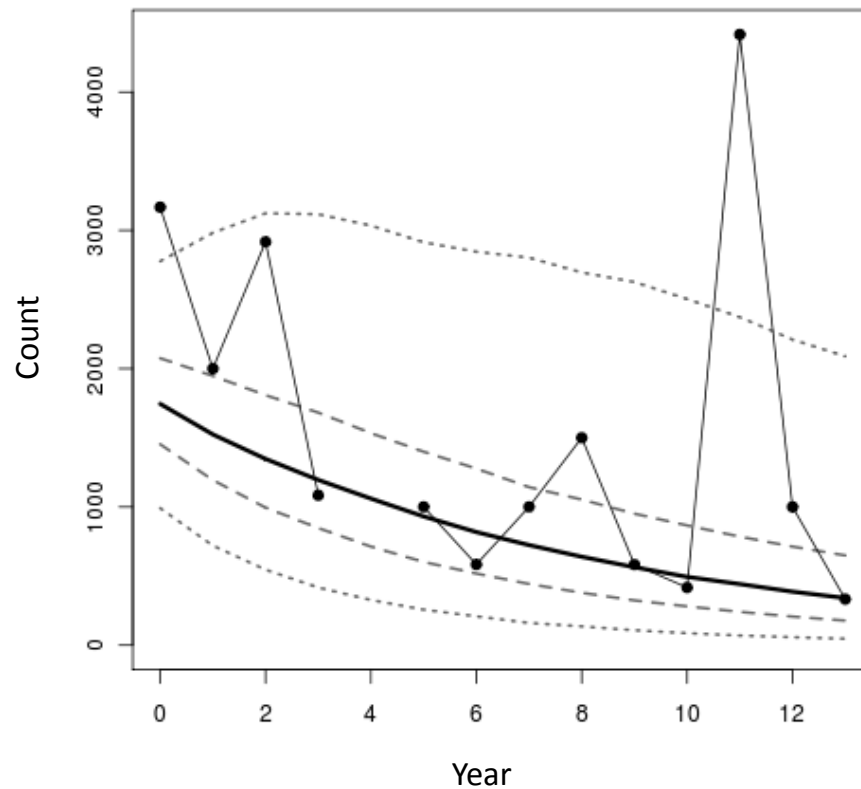


Figure 2: A comparison of the variability in the simulated time series of egg mass counts to the variability in a time series of counts of females from a population of wood frogs in Michigan (Berven 1995). The black circles connected by a solid line represent the time series of counts of females (the count from the fourth year was missing). The dotted line represents the 5<sup>th</sup> and 95<sup>th</sup> percentiles, the dashed line represents the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and the bolded solid line represents the mean of the 20,000 simulated time series.



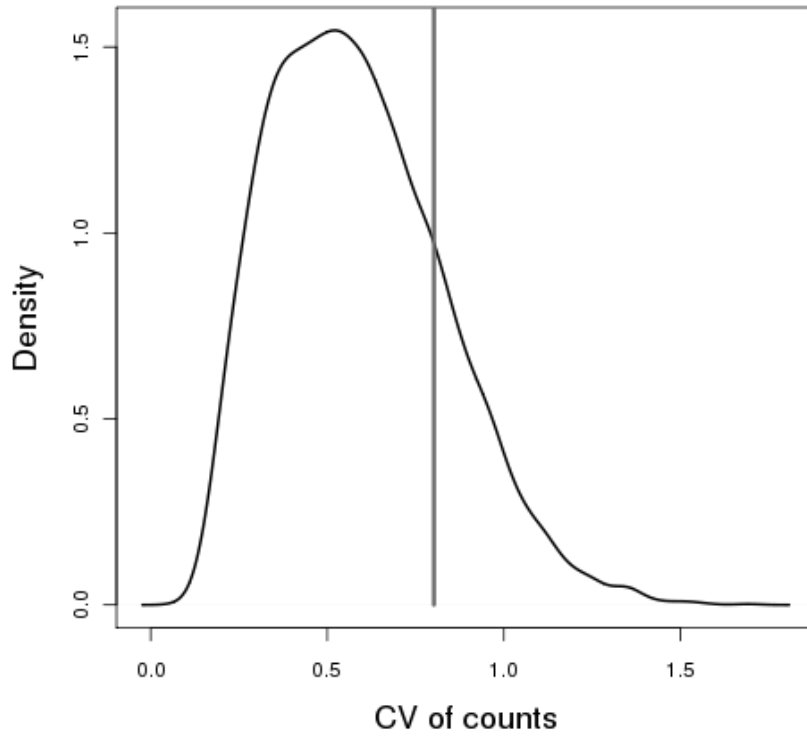


Figure 3: The distribution of coefficients of variation (CV) from 20,000 simulated, 14-year time series. The solid vertical line shows the CV of the 14-year time series of female wood frogs from Berven (1995).

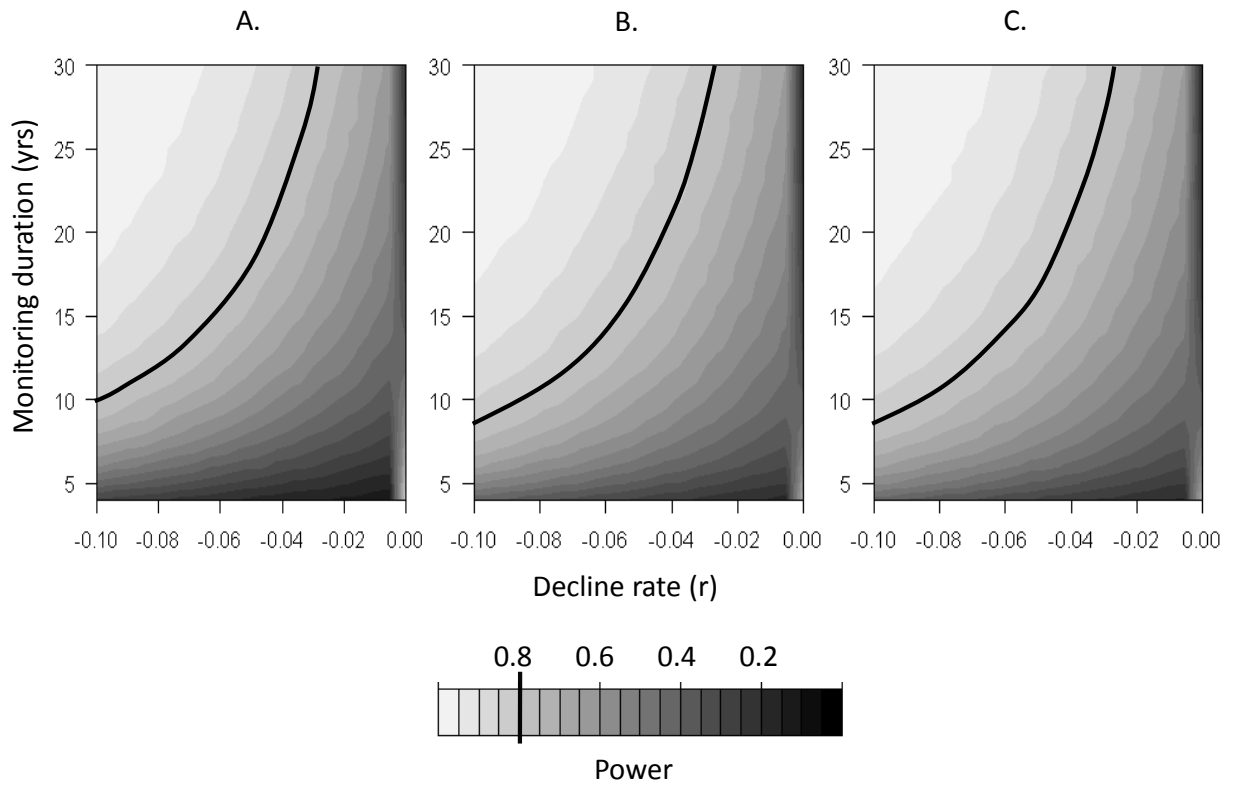


Figure 4: Power to detect negative trends in egg mass counts for different monitoring durations and rates of decline and under three combinations of detection probability and number of workers: A) detection probability,  $p = 0.5$  (low detectability), 1 observer; B)  $p = 0.75$  (medium detectability) and 2 observers; C) and  $p = 0.94$  (high detectability) and 3 observers. As the shading goes from light grey to black on each plot, power decreases. The thick black line represents power of 0.80, and all area above and to the left of the black line represents monitoring conditions of high power.

**CHAPTER 3**  
**OCCUPANCY PATTERNS OF WOOD FROGS AND BOREAL CHORUS**  
**FROGS IN A MONTANE LANDSCAPE**

**Abstract**

For most wildlife populations, basic information with which to assess current status and evaluate potential management actions is sparse or absent. Due to their high visibility and mission to conserve natural resources, this lack of information is especially problematic in national parks in the U.S. I estimated the proportion of wetlands that were occupied by breeding wood frogs (*Rana sylvatica*) and boreal chorus frogs (*Pseudacris maculata*), as well as extinction and colonization probabilities, from 2004 to 2006 in a valley in Rocky Mountain National Park. I also evaluated hypothesized relationships between occupancy and attributes measured at three spatial scales (wetland, seasonal migration, and dispersal). Measurements at the scale of seasonal migration and dispersal were derived using cost-based buffers and least-cost paths. Breeding wood frogs occupied approximately 12% of the wetlands across the three years of the study, while occupancy by boreal chorus frogs increased from approximately 20% in 2004 to 37% in 2006. Occupancy by wood frogs was positively associated with the amount of streamside habitat adjacent to a wetland and negatively associated with the cost-based distance to the

nearest occupied wetland. Occupancy by boreal chorus frogs was positively associated with the number of neighboring, occupied wetlands. For wood frogs, model-averaged estimates of colonization probability were  $< 10\%$  and estimates of extinction probabilities were  $> 30\%$ . Model-averaged estimates of colonization for boreal chorus frogs, on the other hand, were higher ( $> 10\%$ ) than for wood frogs, while estimates of extinction probabilities were much lower ( $< 10\%$ ). The management implications of these results are discussed.

## **Introduction**

Wildlife management is a process in which animal populations and the environment they inhabit are manipulated or protected to achieve goals (e.g., attain a particular population size, spatial distribution, or level of harvest; Williams et al. 2002, Sinclair et al. 2006). Ideally, effective management of a population of organisms begins with: i) goals or objectives for a target population, ii) a set of potential management actions for making progress toward the goals, iii) models that allow predictions to be made of the target population's response to each management action, and iv) a measure of the target population's current state (Nichols et al. 2007). For the vast majority of populations of wildlife, however, too little information is available to determine their current status and make predictions about their response to potential management actions. Given their high visibility, this problem is particularly apparent in national parks in the U.S. Though managers of national parks are charged with conserving populations of wildlife, insufficient resources and conflicting mandates have hindered efforts to collect fundamental information (National Research Council 1992, Stohlgren et al. 1995).

Historically, information regarding status and environmental relationships has been especially sparse for amphibians in national parks (Stohlgren et al. 1995). Yet, amphibians appear to be the most threatened group of animals in the world (Stuart et al. 2004).

In Rocky Mountain National Park, Colorado, historical records include observations of leopard frogs (*Rana pipiens*), boreal toads (*Bufo boreas*), boreal chorus frogs (*Pseudacris maculata*), wood frogs (*Rana sylvatica*) and tiger salamanders (*Ambystoma tigrinum*; Corn et al. 1997). Despite considerable survey effort beginning in 1988, the northern leopard frog has not been observed in the park and is presumed to be extinct (Corn et al. 1997, E. Muths *personal communication*). Of the extant species, only the boreal toad (*Bufo boreas*) has been studied sufficiently such that inference can be made about its current status and predictions can be developed regarding its response to potential management actions. Little is known about the current status and environmental relationships for wood frogs, boreal chorus frogs and tiger salamanders.

The Kawuneeche Valley (KV) is located near the western border of Rocky Mountain National Park (RMNP; Fig. 1). It is a high-elevation valley that contains the headwaters of the Colorado River and is the only location in the park where all extant amphibian species have been observed historically (Corn et al. 1997). With respect to wetlands and amphibians, there have been 2 important alterations to the landscape and biota of the KV: construction of the Grand Ditch and drastically reduced abundance of beaver (*Castor canadensis*). The Grand Ditch is a 22-km water diversion ditch that runs along the western mountainside of the KV. It intercepts runoff from rain and snowmelt and transports the water to areas of higher human population density in eastern Colorado.

Operation of the ditch has reduced the surface flow of water into the KV, reduced the amount and frequency of flooding by streams and rivers and lowered water levels in streams and rivers (Woods 2000, 2001). Each of these processes is an important mechanism for maintaining water table levels of wetlands in the KV, particularly given its location in the relatively arid interior of the western U.S. The beaver population (*Castor canadensis*) in the KV has declined over 90% in the last 60 years (Westbrook et al. 2006). The lack of newly constructed beaver dams and maintenance on existing dams has reduced the frequency, extent and duration of flooding on the floor of the valley (Woods 2000, Westbrook et al. 2006). Park management has expressed interest in understanding the impacts of these alterations on plants and animals in the park (Ozaki 1992), but the lack of information on the historic and current status of populations precludes the identification and quantification of effects for most species. This gap in knowledge also precludes informed management of populations of amphibians and, given the apparent poor state of amphibians in the park and around the world, represents an important gap in our understanding of the park's natural resources.

Occupancy, the proportion of sites or sampling units occupied by a species, is increasingly being used to characterize the state of plant and animal populations over large areas (e.g., Penman et al. 2009, Andelt et al. 2009), and models developed by MacKenzie et al. (2002, 2003, 2006) are commonly being used to derive estimates of occupancy. These models are useful for many reasons. In particular, they account for imperfect detection of the species of interest, facilitate the evaluation of hypothesized relationships between occupancy and elements of a species' environment, and can often

be used for species that are rare or difficult to detect (i.e., many amphibians; MacKenzie et al. 2004).

I used these methods to estimate occupancy of wetlands by wood frogs, boreal chorus frogs, boreal toads, and tiger salamanders in the KV from 2004 to 2006, identify correlations between occupancy patterns and attributes measured at multiple spatial scales (hereafter, environmental covariates), and estimate colonization and extinction rates for each species. I use the term, wetland, in reference to any area that contains standing water for at least a month in most years (e.g., lake, pond or wet meadow).

## **Methods**

### ***Study area***

The study area is comprised of the portion of the Kawuneeche Valley and the adjacent mountainsides up to 3,048 m elevation within Rocky Mountain National Park (Fig. 1). Over 80% of the study area is upland and is dominated by forests of lodgepole pine (*Pinus contorta*) and mixed coniferous species (*Abies lasiocarpa*, *Picea engelmannii*; Salas et al. 2005). The valley bottom is a mix of wetlands dominated by herbaceous vegetation (e.g., *Carex* spp.) and/or shrubs (*Salix* spp.) and riparian areas.

Precipitation in the mountains of Colorado falls primarily in the form of snow (Doesken et al. 2003), thus the timing and duration of runoff from snowmelt plays an important role in determining moisture conditions across the KV. Each year, the majority of the runoff is completed by the end of June, and warmer, drier conditions develop across the valley through the rest of the summer and fall (Doesken et al. 2003). Though thunderstorms over the summer can be common, they often generate small amounts of

patchily-distributed rainfall and periods of little or no rainfall can last several weeks (Woods 2001, Doesken et al. 2003).

### *Amphibian surveys*

I used aerial photographs from 2002 to identify 383 potential wetlands in the study area. I considered them potential wetlands, because identification of wetlands using aerial photography is uncertain (Baker et al. 2006), and I chose to err on the side of inclusion. For example, if a portion of the aerial photograph gave any indication of a wetland (i.e., a wetland or dry wetland basin appeared to be present), I included it among the potential wetlands. I used the set as the sampling frame from which I selected a sample to be surveyed. To ensure the wetlands in the sample were distributed across the entire study area, I ordered the wetlands in the sampling frame from north to south and selected wetlands using a systematic sample with a random start. I surveyed 56 wetlands in 2004 and 2005 and a total of 95 wetlands in 2006. A minimum of two surveys were conducted at the wetlands each year.

Wetlands were grouped depending on their location to minimize travel time. To prevent the introduction of heterogeneity into detection probabilities, I randomly determined the order in which groups of wetlands were surveyed. In addition, I randomly determined the order in which wetlands within groups were surveyed. For groups with wetlands predominantly on the floor of the valley, I randomly determined whether surveys would begin with the northernmost or the southernmost wetland. For groups with wetlands predominantly on a mountainside, I randomly determined whether surveys would proceed from low elevation to high elevation or the opposite direction.



Multiple surveys (i.e., sampling occasions) were conducted at each wetland from approximately mid-May to early July each year (i.e., sampling season). During each sampling occasion, I approached the wetland quietly to detect calling males. In addition, I walked the perimeter of the wetland and as far out as 10 m from the perimeter in search of amphibians of all life stages. I also searched accessible shallows (depth of water less than 60 cm) and haphazardly sampled these shallows for larvae with aquarium nets (10 x 15 cm opening). For each sampling occasion, I noted if a species was detected or not, and if the species was detected, I recorded the number of detections of individuals of all life stages. In addition to the data on species detections, I estimated the proportion of the wetland with emergent vegetation (categorical variable with 4 classes: 0 to 25%, 26 to 50%, 51 to 75% and 76 to 100%) and noted whether the wetland had standing water. Each wetland was mapped by walking the perimeter, recording spatial coordinates using a GPS, and noting distinctive physical features (e.g., downed trees) associated with the wetland. These data and aerial photography of the study area were used to map each wetland and calculate its surface area in ArcGIS 9.2 (ESRI 1999-2006).

### ***Occupancy model***

Presence-absence data have been commonly used to estimate the proportion of sites or area occupied by a species and to identify relationships between species' occurrence patterns and environmental covariates associated with the sites. While recent papers are beginning to account for imperfect detection in presence-absence data (Mazerolle et al. 2005, Tipton et al. 2008), analyses of presence-absence data often fail to explicitly account for imperfect detection of the species of interest. Few species are detected with certainty, and species that are rare or elusive can have very low probabilities of detection

(McDonald 2004). If the data include false absences (i.e., the species was present at a site but not detected), estimates of the proportion of sites occupied will be underestimated (MacKenzie et al. 2003, MacKenzie and Royle 2005) and estimates of other model parameters (e.g., regression coefficients) may be biased as well (Gu and Swihart 2004, Mazerolle et al. 2005, MacKenzie et al. 2006).

MacKenzie et al. (2002, 2003, 2006) developed survey and statistical methods for estimating the proportion of sites occupied and evaluating species-habitat relationships that account for imperfect detection. These models use data from multiple sampling occasions at each site within a sampling season to estimate a variety of parameters. The occupancy model of MacKenzie et al. (2002) was developed for data collected over a single sampling season. This model contains two parameters:  $p_{ij}$ , the probability the species is detected at site  $i$  on sampling occasion  $j$  given that it is present at the site, and  $\psi_i$ , the probability the species occupies site  $i$ . Measurements of environmental covariates that vary among sites or across sampling occasions can also be included in the model (MacKenzie et al. 2002). Inclusion of covariates allows hypothesized relationships between  $\psi_i$  and site characteristics to be evaluated, as well as suspected relationships between  $p_{ij}$  and characteristics of sites and sampling occasions. I used the single-season model and the occupancy data from 2006 to identify correlations between occupancy patterns for wood frogs and boreal chorus frogs and environmental covariates. I did not detect boreal toads or tiger salamanders at enough wetlands to conduct similar analyses for those species.

The occupancy model of MacKenzie et al. (2003) was developed for data collected over multiple sampling seasons, and several demographic parameters can be estimated

using this model. As with the single-season model, detection and occupancy probabilities can be estimated ( $p_{ijk}$  = the probability the species is detected at site  $i$  on sampling occasion  $j$  in sampling season  $k$  given that it is present at the site, and  $\psi_{ik}$  = the probability the species occupies site  $i$  during sampling season  $k$ ). Because data are collected over multiple sampling seasons, parameters that represent the processes that lead to change in occupancy over time can also be estimated with this model. Specifically,  $\gamma_k$  represents the probability that an unoccupied wetland at sampling season  $k$  is occupied at sampling season  $k + 1$  (i.e., the probability of colonization), and  $\varepsilon_k$  represents the probability that an occupied wetland at sampling season  $k$  is unoccupied at sampling season  $k + 1$  (i.e., the probability of extinction; MacKenzie et al. 2003). As with the single season model, hypothesized relationships between these parameters and attributes that vary across sites or sampling seasons can be evaluated through the inclusion of covariates in the model. I used the multiple-seasons model to estimate the proportion of wetlands occupied each year from 2004 to 2006 for wood frogs and boreal chorus frogs and estimate colonization and extinction probabilities of each species in the valley.

The single-season and multiple-seasons occupancy models have several assumptions, which I addressed in both the design and analysis phases of the project. First, the models assume that occupancy status does not change within a sampling season (i.e., whether a site is occupied or unoccupied at the first sampling occasion, it retains that status throughout the remainder of a sampling season; MacKenzie et al 2006). Movements of adult or juvenile frogs into or out of a wetland between sampling occasions could violate that assumption. Therefore, I counted only detections of egg masses and tadpoles in all

analyses. The occupancy model also assumes that the detection of a species during a sampling occasion is independent of detections during other sampling occasions (MacKenzie et al. 2006). I suspected that detection of a species at a wetland would make the detection probability of the species higher in subsequent sampling occasions, because field workers would know where to look. To address this issue, I evaluated a model in which detection probability on sampling occasions after initial detection were different from detection probability on or prior to the sampling occasion of original detection. Finally, the occupancy models assume that detection and occupancy are independent across sites (MacKenzie et al. 2006). The most likely cause of a violation of this assumption in this study is spatial autocorrelation of occupancy status (i.e., the occupancy status of wetlands that are near one another tends to be similar relative to wetlands that are farther apart). I conducted a Mantel test using Program zt (Bonnet and van de Peer 2002) to test for the presence of spatial autocorrelation in occupancy status across wetlands. Mantel tests are used to test for correlations between a matrix of geographic distances and one or more matrices of dissimilarity values (Bonnet and van de Peer 2002, Fortin and Dale 2005). I assigned pairs of wetlands with the same occupancy status a dissimilarity value of 0, whereas pairs of wetlands of different occupancy status were assigned a dissimilarity value of 1. Program zt tests for correlation between matrices using a randomization procedure (Bonnet and van de Peer 2002). A small *P*-value indicates that the occupancy status in wetlands near one another tends to be more (positive spatial autocorrelation) or less (negative spatial autocorrelation) similar than expected by random chance. Spatial autocorrelation of occupancy status could be induced by spatial autocorrelation in environmental attributes that make a wetland suitable or

unsuitable to a species or by movement behavior of the species (e.g., dispersal behavior). In addition to testing for spatial autocorrelation in occupancy status, I evaluated models that represented these processes in the analyses.

***Hypotheses of detection probability – single season***

I identified three covariates that were likely to cause variation in detection probability across sampling occasions in the single-season analysis: the number of workers during a sampling occasion (NUMBWOR), the day of the sampling occasion (SAMPDAY), and the length of the sampling occasion (SAMPTIME). I developed a set of hypothesized relationships between each covariate and detection probability and used the logit link function in all mathematical models of these relationships (MacKenzie et al. 2006):

$$\theta = \frac{e^{\beta_0 + \beta_1(x_1) + \dots + \beta_n(x_n)}}{1 + e^{\beta_0 + \beta_1(x_1) + \dots + \beta_n(x_n)}}, \quad (\text{Equation 1})$$

where  $\theta$  represents the parameter of interest (e.g., detection probability or occupancy probability),  $\beta_0$  is an intercept, the  $\beta_n$  are regression coefficients, and the  $x_n$  are environmental covariates.

I hypothesized that detection probability would increase linearly as NUMBWOR increased and, in addition, that detection probability would increase with increases in NUMBWOR to a threshold. I used the pseudo-threshold form of Franklin et al. (2000) to represent the threshold relationship. All models that included a threshold relationship in this analysis were of the following functional form:

$$\text{logit}(\theta) = \beta_0 + \beta_1 \log_e(x_1 + 0.005), \quad (\text{Equation 2})$$

where  $\theta$  represents the parameter of interest and  $x_1$  represents the covariate of interest. I added 0.005 to covariate values to eliminate values of 0. This form is not a true threshold, because an asymptote is approached but never reached. However, it represents a parsimonious approximation of a true threshold form (Franklin et al. 2000). I denoted these models as  $p(\text{NUMBWOR\_LIN})$  and  $p(\text{NUMBWOR\_THRESH})$  and use this format (the covariate followed by the functional form of the relationship between the covariate and a parameter) to label models.

SAMPDAY represents the day on which a sampling occasion occurred (the first day of sampling in a sampling season was identified as day 1). I hypothesized a linear and quadratic relationship between detection probability and SAMPDAY. I expected detection probability to be highest early in the sampling season when egg masses of both species are present. In particular, wood frogs egg masses are highly detectable (Grant et al. 2005, Scherer 2008). The linear relationship represented the hypothesis that detection probability would be highest for sampling occasions early in a sampling season and decline linearly for subsequent sampling occasions. The quadratic relationship represented the hypothesis that detection probability would be lowest during the middle of each sampling season and higher on sampling occasions that were early and late in each sampling season. Toward the middle of each sampling season, egg masses were less abundant or absent and tadpoles were small. Therefore, I expected detection probability to be lower. All quadratic relationships in this analysis were of the following form (Franklin et al. 2000):

$$\log it(\theta) = \beta_0 + \beta_1(x_1) + \beta_2(x_1^2), \quad (\text{Equation 3})$$

Finally, I hypothesized that the amount of time workers surveyed a pond (SAMPTIME) would affect detection probability and modeled this relationship using the linear and threshold functions. I expected detection probability to be higher for sampling occasions with relatively large values of SAMPTIME. The linear function represented the hypothesis that detection probability would increase at a constant rate with increases in SAMPTIME. The threshold function represented the hypothesis that detection probability would increase at a constant rate with increases in SAMPTIME and, then, approach an asymptote.

In addition to evaluating the effects of the above covariates on detection probability, I also evaluated the hypothesis that detection probability on sampling occasions after initial detection was different from detection probability on or prior to the sampling occasion of initial detection. I identified models that included this effect using the label, INITDET. As noted above, the purpose of evaluating this hypothesis was to determine whether the data met the assumptions of the occupancy models. I also evaluated the hypothesis that detection probability was constant across sampling occasions within the sampling season (denoted  $p(\cdot)$ ).

### ***Hypotheses of occupancy probability - single season***

For pond-breeding amphibians, patterns of occupancy across wetlands are often explained by variation in environmental covariates measured at multiple spatial scales (Knutson et al. 1999, Joly et al. 2001, Knapp et al. 2003, Mazerolle et al. 2005, Compton et al. 2007, Werner et al. 2009). The results of previous studies support the following conceptual model regarding the spatial dynamics of many species of pond-breeding amphibians. Regional populations (i.e., populations over large areas) are comprised of

multiple, local populations. Local populations are comprised of individuals that use a single wetland or cluster of wetlands for breeding (Vos and Stumpel 1995, Semlitsch 2008). Since the embryonic and larval life stages of most species of pond-breeding amphibian are exclusively aquatic, conditions within the wetland must facilitate the survival and development of embryos to metamorphosis if a wetland is to be occupied consistently (Werner and Glennemeier 1999, Stevens et al. 2006, Werner et al. 2009). In some cases, however, conditions at breeding wetlands explain only a small portion of the variation in occupancy across wetlands (Mazzerolle et al. 2005). This lack of explanatory power is likely due to fact that the spatial distribution of individuals in local populations often extends well beyond the boundaries of the breeding wetland (Rittenhouse and Semlitsch 2007a). The juvenile and adult life stages of many species spend more time in terrestrial environments, and individuals migrate to different areas in a landscape to access seasonal resources (e.g., refugia during dry parts of the summer and fall, overwintering sites, areas with a sufficient prey base; Pope et al. 2000, Baldwin et al. 2006; Rittenhouse and Semlitsch 2007a, Semlitsch 2008, Roznik and Johnson 2009). If necessary seasonal resources are not accessible from a wetland, it may not be occupied despite having suitable conditions for breeding and recruitment. Finally, local populations may be connected to one another and to unoccupied areas in the landscape by dispersal (Vos and Stumpel 1995, Smith and Green 2006) such that variation in occupancy across wetlands can be caused by variation in the accessibility of wetlands to other wetlands. Wetlands that are well connected to other occupied wetlands may have higher occupancy probabilities, because dispersing adults or juveniles are more likely to find them (Sjogren Gulve 1994, Vos and Chardon 1998, Knapp et al. 2003, Trenham et al. 2003). Successful



dispersers may lead to higher occupancy by colonizing previously unoccupied wetlands, rescuing local populations from extinction, or ameliorating the effects of inbreeding depression (Sinsch 1997, Trenham et al. 2003, Smith and Green 2006, Werner et al. 2009).

I based the selection of environmental covariates on this conceptual model and, consequently, evaluated covariates from three spatial scales. I measured attributes of: i) individual wetlands, ii) the landscape at the scale of seasonal migration and iii) neighboring wetlands at the scale of dispersal. I define migration as seasonal movements by adults and juveniles between their natal wetland and areas of the landscape with seasonal resources (e.g., food or hibernacula; Marra et al. 2006, Semlitsch 2008). I define dispersal as movement that results in an individual permanently leaving its natal wetland and becoming a member of another local population (Semlitsch 2008). Dispersal tends to be unidirectional and occurs over a larger area than migration.

### **Individual wetlands**

At individual wetlands, I measured three variables: hydroperiod (HYDRO), percent of the surface of the wetland with emergent vegetation (EMERVEG), and the surface area of the wetland (AREA).

I measured the depth of standing water at each sampled wetland from 26 June to 29 June 2006 and combined these data with annual observations of hydroperiod to determine each wetland's relative hydroperiod length and assign each wetland to one of the following categories:

- Category 1 – maximum depth less than 10 cm in late June, 2006. Wetlands have a short hydroperiod and dry annually.

- Category 2 – maximum depth 10 cm or greater but less than 50 cm in late June, 2006. Wetlands have an intermediate hydroperiod and frequently dry.

- Category 3 - maximum depth 50 cm or greater but less than 1 m in late June, 2006. Wetlands have a long hydroperiod and rarely dry.

- Category 4 – maximum depth greater than 1 m in late June, 2006.

Wetlands have the longest hydroperiod and are never dry.

Wood frogs in Colorado use small, ephemeral wetlands for breeding (Corn et al. 1997, Hammerson 1999) and are associated with wetlands of intermediate or short hydroperiod across their geographic range (Knutson et al. 1999, Skelly et al. 1999, Egan and Patton 2004, Hermann et al. 2005, Cunningham et al. 2007). From 2003 to 2006, the date wood frog egg masses were first observed in the study area ranged from 25 April (in 2004) to 20 May (in 2003). In three of the four years, wood frog egg masses were first observed on 12 May or later. Similarly, breeding activity was observed from early- to late-May in wood frog populations in Wyoming (Corn and Livo 1989). In the mountains of Colorado, wood frog eggs require approximately 75 days for development to terrestrial juveniles (Bagdonas 1968). Therefore, a wetland would need to have standing water until at least mid-July for metamorphosis to occur, and wetlands with the shortest hydroperiod (category 1) may be insufficient for wood frog larvae to metamorphose. In addition, previous studies have reported lower occupancy probability and counts of egg masses in wetlands with permanent hydroperiods (i.e., wetlands in category 4; Egan and Patton 2004, Cunningham et al. 2007). Therefore, I evaluated the following hypotheses regarding the relationship between hydroperiod and occupancy in wood frogs: i) The

probability of occupancy will be equal across wetlands of all hydroperiod categories (model denoted as  $\psi[.]$ ), ii) The probability of occupancy will vary between wetlands of different hydroperiod category ( $\psi[\text{HYDRO } 1, 2, 3, 4]$ ), iii) The probability of occupancy in wetlands of category 2 and 3 will be equal and higher than the probability of occupancy in wetlands of category 1 and 4 ( $\psi[\text{HYDRO } 1, 2=3, 4]$ ), and iv) The probability of occupancy in wetlands of categories 1, 2 and 3 will be equal and higher than the probability of occupancy in wetlands of category 4, ( $\psi[\text{HYDRO } 1=2=3, 4]$ ).

I evaluated the same set of hypotheses using the occupancy data for boreal chorus frogs. Boreal chorus frogs, however, use wetlands of temporary and permanent hydroperiod for breeding (Hammerson 1999, Werner et al. 2009). In the study area, they oviposit at approximately the same time as wood frogs and can metamorphose in a shorter amount of time (approximately 50 days; Matthews and Pettus 1966). Therefore, I expected boreal chorus frogs to occupy wetlands within a broader range of hydroperiods than wood frogs.

The presence of emergent vegetation in wetlands may be important for wood frog and boreal chorus frog occupancy for several reasons. Both wood frogs and boreal chorus frogs attach egg masses to emergent vegetation, and emergent vegetation in wetlands provides cover for tadpoles (Corn and Livo 1989, Hammerson 1999, Egan and Paton 2004). In addition, the amount of emergent vegetation in a wetland might be associated positively with levels of dissolved oxygen, abundance of arthropods, and water temperature (Welch and MacMahon 2005). Finally, the presence of emergent vegetation may limit water circulation in a wetland and allow the thermal advantages of communal egg deposition in wood frogs to be realized (Howard 1980). During each sampling

occasion, I estimated the percent of each wetland's surface with emergent vegetation using the following categories: 0 – 25%, 26 – 50%, 51 – 75%, and 76 – 100%. Therefore, I had multiple estimates of the percent of emergent vegetation for each wetland. I categorized each wetland based on the category to which it was most frequently assigned in the field data. I predicted that wetlands with higher percentages of emergent vegetation (EMERGVEG) would have a higher probability of occupancy for both species. Therefore, I evaluated the following hypotheses regarding the relationship between cover from emergent vegetation and occupancy in wood frogs and boreal chorus frogs: i) The probability of occupancy will be equal across wetlands of all four emergent vegetation categories ( $\psi[.]$ ), ii) The probability of occupancy will vary between wetlands in each emergent vegetation category ( $\psi[EMERGVEG 1, 2, 3, 4]$ ), iii) The probability of occupancy in wetlands with greater than 25% emergent vegetation will be higher than in wetlands with lower percentages of emergent vegetation ( $\psi[EMERGVEG 1, 2=3=4]$ ), and iv) The probability of occupancy in wetlands with greater than 50% emergent vegetation will be higher than in wetlands with lower percentages of emergent vegetation ( $\psi[EMERGVEG 1=2, 3=4]$ ).

Finally, wetlands of greater area may have higher probabilities of being occupied by amphibians. Larger wetlands may support bigger populations that are less prone to demographic stochasticity and, consequently, local extinction. In addition, emigrating juveniles and adults may be more likely to find and, therefore, colonize larger wetlands or rescue the local population that uses the wetland for breeding. I hypothesized a linear relationship between occupancy probability and wetland surface area (AREA\_LIN). In

addition, I hypothesized that occupancy probability would increase to a threshold as surface area increased (AREA\_THRESH).

### **Seasonal migration**

Individuals in many populations of animals must migrate to different areas in a landscape to access habitats with seasonal resources (Marra et al. 2006). The purpose of this spatial scale of analysis was to evaluate the hypothesis that sufficient amounts of other seasonal habitat types must be accessible from a wetland for it to be occupied by breeding wood frogs or boreal chorus frogs. Studies of migratory behavior in wood frogs suggest a general pattern of seasonal migration, where after a breeding season or upon metamorphosis for juveniles, frogs migrate to moist forested areas, adjacent wetlands, stream edges and ravines (Vasconcelos et al. 2004, Baldwin et al. 2006, Patrick et al. 2006, Rittenhouse and Semlitsch 2007*b*). These landscape features facilitate hydroregulation and may have higher densities of prey (Rittenhouse and Semlitsch 2007*b*). As winter approaches, wood frogs migrate to areas with unsaturated soils relatively near breeding wetlands (Regosin et al. 2003, 2005). Relative to wood frogs, information on migratory behavior in boreal chorus frogs is sparse. Spencer (1964), however, reported that the majority of adult and juvenile boreal chorus frogs from a high-elevation population in northern Colorado remained near their natal wetland or migrated only a short distance ( $\leq 300$  m) to adjacent wet meadows.

To evaluate relationships between occupancy at a wetland and the amount of seasonal habitat adjacent to the wetland, previous studies have used circular buffers (Fig. 2; Vos and Chardon 1998, Joly et al. 2001). For example, Vos and Chardon (1998) used a series of concentric circles around sampled wetlands. Within each circle, they calculated

the area of various seasonal habitat types and used these measures as predictor variables in models of occupancy. This approach assumes movement from breeding wetlands is isotropic (i.e., migratory movements are equal in all directions from the breeding wetland; Fortin and Dale 2005, Zanini et al. 2008). However, many species of amphibian avoid moving through particular land cover types (deMaynadier and Hunter 1999, Rothermel and Semlitsch 2002), and the cost of movement, in terms of body condition and survival, varies among land cover types (Rothermel and Semlitsch 2002, Mazerolle et al. 2005, Rittenhouse et al. 2008). To more accurately reflect these variable costs of movement, I used cost-based buffers as opposed to concentric circles (Fig. 2; Ray et al. 2002, Theobald 2006, Zanini et al. 2008). Measures of the amount of adjacent seasonal habitat based on cost-based buffers have provided a better explanation of patterns of occupancy in amphibians (Ray et al. 2002, Zanini et al. 2008).

Within each buffer, I derived estimates of the following covariates of occupancy for wood frogs: i) the area of wetland vegetation within 400, 600 and 1000 cost meters of the breeding wetland (WETVEG400, WETVEG600, and WETVEG1000), ii) the length of stream within 400, 600, and 1000 cost meters of the breeding wetland (STREAM400, STREAM600, and STREAM1000), and iii) the amount of upland within 400, 600, and 1000 cost meters of the breeding wetland (UPLAND400, UPLAND600, and UPLAND1000). I used the same covariates for boreal chorus frogs but, because of their body size and evidence that they travel shorter distances from wetlands during seasonal migrations (Rittenhouse and Semlitsch 2007a), used a smaller spatial scale (200, 400 and 800 cost meters). The amount of wetland vegetation and the length of stream were chosen because these landscape elements were most likely to provide the moist, cool conditions

wood frogs and boreal chorus frogs require for survival during the summer and fall in the study area. The third covariate (the amount of upland) was chosen, because it represented potential habitat over the winter for both species (Regosin et al. 2003, 2005). Spencer (1964) noted that boreal chorus frogs also use upland forested and non-forested areas during hibernation. For each covariate, I hypothesized a positive, linear relationship with occupancy. I also hypothesized that occupancy would increase to a threshold with increases in values of each covariate. The measurement units, cost meters, represent the product of the Euclidean distance of a path through a landscape and the relative cost of movement through each land cover type along the path. For example, traveling three meters through a land cover type with a cost of movement of 1 and two meters through a land cover type with a cost of movement of 8 would equate to 19 cost meters.

### **Dispersal**

Finally, I assessed covariates at the scale of dispersal to evaluate the hypothesis that wetlands near occupied wetlands have a higher probability of occupancy than isolated wetlands. Dispersal in pond breeding amphibians differs from seasonal migration in two primary ways: it generally occurs over greater distances (Berven and Grudzien 1990, Baldwin et al. 2006, Semlitsch 2008), and juveniles are the primary dispersers (Berven and Grudzien 1990, Funk et al. 2005, Gamble et al. 2007; but see Smith and Green 2006). Due to their smaller body size and greater ratio of surface area to volume, I expect juveniles to experience a higher cost of movement than adults through land cover types characterized by warm and dry conditions. Therefore, I used different costs of movement in the modeling of the dispersal process (Table 3). I evaluated hypothesized relationships between occupancy and: i) the cost distance to the nearest occupied wetland

(NEAREST), and ii) the number of occupied wetlands within 1500, 3000, and 4500 cost meters for wood frogs (NUMOCC1500, NUMOCC3000, and NUMOCC4500) and the number of occupied wetlands within 1000, 2000 and 3000 cost meters of a sampled wetland for boreal chorus frogs (NUMOCC1000, NUMOCC2000, and NUMOCC3000). I hypothesized a positive, linear and threshold relationship between occupancy and each of these covariates.

### *Estimating the amount of accessible seasonal habitat*

To estimate the amount of seasonal habitat types adjacent to each sample wetland, I developed a cost (or resistance) surface, delineated a cost-based buffer around each wetland, and estimated the amount of seasonal habitat within each buffer. The primary sources of data for these steps were a vegetation map for Rocky Mountain National Park (Salas et al. 2005) and vegetation data from ground-truth plots. The vegetation map partitions the KV into 44 land cover classes. The distinction between some land cover classes was unnecessary for the purposes of this project, and after combining redundant classes, the vegetation map contained 24 classes. The map did not include adequate information on rivers and streams. Therefore, I merged the data from a map of rivers and streams in Rocky Mountain National Park with the vegetation map (see Appendix 1 for details on the merging process) and created two land cover classes for rivers: rivers and large streams with heavy flows and smaller streams with lower flows. I will refer to the final data set of land cover classes ( $n = 26$  classes; 24 classes from the original vegetation map and 2 classes of rivers and streams) in the study area as the merged map.



### **Developing cost surfaces**

The concept of a cost surface recognizes that biotic and abiotic characteristics of land cover classes influence the movement of individuals and requires each land cover class to be assigned a relative cost of movement (Theobald 2005). The relative costs reflect the willingness of an individual to move through a particular land cover class, as well as the effect of the land cover class on the physical condition and survival of the individual (Compton et al. 2007). To develop a cost surface for the study area, I converted the merged map into a raster data set (10 m cells) of land cover classes (Appendix 1 contains additional detail on the development of cost surfaces). I assigned land cover classes to cells based on the land cover class from the merged map that overlapped the center of each cell. Logically, the next step in the process was to assign relative costs of movement to each land cover class. Even with the reduction in the number of land cover classes to 26, however, existing data are inadequate for assigning costs to each class without many arbitrary assignments.

Rather than assign relative costs of movement to each land cover class, I identified broad landscape types to which costs could be assigned based on the results of previous studies. Though movement data on boreal chorus frogs are sparse, previous research provides useful information with which to assign relative costs of movement for wood frogs. Based on a review of this literature, I designated landscape types based on two attributes: type of cover (tree, shrub or grass/forb) and moisture regime (wet or dry). I combined these attributes to form six landscape types: i) Types with canopy cover from trees and a wet moisture regime (Tree-Wet), ii) Types with canopy cover from trees and a dry moisture regime (Tree-Dry), iii) Types with canopy cover from shrubs and a wet

moisture regime (Shrub-Wet), iv) Types with canopy cover from shrubs and a dry moisture regime (Shrub-Dry), v) Types with canopy cover from grasses and forbs and a wet moisture regime (Grass-Wet), and vi) Types with canopy cover from grasses and forbs and a dry moisture regime (Grass-Dry). I assigned the lowest cost of movement to the ‘Tree-Wet’ and ‘Shrub-Wet’ landscape types (Table 1). Several studies have shown that adult and juvenile wood frogs orient toward areas with canopy cover and moist substrates upon leaving breeding ponds (deMaynadier and Hunter 1999, Vasconcelos and Calhoun 2004, Regosin et al. 2005, Baldwin et al. 2006). Coniferous trees dominate the study area and shrubs in the study area have small leaves. Consequently, relative to much of the area in the eastern portion of the wood frog’s geographic range, canopy cover in the KV is relatively sparse. In addition, wood frogs in the western U.S. are not as closely associated with forests as they are in the eastern U.S. (Stebbins 1966). Therefore, I suspect a lack of canopy cover has a smaller impact on costs of movement than moisture regime. Average 24-hr survival probabilities for wood frogs held in enclosures in a forested drainage (comparable to the ‘Tree-Wet’ landscape type) were approximately 0.55, while survival probabilities in enclosures on a forested ridge (comparable to the ‘Tree-Dry’ landscape type) and a clearcut area (comparable to the ‘Grass-Dry’ landscape type) were 0.15 and 0.06 (Rittenhouse et al. 2008). The survival probabilities indicate a relatively large decline between areas with moist and dry substrates, whereas the decline between the two areas with dry substrates but differences in canopy cover was much smaller. Therefore, I assigned a slightly higher cost of movement to the ‘Grass-Wet’ landscape type and made greater increases to the cost of movement through landscape types with a dry regime (Table 1). For the ‘Tree-Dry,’ ‘Shrub-Dry,’ and ‘Grass-Dry’

types, I increased the costs by 4, 6 and 8 cost units relative to the lowest cost landscape types (Table 1). I based these increases on the results from previous studies of movement in wood frogs, as well as the 24-hour survival probabilities from Rittenhouse et al. (2008) described above. For example, Vasconcelos and Calhoun (2004) used drift fences and pitfall traps to capture male, female and juvenile wood frogs as they migrated from a breeding wetland. In pitfall traps 150 m north of the breeding wetland, they caught 8.5, 3 and 22 times the number of males, females and juveniles than in pitfall traps 150 m south of the breeding wetland. The landscape between the breeding wetland and the pitfall traps to the north was a wetland with no canopy cover, while a dry meadow separated the pitfall traps to the south from the breeding wetland. For adults, these results suggest a cost of movement for the 'Grass-Dry' category 3 to 8.5 times higher than for the 'Grass-Wet' category.

Since data to infer movement costs for boreal chorus frogs is sparse, I primarily based relative costs of movement on the fact that they are smaller than wood frogs, and consequently, have a higher surface area to volume ratio. Therefore, I assigned a higher relative cost of movement through dry patch types (Table 1). Boreal chorus frogs also appear to be less closely associated with forests and other areas with canopy cover (Knutson et al. 1999, Trenham et al. 2003), so I did not distinguish between the cost of movement through the 'Tree-Wet' and 'Shrub-Wet' and 'Grass-Wet' landscape types as I did for wood frogs (Table 1).

To translate the movement costs assigned to the 6 landscape types to the 29 land cover types, I sampled the study area by locating 615 ground-truth plots of variable size (0.03 to 7.7 ha). Ground-truth plots were delineated by identifying relatively

homogeneous areas with respect to canopy cover and moisture regime and taking spatial coordinates around the boundary of the plot using a hand-held GPS unit (Garmin eTrex©). I concentrated plots in the 8 land cover classes that comprised nearly 95% of the study area. At each plot, the species of tree or shrub that provided the greatest cover was recorded. Regardless of whether canopy cover from trees or shrubs was present, I also recorded the species of understory plant that provided the greatest cover, as well as other common species in the patch. I assigned each plot to one of the six landscape types based on the data from these surveys. I assigned type of cover ('Tree', 'Shrub' or 'Grass') based on the plant species in the plot. Assignment of moisture regime was based on the 1998 National Wetlands Inventory (<http://www.fws.gov/nwi/plants.htm>). The wetlands inventory classifies plant species into one of five categories: i) obligate wetland (almost always occurs in wetlands), ii) facultative wetland (usually occurs in wetlands), iii) facultative (equally likely to occur in wetlands or non-wetlands), iv) facultative upland (usually occurs in non-wetlands) and v) obligate upland (almost always occurs in non-wetlands). I used this classification to determine if the dominant and common plant species in a plot were associated with wet (obligate or facultative wetland) or dry (obligate or facultative upland) areas. In plots with plant species of differing wetland classifications, I gave priority to the classification of the dominant species and used comments regarding the wetness of the substrate collected during ground-truth surveys.

After the data from the plots were collected, I mapped their locations using ArcGIS 9.2 (ESRI 1999-2006) and overlaid the map of the plots on the merged map. For each land cover class in the merged map, I compiled the data from the plots that were located

within the class. To assign costs of movement to each land cover class,  $LC$ , I calculated a weighted-average cost of movement as below:

$$LC = \sum_{i=1}^6 w_i c_i, \quad (\text{Equation 4})$$

where  $i$  indexes the 6 landscape types ('Tree-Wet', 'Shrub-Wet', ... etc.),  $w_i$  is the proportion of plots of landscape type  $i$  within the land cover class, and  $c_i$  is the estimated cost of movement through landscape type  $i$ . For example, 27 ground-truth plots were located within land cover class 1 (Table 2). Twenty-one of the plots were classified as the 'Grass-Dry' landscape type, which indicates a high cost of movement through the land cover class. Other plots, however, suggested a lower cost of movement through the class (e.g., three plots were classified as the 'Shrub-Wet' land cover class). Therefore, the weighted-average cost of movement is 7.63 (Table 2). Relative costs of movement must be integers. Consequently, all cells of land cover class 1 were assigned a relative cost of movement of 8 in the cost surface. Some land cover classes contained no ground-truth plots. The total area of these land cover classes represents less than 5% of the study area, and in many cases, they were not near sampled wetlands. I used descriptions of the vegetation communities in these land cover classes from Salas et al. (2005) to assign relative costs of movement.

### **Delineating buffers and estimating the amount of seasonal habitat**

I used the 'Cost Allocation' tool in ArcGIS 9.2 (ESRI 1999-2006) to delineate cost-based buffers around each wetland (Appendix 2 contains additional detail on the delineation of cost-based buffers). For each cell in a raster data set, the 'Cost Allocation' tool determines the minimum cost distance from a cell to the nearest source cell. In this

project, source cells represented the sampled wetlands. Starting with the outermost source cells for a wetland, the 'Cost Allocation' tool computes a minimum cost distance from those cells to the next set of adjacent cells (moving away from the wetland). It repeats this procedure for the next set of adjacent cells such that the minimum cost distance for each set of adjacent cells represents a cumulative minimum cost to the nearest source cells. In this way, minimum cost distances are calculated for each ring of cells growing away from the source cells. The algorithm can be stopped when a pre-defined maximum cost distance is reached. For example, when estimating the amount of seasonal habitat within 1000 cost meters of a sampled wetland for wood frogs, the algorithm stopped when the minimum cost distance to an adjacent cell exceeded 1000 cost meters from the nearest source cell. The set of cells within the pre-defined maximum cost distance comprise a cost-based buffer or allocation zone (Fig. 2; Theobald 2006).

After the buffer for each wetland was defined, I summed the amount of available wetland, streamside, and upland habitat within it. This step required that the land cover classes from the merged map be defined as one of the types of seasonal habitat or as non-habitat. For example, data from the ground-truth plots indicated that land cover classes 5, 13, 15, and 120 were wetlands (see Appendix 2 for a complete list of land cover classes and the seasonal habitat types to which they were assigned for wood frogs and boreal chorus frogs). Therefore, I defined them as wetland habitat. If the description of a land cover class and the data from the ground-truth plots were vague with respect to the assignment of seasonal habitat type, I defined the class as non-habitat.

### *Estimating distance to and number of neighboring occupied wetlands*

To estimate the minimum cost distance to the nearest occupied wetland and count the number of occupied wetlands within various cost distances, I used similar methods as described above for delineating cost buffers (Appendix 3 contains additional details). First, however, I created a data set that included the location of every wetland at which breeding by wood frogs was observed in any year from 2004 to 2006. To the breeding wetlands from the occupancy surveys, I added wetlands at which I detected breeding by wood frogs while conducting a genetic study of the population, as well as other breeding wetlands found while conducting fieldwork. Over the three years of the study, I detected breeding by wood frogs at 33 wetlands. I used the same approach to identify 47 wetlands at which breeding by boreal chorus frogs was detected in any year during the study.

As above, I delineated cost-based buffers around sampled wetlands using the 'Cost Allocation' tool. Since the process of dispersal occurs over larger spatial scales (Berven and Grudzien 1990, Semlitsch 2008), I created buffers using larger maximum cost distances than I used for modeling seasonal migration. For wood frogs, I used maximum cost distances of 1500, 3000 and 4500 cost meters. The latter two distances are consistent with the minimum and maximum (930 m and 1513 m straight-line distance) genetic neighborhood sizes estimated for wood frogs in Virginia (Berven and Grudzien 1990). Genetic neighborhood size is a measure of the spatial scale over which dispersal is sufficiently frequent to prevent local populations from genetically diverging. While low dispersal rates may prevent genetic divergence, higher rates may be required for local populations of wood frogs to impact one another demographically. The maximum cost distance of 1500 m, represents shorter Euclidean distances that may be required for

higher rates of dispersal to occur between wetlands. Juvenile wood frogs are the primary dispersers (Berven and Grudzien 1990) and demonstrate a preference for movement toward areas with canopy cover (2-5 juveniles moved toward closed canopy upon emigration from a breeding wetland for every juvenile that move toward open canopy; deMaynadier and Hunter 1999, Vasconcellos and Calhoun 2004, Patrick et al. 2006). Therefore, I developed a cost surface specifically for juvenile wood frogs. This cost surface differed from the surface used in modeling seasonal migration of adults by imposing a greater cost through landscape types without canopy cover and through dry landscape types (Tables 1). The results from Vasconcellos and Calhoun (2004) suggest that the cost of movement for juveniles across dry, open areas is approximately twice the cost experienced by adults. Juvenile wood frogs have a greater surface area to volume ratio and, therefore, will be more vulnerable to desiccation than adults (deMaynadier and Hunter 1999, Vasconcellos and Calhoun 2004). Their smaller body size may also make them vulnerable to a wider array of terrestrial predators (e.g., western terrestrial garter snakes, *Thamnophis elegans*).

I know of no studies that have estimated dispersal distances in boreal chorus frogs or any other *Pseudacris* species. Spencer (1964) assessed movement in boreal chorus frogs in northern Colorado, and found no juvenile moved farther than approximately 250 m from a breeding wetland (Spencer 1964), and one adult moved approximately 680 m. Therefore, I used maximum cost distances of 1000, 2000 and 3000 cost meters for dispersal in boreal chorus frogs. These maximum cost distances were equivalent to maximum straight-line distances of 210, 350, and 630 m, thus they cover the range of distances moved by boreal chorus frogs in the field study. However, the movements



measured by Spencer (1964) may represent seasonal migration and, consequently, may underestimate dispersal distances. As I did with adult boreal chorus frogs, I assigned the same cost of movement to the ‘Tree-Wet,’ ‘Shrub-Wet’ and ‘Grass-Wet’ landscape types (Table 1). Juvenile boreal chorus frogs are similar in size to juvenile wood frogs; therefore I assigned them the same costs through the ‘dry’ landscape types (Table 1). After creating cost-based buffers, I counted the number of occupied wetlands within each buffer. For each sampled wetland, I also computed the minimum cost distance to the nearest occupied wetland using the cost surfaces for juvenile wood frogs and boreal chorus frogs.

### ***Hypotheses - multiple seasons***

In addition to estimating occupancy and identifying correlations between occupancy and environmental covariates, I used the occupancy data from 2004 to 2006 and the multiple seasons model of MacKenzie et al. (2003) to examine the temporal dynamics in occupancy across time and estimate colonization and extinction probabilities at breeding wetlands. Given the short duration of the study (3 years), I chose to evaluate a small set of models with few parameters. I used two parameterizations of the multiple seasons model. The first parameterization includes a parameter for occupancy in the first year of the study ( $\psi_{2004}$ ), as well as parameters for colonization ( $\gamma_k$ ), extinction ( $\varepsilon_k$ ) and detection probabilities ( $p_{ijk}$ ). I used this model to evaluate the temporal dynamics in colonization and extinction probabilities across years. For both species, I assumed  $\psi_{2004}$  was constant across wetlands ( $\psi[.]$ ) and evaluated two hypotheses each for  $\gamma_k$  and  $\varepsilon_k$ : i) both extinction and colonization varied across years ( $\gamma[t]$  and  $\varepsilon[t]$ ), and ii) extinction and colonization did not vary across years ( $\gamma[.]$  and  $\varepsilon[.]$ ). I used the same structure on  $p_{ijk}$

from the top models from the single season analyses ( $p[.]$  for wood frogs and  $p[\text{SAMPTIME\_THRESH}]$  for boreal chorus frogs). The model denoted as  $p(.)$  represents the hypothesis that detection probability was constant across all sampling occasions and sampling seasons. I assumed the relationship between SAMPTIME and detection probability for boreal chorus frogs would be consistent across years (i.e., I did not evaluate an interaction between SAMPTIME and year).

The second parameterization includes parameters for occupancy for all three years of the study ( $\psi_{2004}$ ,  $\psi_{2005}$ , and  $\psi_{2006}$ ), as well as extinction ( $\varepsilon_k$ ) and detection probabilities ( $p_{ijk}$ ). I evaluated the following hypotheses of temporal variation in  $\psi_k$  across the three years of the study: i) occupancy is different across years ( $\psi[t]$ , where year is treated as a categorical variable), ii) occupancy is constant across years ( $\psi[.]$ ), and iii) occupancy is increasing or decreasing linearly across years ( $\psi[T]$ ). As above, I evaluated two models for extinction probability,  $\varepsilon[t]$  and  $\varepsilon[.]$  and used the top models of detection probability from the single season analyses.

### ***Modeling procedure, model selection and goodness-of-fit***

For the analysis using the single-season model, I evaluated the models in two steps. First, I paired each structure on detection probability,  $p_{ij}$ , with  $\psi[.]$ , the structure that represents the hypothesis that occupancy is invariant across wetlands. I used  $\Delta\text{AIC}$ -values,  $\Delta_i$ , and Akaike weights,  $w_i$ , to determine which model  $p_{ij}$  was best supported by the data (due to evidence of overdispersion in the data, I used QAIC in the analysis for boreal chorus frogs). Both  $\Delta_i$  and  $w_i$  quantify the strength of evidence in support of a particular model as the best model in the set of models being evaluated (Burnham and

Anderson 2002). With AIC or QAIC as the model selection criterion, the best model will use fewer parameters to provide good fit to the data relative to other models under consideration (Burnham and Anderson 2002). As  $\Delta_i$  increases, the strength of evidence for model  $i$  decreases. The Akaike weight,  $w_i$ , of a particular model can be interpreted as the probability that the model is the best model (of those models under consideration) given the sampling situation (Burnham and Anderson 2002). After selecting the best structure for  $p_{ij}$ , I paired each of the structures of  $\psi_i$  with the best structure for  $p_{ij}$  and used  $\Delta_i$  and  $w_i$  to determine their strength of evidence. I used a similar procedure in the analysis using the multiple-season model. I evaluated the models of  $p_{ijk}$  before evaluating the models of  $\psi_{ik}$ ,  $\gamma_k$ , and  $\varepsilon_k$ .

I conducted all analyses of the occupancy data in Program PRESENCE (Hines 2006). I rescaled most of the covariates to between zero and one to aid the numerical optimization algorithm in finding the correct parameter estimates. Rescaling was achieved by dividing all the measurements for a particular covariate using a denominator that was greater than the maximum measurement of the covariate.

While AIC is useful for determining the best model from a set of models, it does not assess the adequacy of the fit of models to the data (Burnham and Anderson 2002, MacKenzie and Bailey 2004). For the single season analyses, I used the goodness-of-fit (GOF) test in Program PRESENCE to assess the fit of the most highly parameterized model to the data. The GOF test in Program PRESENCE is based on the Pearson chi-square test statistic (MacKenzie and Bailey 2004) and compares the test statistic ( $\chi^2/\text{degrees of freedom}$ ) from the observed data to the distribution of test statistics from a collection of bootstrapped datasets. I used Program PRESENCE to generate 10,000

bootstrap datasets and compute the probability of observing a value of the test statistic greater than or equal to the test statistic from the observed data (i.e., the  $P$ -value; MacKenzie and Bailey 2004). If the GOF test indicated poor fit of the model to the data, I derived an estimate of the overdispersion parameter,  $\hat{c}$ , by dividing the test statistic from the field data by the average value of the test statistic across all bootstrapped datasets. A goodness-of-fit test is not currently available for the multiple seasons model. Therefore, I applied the results of the GOF test for the single season data to the multiple seasons analysis.

## **Results**

I did not detect northern leopard frogs of any life stage or evidence of breeding by boreal toads at any of the sampled wetlands during the study. However, an adult female boreal toad was detected at one wetland in 2005. I detected evidence of breeding by tiger salamanders at one wetland in 2004 and 2005 and two wetlands in 2006. Breeding by wood frogs was detected at six of 56 wetlands in 2004, nine of 56 wetlands in 2005 and 11 of 95 wetlands in 2006. Boreal chorus frogs were much more common in the KV and were detected at 11 of 56 wetlands in 2004, 19 of 56 in 2005 and 32 of 95 wetlands in 2006.

The results of the Mantel test for both species provided no evidence of positive spatial autocorrelation. Contrary to expectations, wetlands separated by greater geographic distance were more similar with respect to occupancy (negative spatial autocorrelation), though  $P$ -values were not significant ( $P$ -values = 0.19 [wood frogs] and 0.07 [boreal chorus frogs]). Results of the GOF test for the wood frog data suggested

adequate fit of the model ( $P = 0.96$ ). For the boreal chorus frog data, however, the GOF test indicated poor fit ( $P = 0.02$ ), and a  $\hat{c}$  of 2.76.

### *Evaluation of models and parameter estimates - single season*

#### **Wood frogs**

The model selection results indicated that the best structure for detection probability,  $p_{ij}$ , is  $p(\cdot)$ . While other models had  $\Delta_i < 2$ , regression coefficients for the covariates included in these models are imprecisely estimated, and their 95% confidence intervals strongly overlap 0. For example, the model in which detection probability is related to SAMPTIME using a threshold function ( $p(\text{SAMPTIME\_THRESH})$ ) had  $\Delta_i = 1.40$ . However, the estimate of the regression coefficient,  $\hat{\beta}_{\text{SAMPTIME\_THRESH}}$ , was -1.27 (95% CI from -4.52 to 1.98). Across the three sampling occasions in 2006, wood frogs were detected at 9, 8 and 10 wetlands, and they were detected on all three sampling occasions in 7 of the eleven wetlands at which they were observed. These results indicate a high detection probability that varies little between sampling occasions. The estimate of detection probability from the top model was 0.82 (95% CI from 0.64 to 0.92).

The model selection results provided strongest support for a positive relationship between occupancy at a wetland and the length of stream within 1000 cost meters of the wetland (seasonal migration scale; Table 4). The top two models included this covariate, and the combined  $w_i$  for the models was 0.70. The model selection results indicated nearly equivalent support for the linear and threshold form of the relationship (Table 4). The estimated regression coefficient for STREAM1000 from the linear model is 5.78 (95% CI from 2.01 to 9.55) and 2.27 (95% CI from 0.47 to 4.08) from the threshold model. Estimates of occupancy across wetlands,  $\hat{\psi}_i$ , from the top model range from 0.01

(95% CI from 0.002 to 0.08 ) to 0.74 (95% CI from 0.30 to 0.95), and estimates from the 2<sup>nd</sup>-ranked model are similar across much of the range of stream lengths (Fig. 3). The model selection results also provide support for a negative relationship between occupancy at a wetland and the cost distance to the nearest occupied wetland (dispersal scale; Table 4). The estimated regression coefficient from this model,  $\hat{\beta}_{NEAREST}$ , is -0.77 (95% CI from -1.40 to -0.13), which indicates that the probability a wetland is occupied by breeding wood frogs decreases as the cost distance to the nearest occupied wetland increases. Covariates at the scale of wetlands have the weakest support in the data. The best models that included covariates at this scale are  $\psi(\text{EMERGVEG } 1, 2=3=4)$  and  $\psi(\cdot)$ , though the  $\Delta_i$  of these models is  $> 10$  (Table 4). Selection of  $\psi(\text{EMERGVEG } 1, 2=3=4)$  was caused by the fact that wood frog breeding was never detected at wetlands with the least amount of cover from emergent vegetation (category 1).

### **Boreal chorus frogs**

The model selection results provide strong support for  $p(\text{SAMPTIME\_THRESH})$  as the best structure on  $p_{ij}$ , though  $p(\text{SAMPTIME\_LIN})$  also has support in the data (Table 5). Twenty-six of the top 30 models included the threshold form on the relationship between the length of a sampling occasion and the probability of detecting boreal chorus frogs. The estimated regression coefficient,  $\hat{\beta}_{\text{SAMPTIME\_THRESH}}$ , from the top model is 2.28 (95% CI from 1.11 to 3.45), therefore estimates of detection probability increased to a threshold as the length of surveys increased. Estimates of the regression coefficient were similar across all models, and estimates of  $p_{ij}$  from the top model range from 0.13 (95% CI from 0.04 to 0.35) to 0.96 (95% CI from 0.85 to 0.99).

High model selection uncertainty precludes strong inference regarding spatial variation in occupancy in boreal chorus frogs. Seven models have  $\Delta_i < 2$ , and more than 30 models have  $\Delta_i < 3$ . Models that represented hypotheses from all spatial scales are among these models (Table 5). However, only one of these models is ranked higher than the model of no spatial variability in occupancy,  $\psi(\cdot)$ . The top model suggests a positive linear relationship between the probability of occupancy at a wetland and the number of occupied wetlands within 1000 cost meters (dispersal scale; Table 5). The third-ranked model has the same structure on  $\psi_i$  and the estimates of the regression coefficient from these models are nearly identical ( $\hat{\beta}_{NUMOCCI1000\_LIN} = 5.12$  [95% CI from 1.10 to 9.14]). Estimates of  $\psi_i$  from the top model range from 0.27 to 0.74. The top models with covariates measured at the scale of seasonal migration, provided weak evidence of a positive relationship between occupancy and the amount of wetland vegetation adjacent to a wetland (Table 5). There was little to no support for covariates measured at the scale of wetlands.

### *Evaluation of models and parameter estimates - multiple seasons*

#### **Wood frogs**

Though the results suggest that colonization probability varied across years and extinction probability was constant, there is considerable model selection uncertainty. Therefore, I derived model-averaged estimates of  $\psi_k$ ,  $\gamma_k$ , and  $\varepsilon_k$ . The estimate of  $\gamma_k$  for 2004 is 0.09 (95% CI from 0.01 to 0.17) and 0.03 (95% CI from 0 to 0.08) for 2005. These estimates indicate that less than 10% of the unoccupied ponds in the KV were colonized by breeding wood frogs between sampling seasons. Estimates of  $\varepsilon_k$  were

higher and less variable across years ( $\hat{\epsilon}_{2004} = 0.32$  [95% CI: 0.02 – 0.61] and  $\hat{\epsilon}_{2005} = 0.35$  [95% CI: 0.09 – 0.60]) and indicate that over 30% of the breeding wetlands in a given year are unoccupied in the following year. The model selection results also provide weak evidence that occupancy in 2005 was higher than in 2004 and 2006, though confidence intervals on the model-averaged estimates strongly overlap (Fig. 4).

### **Boreal chorus frogs**

The model selection results suggest that extinction probability is constant across years and are equivocal on the temporal dynamics of colonization probability. Model-averaged estimates of colonization probability are higher than for wood frogs ( $\hat{\gamma}_{2004} = 0.17$  [95% CI: 0.07 – 0.28] and  $\hat{\gamma}_{2005} = 0.10$  [95% CI: 0 – 0.21]), and estimates of extinction probability are lower ( $\hat{\epsilon}_{2004} = 0.07$  [95% CI: 0 – 0.24] and  $\hat{\epsilon}_{2005} = 0.09$  [95% CI: 0 – 0.23]). Occupancy probabilities in boreal chorus frogs were more variable across the study period, and the model selection results provide weak evidence of an increasing, linear trend in occupancy (Figure 4).

### **Discussion**

Effective management of a population of plants or animals requires the formulation of goals, a set of potential management actions, reliable information with which to predict the impact of management actions on the population, and estimates of the state of the population (Nichols et al. 2007). Yet, this information often does not exist, most notably for populations of amphibians in national parks (Stohlgren et al. 1995). Even though it is the only location with historic observations of all 4 extant amphibian species in Rocky Mountain National Park (Corn et al. 1997), data on the historic and current



status of populations of these species in the Kawuneeche Valley (KV) are sparse. Several alterations have contributed to change in the KV over the last 50 to 100 years. The Grand Ditch and a large reduction in the abundance of beaver have changed hydrologic conditions in the KV and have likely fragmented the landscape for amphibians by replacing wetlands and areas of saturated soils with more xeric areas (e.g., dry meadows). Examination of soil cores suggests that, historically, the water table was near the surface of the ground, and the soil across much of the KV was waterlogged for extended periods of time each year (Westbrook et al. 2006). Though managers are concerned about the effects of these alterations on animal populations and other park resources (Ozaki 2002), a lack of information on historic and current status have prevented quantification of these effects. I estimated the current state of the populations of wood frogs and boreal chorus frogs in the KV using occupancy as the state variable, evaluated hypothesized relationships between occupancy and environmental attributes measured at multiple spatial scales, and estimated extinction and colonization probabilities.

Over the three years of the study, the proportion of wetlands occupied by breeding wood frogs was relatively constant at approximately 0.12. Reports of high breeding site fidelity in adult wood frogs (Berven and Grudzien 1990; but see Petranka et al. 2004) and the belief that movement in many amphibian species is constrained by their small body size and physiological requirement for moist conditions suggest that wood frogs will tend to breed in the same wetlands across years. Estimates from this study, however, indicate relatively high levels of turnover in the wetlands used for breeding. Of the wetlands that were occupied by breeding wood frogs, approximately 33% were not occupied the following year. The cessation of breeding at a wetland does not necessarily represent the

extinction of a local population. Petranka et al. (2004) observed cases of low site fidelity in wood frogs and hypothesized that individuals evaluate wetlands prior to ovipositing and avoid wetlands of low quality to future offspring. Therefore, estimates of extinction probability from occupancy studies of pond-breeding amphibians may be a combination of local population extinctions and movement of local populations to neighboring wetlands for breeding. While wood frogs abandoned some wetlands, they bred in 3% to 9% of wetlands not used for breeding in a previous year (i.e., colonizations). Hecnar and M'Closkey (1996) and Trenham et al. (2003) also reported unexpectedly high rates of turnover in the wetlands used by wood frogs for breeding across years.

Occupancy by breeding wood frogs was correlated with environmental covariates measured at multiple spatial scales, a pattern that has been observed in many amphibian populations and reflects the fact that multiple processes affect patterns of abundance and occupancy across a landscape (Compton et al. 2007). Mazerolle et al. (2005) found that occupancy patterns of the green frog (*R. clamitans*) in New Brunswick were correlated with measurements of vegetation structure taken from wetlands, as well as measurements taken over much larger areas (e.g., the percent of forest cover within 1000 m of a wetland). Knapp et al. (2003) reported a strong correlation between occupancy by larvae of the mountain yellow-legged frog (*R. muscosa*) and the depth of a pond or lake and also demonstrated a correlation between occupancy by larvae and the number of fishless bodies of water within 1 km of the wetland. At the scale of seasonal migration, occupancy by breeding wood frogs in the KV was positively related to the length of rivers and streams within 1000 cost meters of a wetland (Table 4). Support for the linear and threshold forms of the relationship was similar. On average, 1000 cost meters from a

wetland is equivalent to a maximum straight-line distance of approximately 350 meters. Rittenhouse and Semlitsch (2007a) reported that 95% of radio-tagged wood frogs from a study in Missouri remained within 300 m of a wetland after breeding. This spatial scale appears to be appropriate for the management of seasonal habitat types for populations of wood frogs in Colorado as well. After breeding, wood frogs migrate to moist, cool areas and spend the drier portions of the year (summer and early fall) in these areas (Vasconcelos et al. 2004, Baldwin et al. 2006, Patrick et al. 2006, Rittenhouse and Semlitsch 2007b). Streamside locations may provide wood frogs with these conditions (Baldwin et al. 2006) and, given the recent hydrologic alterations, may be one of a few reliable sources of these conditions in the KV. The results of the analysis also indicate that wetlands with lower cost distances to the nearest occupied wetland have higher probabilities of occupancy by wood frogs (Table 4). Dispersal among wetlands that are near one another may lead to higher occupancy as adjacent unoccupied wetlands are more likely to be colonized, populations at neighboring wetlands are more likely to be rescued, and the deleterious effects of inbreeding depression are less likely to occur (Green 2003, Trenham et al. 2003). Alterations to landscapes that reduce or eliminate dispersal between local populations and between occupied and unoccupied wetlands may threaten the persistence of amphibian populations. For example, Funk et al. (2005) reported high dispersal rates in a population of Columbia spotted frogs (*R. luteiventris*) in the mountains of Montana and argued that managing landscapes to maintain connectivity between local populations may be necessary to reduce extinction rates. A concurrent study of the genetic structure of the wood frogs in the KV detected significant genetic differentiation between wood frogs in the northern and southern part of the valley

(Scherer, *unpublished data*). This result supports the hypothesis that alterations to the valley have reduced the degree of connectivity between local populations of wood frogs and may be an early warning of a threat to the regional population's long-term persistence.

Previous studies of the spatial distribution and patterns of abundance in wood frogs have reported a strong influence of covariates measured at the scale of individual wetlands (e.g., hydroperiod; Knutson et al 1999, Hermann et al. 2005, Cunningham et al. 2007). Although models that included covariates measured at this scale were not included among the best models in this study, estimates from models that included those covariates were consistent with predictions and indicate that wood frogs breed in ponds with moderate to high cover from emergent vegetation and short to moderate hydroperiod.

Relative to wood frogs, boreal chorus frogs abandoned a small proportion (~ 8%) of wetlands between years and colonized unoccupied wetlands at a high rate (10% – 17%). Consequently, estimates of the proportion of wetlands occupied by breeding boreal chorus frogs increased from 2004 to 2006 (from 0.20 to 0.37; Fig. 4). This increase in occupancy occurred approximately 3 years after a severe drought and is consistent with patterns in occupancy for western chorus frogs (*P. triseriata*) reported by Werner et al. (2009). Colorado experienced one of the driest years on record in 2002, and unusually warm temperatures in April and May caused the snowpack to melt earlier in the year than usual. With only a short “meltout” period and a relatively long “dry” period, more wetlands in the KV dried completely, and they dried earlier. While these conditions were likely detrimental to the population of boreal chorus frogs, they also had negative impacts on the aquatic predators that prey on eggs and larvae. Werner et al. (2009) provided

evidence that the spatial distribution of populations of western chorus frogs in Michigan was constrained by predators. Three years after a severe drought in their study area, they observed increases in the density and spatial distribution of western chorus frogs. They argued that the drought caused a reduction in the density and spatial distribution of aquatic predator populations, which led to an increase in western chorus frogs. The current increases in boreal chorus frogs in the KV may have a similar cause.

The top model of occupancy indicated a positive relationship between the probability of occupancy at a wetland and the number of occupied wetlands within 1000 cost meters (Table 5). Though models that included other covariates had similar support from the data, the 95% confidence intervals for the regression coefficients associated with those covariates were wide and overlapped zero. On average, 1000 cost meters was equivalent to a maximum straight-line distance of 210 m, which is consistent with the spatial scale over which dispersal affected occupancy patterns of western chorus frogs in Michigan (Werner et al. 2009). It is important to note that previous research on movement and habitat use in boreal chorus frogs is sparse, particularly when compared to the abundance of information on wood frogs. Consequently, I had less information on which to base hypothesized relationships between occupancy and environmental covariates and to parameterize cost surfaces. In addition, wood frog breeding sites in the KV were better known and this information was used to estimate the number of occupied wetlands within the various cost buffers. This relative dearth of information probably contributed to the lack of strong inferences from the evaluation of hypothesized relationships between occupancy by breeding boreal chorus frogs and environmental covariates.

### *Management implications*

This study has provided managers with estimates of wetland occupancy by breeding wood frogs and boreal chorus frogs over three years in the KV. Currently, active management of amphibians in Rocky Mountain National Park is concentrated on boreal toads and was prompted by reports of declines in populations of toads in the park and across the southern Rocky Mountains (Carey 1993, Muths et al. 2003). Future management of other populations of amphibian species in the park should begin with the specification of goals and identification of potential management actions to achieve those goals. Once those steps are complete, managers and scientists should work together to implement management actions in such a way that progress toward goals can be evaluated and knowledge of the system is advanced (Williams et al. 2007).

Without historical context, the estimates of occupancy for wood frogs and boreal chorus frogs are difficult to interpret. Though I found no evidence that occupancy by wood frogs declined from 2004 to 2006, they occupy a small proportion of wetlands in the KV and the number of individuals breeding at wetlands appears to be small. In 21 wetlands with observations of breeding by wood frogs in 2006, six or fewer egg masses were detected in 13 of the wetlands, and the highest count of egg masses was 35. Over the three years of the study, the vast majority of counts of wood frog egg masses indicate the number breeding adult wood frogs at wetlands is small. Wood frog egg masses are highly conspicuous; therefore it is unlikely that low detectability is the cause of the small counts (Grant et al. 2005, Scherer 2008). Boreal chorus frogs, on the other hand, occupy a higher proportion of wetlands, and their spatial distribution appears to be expanding. Breeding choruses at several wetlands indicate large adult populations.

Previous studies have highlighted the importance of terrestrial habitats in the management of populations of pond-breeding amphibians (e.g., Semlitsch and Bodie 2003, Harper et al. 2008). This study provided evidence of the importance of considering the terrestrial environment in management plans for wood frogs and boreal chorus frogs in the KV. Occupancy by breeding wood frogs was higher in wetlands that were near streams, and occupancy in both species was higher in wetlands that were relatively near neighboring wetlands with breeding conspecifics. In addition to habitat manipulations at individual wetlands, therefore, managers should consider actions at broader spatial scales. Given the relatively limited spatial distribution of wood frogs, maintaining and enhancing connectivity between wetlands, as well as between wetlands and other seasonal habitat types in the KV, may be necessary for their long-term persistence. Management actions that restore historic hydrologic processes in the KV (e.g., enhancement of the beaver population, construction of artificial dams on rivers and streams, changes in the operation of the Grand Ditch) may be particularly beneficial.

### **Literature cited**

- Andelt, W.F., G.C. White, P.M. Schnurr, and K.W. Navo. 2009. Occupancy of random plots by white-tailed and Gunnison's prairie dogs. *Journal of Wildlife Management* 73:35-44.
- Bagdonas, K.R. 1968. Variation in Rocky Mountain wood frogs. M.S. thesis. Colorado State University. Fort Collins, Colorado, U.S.A.

- Baker, C., R. Lawrence, C. Montagne, and D. Patten. 2006. Mapping wetlands and riparian areas using Landsat ETM+ imagery and decision-tree-based models. *Wetlands* 26:465-474.
- Baldwin, R.F., A.J.K. Calhoun, and P.G. deMaynadier. 2006. Conservation planning for amphibian species with complex habitat requirements: a case study using movements and habitat selection of the wood frog *Rana sylvatica*. *Journal of Herpetology* 40:443-454.
- Berven, K.A., and T.A. Grudzien. 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. *Evolution* 44:2047-2056.
- Bonnet, E., and Y. Van de Peer. 2002. zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software* 7:2-12.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. 2nd edition. Springer-Verlag. New York, New York, U.S.A.
- Carey, C., 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. *Conservation Biology* 7: 355–362.
- Compton, B.W., K. McGarigal, S.A. Cushman, and L.R. Gamble. 2007. A resistant-kernel model of connectivity for amphibians that breed in vernal pools. *Conservation Biology* 21:788-799.
- Corn, P.S., L.J. Livo. 1989. Leopard frog and wood frog reproduction in Colorado and Wyoming. *Northwestern Naturalist* 70:1-9.
- Corn, P.S., M.L. Jennings, and E. Muths. 1997. Survey and assessment of amphibian populations in Rocky Mountain National Park. *Northwestern Naturalist* 78:34-55.



- Cunningham, J.M., A.J.K. Calhoun, and W.E. Glanz. 2007. Pond-breeding amphibian species richness and habitat selection in a beaver-modified landscape. *Journal of Wildlife Management* 71:2517-2526.
- deMaynadier, P.G., and M.L. Hunter, Jr. 1999. Forest canopy closure and juvenile emigration by pool-breeding amphibians in Maine. *Journal of Wildlife Management* 63:441-450.
- Doesken, N.J., R.A. Pielke, Sr, and O.A.P. Bliss. 2003. *Climate of Colorado. Climatology of the United States #60.* Colorado Climate Center. Colorado State University. Fort Collins, Colorado, U.S.A.
- Egan, R.S., and P.W.C. Paton. 2004. Within-pond parameters affecting oviposition by wood frogs and spotted salamanders. *Wetlands* 24:1-13.
- Fortin, M.-J., and M. Dale. 2005. *Spatial analysis: a guide for ecologists.* Cambridge University Press. Cambridge, U.K..
- Franklin, A.B., D.R. Anderson, R.J. Gutierrez, and K.P. Burnham. 2000. Climate, habitat quality, and fitness in northern spotted owl populations in northwestern California. *Ecological Monographs* 70:539-590.
- Funk, W.C., A.E. Greene, P.S. Corn, and F.W. Allendorf. 2005. High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biology Letters* 1:13-16.
- Gamble, L.R., K. McGarigal, and B.W. Comptom. 2007. Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: implications for spatio-temporal population dynamics and conservation. *Biological Conservation* 139:247-257.

- Grant, E. H. C., R. E. Jung, J. D. Nichols, and J. E. Hines. 2005. Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. *Wetlands Ecology and Management* 13:305–320.
- Green, D.M. 2003. The ecology of extinction: population fluctuation and decline in amphibians. *Biological Conservation* 111:331-343.
- Gu, W., and R.K. Swihart. 2004. Absent or undetected? Effects of non-detection of species occurrence on wildlife-habitat models. *Biological Conservation* 116:195-203.
- Hammerson, G.A. 1999. *Amphibians and reptiles in Colorado*. University Press of Colorado. Niwot, Colorado, U.S.A.
- Harper, E.B., T.A.G. Rittenhouse, and R.D. Semlitsch. 2008. Demographic consequences of terrestrial habitat loss for pool-breeding amphibians: predicting extinction risks associated with inadequate size of buffer zones. *Conservation Biology* 22:1205-1215.
- Hecnar, S.J., and R.T. M'Closkey. 1996. Regional dynamics and the status of amphibians. *Ecology* 77:2091-2097.
- Herrmann, H.L., K.J. Babbitt, M.J. Baber, and R.G. Congalton. 2005. Effects of landscape characteristics on amphibian distribution in a forest-dominated landscape. *Biological Conservation* 123:139-149.
- Hines, J.E. 2006. PRESENCE2 – software to estimate patch occupancy and related parameters. USGS-PWRC. <http://www.mbr-pwrc.usgs.gov/software/presence.html>.
- Howard, R.D. 1980. Mating behaviour and mating success in woodfrogs, *Rana sylvatica*. *Animal Behaviour* 28:705-716.

- Joly, P., C. Miaud, A. Lehmann, and O. Grolet. 2001. Habitat matrix effects on pond occupancy in newts. *Conservation Biology* 15:239-248.
- Knapp, R.A., K.R. Matthews, H.K. Preisler, and R. Jellison. 2003. Developing probabilistic models to predict amphibian site occupancy in a patchy landscape. *Ecological Applications* 13:1069-1082.
- Knutson, M.G., J.R. Sauer, D.A. Olsen, M.J. Mossman, L.M. Hemesath, and M.J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, U.S.A. *Conservation Biology* 13:1437-1446.
- MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-2255.
- MacKenzie, D.I., J.D. Nichols, J.E. Hines, M.G. Knutson, and A.B. Franklin. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology* 84:2200-2207.
- MacKenzie, D.I., J.A. Royle, J.A. Brown, and J.D. Nichols. 2004. Occupancy estimation and modeling for rare and elusive populations. Pages 149-172 *In* W.L. Thompson (ed.). *Sampling rare or elusive species*. Island Press, Washington, D.C., U.S.A.
- MacKenzie, D.I., and L.L. Bailey. 2004. Assessing the fit of site-occupancy models. *Journal of Agricultural, Biological and Environmental Statistics* 9:300-318.
- MacKenzie, D.I., and J.A. Royle. 2005. Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology* 42:1105-1114.

- Mackenzie, D.I., J.D. Nichols, J.A. Royle, K.H. Pollock, L.L. Bailey, and J.E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press. Burlington, Massachusetts, U.S.A.
- Marra, P.P., D.R. Norris, S.M. Haig, M. Webster, and J.A. Royle. 2006. Migratory connectivity. Pages 157-183 *In* K.R. Crooks and M. Sanjayan (eds.). Connectivity Conservation. Cambridge University Press. Cambridge, U.K.
- Matthews, T., and D. Pettus. 1966. Color inheritance in *Pseudacris triseriata*. *Herpetologica* 22:269-275.
- Mazerolle, M.J., A. Desrochers, and L. Rochefort. 2005. Landscape characteristics influence pond occupancy by frogs after accounting for detectability. *Ecological Applications* 15:824-834.
- McDonald, L.L. 2004. Sampling rare populations. Pages 11-42 *In* W.L. Thompson (ed.). Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters. Island Press. Washington, D.C., U.S.A.
- Muths, E., P.S. Corn, A.P. Pessier, and D.E. Green. 2003. Evidence for disease-related amphibian decline in Colorado. *Biological Conservation* 110:357-365.
- National Research Council. 1992. Science and national parks. National Academy Press. Washington, D.C., U.S.A.
- Nichols, J.D., M.C. Runge, F.A. Johnson, and B.K. Williams. 2007. Adaptive harvest management of North American waterfowl populations: a brief history and future prospects. *Journal of Ornithology* 148 (Supplement 2):S343-S349.
- Ozaki, V. 2002. Geoindicators scoping report for Rocky Mountain National Park. GPRA Goal IB4. Data Set Report. Rocky Mountain National Park, Colorado, U.S.A.

- Patrick, D.A., M.L. Hunter, Jr., and A.J.K. Calhoun. 2006. Effects of experimental forestry treatments on a Maine amphibian community. *Forest Ecology and Management* 234:323-332.
- Penman, T.D., D.L. Binns, and R.P. Kavanagh. 2009. Patch-occupancy modeling as a method for monitoring changes in forest floristics. *Conservation Biology* 23:740-749.
- Petranka, J.W., C.K. Smith, and A.F. Scott. 2004. Identifying the minimal demographic unit for monitoring pond-breeding amphibians. *Ecological Applications* 14:1065-1078.
- Pope, S.E., L. Fahrig, and H.G. Merriam. 2000. Landscape complementation and metapopulation effects on leopard frog populations. *Ecology* 81:2498-2508.
- Ray, N., A. Lehmann, and P. Joly. 2002. Modeling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. *Biodiversity and Conservation* 11:2143-2165.
- Regosin, J.V., B.S. Windmiller, and J.M. Reed. 2003. Terrestrial habitat use and winter densities of the wood frog (*Rana sylvatica*). *Journal of Herpetology* 37:390-394.
- Regosin, J.V., B.S. Windmiller, R.N. Homan, and J.M. Reed. 2005. Variation in terrestrial habitat use by four pool-breeding amphibian species. *Journal of Wildlife Management* 69:1481-1493.
- Rittenhouse, T.A.G., and R.D. Semlitsch. 2007a. Distribution of amphibians in terrestrial habitat surrounding wetlands. *Wetlands* 27:153-161.
- Rittenhouse, T.A.G., and R.D. Semlitsch. 2007b. Postbreeding habitat use of wood frogs in a Missouri oak-hickory forest. *Journal of Herpetology* 41:645-653.

- Rittenhouse, T.A.G., E.B. Harper, L.R. Rehard, and R.D. Semlitsch. 2008. The role of microhabitats in the dessication and survival of anurans in recently harvested oak-hickory forest. *Copeia* 2008:807-814.
- Rothermel, B.B., and R.D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. *Conservation Biology* 16:1324-1332.
- Roznik, E.A., and S.A. Johnson. 2009. Canopy closure and emigration by juvenile gopher frogs. *Journal of Wildlife Management* 73:260-268.
- Salas, D., J. Stevens, and K. Schulz. 2005. Rocky Mountain National Park, Colorado: 2001-2005 vegetation classification and mapping. Technical Memorandum 8260-05-02. U.S. Bureau of Reclamation. Denver, Colorado, U.S.A.
- Scherer, R.D. 2008. Detection of wood frog egg masses and implications for monitoring amphibian populations. *Copeia* 2008:669-672.
- Semlitsch, R.D. 2008. Differentiating migration and dispersal processes for pond-breeding amphibians. *Journal of Wildlife Management* 72:260-267.
- Semlitsch, R.D., and J.R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology* 17:1219-1228.
- Sinclair, A.R.E., J.M. Fryxwell, and G. Caughley. 2006. *Wildlife ecology, conservation and management*. 2<sup>nd</sup> edition. Blackwell Publishing. Malden, Massachusetts, U.S.A.
- Sinsch, U. 1997. Postmetamorphic dispersal and recruitment of first breeders in a *Bufo calamita* metapopulation. *Oecologia* 112:42-47.

- Sjorgen Gulve, P. 1994. Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. *Ecology* 75:1357-1367.
- Skelly, D.K., E.E. Werner, and S.A. Cortwright. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. *Ecology* 80:2326-2337.
- Smith, M.A., and D.M. Green. 2006. Sex, isolation and fidelity: unbiased long-distance dispersal in a terrestrial amphibian. *Ecography* 29:649-658.
- Spencer, A.W. 1964. Movements of boreal chorus frogs. Ph.D. dissertation. Colorado State University. Fort Collins, Colorado, U.S.A.
- Stebbins, R.C. 1966. A field guide to western reptiles and amphibians. Houghton Mifflin Company. Boston, Massachusetts, U.S.A.
- Stevens, C.E., C.A. Paszkowski, G. Scrimgeour. 2006. Older is better: beaver ponds on boreal streams as breeding habitat for wood frogs. *Journal of Wildlife Management* 70:1360-1371.
- Stohlgren, T.J., J.F. Quinn, M. Ruggiero, and G.S. Waggoner. 1995. Status of biotic inventories in U.S. national Parks. *Biological Conservation* 71:97-106.
- Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783-1786.
- Theobald, D.M. 2005. GIS concepts and ArcGIS methods. 2<sup>nd</sup> edition. Conservation Planning Technologies. Fort Collins, Colorado, U.S.A.
- Theobald, D.M. 2006. Exploring the functional connectivity of landscapes using landscape networks. Pages 416-443 *In* K.R. Crooks and M. Sanjayan (eds.). *Connectivity Conservation*. Cambridge University Press, Cambridge, U.K.

- Tipton, H.C., V.J. Dreitz, and P.F. Doherty, Jr. 2008. Occupancy of mountain plover and burrowing owl in Colorado. *Journal of Wildlife Management* 72:1001-1006.
- Trenham, P.C., W.D. Koenig, M.J. Mossman, S.L. Stark, and L.A. Jagger. 2003. Regional dynamics of wetland-breeding frogs: turnover and synchrony. *Ecological Applications* 13:1522-1532.
- Vasconcelos, D., and A.J.K. Calhoun. 2004. Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. *Journal of Herpetology* 38:551-561.
- Vos, C.C., and A.H.P. Stumpel. 1995. Comparison of habitat isolation parameters in relation to fragmented distribution patterns in the tree frog (*Hyla arborea*). *Landscape Ecology* 11:203-214.
- Vos, C.C., and J.P. Chardon. 1998. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *Journal of Applied Ecology* 35:44-56.
- Welch, N.E., and J.A. MacMahon. 2005. Identifying habitat variables important to the rare Columbia spotted frog in Utah (U.S.A.): an information-theoretic approach. *Conservation Biology* 19:473-481.
- Werner, E.E., and K.S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia* 1999:1-12.
- Werner, E.E., R.A. Relyea, K.L. Yurewicz, D.K. Skelly, and C.J. Davis. 2009. Comparative landscape dynamics of two anuran species: climate-driven interaction of local and regional processes. *Ecological Monographs* 79:503-521.



- Westbrook, C.J., D.J. Cooper, and B.W. Baker. 2006. Beaver dams and overbank floods influence groundwater-surface water interactions of a Rocky Mountain riparian area. *Water Resources Research* 42. doi:10.1029/2005WR004560.
- Williams, B.K., J.D. Nichols, and M.J. Conroy. 2002. Analysis and management of animal populations. Academic Press. San Diego, California, U.S.A.
- Williams, B.K., R.C. Szaro, and C.D. Shapiro. 2007. Adaptive management: the U.S. Department of the Interior technical guide. Adaptive Management Working Group. U.S. Department of the Interior. Washington, D.C., U.S.A.
- Woods, S.W. 2000. Hydrologic effects of the Grand Ditch on streams and wetlands in Rocky Mountain National Park, Colorado. M.S. Thesis. Colorado State University. Fort Collins, Colorado, U.S.A.
- Woods, S.W. 2001. Ecohydrology of subalpine wetlands in the Kawuneeche Valley, Rocky Mountain National Park, Colorado. Ph.D. dissertation. Colorado State University. Fort Collins, Colorado, U.S.A.
- Zanini, F., A. Klingemann, R. Schlaepfer, and B.R. Schmidt. 2008. Landscape effects on anuran pond occupancy in an agricultural countryside: barrier-based buffers predict distributions better than circular buffers. *Canadian Journal of Zoology* 86:692-699.

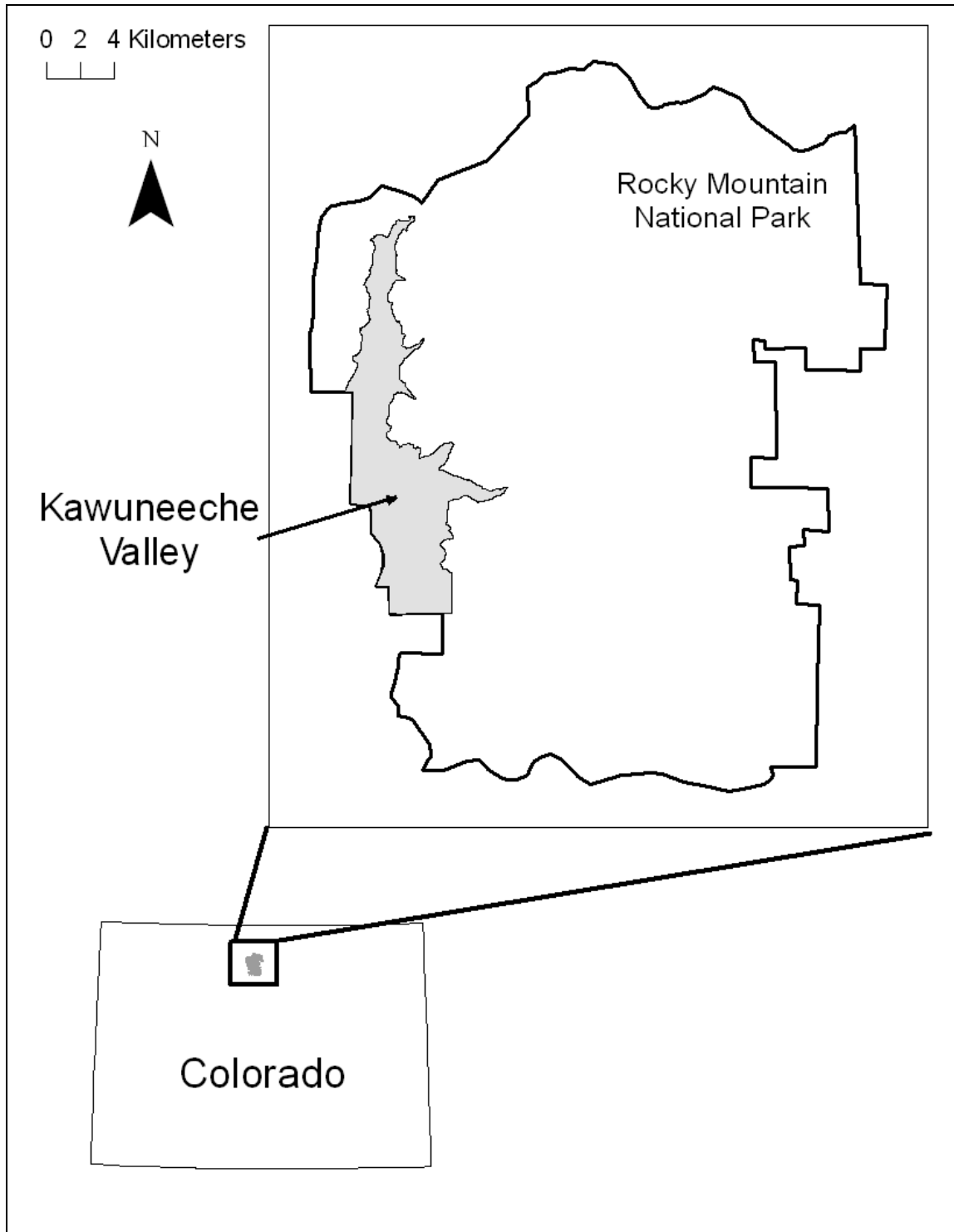


Figure 1: Map of the study area, the Kawuneeche Valley, in Rocky Mountain National Park, Colorado.

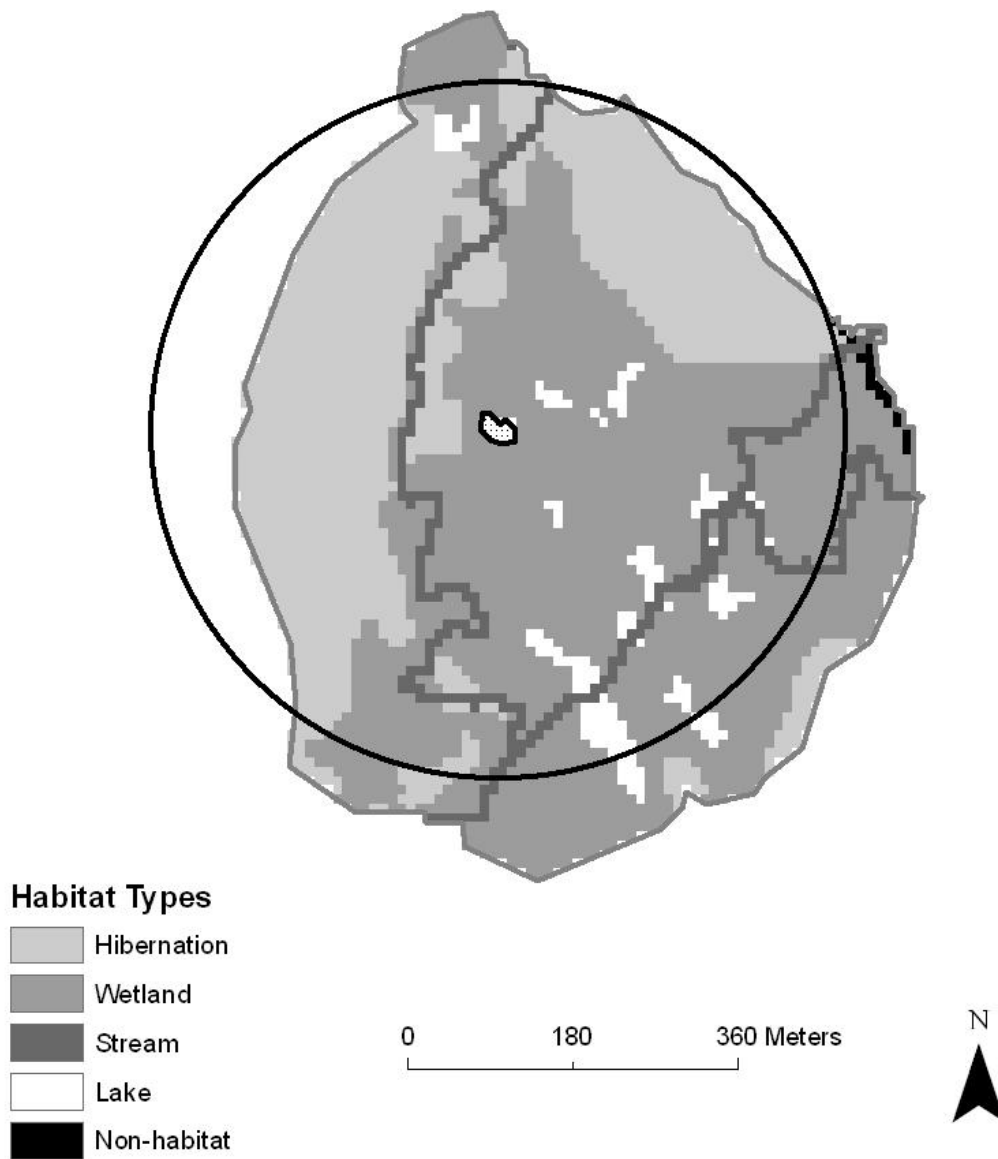


Figure 2: Circular and cost-based buffers for estimating the amount of seasonal habitat types adjacent to breeding wetlands. The small, stippled area with a black outline in the center of the figure is wetland 5048. The black circle represents a circular buffer around the wetland, and the gray line delineates a cost-based buffer. Within the buffer, cells of different color represent different habitat types or areas of non-habitat.

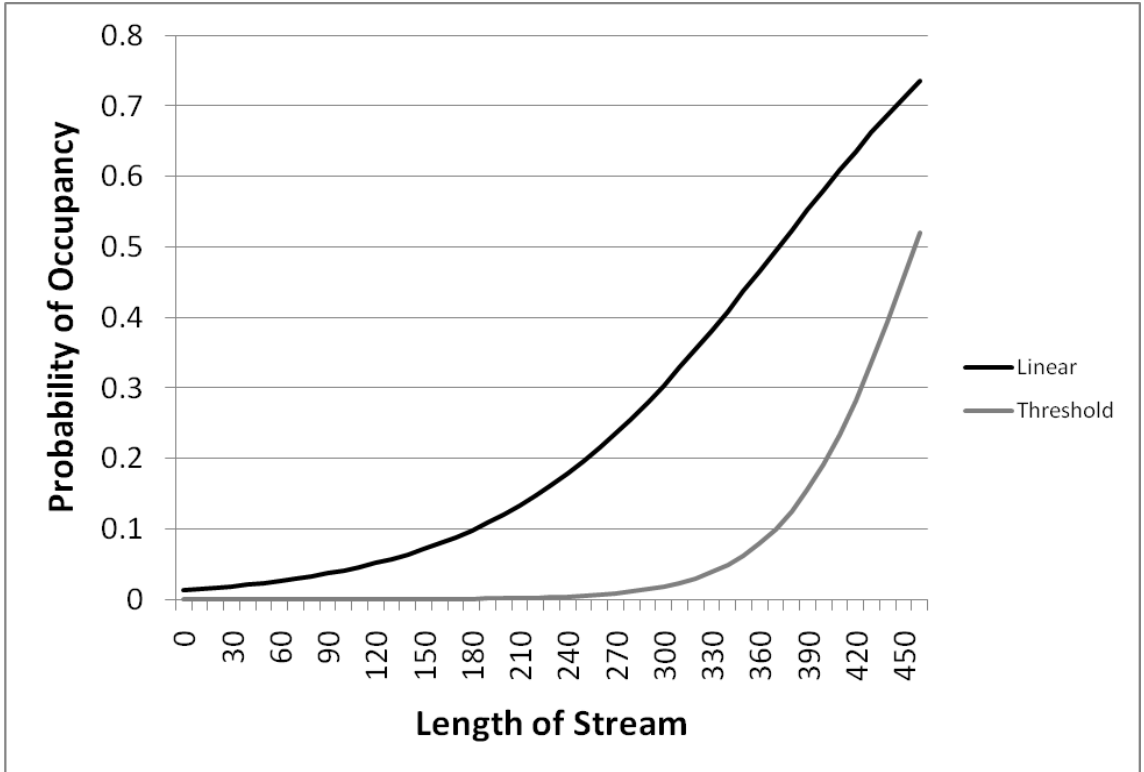


Figure 3: The relationship between the probability of occupancy by breeding wood frogs and the length of stream within 1000 cost meters of a wetland from the top two models. The highest ranked model is (p[.],  $\psi$ [STREAM1000\_LIN]; black line) and the 2<sup>nd</sup>-ranked model is (p[.],  $\psi$ [STREAM1000\_THRESH]; gray line).

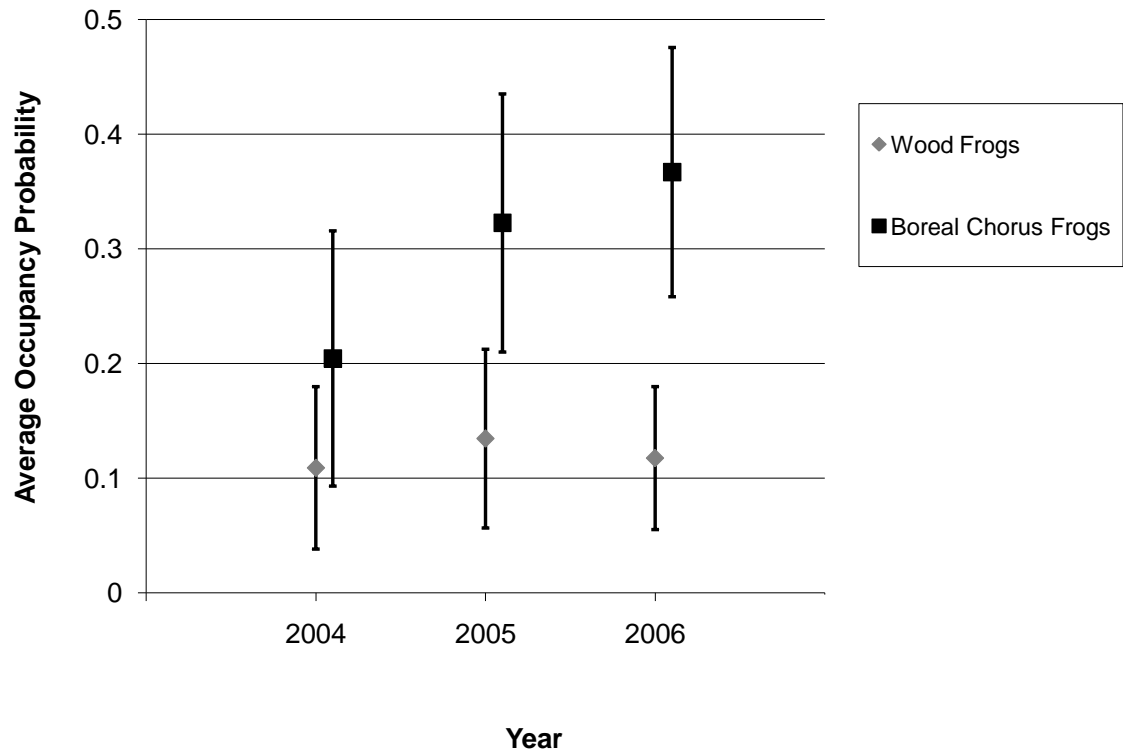


Figure 4: Model-averaged estimates of occupancy probability for wood frogs (gray diamonds) and boreal chorus frogs (black squares) from 2004 to 2006 in the study area.

Error bars represent 95% confidence intervals.

Table 1: Costs of movement for wood frogs, *Rana sylvatica*, and boreal chorus frogs, *Pseudacris maculata*, adults and juveniles (in parentheses) through each of 6 landscape types.

Landscape Type	Cost – Wood Frog	Cost – Chorus Frog
Tree-Wet	1 (1)	1 (1)
Shrub-Wet	1 (1)	1 (1)
Grass-Wet	2 (3)	1 (1)
Tree-Dry	5 (10)	7 (10)
Shrub-Dry	7 (14)	9 (14)
Grass-Dry	9 (18)	11 (18)

Table 2: An illustration of the approach used to assign relative costs of movement to land cover classes using data from ground-truth plots. The table shows the number and proportion of ground-truth plots of each landscape type that were located within land cover class 1. Equation 4 was used to calculate the weighted-average cost of movement, *LC*. Costs of movement are for wood frogs.

Landscape Type	Number of Plots	Proportion of Plots	Cost of Movement
Tree-Wet	0	0.00	1
Tree-Dry	1	0.04	5
Shrub-Wet	3	0.11	1
Shrub-Dry	1	0.04	7
Grass-Wet	1	0.04	2
Grass-Dry	21	0.78	9
Weighted-average Cost of Movement, <i>LC</i>			7.63

Table 3: Weighted-average costs of movement (Equation 4) for wood frogs, *Rana sylvatica*, and boreal chorus frogs, *Pseudacris maculata*, adults and juveniles (in parentheses) through 7 of the 26 land cover classes in the study area. These land cover classes were selected to show a range of costs (see Appendix 1 for the full list of land cover classes with movement costs). Classes 22 and 23 are the most common land cover classes and comprise over 80% of the study area.

Land Cover Class	Brief Class Description	Cost - Wood Frog	Cost - Chorus Frog
5	Herbaceous wetland	3 (6)	3 (5)
13	Shrub upland	1 (2)	1 (1)
22	Sub-alpine mixed conifer	4 (8)	6 (8)
23	Lodgepole pine	4 (9)	6 (9)
52	Lakes and reservoirs	Barrier	Barrier
501	Small streams	4 (6)	4 (6)
502	Rivers and large streams	8 (14)	10 (14)



Table 4: Model selection results for analysis of the occupancy data for wood frogs, *Rana sylvatica*. Models are arranged according to the spatial scale at which environmental covariates were measured (wetland, seasonal migration, and dispersal). All models with  $\Delta_i < 6$  are shown, as well as the top models at each spatial scale. For all models in the table, the structure on detection probability is  $p(\cdot)$ . The column labeled ‘ $k$ ’ contains the number of parameters in each model and the column labeled ‘ $-2(\ln(\mathcal{L}))$ ’ is -2 times the value of the log likelihood function at its maximum. ‘\*’ denotes the top model.

Spatial Scale	Model	AIC	$\Delta_i$	$w_i$	$k$	$-2(\ln(\mathcal{L}))$
Wetland	$\psi(\text{EMERGVEG } 1, 2=3=4)$	103.2	10.2	< 0.01	3	97.2
	$\psi(\cdot)$	103.3	10.3	< 0.01	2	99.3
Migration	$\psi(\text{STREAM1000\_LIN})^*$	93.0	0.0	0.41	3	87.0
	$\psi(\text{STREAM1000\_THRESH})$	93.7	0.7	0.29	3	87.7
	$\psi(\text{WETVEG400\_LIN})$	97.8	4.8	0.04	3	91.8
Dispersal	$\psi(\text{NEAREST\_THRESH})$	96.9	3.9	0.06	3	90.9
	$\psi(\text{NUMOCC3000\_THRESH})$	98.4	5.4	0.03	3	92.4
	$\psi(\text{NUMOCC4500\_THRESH})$	98.8	5.8	0.02	3	92.8

Table 5: Model selection results for analysis of the occupancy data for boreal chorus frogs, *Pseudacris maculata*. Models are arranged according to the spatial scale at which environmental covariates were measured (wetland, seasonal migration, and dispersal). All models with  $\Delta_i < 2$  are shown, as well as the top models at each spatial scale. The column labeled ‘ $k$ ’ contains the number of parameters in each model and the column labeled ‘ $-2(\ln(\mathcal{L}))$ ’ is  $-2$  times the value of the log likelihood function at its maximum. ‘\*’ denotes the top model

Spatial Scale	Model	QAIC	$\Delta_i$	$w_i$	$k$	$-2(\ln(\mathcal{L}))$
Wetland	$\psi(\cdot),$ $p(\text{SAMPTIME\_THRESH})$	87.2	0.7	0.04	3	224.1
	$\psi(\cdot),$ $p(\text{SAMPTIME\_THRESH})$	88.3	1.8	0.02	3	227.04
Migration	$\psi(\text{WETVEG400\_LIN}),$ $p(\text{SAMPTIME\_THRESH})$	88.5	2.0	0.02	4	222.2
	$\psi(\text{WETVEG800\_LIN}),$ $p(\text{SAMPTIME\_THRESH})$	88.6	2.1	0.02	4	222.3
Dispersal	$\psi(\text{NUMOCC1000\_LIN}),$ $p(\text{SAMPTIME\_THRESH})^*$	86.5	0.0	0.06	4	216.7
	$\psi(\text{NUMOCC1000\_LIN}),$ $p(\text{SAMPTIME\_LIN})$	87.6	1.1	0.03	4	219.7

	$\psi(\text{NUMOCC1000\_THRESH}),$ $p(\text{SAMPTIME\_THRESH})$	87.9	1.4	0.03	4	220.4
	$\psi(\text{NUMOCC2000\_LIN}),$ $p(\text{SAMPTIME\_THRESH})$	87.9	1.4	0.03	4	220.6

Appendix 1: A detailed description of the processes used to develop spatial data and a cost surface for modeling occupancy in wood frogs (*Rana sylvatica*) and boreal chorus frogs (*Pseudacris maculata*). All procedures were conducted in ArcGIS 9.2 (ESRI 1999-2006).

- 1) I started with a polygon shapefile of the study area, 'study\_area\_boundary.' The study area was defined by the RMNP boundary on the south side and southern half on the west side. A contour line at 3,048 m in elevation defined the boundary everywhere else, except near the southeast corner. The straight line at the southeast corner of the study area excludes Summerland Park, which could not be included due to constraints in resources.
- 2) I created a buffer around the study area. The purpose of this step was to create a polygon shapefile that could be used to clip datasets that covered larger areas. I chose a buffer of 3,000 meters and named the shapefile 'study\_area\_buffer.'
- 3) I clipped the land cover data of Salas et al. (2005) using 'study\_area\_buffer.' They partitioned the study area into 44 land cover classes. I named the new dataset 'kv\_buffer\_veg.'
- 4) I clipped a dataset of streams using 'study\_area\_buffer' and named the resulting shapefile 'kv\_buffer\_strm.'
  - a. I also added a field to the attribute table of 'kv\_buffer\_stream' to distinguish the stream sections that made up the Colorado River, Tonahutu

Creek, and Onahu Creek from all other stream sections. These streams tended to be much wider and have higher flows than other streams in the study area. I named the field ‘river\_stream’ and labeled stream sections that were part of the Colorado River, Tonahutu Creek or Onahu Creek with a ‘2’ and stream sections not part of one of those streams with a ‘1.’

- 5) I reclassified ‘kv\_buffer\_veg’ to reflect the fact that I combined some land cover classes into a single class if they were similar in vegetation or were expected to have similar effects on amphibian movement (see table below). For example, classes 1, 2 and 4 in the original classification are different kinds of ‘Herbaceous Upland.’ Each class was characterized by relatively low-growing shrubs, herbs and grasses. I reclassified them to a single class (land cover class 1). Similarly, classes 5, 6 and 7 were different kinds of ‘Wetlands.’ I reclassified them into a single class (land cover class 5).

veg_code	revised_code		veg_code	revised_code
1	1		38	38
2	1		39	39
4	1		41	41
5	5		43	43
6	5		46	46
7	5		47	9
9	9		48	48
10	9		49	48

11	9		51	51
13	13		52	52
14	13		53	52
15	15		120	120
18	15		121	120
20	20		141	141
22	22		142	142
23	23		162	162
24	23		163	162
26	23		164	162
33	33		190	190
34	34		191	191
35	34		400	39
36	34		999	999

Reclassification scheme used to combine similar land cover classes. The columns titled 'veg\_code' show the original land cover classes, and the columns titled 'revised\_code' show the final land cover classes.

- 6) I converted the shapefile, kv\_buffer\_veg, into a raster dataset. The conversion was necessary, because the 'Cost Distance' algorithm requires a raster file of relative costs of movement.
  - a. The conversion created a grid with 10-meter cells over 'kv\_buffer\_veg.' To each cell in the grid, it assigned the land cover class of the polygon that contains the center of the cell. For example, if the center of a cell is

located within a polygon of land cover class 1, the cell is assigned a 1. I named the raster version of the vegetation data 'kv\_veg.'

- 7) Originally, 'kv\_buffer\_veg' included polygons of the Colorado River. However, the entire river was not digitized, and other streams in the valley were not digitized. Therefore, the raster dataset, 'kv\_veg,' did not include all rivers and streams in the study area. I used the following steps to merge all rivers and streams into the land cover data:
- a. I used the following 'con' statement in Map Algebra to assign all river pixels in 'kv\_veg' to 'NoData': `con(kv_veg <> 51, kv_veg)`
    - i. If the value in a pixel in kv\_veg is not equal to 51 (the stream class), return the value in kv\_veg to the cell. For each stream pixel, it assigned 'NoData.'
  - b. I reassigned all cells classified as a stream (land cover class 51) in 'kv\_veg' to the land cover class of the nearest cell using the 'Euclidean Allocation' tool. I named the file 'kvveg\_nostrm.'
  - c. I converted the streams data set ('kv\_buffer\_strm') into a raster. All cells that represented larger streams (Colorado River, Tonahutu Creek, and Onahu Creek) were assigned a value of '502,' while all cells that represented smaller streams were assigned a value of '501.' I chose these values, because they were different from any of the values used to represent land cover classes. I named the file 'kv\_strm\_riv.'
  - d. I merged the data from the raster of land cover types, 'kvveg\_nostrm,' and the raster of rivers and streams, 'kv\_strm\_riv.'

i. I used the following ‘con’ statement in Map Algebra:

con(isnull(kv\_strm\_riv), kvveg\_nostrm, kv\_strm\_riv). I named the resulting file ‘kv\_veg\_strm’ and refer to this file as the merged map. At this point, every cell in ‘kv\_veg\_strm’ (the study area plus the 3,000 m buffer; i.e., the merged map) contained an integer that represented a land cover class or stream value.

8) I reclassified ‘kv\_veg\_stream’ so that each land cover or stream class for each cell was converted to a cost. The costs I used are in the table below (Note: a cost surface can only take on integer values. For classes where the weighted-average cost of movement was a real number, I rounded the cost to an integer.):

Land Cover Class	Brief Class Description	Cost	Cost
		Wood Frog	Chorus Frog
1	Herbaceous upland	8 (15)	9 (15)
5	Herbaceous wetland	3 (6)	3 (5)
9	Alpine ice and rock fields	Barrier	Barrier
13	Shrub upland	1 (2)	1 (1)
15	Aspen	2 (4)	3 (4)
20	Montane douglas fir	5 (10)	7 (10)
22	Sub-alpine mixed conifer	4 (8)	6 (8)
23	Lodgepole pine	4 (9)	6 (9)
33	Juniper	7 (14)	9 (14)
34	Ponderosa pine	5 (10)	7 (10)



38	Limber pine	5 (10)	7 (10)
39	Ribbon forests	5 (10)	7 (10)
41	Disturbed area	9 (18)	11 (18)
43	Blue spruce	5 (10)	7 (10)
46	Talus	8 (15)	9 (15)
48	Exposed soil and cliff faces	6 (12)	7 (11)
52	Lakes and reservoirs	Barrier	Barrier
120	Riparian shrub	2 (3)	2 (3)
141	Upland shrub – sagebrush	5 (9)	5 (8)
142	Upland shrub – bitterbrush	7 (14)	9 (14)
162	Mixed conifer with aspen	4 (7)	4 (7)
190	Riparian mixed conifer, high elevation	3 (5)	4 (5)
191	Riparian mixed conifer, low elevation	1 (1)	1 (1)
501	Small streams	4 (6)	4 (6)
502	Rivers and large streams	8 (14)	10 (14)
999	Un-vegetated surfaces	18 (22)	22 (22)

Relative costs of movement for wood frogs, *Rana sylvatica*, and boreal chorus frogs, *Pseudacris maculata*, adults and juveniles (in parentheses) through 26 land cover classes in the study area.

Appendix 2: Description of the processes used to delineate cost-based buffers and derive estimates of the amount of seasonal habitat for wood frogs (*Rana sylvatica*) and boreal chorus frogs (*Pseudacris maculata*) around each wetland. All procedures were conducted in ArcGIS 9.2 (ESRI 1999-2006).

- 1) As part of the preparation for this analysis, I digitized each of the sampled wetlands. I also created shapefiles for individual wetlands (as opposed to a single shapefile that included every wetland).
- 2) I used the ‘Cost Allocation’ tool to delineate a cost-based buffer around each wetland.
  - a. For each wetland raster, I used ‘Cost Allocation’ to define an area around the wetland in which every cell is within 200 (or 400 or 800 for boreal chorus frogs, and 400, 600 or 1000 for wood frogs) cost-distance meters of the wetland.
- 3) I developed a map of habitat types across the study area. In order to estimate the amount of wetland, streamside, and upland habitat within each buffer, I reclassified kv\_veg\_strm so that each cell was labeled as being one of the three seasonal habitat types, or as non-habitat (see table below).

Land Cover Class	Habitat Type
1	Upland
5	Wetland
9	Non-habitat
13	Wetland
15	Wetland
20	Upland
22	Upland
23	Upland
33	Upland
34	Upland
38	Upland
39	Non-habitat
41	Non-habitat
43	Upland
46	Non-habitat
48	Non-habitat
52	Non-habitat
120	Wetland
141	Non-habitat
142	Upland
162	Non-habitat
190	Non-habitat

191	Non-habitat
501	Streamside
502	Streamside
999	Non-habitat

Classification scheme used to convert land cover types into one of the three seasonal habitat types (wetland, streamside, and upland).

- 4) I used the 'Combine' tool to generate a count of the number of cells of each seasonal habitat type within each buffer and transferred those counts into a spreadsheet.

Appendix 3: Description of the processes used to derive estimates of the least cost distance to the nearest neighboring occupied wetland from each sampled wetland, as well as the number of occupied wetlands within various cost distances of each sampled wetland. All procedures were conducted in ArcGIS 9.2 (ESRI 1999-2006).

- 1) I created polygon shapefiles that included the location of every wetland at which breeding by wood frogs (*Rana sylvatica*) was observed in any year from 2004 to 2006. Over the three years of the study, I detected breeding by wood frogs at 33 wetlands. I used the same approach to identify 47 wetlands at which breeding by boreal chorus frogs (*Pseudacris triseriata*) was detected in any year during the study. I named these files ‘rasy\_wetlands\_all’ (for wood frogs) and ‘psma\_wetlands\_all’ (for boreal chorus frogs).
- 2) I converted ‘rasy\_wetlands\_all’ and ‘psma\_wetlands\_all’ into raster data sets named ‘rasy\_wetlands’ and ‘psma\_wetlands.’
- 3) To obtain counts of the number of occupied wetlands within 1500, 3000, and 4500 cost meters of a sampled wetland (for wood frogs; 1000, 2000 and 3000 cost meters were the maximum cost distances for boreal chorus frogs), I used the ‘Cost Allocation’ tool and the cost surfaces for wood frog and boreal chorus frog juveniles to delineate allocation zones around each sampled wetland. Then, I converted the cost allocation zones (which are rasters) to polygon shapefiles. I

conducted this step to make it easier to see the boundary of the allocation zone, and consequently, count the number of occupied wetlands within the zone.

Finally, I displayed the allocation zone for a single wetland and counted the number of breeding wetlands within the allocation zone.

- 4) To obtain an estimate of the cost distance to the nearest occupied wetland, I used the 'Cost Distance' tool individually for each wetland (I used the cost surfaces for wood frog and boreal chorus frog juveniles). I did not set a maximum distance, because I wanted the cost distance algorithm to grow out to the boundaries of the study area.
- 5) I used the 'Sample' tool and the raster data sets of all wetlands known to be occupied by breeding wood frog and boreal chorus frog between 2004 and 2006 ('rasy\_wetlands' and 'psma\_wetlands') to create a table of minimum cost distances from a sampled wetland to wetlands known to be occupied by wood frogs or boreal chorus frog. The 'Sample' tool assigns to the cell of one raster the value in the cell of another raster that is nearest to it. For example, the value in the cells of the 'Cost Distance' raster I created for each sampled wetland was the minimum cost distance from the cell to a sampled wetland. In the raster datasets of occupied wetlands, the cells that represent each wetland were assigned a pond identification number, and all other cells were assigned 'NoData.' I used the 'Sample' tool to assign the cost distance values in the cost distance raster for each sampled wetland to the cells of the occupied wetlands. Those steps provided me with the minimum cost distance from a source cell (the sampled wetlands) to every cell that represented an occupied wetland. I selected the minimum value.

## CHAPTER 4

### THE GENETIC STRUCTURE OF A RELICT POPULATION OF WOOD FROGS

#### Abstract

Habitat fragmentation and the associated reduction in connectivity between habitat patches are commonly cited causes of genetic differentiation and reduced genetic variation in animal populations. We used eight microsatellite markers to test hypotheses regarding the genetic structure and levels of genetic variation in a relict population of wood frogs (*Rana sylvatica*) in Rocky Mountain National Park, Colorado. We also tested for a pattern of isolation-by-distance, as well as evidence of a recent population bottleneck. The results from the clustering algorithm in Program STRUCTURE indicate the population is partitioned into two genetic clusters (subpopulations), and an estimate of  $F_{ST}$  provides strong evidence of differentiation between the clusters. Though we found support for a pattern of genetic isolation-by-distance, other processes also may have affected levels of genetic differentiation. Genetic variability in the population is low relative to other studies. However, we found no evidence that a recent bottleneck was responsible for the low variation. Management objectives should include increasing the size and spatial distribution of the population and improving gene flow between the genetic clusters. Construction or restoration of wetlands in the landscape between the clusters has the potential to address each of these objectives.

## **Introduction**

Anthropogenic alterations to landscapes and the subsequent fragmentation of habitat are frequently cited as causes of reduced connectivity among local populations of animals (Shepard et al. 2008, Ford and Fahrig 2008, Pruett et al. 2009). A reduction in connectivity can lead to genetic differentiation among local populations and lower genetic variation within them (Frankham 2006, Allentoft et al. 2009). Relative to other terrestrial vertebrates, amphibians may be particularly vulnerable to landscape changes that fragment habitat due to their small body size and physiological requirement for moist conditions (Gibbs 1998; but see Green 2003). Change in the permeability of the landscape between habitat patches can have important deleterious impacts on amphibian populations (Funk et al. 2005). Several studies have examined the effects of habitat fragmentation on amphibian populations and detected reduced genetic variability in local populations, as well as significant genetic differentiation between them (Andersen et al. 2004, Arens et al. 2007, Noel et al. 2007, Spear and Storfer 2008).

Wood frogs (*Rana sylvatica*) are one of the most widespread amphibian species in North America, ranging from southern portions of the eastern seaboard in the U.S. to beyond the Arctic Circle in Canada (Stebbins 2003). In northern Colorado and southern Wyoming, however, wood frog populations occur in a few, relatively small areas (Hammerson 1999) and are relicts from a population that may have once been distributed across much of the southwestern U.S. (Lee-Yaw et al. 2008). Primarily due to their restricted ranges, they are currently listed as a species of concern in Colorado and a species of “greatest conservation need” in Wyoming (CDOW 2005, WGFD 2005). The



wood frog is one of only 4 extant amphibian species in Rocky Mountain National Park, Colorado (Corn et al. 1997) and is restricted to the Kawuneeche Valley (KV) on the western side of the park (Fig. 1). Therefore, natural and anthropogenic disturbances in the KV may threaten the long-term persistence of the species in the park.

With respect to wetlands and amphibians, there have been 2 important disturbances to the landscape and biota of the KV over the last 50 to 100 years: construction of the Grand Ditch and significant reduction in the abundance of beaver (*Castor canadensis*). The Grand Ditch is a water diversion ditch that is over 22 km long and runs along the western hillslope of the KV at an elevation of approximately 3,100 m (Fig. 1). Construction of the ditch and diversion of water started in the 1890s, and diversion over its entire length began in 1936 (Perry 2008). Woods (2000) estimates the ditch intercepts an average of 29% of the total runoff from the watershed each year. Consequently, operation of the ditch has reduced the surface flow of water into the KV, reduced the amount and frequency of flooding by streams and rivers and lowered water levels in streams and rivers (Woods 2000, 2001). Each of these processes is an important mechanism for maintaining water table levels in the KV, particularly given its location in the relatively arid interior of the western U.S.

In addition to the disturbance caused by the ditch, the beaver population in the KV has declined over 90% in the last 60 years (Westbrook et al. 2006). The lack of newly constructed beaver dams and maintenance on existing dams has reduced the frequency, extent and duration of flooding on the floor of the valley (Woods 2000, Westbrook et al. 2006). In another mountain valley in Rocky Mountain National Park, a comparison of aerial photographs taken in 1947 and 1964 shows a dramatic reduction in the area

inundated by beaver ponds and canals as beaver populations declined (Baker 2003). In addition to the effect of beaver on flooding regimes, water table levels tend to be higher near the ponds they construct (Westbrook et al. 2006). Although historic data on water tables do not exist, current levels of the water table in the KV appear to be relatively low. Examination of soil cores indicates that, historically, the water table was near the surface of the ground, and the soil across much of the KV was waterlogged for extended periods of time each year (Westbrook et al. 2006). Therefore, operation of the Grand Ditch and the reduction in beaver appear to have fragmented wetlands (i.e., lakes, ponds, wet meadows) and increased the spatial extent of more xeric areas (e.g., dry meadows).

Though these disturbances appear to have fragmented wetland habitat, effects on the population of wood frogs depend on levels of gene flow between fragments (Frankham et al. 2002). If fragmentation has reduced the rate at which wood frogs move between wetlands in the KV, we hypothesized two effects. We hypothesized the population would be structured as multiple subpopulations, and the subpopulations would be genetically differentiated. We also hypothesized that subpopulations would be characterized by relatively low levels of genetic variation. The primary purpose of this study was to evaluate these hypotheses. The results of the analyses provided several insights regarding potential management action for the population of wood frogs in the KV.

## **Methods**

### ***Study area***

The study area is the Kawuneeche Valley (KV) and the adjacent mountainsides up to 3,048 m elevation in Rocky Mountain National Park (Fig. 1). It contains the headwaters

of the Colorado River and some of the largest remaining sub-alpine wetland complexes in Colorado (Woods 2000). Over 80% of the study area is upland and is dominated by forests of lodgepole pine (*Pinus contorta*) and mixed coniferous species (*Abies lasiocarpa*, *Picea engelmannii*; Salas et al. 2005). The valley bottom is a mix of wetlands dominated by herbaceous vegetation (e.g., *Carex* spp.) and/or shrubs (*Salix* spp.) and riparian areas.

### ***Field methods***

Wood frogs deposit their eggs in clumps (i.e., egg masses) that are approximately the size and shape of a baseball (Hammerson 1999), and individual egg masses can be easily distinguished from one another. In the southern Rocky Mountains, reproducing females deposit a single egg mass in the spring (Corn and Livo 1989). Therefore, collecting eggs from different egg masses minimizes the probability of sampling known siblings.

The sampling plan was to sample most of the egg masses in the KV in 2006. Based on extensive surveys in the KV in 2004 to 2005, we developed a list of more than 40 wetlands to search for egg masses. The list included wetlands at which wood frog reproduction had been observed during previous surveys, as well as wetlands at which adults or juveniles were found but evidence of reproduction (i.e., egg masses or tadpoles) was not. In the spring of 2006, each of these wetlands was surveyed for egg masses by at least two field workers. From each egg mass, a minimum of two eggs or hatchlings was collected. Only one egg from each egg mass was used in the subsequent genetic analyses. Oviposition in populations of wood frogs often occurs over a short period of time (5 to 10 days; Petranka et al. 2004) in the spring. To maximize the probability that oviposition at each wetland was complete at the time it was surveyed, surveys were conducted a

minimum of six days after observing the first egg mass in the KV. The fact that no new egg masses were observed on subsequent visits to a subset of the wetlands suggests a high percentage of egg masses were present at the time of the surveys. Wood frog egg masses are highly detectable (Grant et al. 2005, Scherer 2008); therefore, we think a high percentage of the egg masses in the study area was sampled.

### ***Genetic processing of samples***

DNA was extracted using the Wizard Genomic DNA Purification System (Promega) following manufacturer's specifications. All 160 wood frog samples were screened using eight nuclear microsatellite loci. We used eight microsatellite markers (*RsyC11*, *RsyC63*, *RsyD20*, *RsyD32*, *RsyD40*, *RsyD55*, *RsyD70*, and *RsyD88*) that were isolated and characterized for *R. sylvatica* (Julian and King 2003). Amplifications were performed using a M13-tailed forward primer as described by Boutin-Ganache et al. (2001). Each 12.5 $\mu$ l reaction contained 125 $\mu$ M each dNTP, 1X *Taq* buffer (Kahn et al. 1998), 0.034 $\mu$ M M13-tailed forward primer, 0.5 $\mu$ M non-tailed reverse primer, 0.5 $\mu$ M M13 dye-labeled primer with Beckman Coulter dyes D2, D3 or D4 (Proligo), and 0.31U *Taq* polymerase (Promega). The thermal profile for the M13 dye-labeled reactions was as follows with the annealing temperature varying: preheat at 94 $^{\circ}$ C for 2 min, denature at 94  $^{\circ}$ C for 40 sec, anneal for 1 min, and extend at 72  $^{\circ}$ C for 1 min. Each PCR had 35 amplification cycles (MJ Research PTC-200, Bio-Rad) and concluded with a 10 min post heat at 72  $^{\circ}$ C for 5 min. The annealing temperature for *RsyD20* was 53  $^{\circ}$ C , for *RsyC11* and *Rsy D40* was 58  $^{\circ}$ C, and for all other loci was 55  $^{\circ}$ C. The PCR products were diluted and run on the CEQ8000 XL DNA Analysis System (Beckman Coulter). All loci were run with the S400 size standard (Beckman Coulter) and analyzed using the Frag 3 default method.

### ***Genetic analyses***

Initially, we treated samples from individual wetlands as “populations” and used ARLEQUIN 2.0 to test for Hardy-Weinberg (H-W) equilibrium (Schneider et al. 2000). ARLEQUIN 2.0 uses a modified version of the Monte Carlo random walk algorithm of Guo and Thompson (1992). The algorithm was run for 100,000 steps after 1,000 dememorization steps. To determine if *P*-values indicated a significant departure from H-W equilibrium, we used the sequential Bonferroni technique (Rice 1989) and an overall  $\alpha = 0.05$ . Linkage disequilibrium for each pair of loci was evaluated in each wetland in GENEPOP (Raymond and Rousset 1995; Markov chain parameters: 10,000 dememorization steps, 1,000 batches, 10,000 iterations per batch). We also used a Mantel test to determine if a pattern of genetic isolation-by-distance was present in the data and conducted the test using the software Alleles in Space (Miller 2005) with 1000 permutations.

We used Program STRUCTURE 2.0 to evaluate the genetic structure of the population of wood frogs. Specifically, we estimated the number of genetically-distinct population clusters (Pritchard et al. 2000). Program STRUCTURE uses a model-based clustering algorithm to estimate the number of clusters, *K*, given the data (Pritchard et al. 2000). In addition to estimating the number of genetic clusters, we also used the results of this analysis to gain insights into relative rates of gene flow across the study area. We set the range of *K* from 1 to 12. Final runs were performed using the admixture model with correlated allele frequencies at burn-in length of 100,000 and MCMC of 250,000 (20 runs per *K*). We used the estimated average log likelihood of the data given *K*,  $\text{Ln}(X|K)$ , and  $\Delta K$  (Evanno et al. 2005) as the metrics for selecting the number of genetic clusters given

the data. Higher values of these metrics indicate greater support for a given number of genetic clusters (Evanno et al. 2005, Allentoft et al. 2009).

After identifying genetic clusters in Program STRUCTURE, we treated each cluster as a subpopulation and used ARLEQUIN 2.0 to estimate genetic variability within each subpopulation and  $F_{ST}$  between them. We also tested each subpopulation for evidence of a bottleneck using the software BOTTLENECK (Cornuet and Luikart 1996) and the Wilcoxon test under the two-phased model of mutation (TPM) with 1,000 replications. Since estimates of genetic variability in each subpopulation were low, the purpose of this test was to evaluate the possibility that a recent bottleneck was the cause. Short periods of time at small population size causes reduced genetic variation in animal populations (Frankham et al. 2002).

## Results

We collected embryos or hatchlings from 160 egg masses at 19 wetlands in the study area and from one wetland on private property west of the KV (WRR Pond; Fig. 1 and Table 1). All microsatellite loci were polymorphic, and the number of alleles for each locus ranged from 3 to 18 (Table 2). We found no evidence of loci that were out of H-W equilibrium; therefore, all loci were used in subsequent analyses. We observed no evidence of linkage disequilibrium across all pairs of loci (smallest  $P$ -value = 0.16), and little to no evidence of linkage disequilibrium has been detected in other studies that have used these microsatellite loci (Julian and King 2003, Crosby et al. 2009).

The data provided the strongest support for two genetic clusters of individuals in the KV, a northern and southern cluster ( $\text{Ln}[X|K] = -2831.6$ ,  $\Delta K = 499.2$ ; Table 3, Fig. 2).

Hereafter, we will refer to these clusters as the northern and southern subpopulations. Of the 77 individuals sampled from wetlands in the northern part of the KV, 65 individuals (84%) had a high probability ( $> 0.80$ ) of assignment to the northern subpopulation (Fig. 1 and 2). Sixty-nine of 83 individuals (83%) sampled from southern wetlands had a high probability of assignment to the southern subpopulation. It is important to note, however, that a few individuals had high probabilities of assignment to subpopulations that were not consistent with the location from which they were sampled (e.g., the last individual in wetland #5050 had a high probability of assignment to the southern subpopulation even though it was sampled from a northern wetland; Fig. 2). After the initial analysis, we looked for evidence of genetic structure at finer resolution by separately analyzing the data from the northern and southern subpopulations in Program STRUCTURE. The data provided no evidence of finer genetic structure (the model of  $K = 1$  in the northern and southern subpopulations received the most support). The results of the Mantel test suggested a pattern of increased isolation with geographic distance ( $P$ -value  $< 0.001$ , Pearson's correlation coefficient,  $r = 0.22$ ).

The average numbers of alleles across loci in each subpopulation were 5.5 (northern) and 7.63 (southern; Table 1), though there was no evidence that observed heterozygosity varied between the subpopulations. Estimates of  $F_{ST}$  indicate high levels of genetic differentiation between the northern and southern subpopulations ( $F_{ST} = 0.0675$ ,  $P$ -value  $< 0.0001$ ), and we found no evidence of recent bottlenecks in either subpopulation (northern subpopulation,  $P$ -value = 0.98; southern subpopulation,  $P$ -value = 0.81).

## Discussion

Anthropogenic disturbance of landscapes can have important effects on the genetic structure and variability in populations of animals and, in cases where disturbances fragment suitable habitat and reduce gene flow between the fragments, can threaten the long-term persistence of populations (Frankham 2006). Water diversion and storage projects and the loss of beaver have heavily modified many riparian systems in the western U.S. (Pepin et al. 2002). In the Kawuneeche Valley of Rocky Mountain National Park, these disturbances have altered hydrologic conditions, and negative impacts on peatlands, populations of willow (*Salix* spp.) and benthic invertebrates have been observed or hypothesized (Woods 2000, 2001, Chimner 2000, Westbrook et al. 2006, Clayton and Westbrook 2008). In addition, these disturbances appear to have fragmented wetlands in the KV (Baker 2003, Westbrook et al. 2006) and, consequently, may have negative impacts on amphibian populations. The primary purpose of this study was to evaluate hypothesized effects of changes to the hydrologic conditions of the valley on the genetic structure and levels of genetic variation in a relict population of wood frogs. In other locations fragmented by human activities, lower levels of genetic variation and gene flow have been observed in amphibian populations (Andersen et al. 2004, Arens et al. 2007, Noel et al. 2007, Spear and Storfer 2008). Arens et al. (2007) analyzed genetic data from populations of Moor frogs (*R. arvalis*) in areas with different levels of fragmentation and reported greater genetic differentiation and lower genetic diversity in the area of higher fragmentation. Similarly, Noel et al. (2007) compared levels of differentiation and genetic variability between populations of eastern red-backed salamanders (*Plethodon cinereus*) in a landscape fragmented by urban development and a



landscape of continuous habitat. They reported higher levels of differentiation and lower levels of genetic variation in the fragmented landscape.

The genetic clustering algorithm in Program STRUCTURE indicated that the wood frogs in the KV are structured as two subpopulations. However, a few individuals had high probabilities of assignment to a subpopulation that differed from the location from which they were sampled. This result suggests individuals are capable of moving between the subpopulations, though at a lower rate than movement between wetlands within a subpopulation. One possible cause of the relatively low rates of movement between the northern and southern subpopulations is the geographic distance between them (approximately 4 km). The Mantel test indicated a significant pattern of isolation-by-distance, and previous genetic studies have reported evidence of stepping-stone dispersal in populations of montane amphibians (Shaffer et al. 2000, Spear et al. 2005). Despite extensive surveys from 2004 – 2006, evidence of breeding by wood frogs was only observed at one wetland in one year in the area between the subpopulations in the KV (R. Scherer, *unpublished data*; Fig. 1). Corn et al. (1997) conducted amphibian surveys in the KV from 1988 to 1994 and did not detect breeding by wood frogs at any wetlands in the area between the subpopulations. Within subpopulations, on the other hand, the density of occupied wetlands is much higher (Fig. 1). The absence of persistent occupancy by wood frogs in the area between the northern and southern subpopulations suggests the intervening landscape is unsuitable for wood frog occupancy which may greatly limit stepping-stone dispersal and lead to genetic differentiation.

Though there is evidence that isolation-by-distance is one cause of the genetic structure of wood frogs in the KV, low permeability of the landscape between the

northern and southern subpopulations may also be a cause of the relatively low rates of movement and genetic differentiation. Several previous studies have reported little evidence of genetic differentiation in populations of wood frogs, despite distances between subpopulations as great as 20 km (Newman and Squire 2001, Squire and Newman 2002, Julian and King 2003). The results of these studies suggest that a geographic distance of 4 km is insufficient to produce genetic differentiation in populations of wood frogs. However, Crosby et al. (2009) examined genetic population structure in populations of wood frogs separated by 0.95 to 50 km in an area highly fragmented by agriculture and urban development and detected significant genetic differentiation in approximately half of these comparisons. Though they reported significant genetic differentiation, estimates of  $F_{ST}$  in Crosby et al. (2009) were small compared to the present study (estimates of  $F_{ST}$  from significant pairwise comparisons ranged from 0.001 to 0.011 compared to 0.0675 from the present study). Given these results, the significant genetic differentiation between populations separated by only 4 km in the KV suggests that the permeability of the landscape between the northern and southern populations is lower than a landscape dominated by high levels of human development and infrastructure. Based on visual inspection of a map of land cover types in the KV (Salas et al. 2005), the majority of the intervening landscape is herbaceous wetland that lacks canopy cover from shrubs or trees. The landscape between wetlands within each subpopulation, on the other hand, has higher amounts of canopy cover. Several studies have shown that movement of adult and juvenile wood frogs is preferentially directed toward areas with canopy cover and moist substrates (deMaynadier and Hunter 1999, Vasconcelos and Calhoun 2004, Regosin et al. 2005,

Baldwin et al. 2006). The lack of canopy cover in the landscape between the northern and southern subpopulations may constrain movement and, consequently, gene flow.

While landscape change is one cause of population fragmentation and genetic differentiation, other processes can also cause genetic differentiation between populations. For example, it can result from genetic drift when populations have small population sizes (Newman and Squire 2001, Squire and Newman 2002). Counts of egg masses from surveys in the KV from 2004 to 2006 indicate that the number of wood frogs breeding at individual wetlands is small. At wetlands where wood frog egg masses were detected, counts of egg masses ranged from 1 to 32, and most counts were less than 10 (Table 2). Wood frog egg masses are highly conspicuous (Grant et al. 2005, Scherer 2008); therefore, it is unlikely that low detectability is the cause of the small counts. Genetic differentiation can also occur when subpopulations experience bottlenecks or periodic extinction followed by recolonization (Newman and Squire 2001). Drought is common in the recent climatic history of Colorado (McKee et al. 1999), and large declines in amphibian populations are often associated with years of low precipitation (Pechmann et al. 1991, Berven 1995). However, we did not detect evidence of recent bottlenecks in the northern and southern subpopulations, though the lack of evidence may be a consequence of low power. Finally, the fact that we based estimates of  $F_{ST}$  on genetic samples from embryos, as opposed to samples of adults, may overestimate levels of differentiation between subpopulations. If a small proportion of adults are responsible for the eggs produced in a particular year, inferences regarding the genetic composition of a population may be biased (Goldberg and Waits 2010).

Genetic variation (alleles per locus) in both subpopulations was low relative to other populations of wood frogs (Table 4). Julian and King (2003) collected genetic samples from 113 individuals at three wetlands and used the same microsatellite markers as used in this study. Despite a smaller total sample size, they observed considerably higher numbers of alleles at every locus (Table 4). Crosby et al. (2009) had four markers in common with this study. They sampled from 29 to 96 adults at each of 9 wetlands in areas of high human density. In most cases, the number of alleles at each wetland was higher than the number of alleles across all individuals sampled from the KV. Though recent fragmentation of wetlands in the KV may have contributed to the reduced genetic variation, other factors have also contributed. For example, genetic variation is generally lower in areas that have been colonized by a species since the last period of wide-spread glaciation (10,000 to 15,000 years ago; Beebee and Rowe 2000, Newman and Squire 2001, Ficetola et al. 2007, Allentoft et al. 2009). Ficetola et al. (2007) found that genetic variation within populations of the frog, *R. latastei*, was affected by patterns of postglacial colonization, as well as contemporary isolation. Wood frog populations in Colorado occur in a few isolated mountain valleys (Hammerson 1999) and appear to be the result of colonization from populations in present-day Wisconsin following the last glaciation (Lee-Yaw et al. 2008). In addition, the colonization and extinction events associated with metapopulation dynamics can cause reduced genetic variation (Newman and Squire 2001, Frankham et al. 2002). Many authors have argued that amphibian populations are spatially structured as metapopulations (Pechmann and Wilbur 1994, Alford and Richards 1999), though there is disagreement over the generality of this characterization (Marsh et al. 1999, Petranka et al. 2004, Smith and Green 2005).

Regardless of the causes, the low genetic variation of wood frogs in the KV is a concern. Weyrauch and Grubb (2006) reported higher mortality rates for wood frog eggs and larvae from populations with lower genetic variability. In addition, they found that larvae from populations with lower genetic variation were more susceptible to the deleterious effects of UV-B radiation. Similarly, Johansson et al. (2007) reported lower larval survival and body size in common frogs (*R. temporaria*) from wetlands with lower levels of genetic variation.

The results of this analysis provide several useful insights regarding the management and conservation of wood frogs in the KV. The degree to which habitat fragmentation causes deleterious genetic effects on any population of animals largely depends on the number of subpopulations into which a population is divided, the size of the subpopulation on each habitat fragment, and the level of gene flow between the fragments (Frankham 2006). The genetic clustering algorithm indicated that wood frogs in the KV are subdivided into two subpopulations. Evidence of significant genetic differentiation suggest gene flow between the subpopulations is limited, and compared to other populations of wood frogs, levels of genetic variation in each subpopulation are low. Further subdivision of the population would lead to smaller subpopulation sizes and further loss of genetic variability and, consequently, may pose a considerable threat to the long-term persistence of the population. Therefore, management and conservation of the wood frogs in the KV should focus on increasing the size and spatial distribution of the subpopulations, maintaining the current levels of gene flow within subpopulations, and enhancing gene flow between them. The results of a concurrent study of occupancy patterns of wood frogs in the KV indicated that occupied wetlands tended to be located

near streams and other wetlands occupied by wood frogs (R. Scherer, *unpublished data*). One management option is to construct or restore wetlands between the existing subpopulations but near streams and other wetlands used by breeding wood frogs. This option may be beneficial in many ways, most notably in increasing abundance and spatial distribution of the subpopulations and providing habitat in the area between them. The latter benefit may increase gene flow between the subpopulations by restoring stepping-stone dispersal. Because of its location in a national park, however, direct manipulation of the landscape in the KV may not be feasible. Other management options include translocating wood frogs between subpopulations, restoring beaver populations, and altering the operation of the Grand Ditch. While vegetation conditions are currently unsuitable for reintroduction of beaver in most of Rocky Mountain National Park, managers are continuing to evaluate the possibility of reintroduction in the future (M. Watry and T. Johnson, *personal communication*). Clayton and Westbrook (2008) argued that the Grand Ditch could be decommissioned or operated such that its impacts on important hydrologic processes are minimized.

**Acknowledgements** I thank my co-authors on this chapter, E. Muths, B.R. Noon, and S.J. Oyler-McCance. I also thank M.B. Albrechtsen and C. Knopf for collecting samples. Funding for this project was provided by the U.S.G.S. Amphibian Research and Monitoring Initiative, Denver University's Partners in Scholarship, and Rocky Mountain National Park. The methods in this study were approved by Colorado State University's Animal Care and Use Committee (permit numbers 05-012A-01).

## Literature cited

- Alford, R.A., and S.J. Richards. 1999. Global amphibian declines: a problem in applied ecology. *Annual Review of Ecology and Systematics* 30:133-165.
- Allentoft, M.E., H.R. Siegismund, L. Briggs, L.W. Andersen. 2009. Microsatellite analysis of the natterjack toad (*Bufo calamita*) in Denmark: populations are islands in a fragmented landscape. *Conservation Genetics* 10:15-28.
- Andersen, L.W., K. Fog, and C. Damgaard. 2004. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society of London B* 271:1293-1302.
- Arens, P., T. van der Sluis, W.P.C. van't Westende, B. Vosman, C.C. Vos, and M.J.M. Smulders. 2007. Genetic population differentiation and connectivity among fragmented Moor frog (*Rana arvalis*) populations in The Netherlands. *Landscape Ecology* 22:1489-1500.
- Baker, B.W. 2003. Beaver (*Castor canadensis*) in heavily-browsed environments. *Lutra* 46:173-181.
- Baldwin, R.F., A.J.K. Calhoun, and P.G. deMaynadier. 2006. Conservation planning for amphibian species with complex habitat requirements: a case study using movements and habitat selection of the wood frog *Rana sylvatica*. *Journal of Herpetology* 40:443-454.
- Beebee, T.J.C., and G. Rowe. 2000. Microsatellite analysis of natterjack toad *Bufo calamita* Laurenti populations: consequences of dispersal from a Pleistocene refugium. *Biological Journal of the Linnean Society* 69:367-381.

- Berven, K.A. 1995. Population regulation in the wood frog, *Rana sylvatica*, from three diverse geographic localities. *Australian Journal of Ecology* 20:385-292.
- Boutin-Ganache, I., M.Raposo, M. Raymond, and C.F. Deschepper. 2001. M-13 tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques* 31:24-28.
- Chimner, R.A. 2000. Carbon dynamics of Southern Rocky Mountain fens. Ph.D. Dissertation. Colorado State University. Fort Collins, Colorado.
- Clayton, J.A., and C.J. Westbrook. 2008. The effect of the Grand Ditch on the abundance of benthic invertebrates in the Colorado River, Rocky Mountain National Park. *River Research and Applications* 24:975-987.
- Colorado Division of Wildlife. 2005. Colorado's comprehensive wildlife conservation strategy. Colorado Division of Wildlife. Denver, Colorado, U.S.A.
- Corn, P.S., and L.J. Livo. 1989. Leopard frog and wood frog reproduction in Colorado and Wyoming. *Northwestern Naturalist* 70:1-9.
- Corn, P.S., M.L. Jennings, and E. Muths. 1997. Survey and assessment of amphibian populations in Rocky Mountain National Park. *Northwestern Naturalist* 78:34-55.
- Cornuet, J.M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144:2001-2014.
- Crosby, M.K.A., L.E. Licht, and J. Fu. 2009. The effect of habitat fragmentation on finescale population structure of wood frogs (*Rana sylvatica*). *Conservation Genetics* 10:1707-1718.



- deMaynadier, P.G., and M.L. Hunter, Jr. 1999. Forest canopy closure and juvenile emigration by pool-breeding amphibians in Maine. *Journal of Wildlife Management* 63:441-450.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611-2620.
- Ficetola, G.F., T.W.J. Garner, and F. DeBernardi. 2007. Genetic diversity, but not hatching success, is jointly affected by postglacial colonization and isolation in the threatened frog, *Rana latastei*. *Molecular Ecology* 16:1787-1797.
- Ford, A.T., and L. Fahrig. 2008. Movement patterns of eastern chipmunks (*Tamias striatus*) near roads. *Journal of Mammalogy* 89:895-903.
- Frankham, R. 2006. Genetics and landscape connectivity. Pages 72-96 *In* K.R. Crooks and M. Sanjayan (eds.). *Connectivity Conservation*. Cambridge University Press, Cambridge, U.K.
- Frankham, R., J.D. Ballou, and D.A. Briscoe. 2002. *Introduction to conservation genetics*. Cambridge University Press. Cambridge, U.K.
- Funk, W.C., A.E. Greene, P.S. Corn, and F.W. Allendorf. 2005. High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biology Letters* 1:13-16.
- Gibbs, J.P. 1998. Amphibian movements in response to forest edges, roads and streambeds in southern New England. *Journal of Wildlife Management* 62:584-589.

- Goldberg, C.S., and L.P. Waits. 2010. Quantification and reduction of bias from sampling larvae to infer population and landscape genetic structure. *Molecular Ecology Resources* 10:304-313.
- Grant, E. H. C., R. E. Jung, J. D. Nichols, and J. E. Hines. 2005. Double-observer approach to estimating egg mass abundance of pool-breeding amphibians. *Wetlands Ecology and Management* 13:305–320.
- Green, D.M. 2003. The ecology of extinction: population fluctuation and decline in amphibians. *Biological Conservation* 111:331-343.
- Guo, S.W., and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361-372.
- Hammerson, G.A. 1999. *Amphibians and reptiles in Colorado*. University Press of Colorado. Niwot, Colorado, U.S.A.
- Johansson, M., C.R. Primmer, and J. Merila. 2007. Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). *Molecular Ecology* 16:2693-2700.
- Julian, S.E., and T.L. King. 2003. Novel tetranucleotide microsatellite DNA markers for the wood frog, *Rana sylvatica*. *Molecular Ecology Notes* 3:256-258.
- Kahn, N.W., J. St. John, and T.W. Quinn. 1998. Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. *Auk* 115:1074-1078.
- Lee-Yaw, J.A., J.T. Irwin, and D.M. Green. 2008. Postglacial range expansion from northern refugia by the wood frog, *Rana sylvatica*. *Molecular Ecology* 17:867-884.

- Marsh, D.M., E.H. Fegraus, and S. Harrison. 1999. Effects of breeding pond isolation on the spatial and temporal dynamics of pond use by the tungara frog, *Physalaemus pustulosus*. *Journal of Animal Ecology* 68:804-814.
- McKee, T.B., N.J. Doesken, and J. Kleist. 1999. Historical dry and wet periods in Colorado. Climatology Report 99-1, Part A: Technical Report, Part B. Colorado State University. Fort Collins, Colorado, U.S.A.
- Miller, M.P. 2005. Alleles in space: computer software for the joint analysis of inter-individual spatial and genetic information. *Journal of Heredity* 96:722-724.
- Newman, R.A., and T. Squire. 2001. Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology* 10:1087-1100.
- Noel, S., M. Ouellett, P. Galois, and F.-J. Lapointe. 2007. Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. *Conservation Genetics* 8:599-606.
- Pechmann, J.H.K., D.E. Scott, R.D. Semlitsch, J.P. Caldwell, L.J. Vitt, and J.W. Gibbons. 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. *Science* 253:892-895.
- Pechmann, J.H.K., and H.M. Wilbur. 1994. Putting declining amphibian populations in perspective: natural fluctuations and human impacts. *Herpetologica* 50:65-84.
- Pepin, D.M., N.L. Poff, and J.S. Baron. 2002. Ecological effects of resource development in running waters. Pages 113-132 *In* J.S. Baron (ed.). *Rocky Mountain futures: an ecological perspective*. Island Press. Washington, D.C., U.S.A.
- Perry, P.J. 2008. *It happened in Rocky Mountain National Park*. Morris Book Publishing. Kearney, Nebraska, U.S.A.

- Petranka, J.W., C.K. Smith, and A.F. Scott. 2004. Identifying the minimal demographic unit for monitoring pond-breeding amphibians. *Ecological Applications* 14:1065-1078.
- Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- Pruett, C.L., M.A. Patten, and D.H. Wolfe. 2009. Avoidance behavior by prairie grouse: implications for development of wind energy. *Conservation Biology* 23:1523-1529.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.
- Regosin, J.V., B.S. Windmiller, R.N. Homan, and J.M. Reed. 2005. Variation in terrestrial habitat use by four pool-breeding amphibian species. *Journal of Wildlife Management* 69:1481-1493.
- Salas, D., J. Stevens, and K. Schulz. 2005. Rocky Mountain National Park, Colorado: 2001-2005 vegetation classification and mapping. Technical Memorandum 8260-05-02. U.S. Bureau of Reclamation. Denver, Colorado, U.S.A.
- Scherer, R.D. 2008. Detection of wood frog egg masses and implications for monitoring amphibian populations. *Copeia* 2008:669-672.
- Schneider S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN ver 2.000: a software population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Shaffer, H.B., G.M. Fellers, A. Magee, and S.R. Voss. 2000. The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite Toad, *Bufo canorus* (Anura, Bufonidae) based on single-strand conformation

- polymorphism analysis (SSCP) and mitochondrial DNA sequence data. *Molecular Ecology* 9:245-257.
- Shepard, D.B., A.R. Kuhns, M.J. Dreslik, and C.A. Phillips. 2008. Roads as barriers to animal movement in fragmented landscapes. *Animal Conservation* 11:288-296.
- Smith, M.A., and D.M.Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* 28:110-128.
- Spear, S.F., C.R. Peterson, M.D. Matocq, and A. Storfer. 2005. Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology* 14:2553-2564.
- Spear, S.F., and A. Storfer. 2008. Landscape genetic structure of coastal tailed frogs (*Ascaphus truei*) in protected vs. managed forests. *Molecular Ecology* 17:4642-4656.
- Squire, T. and R.A. Newman. 2002. Fine-scale population structure in the wood frog (*Rana sylvatica*) in a northern woodland. *Herpetologica* 58:119-130.
- Stebbins, R.C. 2003. A field guide to western reptiles and amphibians. 3<sup>rd</sup> edition. Houghton Mifflin Company. New York, New York, U.S.A.
- Vasconcelos, D., and A.J.K. Calhoun. 2004. Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. *Journal of Herpetology* 38:551-561.
- Westbrook, C.J., D.J. Cooper, and B.W. Baker. 2006. Beaver dams and overbank floods influence groundwater-surface water interactions of a Rocky Mountain riparian area. *Water Resources Research* 42. doi:10.1029/2005WR004560.

- Weyrauch, S.L., and T.C. Grubb, Jr. 2004. Patch and landscape characteristics associated with the distribution of woodland amphibians in an agricultural fragmented landscape: an information-theoretic approach. *Biological Conservation* 115:443-450.
- Weyrauch, S.L., and T.C. Grubb, Jr. 2006. Effects of the interaction between genetic diversity and UV-B radiation on wood frog fitness. *Conservation Biology* 20:802-810.
- Woods, S.W. 2000. Hydrologic effects of the Grand Ditch on streams and wetlands in Rocky Mountain National Park, Colorado. M.S. Thesis. Colorado State University. Fort Collins, Colorado, U.S.A.
- Woods, S.W. 2001. Ecohydrology of subalpine wetlands in the Kawuneeche Valley, Rocky Mountain National Park, Colorado. Ph.D. Dissertation. Colorado State University. Fort Collins, Colorado, U.S.A.
- Wyoming Game and Fish Department. 2005. A comprehensive wildlife conservation strategy for Wyoming. Wyoming Game and Fish Department. Cheyenne, Wyoming, U.S.A.

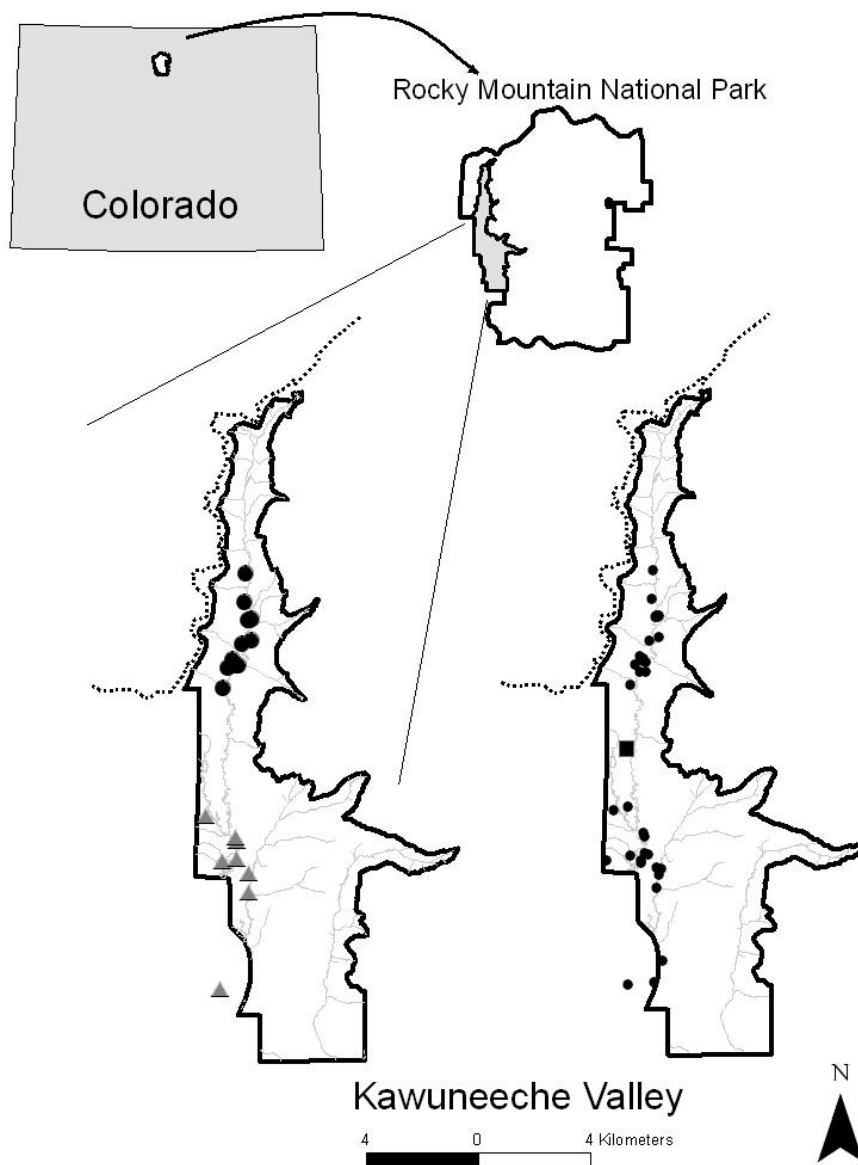


Figure 1: Map of the Kawuneeche Valley (KV) in Rocky Mountain National Park, Colorado. The Grand Ditch is represented by the bold, dotted line. The map on the left shows the wetlands from which genetic samples were collected. The results from Program STRUCTURE suggest two genetic clusters in the KV: a northern (black circles) and southern (gray triangles) cluster. The map of the KV on the right shows the locations of wetlands (solid, black circles) where wood frogs were detected during surveys from

2004 to 2006 (the black square in the center represents the only wetland at which breeding by wood frogs was detected in the area between genetic clusters).



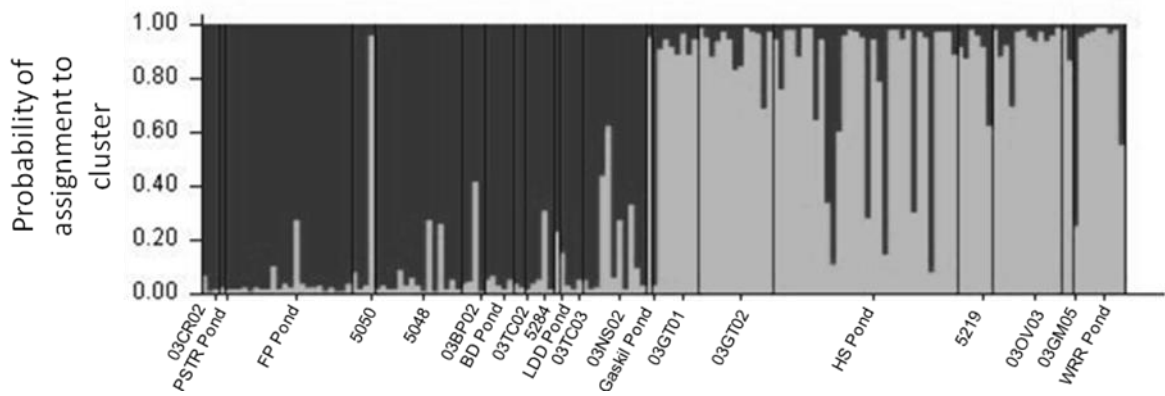


Figure 2. The results of the genetic clustering algorithm in Program STRUCTURE.

Wetlands are listed along the x-axis from the northernmost (03CR02) to the southernmost (WRR Pond). The narrow black lines partition individuals into the wetlands from which they were sampled. Each colored bars across the figure represents an individual. The proportion of each bar colored in dark gray indicates the probability of assignment to the northern population, and the proportion colored in light gray indicates the probability of assignment to the southern population.

Table 1: Number of sampled egg masses and genetic variation across the 20 sampled wetlands in the Kawuneeche Valley (KV). Results from Program STRUCTURE indicated the population was comprised of two genetic clusters (a northern and southern cluster); therefore, the number of sampled egg masses and genetic variation for the northern (03CR02 to 03NS02) and southern (Gaskil Pond to WRR Pond) clusters are also shown. N = number of sampled egg masses, mean  $N_{\text{mean}}$  = mean number of alleles across loci, mean  $H_O$  = mean observed heterozygosity across loci, mean  $H_E$  = mean expected heterozygosity across loci.

Wetland	N	$N_{\text{mean}}$	Mean $H_O$ (SE)	Mean $H_E$ (SE)
03CR02	3	1.63	0.438 (0.151)	0.262 (0.081)
PSTR Pond	1	1.25	0.250 (0.164)	0.125 (0.082)
FP Pond	22	4.25	0.382 (0.069)	0.397 (0.082)
5050	4	2.63	0.469 (0.153)	0.430 (0.101)
5048	15	3.25	0.381 (0.088)	0.452 (0.081)
03BP02	4	2.63	0.438 (0.091)	0.426 (0.071)
BD Pond	5	3.00	0.588 (0.114)	0.500 (0.074)
03TC02	2	1.88	0.500 (0.134)	0.344 (0.081)
5284	5	2.63	0.450 (0.118)	0.435 (0.089)
LDD Pond	1	1.00	0.250 (0.164)	0.125 (0.082)
03TC03	4	2.63	0.542 (0.130)	0.469 (0.083)
03NS02	11	3.88	0.456 (0.097)	0.483 (0.094)
Gaskil Pond	1	1.38	0.375 (0.183)	0.188 (0.091)

03GT01	8	3.63	0.398 (0.113)	0.483 (0.110)
03GT02	13	5.38	0.617 (0.077)	0.588 (0.064)
HS Pond	32	6.00	0.567 (0.069)	0.593 (0.076)
5219	6	3.75	0.504 (0.079)	0.520 (0.076)
03OV03	12	4.50	0.711 (0.082)	0.621 (0.051)
03GM05	2	2.38	0.563 (0.148)	0.469 (0.081)
WRR Pond	9	3.00	0.424 (0.076)	0.490 (0.081)
Northern cluster	77	5.50	0.428 (0.069)	0.495 (0.083)
Southern cluster	83	7.63	0.561 (0.054)	0.614 (0.068)

Table 2: Genetic variation across the microsatellite loci.  $N_A$  = total number of alleles at a locus across wetlands,  $N_{\min}$  = the minimum number of alleles per locus at a wetland,  $N_{\max}$  = the maximum number of alleles per locus at a wetland, and  $N_{\text{mean}}$  = the average number of alleles per locus across wetlands. *RsyD20* and *RsyD32* did not amplify from the single egg mass collected at LDD Pond; therefore, the minimum number of alleles for those microsatellite loci was 0.

Locus	$N_A$	$N_{\min}$	$N_{\max}$	$N_{\text{mean}}$
<i>RsyC11</i>	14	1	9	4.15
<i>RsyD20</i>	7	0	6	3.2
<i>RsyD32</i>	4	0	4	2.1
<i>RsyD40</i>	3	1	3	1.6
<i>RsyD55</i>	6	1	6	2.6
<i>RsyD88</i>	7	1	6	2.75
<i>RsyC63</i>	18	1	13	4.9
<i>RsyD70</i>	9	1	6	2.95

Table 3: Results of the analysis in Program STRUCTURE. For  $K = 1$  to 12, estimates of the average probability of the data given  $K$ ,  $\text{Ln}(X|K)$  and  $\Delta K$  are provided.

$K$	$\text{Ln}(X K)$	$\Delta K$
1	-3038.2	0.0
2	-2831.6	499.2
3	-2865.4	2.7
4	-2851.4	1.7
5	-2899.2	2.1
6	-3020.4	1.4
7	-3074.6	0.9
8	-3185.7	0.6
9	-3251.7	0.7
10	-3216.2	1.7
11	-3285.2	0.3
12	-3327.6	0.0

Table 4: Levels of genetic diversity from the wood frog population in the KV relative to levels of genetic diversity from other genetic studies of wood frogs. Crosby et al. (2008) collected samples from between 29 and 96 individuals at 9 wetlands; therefore, I provide a range of values across sampled wetlands for the number of genetic samples, n, and the number of alleles per locus,  $N_A$ .

Locus	Julian and King (2003)		Crosby et al. (2008)		This study	
	n	$N_A$	n	$N_A$	n	$N_A$
<i>RsyC11</i>	111	24	29-96	11-23	158	14
<i>RsyD20</i>	106	23	29-96	-	118	7
<i>RsyD32</i>	112	16	29-96	-	140	4
<i>RsyD40</i>	110	34	29-96	6-18	159	3
<i>RsyD55</i>	110	17	29-96	-	156	6
<i>RsyD88</i>	112	25	29-96	10-14	158	7
<i>RsyC63</i>	111	26	29-96	19-35	146	18
<i>RsyD70</i>	110	33	29-96	-	155	9