THESIS

INVESTIGATING THE DIRECT AND INDIRECT EFFECTS OF INTRODUCED GREENBACK CUTTHROAT TROUT ON BOREAL TOAD RECRUITMENT

Submitted by

Wendy Elizabeth Lanier

Department of Fish, Wildlife, and Conservation Biology

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Master's Committee:

Advisor: Larissa Bailey

Kevin Bestgen
Chris Funk

ABSTRACT

INVESTIGATING THE DIRECT AND INDIRECT EFFECTS OF INTRODUCED GREENBACK CUTTHROAT TROUT ON BOREAL TOAD RECRUITMENT

Worldwide, numerous amphibian species are at great risk of extinction. Currently, a third of all amphibian species are listed by the International Union for Conservation of Nature as threatened and this number is likely underestimated as there are many species whose status is unknown due to insufficient data. Species in the family *Bufonidae*, the true toads, are one of the most threatened groups of amphibians. One such declining bufonid is the boreal toad, *Anaxyrus boreas boreas*.

While much of the decline in boreal toad populations can be attributed to the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), not all populations are impacted by this pathogen. Declining populations of boreal toads in protected areas in the absence of Bd are largely enigmatic. However, there is one hypothesis that might explain these declines. The greenback cutthroat trout, a federally threatened species, have been introduced into alpine lakes in an effort to bolster their population. These trout were introduced into some lakes that supported breeding populations of toads and could be causing the declines observed in the toad populations.

Adult boreal toads are mostly terrestrial and beyond the gape limitation of greenback cutthroat trout. As such, trout are not likely to reduce adult toad survival. What is more likely is that introduced trout are reducing or eliminating recruitment of individuals to the adult population. The life stages where trout could interrupt recruitment are: embryos, tadpoles, and postmetamorphic individuals. Among these life stages, trout may reduce recruitment via various

direct or indirect effects. The direct effect of predation is unlikely as boreal toads contain bufotoxins at all life stages and are, therefore, unpalatable to trout. However, trout may not recognize palatable verses unpalatable prey and must taste a prey individual to determine if it is palatable. For early developmental stages, this "tasting" process could result in physical damage and stress to those individuals and reduce survival. Trout presence may also indirectly affect survival, growth, and development of toads. One indirect effect is a reduction in tadpole activity, thereby reducing time spent foraging. Trout presence could also cause tadpoles to shift habitat use away from energetically favorable habitat and towards habitat that offers protection but is less energetically favorable. Lastly, in the presence of trout, a tadpole might expedite its development in an attempt to emerge out of the water and away from the trout pressure. The impacts of trout on the growth and development of tadpoles could also result in carry-over effects in the postmetamorphic stage and influence subsequent vital rates such as survival and growth.

I used field and laboratory studies to investigate various direct and indirect effects of trout on early life stage survival, growth, and development of toads. In the first chapter, I investigated the effect of trout presence on embryo survival in the field. I also analyzed trout microhabitat use to determine the probability of trout presence around the toad egg masses. I found no effect of trout presence on embryo survival but sample sizes were small. Furthermore, I found that trout are unlikely to use the areas around the egg masses. Adult toads select protective habitat to deposit their egg masses, and it is likely this careful placement of the egg masses that confers protection to the embryos.

Chapter two focuses on trout effects on tadpole and postmetamorphic life stages of boreal toads. I used laboratory experiments to test impacts of trout presence on tadpole survival,

growth, and development as well as postmetamorphic survival and growth. I found that trout exposure reduced tadpole survival by 10-20% despite the fact that only one tadpole was consumed by the trout. The likely driver behind this reduction in survival is the process of tasting by the trout. In a four hour exposure period, which occurred every day throughout the tadpoles' development, an individual tadpole would be tasted on average 0.84 times during an exposure period. I also found that exposure to trout delayed metamorphosis. I saw no carry-over effects of trout exposure on postmetamorphic survival or growth. This experiment was conducted on wild-bred and captive-bred tadpoles. I also found that captive-bred tadpoles suffered higher mortality and reduced growth in both the tadpole and postmetamorphic stages than wild-bred tadpoles.

Managing imperiled species becomes difficult when the strategies to conserve the two species conflict. I have demonstrated that the introduction of greenback cutthroat trout can reduce recruitment in boreal toad populations. This new knowledge can be used to inform conservation of toads as well as trout. A common strategy of managers for both species is to establish new populations through reintroductions. My results can be used to help managers select sites for those reintroductions. Furthermore, this better understanding of the intricate dynamics between these two species can help inform management in areas where the two currently occur together due to previous trout stocking. For example, managers could isolate trout from toad breeding habitat during the late spring and summer months

Conservation of any species requires detailed knowledge of the vital rates that drive the population dynamics of the species. Due to the reproductive biology of many species, it is often best to use female-specific parameters to understand the dynamics of a population. However, in many imperiled populations, it is difficult to obtain the quantity of data necessary to estimate those parameter estimates. This problem is exacerbated in species like the boreal toad when

females skip breeding opportunities, thereby reducing their availability for detection. My last chapter explores possible survey strategies to improve female parameter estimation. I simulated two boreal toad populations, an easily accessible one and an inaccessible population, and compared the ability of different survey designs to estimate female survival and breeding probability in each simulated population. For the accessible population, I found that any survey design that is nonrandom (i.e., surveys are conducted according to a designated schedule) and has at least four surveys during the breeding season is able to adequately estimate female parameters. However, in the inaccessible population, designs consisting of surveys conducted on consecutive days early in the season produced the best parameter estimates when compared with other survey designs.

Boreal toad conservation will require detailed knowledge about the local drivers of their decline as well as accurate and precise estimates to describe the status of the populations. Through this thesis, managers will better understand the intricate dynamics between greenback cutthroat trout and boreal toads and have a framework to ensure they are able to collect enough data to monitor their toad populations successfully.

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LIST OF KEY WORDS

Embryo survival; Microhabitat use; Occupancy analysis; Amphibian decline; Direct predatory effects; Indirect predatory effects; Tadpole survival; Tadpole growth; Tadpole development; Recruitment; Parameter optimization; Multistate open robust design; Anaxyrus boreas boreas; Oncorhynchus clarkii stomias

CHAPTER ONE:

IN SITU EXPLORATION OF THE INTERACTIONS BETWEEN INTRODUCED TROUT AND AN ALPINE TOAD

Across the world, introduced trout have negatively impacted amphibian populations. One such introduced trout, the greenback cutthroat trout (*Oncorhynchus clarkii stomias*), may be exerting negative effects on boreal toad, *Anaxyrus boreas boreas*, populations. Here I present two studies, one experimental and one observational, that investigate the potential impacts of introduced greenback cutthroat trout on boreal toad embryos. I experimentally tested whether toad embryo survival is reduced in the presence of trout and I also analyzed trout microhabitat use to determine if there is overlap between where toad egg masses are laid and the microhabitat that the trout use. I found no evidence that trout presence reduced embryo survival. In addition, my analysis revealed that trout do not use areas where toad eggs are laid but may overlap areas used by other toad life stages.

Introduction

Amphibian populations are declining worldwide. Some estimates suggest that amphibians are facing extinction rates 211 times higher than the background extinction rate (McCallum 2007) and in the United States, amphibian occupancy is decreasing at a rate of 3.7% each year (Adams et al. 2013). Several factors have been implicated in these declines: alien species, over-exploitation, land use change, global climate change, environmental contaminants, and emerging infectious diseases (Collins and Storfer 2003). One group of alien species, introduced salmonids, have negatively impacted alpine amphibians in the western United States (Bradford 1989, Knapp 2005, Pilliod et al. 2010).

Trout are often introduced for recreation as well as conservation purposes (Bahls 1992). However, trout can severely alter ecosystems into which they are introduced (Epanchin et al. 2010, Finlay and Vredenburg 2007, Knapp 2005). While trout are unlikely to feed directly on adult amphibians, they can reduce embryo and larval survival through direct predation (Bull and Marx 2002, Gillespie 2001, Pearson and Goater 2009). Trout presence may also indirectly affect early toad life stages by causing reductions in activity, habitat shifts, and alter rates of development (Currens et al. 2007, Relyea 2001, Orizaola and Brana 2005).

To date, most trout-amphibian interaction studies typically report the common outcome of reduced amphibian distribution and abundance (i.e., Bradford et al. 1998, Knapp 2005, Welsh et al. 2006). Unfortunately, few studies have attempted to experimentally investigate the potential direct or indirect mechanisms by which the trout impact different life stages (but see Tyler et al. 1998, Kiesecker et al. 2001, Vredenburg 2004, Pearson and Goater 2009). Given the many ways trout impact amphibian populations, knowledge of the mechanisms by which trout negatively affect amphibian life stages may assist managers tasked with conserving declining populations.

Spruce Lake in Rocky Mountain National Park (RMNP) is one of many historically fishless alpine lakes that now contain introduced trout (Bahls 1992). It is home to one of the few remaining boreal toad (*Anaxyrus boreas boreas*) breeding populations in the park. Research that began in 2001 revealed high annual adult survival (>0.90) but virtually no recruitment, resulting in a population decline of 5% annually (Muths and Scherer 2011). In 20 years of monitoring at Spruce Lake (1991-2010) only one postmetamorphosis individual was documented (Muths and Scherer 2011). Unlike many populations of boreal toads in the Southern Rocky Mountains, Spruce Lake has a very low prevalence of the amphibian chytrid fungus, *Batrachochytrium*

dendrobatidis (Muths et al. 2003, Muths and Scherer 2011). Thus, another factor is apparently responsible for population decline of boreal toad in Spruce Lake.

It is plausible that the introduced trout in Spruce Lake are responsible for the low recruitment in the toad population via predation or other indirect effects. Here I use both experimental and observational studies to determine if trout are impacting one aspect of toad recruitment, embryo survival. I estimated embryo survival in both trout and trout-free habitats using a manipulative field experiment. In addition, I conducted a trout microhabitat use study to determine the probability of trout occurrence in the areas where the toads deposit their eggs. My aim was to determine if trout share microhabitats with toad embryos and test the possible effects of trout presence on embryo survival.

Methods

Species Description

The boreal toad is an alpine species that ranges across Utah, Wyoming, Colorado, Idaho, and Nevada, US. Their breeding ponds, summer range, and overwinter refugia, all occur in higher elevation lodgepole pine or spruce-fir forests from 2,100 to 3,600 meters (Campbell 1970). While males occasionally skip breeding opportunities, they usually return to the breeding area every year (Muths et al. 2006). In contrast, females of reproductive age subsequently skip one or more breeding opportunities (Muths et al. 2010). Breeding occurs in the spring after iceoff in shallow margins of wetlands, and eggs and tadpoles develop throughout the summer months to metamorphose and emerge before the area freezes again. The Southern Rocky Mountain (SRM) population of boreal toads is declining (Carey 1993, Scherer et al. 2005, Muths and Scherer 2011) and is currently "warranted but precluded" from listing under the Endangered

Species Act. A listing decision by the U.S. Fish and Wildlife Service is slated for 2017 (U.S. Fish and Wildlife Service 2012).

Study Areas

We sampled two boreal toad breeding areas in RMNP: Spruce Lake and a wetland complex near Fay Lakes (Figure 1.1). In 1991, as part of recovery efforts, greenback cutthroat trout, *Oncorhynchus clarkii stomias*, were introduced to Spruce Lake to bolster populations of this federally threatened species (U.S. Fish and Wildlife Service 1998). Due to natural downstream barriers, Spruce Lake offered introduced greenback cutthroat trout a habitat free from competition and hybridization from other non-native trout species and the cutthroat population has thrived. As such, this lake served as my "trout" site. The breeding area near Fay Lakes (Fay Lakes) was recently discovered in 2003 and, like Spruce Lake, is one of the few remaining boreal toad breeding areas in the park. It is a shallow, seasonal wetland and is, therefore, free from trout. This site served as my "control" site.

Embryo Survival

Embryo survival studies required manipulation of wild egg masses in control and trout treatment sites. To accomplish this, arrival of adult toads at the breeding areas was monitored closely in late spring, 2013 and 2014. Once egg masses were laid, I divided each into two approximately equal halves. Each half was gently placed over a white background board and photographed. I used these photographs and the cell counter function in ImageJ to census the number of eggs in each half (Rasband 2009).

One randomly selected egg mass half was caged and the other was left exposed. Mesh cages measuring 40x40x15 cm were constructed of polyvinyl chloride pipe (PVC) that were filled with sand and sealed with aquarium-safe silicone sealant (Figure 1.2). Screen material was

stitched to the frame so that five sides of the cages were enclosed in mesh while the bottom remained open to the substrate. If the cage did not contact the substrate such that fish were confidently excluded, plastic sheeting was added to create a skirt that was covered with gravel, mud, and rocks. The exposed half of the egg mass was not caged and in Spruce Lake remained accessible to predation or disturbance by trout. I repeated this experimental set up at the control site for all egg masses.

The embryos were monitored at least twice per day during embryo development. When individuals from an egg mass hatched (Gosner stage 25), a temporary removal method was used to estimate the number of hatched tadpoles. I used a dip net (12 x 9.5 cm) to sweep the area occupied by recently hatched tadpoles (usually about 1.5 x 1.5 m), being careful to not disturb or disperse undetected tadpoles. I counted the number of tadpoles in the net after each sweep and placed them in a separate enclosure. I continued removals, maintaining constant effort (i.e. area covered in one sweep), until less than ten individuals were captured in successive net sweeps. Sampling was similar for the caged halves, but I simply cut the mesh off the top of the cage and sampled within the cage. Upon completion of the sampling, tadpoles were returned to the original area of the egg mass. This temporary removal sampling design was employed for all egg masses in the experiment.

Trout Microhabitat Use

During embryo development, I collected microhabitat use data for greenback cutthroat trout near, but not including, the area where the egg masses were deposited. This area was divided into four strata of increasing distance from the edge of the shore (0-3, 3-6, 6-9, and 9-12 m). Sampling plots (2 x 2 m) were randomly chosen within each stratum. A ruler was also placed in each plot for reference when estimating the length of trout observed in the plot. Plots were

established in the morning or evening and then surveyed the following evening or morning, respectively (after a minimum of 8 hours) when the trout are most likely to be active and feeding (Cuenca and de la Higuera 1994, Sánchez-Vázquez and Tabata 1998). I conducted multiple (2-3) 5-minute surveys from 4-7 m away with at least 15 minutes between surveys to ensure independence of the surveys. Eight new plots were surveyed each day, 4 in the morning and 4 in the evening until the egg masses hatched. Because smaller trout likely use different microhabitats than larger trout, the lengths of the observed trout were estimated using the ruler placed in each plot. Once all surveys were completed in a morning or evening, covariates including depth, vegetation density (percent of the plot with vascular plant matter), temperature, dissolved oxygen, and a qualitative measure of connectivity to the rest of the lake (range: 0 = completely isolated by islands, 5 = completely open to the body of the lake) were recorded for the plot (Table 1.1). These same covariates were also measured at the egg mass locations so habitat could be compared to that used by trout.

Analysis

We used robust design closed capture models to estimate embryo survival probability for each egg mass half (Pollock et al. 1990, Kendall and Nichols 1995). I considered each egg mass half a "population" with the known number of animals "released" during the first primary period based on photograph census counts. The second primary period consisted of the count of tadpoles during each removal sweep (for an example of the data, see Appendix 1.). To account for the removal design, I set recapture probabilities (c) to zero in the second primary period. Embryo survival estimates represent the probability that an egg survived through hatching, i.e., the probability that an individual egg 'released' during the first primary period survived to the

second primary period. Analysis was conducted using Program MARK (White and Burnham 1999).

To control for background heterogeneity in survival between egg masses I calculated the proportional difference in survival probability between the caged and exposed halves,

$$Prop.Diff = \frac{\hat{S}_{caged} - \hat{S}_{exposed}}{\hat{S}_{caged}}. \qquad \text{Eqn (1)}$$

This allowed us to analyze the proportional reduction in embryo survival that resulted from exposure. I used the delta method to calculate the variance associated with the proportional difference:

$$var(Prop.Diff) = \frac{var(\hat{S}_{exposed})}{\hat{S}_{caged}^{\,2}} + \frac{var(\hat{S}_{caged}) * \hat{S}_{exposed}^{\,2}}{\hat{S}_{caged}^{\,4}}. \qquad \text{Eqn (2)}$$

We used Program Contrast (Hines and Sauer 1989) to compare the proportional differences from the trout site and the control site to determine if the presence of trout influenced embryo survival. Program Contrast is useful because it tests specific hypotheses about estimates while accounting for their variances and covariances using the methods described by Sauer and Williams (1989). I tested and confirmed the assumption that my estimates of proportional differences were normally distributed.

We used single season occupancy models in Program MARK to model trout microhabitat use (White and Burnham 1999, MacKenzie et al. 2005). These models contain two parameters: ψ, the probability the plot was used by trout during the survey period and p, the probability of detecting a trout in the plot during a 5-minute survey, given the plot was used. I expected that microhabitat use might be different for different sized trout, so I grouped the observations by size class according to body length. I did not detect any 11-17 cm trout, thus my data had a natural

break which I used to split the size classes. I categorized trout <11 cm as "small" and those >17 cm as "large", with the smallest trout being 5 cm and the largest 23 cm. I hypothesized that the effect of depth and vegetation density on the probability of use would be different for large and small trout, so I modeled interactions between length class and these covariates. I tested for correlation among my microhabitat variables to reduce the number of covariates.

To create my occupancy models, I first determined the most parsimonious structure for detection probability, *p*. The structures I considered were: constant, trout length dependent, vegetation density dependent, and an additive structure with length and vegetation density. Using the most parameterized occupancy structure, I constructed models using these four structures for *p* and ranked the models using AIC_c to determine the best detection structure. Retaining the best detection structure, I fit all combinations of covariates for occupancy probability including: univariate models for all uncorrelated covariates, additive effects of length and covariates, and interactions for length and depth and length and vegetation density (length*depth; length*vegetation density; Doherty et al. 2012). Models were ranked using AIC_c. I set my effective sample size as the number of sampling plots to avoid overinflating my sample size. Using the best structure for occupancy (ψ) I repeated the selection process for my four detection structures to confirm that the best *p* structure was still supported with my top occupancy structure.

I used the top ranked model and the microhabitat covariates I measured near the egg masses to predict the probability of trout habitat use near the egg masses. Water temperature and dissolved oxygen were measured twice daily, once in the morning and once in the evening, and because these metrics vary, I averaged the measurements for each period and used these averages to predict trout use in the morning as well as the evening.

Results

Embryo Survival

We discovered ten egg masses from the two sites over the two years: six at the control site and four at the trout site. It typically took an egg mass 5-9 days from discovery to hatching (Gosner stage 25). Some of the egg masses were discovered as they were being deposited while others were discovered after deposition (about 1-3 days after), making it difficult to assess development times. Embryo survival for both caged and exposed halves was much higher at the trout site (range: 0.13-0.72) than the control site (range: 0.01-0.40; Table 1.2). This was primarily due to an outbreak of the water mold *Saprolegnia ferax* at the control site in 2013. Survival probabilities observed for the control site during 2014 were within the range of those found at the trout site (Table 1.2). Despite this, I found no difference in proportional survival between the trout and the control site ($\chi^2 = 1.68$, df = 1 p-value= 0.19). Therefore exposed eggs (not caged) at the trout site did not show a greater decreases in embryo survival than those at the control site.

Trout Microhabitat Use

We surveyed 85 plots near the toad breeding area at the trout site. There was a high degree of correlation between the covariates of water depth, strata, and connectivity (Pearson's correlation coefficients: 0.72, depth and strata; 0.77, connectivity and depth; and 0.92 strata and connectivity). Based on highly correlated data, strata and connectivity were excluded from the analysis leaving just water depth.

Model selection results suggested that detection probability was constant across plots and size classes (w = 0.45). This remained the best structure for detection probability when I reiterated the selection process with the most supported model for occupancy. I fit 105 models to

the data and, despite considerable model uncertainty, trout use was most influenced by trout length class, depth, and vegetation density (Table 1.3). The top ranked model consisted of additive effects of these variables and temperature (w = 0.10, Table 1.4). This top model suggested trout use increased with increasing depth (Figure 1.3), decreased with increasing vegetation density (Figure 1.4), and increased with increasing temperature (Figure 1.5).

To account for model uncertainty, I model-averaged the estimates of detection probability and habitat use in an average plot for both trout length classes. These estimates indicate that larger trout were more likely to use the sampled areas than smaller trout ($\hat{\psi}_{large}$= 0.15, SE = 0.065; $\hat{\psi}_{small}$ = 0.024, SE = 0.016). The model-averaged detection probability was $\hat{p} = 0.23$ (SE = 0.064).

Predicted Trout Use Around the Egg Mass Habitats

We collected microhabitat covariate data for three egg mass locations at Spruce Lake: one in 2013 and two in 2014. A third egg mass was deposited at Spruce Lake in 2014 but due to its late deposition, I was logistically unable to collect microhabitat covariates for this egg mass. Predicted trout microhabitat use was very low for both length classes in the vicinity of the three egg masses (range = 0.00011 – 0.015) (Table 1.5).

Discussion

We found little evidence that trout influence survival of toad embryos. Comparisons of proportional differences in embryo survival showed no difference between the trout and control sites. Furthermore, my microhabitat use study suggested that trout are unlikely to use habitat near the egg masses. Predicted use of the areas near the egg masses was an order of magnitude lower than the average predicted use of the entire sampled area. The predicted use around the egg masses represents only a snapshot in time (15 minutes) and the probability that a trout could be

near an egg mass becomes greater if one considers a 14 hour period of daylight. The habitat use estimates also represent a snapshot in space, and in order to reach an egg mass, a trout must pass through a matrix of shallow water and high vegetation density similar to the habitat at the egg mass locations. The microhabitat in this matrix would have low predicted trout use, serving as a barrier to trout movement towards the egg masses.

Embryo survival was much lower at my control site, Fay Lakes, in 2013. The lower survival was likely due to a stochastic disease event. During that breeding season, all of the egg masses at Fay Lakes became infected with the water mold *Saprolegnia ferax*, which can reduce boreal toad embryo survival (Blaustein et al. 1994). Mold infestations reduced survival of both the caged and exposed embryos. I attempted to account for this event by comparing the proportional differences in embryo survival rather than the unadjusted differences. Given that five of six control egg masses were exposed to the water mold in 2013, this event influenced my results. Reduced embryo survival at Fay Lakes in 2013 could have diminished any possible effects from the cages independent of trout effects (e.g., warmer water due to the dark mesh of the caging structures). As a result, the cage effects at Spruce Lake that resulted in higher survival might not have been reflected in the caged embryos at Fay Lakes. In addition, low embryo survival at Fay Lakes produced highly variable proportional differences, which is evident by the fact that the largest proportional differences (both positive and negative) are from this site in 2013.

Though I was limited to few egg masses, my results suggest that embryo survival was comparable at the trout and control sites when not affected by water mold. Adjusting for lower embryo survival for egg masses affected by the mold, I also found that proportional survival differences between caged and exposed eggs were similar between the two study areas.

Moreover, trout are unlikely to use areas near the toad egg masses, and, thus, are unlikely to influence embryo survival. Any negative impacts of introduced trout on boreal toad recruitment are unlikely to occur during the toad's embryonic stage. While the toads are unpalatable to trout at all life stages (Licht 1968, Brodie and Formanowicz 1987), selection of egg mass placement sites by breeding adult toads appears to convey additional protection for the developing embryos at Spruce Lake. However, this pattern might not hold at all boreal toad breeding sites. Breeding sites with less diversity in microhabitat structure offer fewer options for adult toads to deposit fertilized eggs in a protective microhabitat. If these sites also contain introduced trout, the egg masses might be subjected to physical disturbance by the trout, direct predation, or exploratory gustation of the egg masses to determine palatability. Impacts such as these could have the potential to reduce embryo survival.

Once the embryos hatch, the tadpoles disperse away from egg deposition sites to areas with higher trout use (W. Lanier personal observation). In these areas, tadpoles are vulnerable to physical disturbance and stress resulting from repeated gustation by the trout (see Chapter 2). This repeated gustation can reduce tadpole survival (Chapter 2). Furthermore, trout exposure might delay metamorphosis and limit the time that recently metamorphosed individuals have to prepare to overwinter (Chapter 2). Both of these effects of trout on the tadpole stage could explain the low recruitment at Spruce Lake.

While my results from this field study do not explain the low recruitment in the boreal toad population at Spruce Lake, they do serve as an example of research aimed at understanding the mechanisms by which introduced salmonid species can impact amphibian populations. To date, the body of literature focused on the interactions between introduced salmonids and amphibians is lacking this kind of research. Furthermore, the research I present here is unique in

that it pairs an experimental study of the potential prey with an observational study of the potential predator. I quantified the likelihood that trout occurred in areas commonly used for egg deposition and examined the potential impact of trout on embryo survival. While I found no evidence that introduced trout negatively impact toad embryos, my study did suggest that interactions between trout and subsequent aquatic life stages of the toads are possible. Thus, my study revealed a better understanding of the system and should focus future studies to inform management for both boreal toads and greenback cutthroat trout.

Table 1.1. Abbreviations, descriptions, and the observed ranged for each microhabitat covariate collected at plots (2 x 2 m) sampled to estimate greenback cutthroat trout microhabitat use around a boreal toad breeding area in Rocky Mountain National Park.

Microhabitat Covariate	Abbreviation	Variable Type	Range or Categories	Description
Water depth	Depth	Continuous	0.15-0.55 m	Water depth
Vegetation Density	Veg	Continuous	0-0.95	Percent of plot vegetation
Temperature	Temp	Continuous	7.6-20.7°C	Water temperature taken at the center of the plot in the middle of the water column
Dissolved Oxygen	DO	Continuous	2.42-9.21 mg/L	Dissolved oxygen concentration taken in the center of the plot in the middle of the water column
Connectivity	Conn	Continuous	0-5	Qualitative measure of how connected the plot was to the main body of the lake
Strata	Strata	Continuous	1-4	Predefined strata of increasing distance from shore in which the sampling plots were placed.
Time of Day	Time	Categorical	morning or evening	Crepuscular period in which the surveys took place. 1 = AM, 0 = PM

Table 1.2. Estimates of boreal toad embryo survival and standard errors (in parentheses) for egg mass halves that were caged and those that were exposed to greenback cutthroat trout presence. There were ten egg masses found at the control and trout sites in Rocky Mountain National Park in 2013 and 2014. The proportional difference, defined as the difference in survival probability between the caged and exposed halves divided by the caged survival probability, and associated variance are also given.

Site and Year		Egg Mass	Caged	Exposed	Proportional Difference	Proportional Difference Variance
Control Site	2013	1	0.01 (0.0021)	0.02 (0.0024)	-0.47	0.083
		2	0.12 (0.0050)	0.14 (0.0069)	-0.17	0.0052
		3	0.08 (0.016)	0.04 (0.0041)	0.44	0.016
		4	0.05 (0.0038)	0.02 (0.0023)	0.58	0.0030
		5	0.08 (0.0058)	0.01 (0.0020)	0.86	0.00072
	2014	6	0.40 (0.0060)	0.16 (0.0038)	0.61	0.00010
Trout Site	2013	7	0.34 (0.012)	0.29 (0.012)	0.13	0.0020
	2014	8	0.38 (0.017)	0.13 (0.011)	0.66	0.0012
		9	0.72 (0.038)	0.52 (0.036)	0.28	0.0039
		10	0.32 (0.0077)	0.17 (0.0061)	0.47	0.00054

Table 1.3. Cumulative weights for the covariates used in the analysis of trout microhabitat use around a boreal toad breeding area in Rocky Mountain National Park. The cumulative weight is the sum of the weights of all the models that contain a given covariate.

Covariate	Cumulative *w*
Length	1.00
Depth	0.99
Veg	1.00
Temp	0.50
DO	0.28
Time	0.31

Table 1.4. Model selection results for the analysis of greenback cutthroat trout microhabitat use around a boreal toad breeding area in Rocky Mountain National Park. I only present the top models ($w > 0.01$) of the 105 models that were fit to the trout microhabitat use data. Interactions between trout length classes (large and small) and vegetation density or depth are indicated with a star (*) and separated within parentheses, additive effects are indicated with a plus sign (+). Detection probability was constant for all these models, *p(.)*. The columns present the model notation, Akaike's information criterion values adjusted for sample size (AIC_c), the difference between the model's AICc value and that of the top model (ΔAIC_c), AIC_c weights (w), number of parameters (K), and the deviance of the model.

Model	AIC_c	ΔAIC_c	w	K	Deviance
Length+Depth+Veg+Temp	314.41	0.00	0.10	6	301.33
Length+Depth+Veg	314.44	0.03	0.09	5	303.68
(Length*Veg)+Depth+Temp	315.10	0.69	0.07	7	299.64
(Length*Depth)+Veg+Temp	315.13	0.72	0.07	7	299.68
Length+Depth+Veg+Time	315.37	0.96	0.06	6	302.29
(Length*Veg)+Depth	315.63	1.22	0.05	6	302.55
Length+Depth+Veg+DO	315.75	1.34	0.05	6	302.67
(Length*Depth)+Veg	315.96	1.55	0.04	6	302.88
(Length*Veg)+(Length*Depth)+Temp	316.51	2.10	0.03	8	298.61
(Length*Veg)+Depth+Time	316.51	2.10	0.03	7	301.06
Length+Depth+Veg+Time+Temp	316.56	2.15	0.03	7	301.11
Length+Depth+Veg+DO+Temp	316.65	2.24	0.03	7	301.19
(Length*Depth)+Veg+Time	316.75	2.34	0.03	7	301.30
(Length*Depth)+Veg+Time+Temp	316.91	2.50	0.03	8	299.01
(Length*Veg)+Depth+Time+Temp	316.98	2.57	0.03	8	299.08
(Length*Veg)+Depth+DO	317.10	2.69	0.02	7	301.64
(Length*Depth)+Veg+DO	317.39	2.98	0.02	7	301.94
(Length*Veg)+Depth+DO+Temp	317.50	3.09	0.02	8	299.61
Length+Depth+Veg+Time+DO	317.51	3.09	0.02	7	302.05
(Length*Depth)+Veg+DO+Temp	317.52	3.11	0.02	8	299.63
(Length*Veg)+(Length*Depth)	317.64	3.23	0.02	7	302.19
(Length*Veg)+(Length*Depth)+Time+Temp	318.00	3.59	0.02	9	297.60

Table 1.5. The predicted microhabitat use for the large and small greenback cutthroat trout and the associated standard errors in parentheses for the areas where boreal toad egg masses were laid. These predictions were calculated by plugging in microhabitat covariate values measured at each egg mass into the model: $Logit(\psi) = \beta_0 + \beta_1(Size) + \beta_2(depth) + \beta_3(veg) + \beta_4(temp)$, assuming constant detection probability. The morning and evening estimates differed only in the temperature covariate used in their estimation.

		Microhabitat Use	
		Small	Large
2013 Egg Mass 1	morning	0.00011 (0.00019)	0.0011 (0.0015)
	evening	0.00049 (0.00068)	0.0049 (0.0052)
2014 Egg Mass 1	morning	0.00076 (0.0010)	0.0076 (0.0072)
	evening	0.0015 (0.0016)	0.015 (0.011)
2014 Egg Mass 2	morning	0.00030 (0.00052)	0.0030 (0.0039)
	evening	0.0012 (0.0015)	0.012 (0.011)

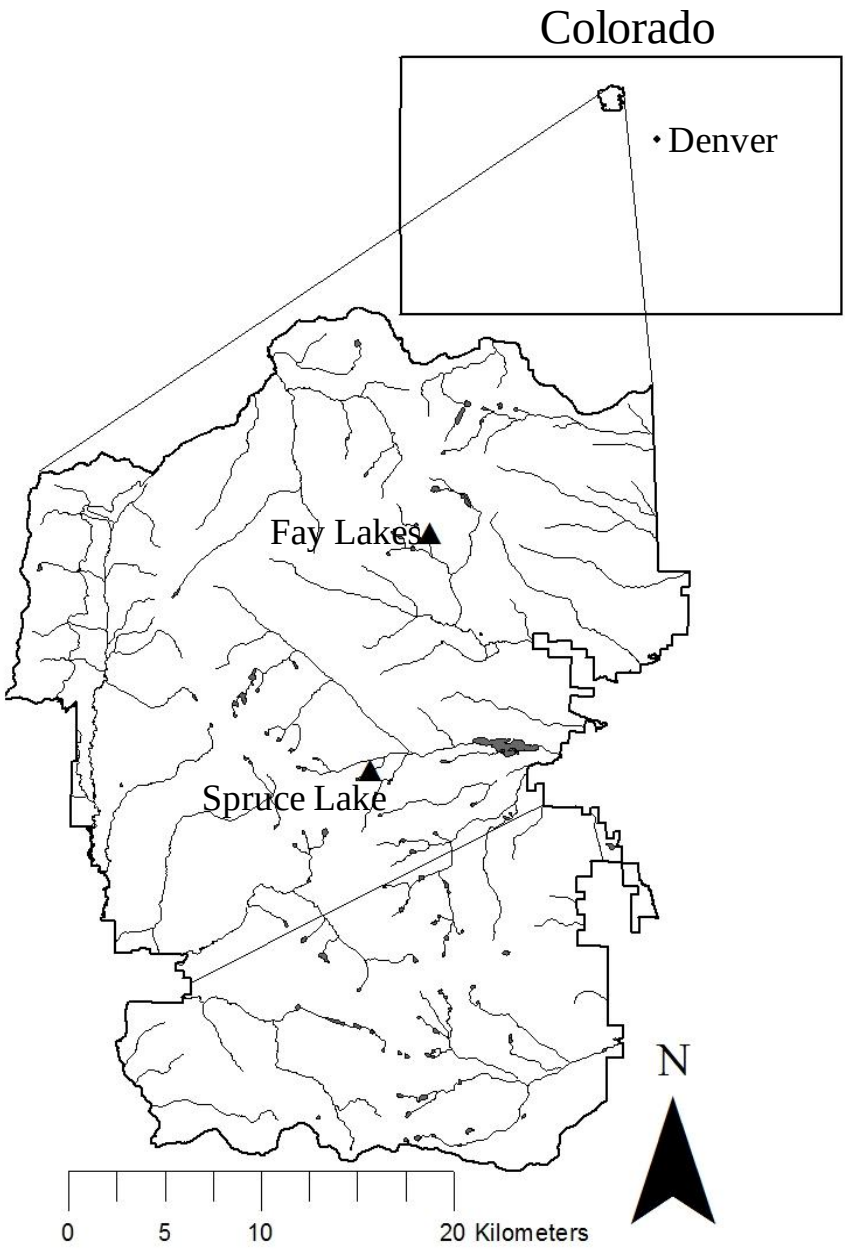

Figure 1.1. Map of Rocky Mountain National Park and its major water bodies. The two study sites, Spruce Lake and Fay Lakes, are denoted by black triangles.

Figure 1.2. Photograph of two caged boreal toad egg mass halves at the trout site, Spruce Lake. The paired exposed egg mass halves are not visible but located directly adjacent to each cage.

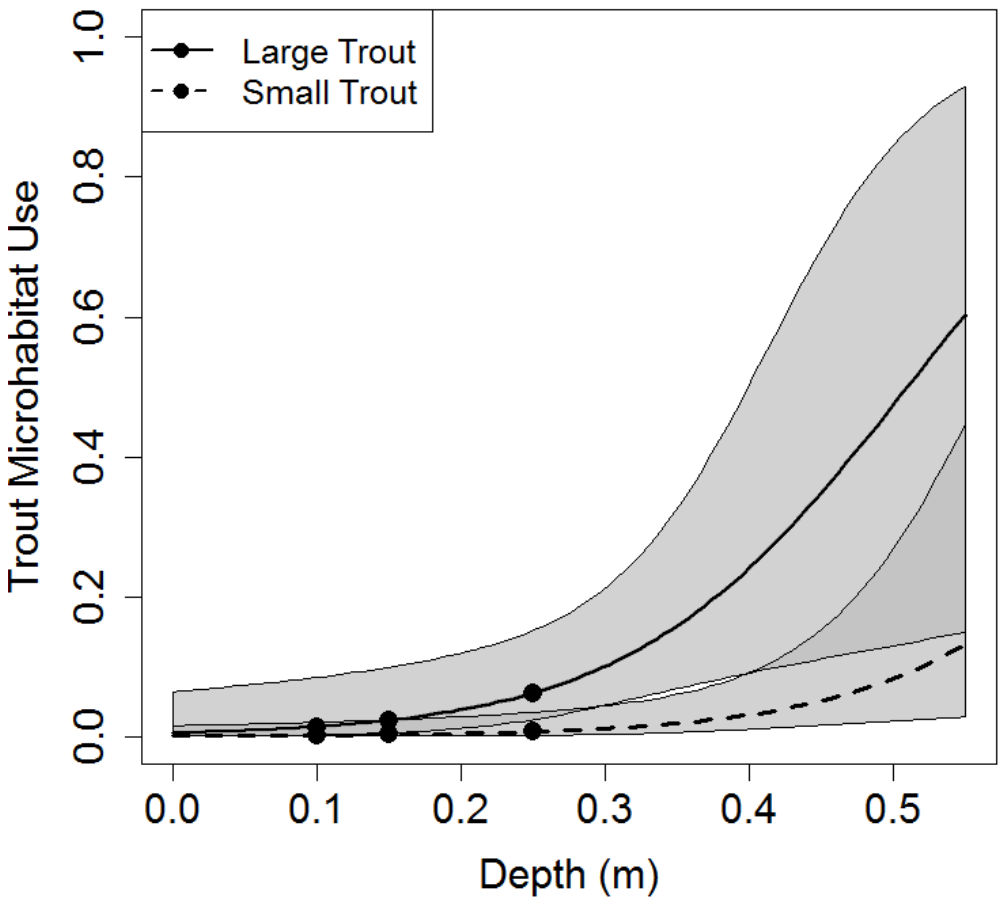

Figure 1.3. Relationship of estimated greenback cutthroat trout microhabitat use as a function of depth using the highest weighted occupancy model, ψ(size+depth+veg+temp), *p*(.), and the average values of vegetation density and temperature. The predicted use of the small length class of trout is designated by the dashed line and the solid line represents the predicted use of the large length class. Shaded areas represent 95% confidence intervals for the respective fitted lines. The circles indicate the depth and predicted use at the three egg mass locations.

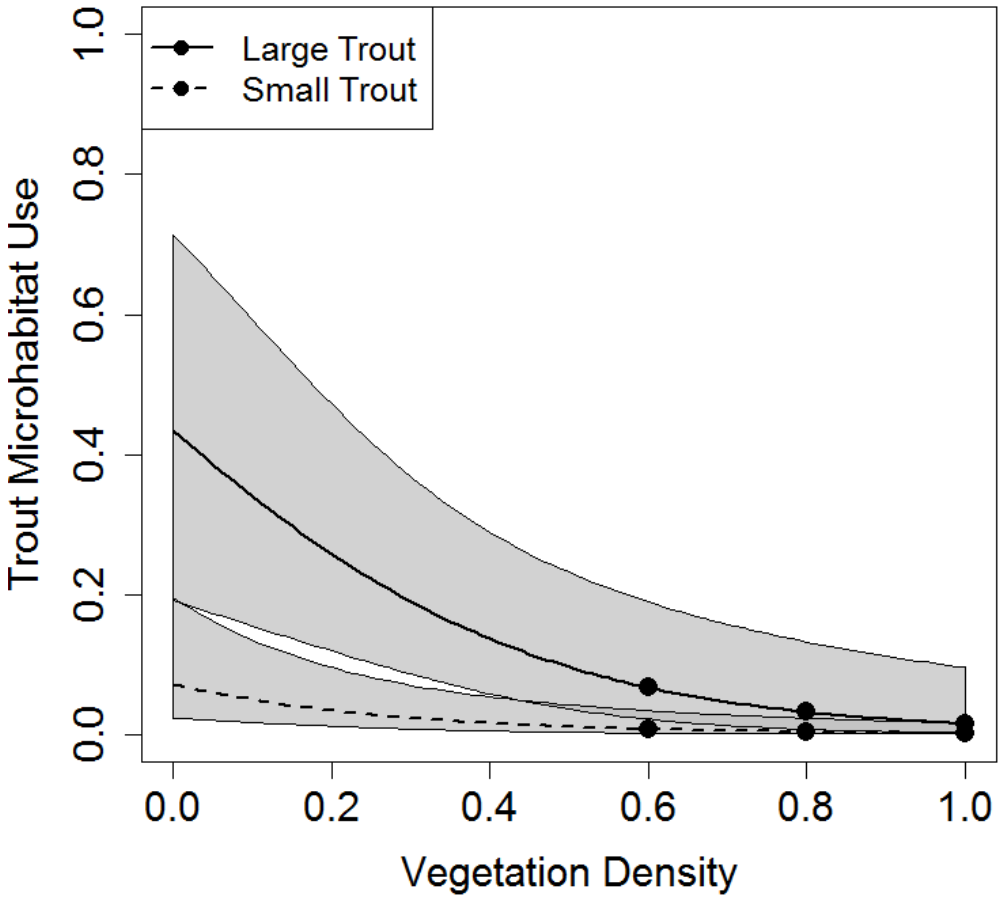

Figure 1.4. Relationship of estimated greenback cutthroat trout microhabitat use as a function of vegetation density using the highest weighted occupancy model, ψ(size+depth+veg+temp), *p*(.), and the average values of vegetation density and temperature. The predicted use of the small length class of trout is designated by the dashed line and the solid line represents the predicted use of the large length class. Shaded areas represent 95% confidence intervals for the respective fitted lines. The circles indicate the vegetation density and predicted use at the three egg mass locations.

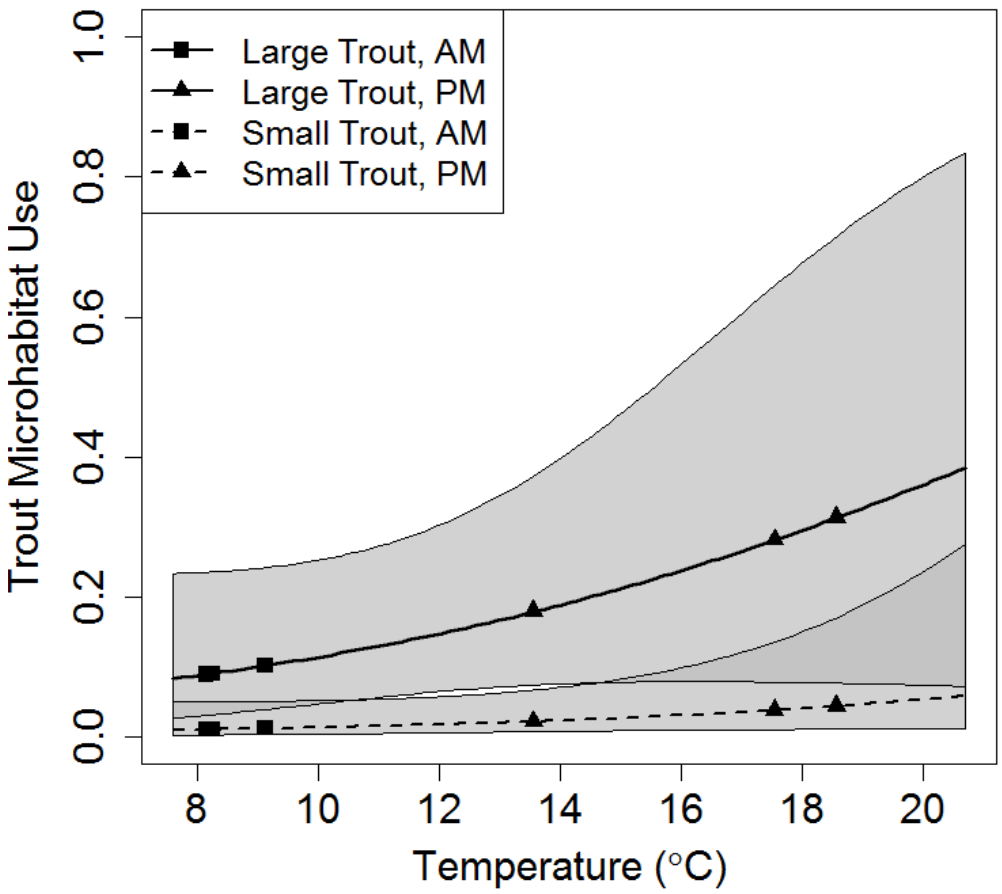

Figure 1.5. Relationship of estimated greenback cutthroat trout microhabitat use as a function of temperature using the highest weighted occupancy model, ψ(size+depth+veg+temp), *p*(.), and the average values of vegetation density and temperature. The predicted use of the small length class of trout is designated by the dashed line and the solid line represents the predicted use of the large length class. Shaded areas represent 95% confidence intervals for the respective fitted lines. The squares indicate the average morning temperatures and predicted use at the three egg mass locations. The triangles represent the same for the average evening temperatures.

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CHAPTER TWO:

NEGATIVE IMPACTS OF TROUT EXPOSURE ON AN UNPALATABLE TOAD

Here, I present experimental research investigating the direct and indirect effects of hatchery reared greenback cutthroat trout on boreal toad tadpole survival, growth, and development as well as post metamorphosis survival and growth. I also explored differences among individuals hatched from captive-bred or wild eggs. I found that exposure to trout reduced tadpole survival by 10-20%, despite the fact that only one tadpole was consumed. Trout exposure also delayed metamorphosis by 1-2.5 days. Additionally, I found that captive-bred individuals had lower tadpole survival, reduced tadpole growth, and greater loss in body condition following metamorphosis regardless of whether they were exposed to trout or not.

Boreal toads, like many bufonids, are unpalatable during all life stages. However, my results suggest that hatchery reared trout do not innately avoid boreal toads; instead they must taste the tadpoles to determine prey palatability. This repeated gustation likely caused the low survival probabilities that I observed. My results shift the current understanding of the interactions between introduced trout and unpalatable amphibian species by illustrating the non-consumptive negative impacts of these fish, indicating that effective conservation of boreal toads requires habitat free from introduced trout.

Introduction

Amphibian populations are experiencing declines worldwide (Stuart et al. 2004, Bishop et al. 2012, Adams et al. 2013) with some estimates indicating that amphibians are currently facing extinction rates 211 times the background rate (McCallum 2007). Collins and Storfer (2003) detail six hypotheses explaining global amphibian declines: over-exploitation, land use

change, global climate change, environmental contaminants, emerging infectious diseases, and alien species. Introduced trout are one such alien species that are commonly released into alpine ecosystems across the United States (Bahls 1992) and are known to negatively impact palatable amphibian species (Knapp 2005, Welsh et al. 2006, Pilliod et al. 2010). However, bufonid eggs and tadpoles contain bufotoxins, making them unpalatable to many vertebrate predators, including trout (Licht 1968, Kats et al. 1988, Crossland and Alford 2006). Still, bufonids are declining more rapidly than other amphibian families (Stuart et al. 2004), and while their declines are primarily caused by disease (Muths et al. 2003), there are cases where disease prevalence is low yet toad populations continue to decline (Muths and Scherer 2011). These cases prompt a more detailed investigation into the direct and indirect effects of introduced fish on this important group of amphibians.

Trout can severely alter ecosystems into which they are introduced (Epanchin et al. 2010; Finlay and Vredenburg 2007; Knapp 2005). While trout are unlikely to feed directly on adult amphibians, they can reduce embryo and larval survival through direct predation (Bull and Marx 2002; Gillespie 2001; Pearson and Goater 2009). Trout presence may also indirectly affect tadpole growth, development, and survival probability (Currens et al. 2007). For example, predator presence has been shown to reduce the activity of anuran tadpoles, causing them to spend more time hiding and less time foraging, thereby hindering development, growth, and survival (Lawler 1989, Relyea 2001). Predator presence can also impact the rate of development in larval amphibians (Orizaola and Brana 2005). These indirect effects could impact tadpole survival but also size and time to metamorphosis, which are thought to influence post-metamorphic vital rates (Smith 1987, Semlitsch et al. 1988, Goater 1994).

Often the toxicity of a species when consumed or engulfed by a predator is assumed to offer immunity from predators (Welsh et al. 2006). Bufonids have been found to be largely immune to the effects of predatory fish due to their bufotoxins (Bull and Marx 2002; Knapp 2005; Welsh et al. 2006), but some fish, including trout, must taste prey to determine if it is palatable (Lawler and Hero 1997, Grasso et al. 2010). Tadpoles can be engulfed and rejected by trout many times during their development, for example, Grasso et al. (2010) wrote "During one [one hour] observational trail, five tadpoles of [*Anaxyrus*] *canorus* were engulfed and rejected over 111 times". This gustation has the potential to directly reduce tadpole survival.

In this chapter I present work that explores the potential direct and indirect effects of introduced greenback cutthroat trout (*Oncorhynchus clarkii stomias*) on wild boreal toad (*Anaxyrus boreas boreas*) tadpoles as well as for tadpoles from egg masses produced by captive-bred toads. I performed a laboratory experiment to test the effects of trout exposure on the survival, growth and development of the tadpoles, as well as residual effects of trout presence on the survival and growth of post-metamorphic individuals. I present evidence for non-consumptive negative effects of these trout on boreal toad recruitment. This new evidence has management implications for both boreal toads and greenback cutthroat trout and suggests that effective conservation of boreal toads requires isolation from introduced trout.

Methods

Study Species

The Southern Rocky Mountain population of boreal toads once ranged through the mountains of Wyoming, Colorado, and New Mexico but is now in decline (Carey 1993, Scherer et al. 2005, Muths and Scherer 2011). Much of the decline can be attributed to the chytrid fungus *Batrachochytrium dendrobatidis* (Muths et al. 2003, Carey et al. 2005); however, this pathogen

cannot explain declines for all populations of boreal toads. For example, Spruce Lake in Rocky Mountain National Park supports one of the few remaining breeding populations in the park and has a very low prevalence of the fungus. Yet this population declined ~ 5% annually during the period 2001-2010 (Muths and Scherer 2011) despite high annual adult survival probabilities (>0.90 in all years). Per capita recruitment rates were extremely low (<0.05), substantially lower than values reported for other boreal toad populations (Muths et al. 2011). While lack of recruitment is believed to be the major contributor to continued boreal toad population decline at Spruce Lake (Muths and Scherer 2011), the reason for such low recruitment is unknown.

Spruce Lake is one of many historically fishless alpine lakes of the western United States that have been stocked with nonnative salmonids (Bahls 1992, Knapp et al. 2001). In 1991, as part of recovery efforts, greenback cutthroat trout were introduced to Spruce Lake to bolster populations of this federally listed species (threatened status). Due to natural downstream barriers, Spruce Lake offered the introduced greenback cutthroat trout a habitat free from competition and hybridization from other non-native trout species and the cutthroat population has thrived.

Tadpole-Trout Experiment

We obtained three hundred boreal toad tadpoles (Gosner stages 26-30, 18-29 mm) from the Colorado Parks and Wildlife Fish Research Hatchery (Bellvue, CO). These individuals were from egg masses collected from wild breeding populations. The wild-bred egg masses were brought into a captive rearing facility to develop until the time I received them. I randomly selected 108 individuals and assigned six to each of 18 tanks (9.5 L, Figure 2.1a). An additional one hundred and fifty boreal toad tadpoles (Gosner stages 19-20, 7-9 mm) were obtained from the Colorado Parks and Wildlife Native Aquatic Species Restoration Facility (Alamosa, CO).

These individuals were from egg masses produced by breeding captive adults and were considered captive-bred in my experiment. Similarly, I randomly selected 108 of these individuals and assigned them in groups of six to 18 tanks. I randomly assigned each tank as a treatment (trout exposure) or control, with twice as many treatment groups as controls yielding 24 groups assigned to the trout exposure treatment (12 with captive-bred tadpoles and 12 with wild-bred tadpoles) and 12 groups assigned as controls (6 with captive-bred tadpoles and 6 with wild-bred tadpoles). The tanks containing the groups were randomly placed within one of four troughs that allowed water to continuously flow around the tanks to regulate temperature (average temperature 17.7^{o} C). Tadpoles were fed a combination of Hakari Algae Wafers and frozen vegetable cubes ad libitum (Scherff-Norris et al. 2002).

Hatchery reared greenback cutthroat trout (87-177 mm) were obtained from Colorado Parks and Wildlife Fish Research Hatchery (Bellvue, CO). The genetics of cutthroat trout subspecies in Colorado are currently under question (Metcalf et al. 2007, Metcalf et al. 2012). To maintain as much realism as possible, I used the "greenback strain" trout that have been stocked into alpine lakes in Colorado. Trout were obtained several weeks prior to the experiment and held in large circular holding tanks (900 L) with a continuous flow of water. During this acclimation period, the trout were fed #2-4 Trout Diet (manufacturer Rangen) at ~4% of their body weight via automatic feeders; water temperature was 16.4^{o} C and flow though tanks (~ 5 L/min) maintained high water quality. Trout were also introduced to live prey by feeding earthworms and boreal chorus frog (*Pseudacris maculata*) tadpoles ad libitum. The boreal chorus frog is a palatable amphibian species that is often found in the same habitats as boreal toads and was readily consumed by trout. Once the experiment began, the total amount of pelletized food

offered to the trout remained the same, however, it was delivered in two discrete feedings every evening.

In a separate laboratory area, 36 experimental tanks (19 L) were placed in two rectangular troughs, these also were flow-through tanks (water replaced >8 times per day), where the trout exposures (treatments) took place (Figure 2.1b). I wrapped each tank in white plastic sheeting to eliminate visual cues between control and treatment groups. Every day, before the trout were fed, 24 were randomly selected from the main holding tank and one was transferred to each of the experimental tanks for the trout exposure treatment. No trout were placed in the control tanks. Experimental trout were allowed 16 hours to acclimate to the smaller tanks, during which time they were not fed. This ensured that the trout recovered from any stress related to transfer and were hungry prior to the daily exposure period. Following the 16-hour trout fasting period, the tadpoles groups were transferred from their holding tanks into the corresponding experimental tanks. Tadpoles were introduced into experimental tanks by submerging the lip of a plastic cup into the tank to allow it to gradually fill with water and then gently tipping the cup to release the tadpoles into the tank. This gentle introduction of tadpoles eliminated the risk of the trout striking due to feeding reflex and minimized stress to the tadpoles. Six of the trout-exposed tadpole groups, three captive-bred and three wild-bred tadpole groups, were randomly selected for video recording of the daily trout exposures via GoPro cameras. These cameras were submerged in the experimental tanks to record any interactions that occurred between trout and tadpoles.

Tadpoles remained in experimental tanks for four hours and then were removed and placed back into their holding tanks. Next, I introduced a palatable prey item into the trout exposure tanks to gauge their willingness to feed in these smaller tanks. At the beginning of the

experiment, I used wild-caught boreal chorus frog tadpoles, but switched to earthworms after all frog tadpoles had metamorphosed. These feeding trials lasted two hours and any uneaten prey items were collected and the number recorded. The trout were then removed from the exposure tanks and transferred to a temporary holding tank. Experimental tanks were cleaned, and a new set of 24 trout were randomly chosen and transferred to begin their 16-hour acclimation before the following day's exposure. Only then were previously used trout transferred from the temporary holding tank to the main holding tank, which ensured that no trout would be used in exposure trials in two consecutive days. This daily cycle repeated until all the tadpoles began metamorphosis, which I defined as the appearance of a forelimb (Gosner stage 42). Every four days I measured the total length of all surviving tadpoles.

The experiment and all culture activities were conducted in the Aquatic Research Lab at Colorado State University. Untreated well water (16-18°C) was used in all holding and experimental tanks. All protocols for animal care and use in this experiment were approved by the Animal Care and Use Committee at Colorado State University.

Post-metamorphosis monitoring

Once an individual's forelimbs emerged (Gosner stage 42), I clipped a single toe and placed the individual into a transitional container (22.0 x 22.0 x 9.8 cm) with a few centimeters of water and a stone for toads to emerge from the water (Figure 2.1c). Tadpoles maintained their original group assignments in these transitional containers and no more than three individuals were place in each of these containers. Thus, groups with more than three surviving tadpoles were split into two containers. The combination of container number and toe-clip position gave each individual a unique identifier. Once an individual emerged (Gosner stage 45-46), it was weighed, measured (snout-vent length), and transferred to a terrestrial habitat container (22.0 x

22.0 x 9.8 cm) with Eco Earth substrate (manufacturer Zoo Med), autoclaved moss for cover, and a small petri dish with water (Figure 2.1d). The moss and the substrate were kept moist by misting with well water and the metamorphs were fed wingless fruit flies ad libitum. I monitored the survival and growth of individuals for four weeks following metamorphosis to assess if there were any lingering effects of trout exposure. After four weeks I recorded a final weight and snout-vent length.

Video Processing

We randomly selected 20 days of footage (~34% of total days) to view and score from each of the six tadpole groups. Specifically, I recorded the number of gustation events when a trout struck a tadpole and the total footage time (battery life typically permitted three hours of footage). I defined the strike rate per tadpole as the number of strikes observed in one video session per hour, divided by the number of tadpoles in the group on the day the video was recorded. I calculated an average strike rate per tadpole for each of the six groups over the course of the entire experiment.

Analysis

We tested the effect of trout exposure and tadpole source (captive-bred or wild-bred) on five tadpole and three post-metamorphic stage response variables. With the exception of tadpole growth rate (see below), for each analysis I created models with all possible combinations of the fixed effects of *Treatment* (control and trout exposure) and *Source*, including additive and interactive models. Models were fit to the described response variables and ranked using an information theoretic approach (Burnham and Anderson 2002). I then added a random effect to the best fitting fixed-effects model in each analysis to account for random variation among tanks or containers and interpreted the resulting mixed model. I chose this method over model

averaging because I felt it was important to include the random effect of tank or container. Furthermore, the methods for model averaging mixed models are complicated and disputed.

The captive-bred tadpoles were obtained at a younger age (3 days post hatching) than the wild-bred tadpoles (24 days post-hatching) and thus, were exposed to trout for a longer period of time. To account for different lengths of exposure, I split tadpole survival into two survival periods: survival from age 3-24 days (S_{T1}) and from age 24 days to metamorphosis (S_{T2}) . In both periods, tadpole survival probability was defined as the probability that an individual tadpole survived through that period. The experimental units were the individual tadpoles and the random effect of their housing was tadpole tank nested with trough. For example, the interactive model with all possible fixed effects and the random effect for survival in the second period (from 24 days to metamorphosis) is:

$$Logit\ (S_{T2}) = \mu + \alpha(Source) + \beta(Treatment) + \alpha\beta(Source * Treatment) + \gamma_{Ta(Tr)}, \quad \text{Eqn (1)}$$

where tadpole survival, S_{T2}, is a function of an intercept, μ, the coefficient for *Source*, α, (*Source*: 0 = captive, 1 = wild), the coefficient for *Treatment*, β, (*Treatment:* 0 = control, 1 = trout exposed), the coefficient for the interaction, $\alpha\beta$, and the random effect of tadpole tank nested in trough, $\gamma_{Ta(Tr)}$. The model for tadpole survival during the first period, S_{T1}, is identical except that *Source* and the interaction of *Source* and *Treatment* is not included because the only source were captive-bred tadpoles.

A third tadpole survival analysis was performed for the six tadpole groups that were filmed. Similar to other analyses, I estimated tadpole survival using a logistic regression model with the added continuous predictor variable of average strike rate (*r*) as a fixed effect.

Treatment was dropped from this model since all six tadpole groups were trout-exposed. The resulting interactive model is:

$$Logit(S_{T2}) = \mu + \alpha(Source) + \varphi(r) + \alpha\varphi(Source * r) + \gamma_{Ta(Tr)}, \quad \text{Eqn (2)}$$

with a new coefficient for average strike rate, φ.

Tadpole growth was measured with two metrics: daily growth rate and body condition at emergence. Because the tadpoles were not individually marked until Gosner stage 42, I calculated the average length of the tadpoles in each group during each measurement occasion (every four days). I calculated the change in this average length variable between two measurement occasions and divided by four to obtain a daily growth rate for each group. If any tadpole died during a four day period, the growth rate for that group was discarded for that period as the mortality would skew the length averages. Growth rate was not calculated after the tadpoles reached the age of 50 days, when most individuals in my experiment began to shrink as they absorbed their tails as part of metamorphosis.

We used a repeated measures analysis for these growth rate data. First, I used AIC to select the best of four different covariance structures: unstructured covariances, compound symmetry, autoregressive covariances, and autoregressive covariances with heterogeneous variances. I fit a quadratic regression model using the selected covariance structure to analyze the additive effects of *Source* and *Treatment* on tadpole growth rate. The additive model,

$$Growth = \mu + \theta_1(age) + \theta_2(age^2) + \alpha(Source) + \beta(Treatment), \quad \text{Eqn (3)}$$

included an intercept, a quadratic age effect (θ_1 and θ_2) and terms for *Source* and *Treatment* similar to the previous models. I did not perform model selection but rather interpreted the estimated coefficients from this model.

We also explored whether trout exposure influenced time to metamorphosis (development rate) or body condition at emergence (Gosner stage 46). Time to metamorphosis was defined as the number of days between when the tadpole hatched and when it developed a forelimb (Gosner stage 42). Body condition is calculated by dividing tadpole weight (g) by its snout-vent length (mm). After tadpoles metamorphosed, individual identification was possible, so in these analyses, and all subsequent analyses, each individual is considered a sample unit. I analyzed these continuous response variables using ANOVA models with the fixed effects of *Treatment* and *Source*. Following metamorphosis, an individual was housed in a transition container; therefore, the random effect in the body condition analysis is container, nested within tank, nested within trough.

Survival following metamorphosis was also divided into two periods: survival from metamorphosis to emergence (S_{M1}) and survival from emergence to four weeks post metamorphosis (S_{M2}). I estimated both of these post metamorphosis survival probabilities using logistic regression models with the fixed effects of *Treatment* and *Source* and a random effect of container, nested within tank, nested within trough.

We used the change in body condition in the first four weeks following metamorphosis as the metric for post-metamorphic growth. I calculated this by subtracting the body condition at emergence from the final body condition. I analyzed this change in body condition with ANOVA models using the fixed effects of *Treatment* and *Source* and a random effect of container, nested within tank, nested within trough.

We used the lme4 package in Program R to fit all the fixed effect models and mixed models (Bates et al. 2014). I also confirmed that models used in these analyses met their appropriate assumptions (e.g., homoscedasticity and normality of errors in the ANOVA and quadratic regression models).

Results

Interaction Videography

We collected over 1000 video hours of interactions between the trout and the six tadpole groups. The 20 randomly selected video events (349 hours) showed strike rates of 0 to 1.73 strikes per hour per tadpole. The average strike rate was 0.21/hr/tadpole (SD = 0.33), meaning that during the course of a daily four hour exposure period, a tadpole would be engulfed an average of 0.84 times.

Tadpole Survival

The top ranked fixed-effect model for tadpole survival in both periods included only *Treatment* (Appendix 2.i. and 2.ii., Table 2.1). The top model for the first period, age 3-24 days, produced imprecise estimates and when I attempted to add the random effect of tank the model was unidentifiable, so I report estimates based on the best fixed effects model only (Appendix 2.i). Survival probabilities were high for the control individuals ($\hat{S}_{T1,control}$= 1.0, SE = 0.00) and lower for the trout-exposed individuals ($\hat{S}_{T1,trout}$= 0.88, SE = 302.77). The standard errors of these estimates suggest poor fit of the model to the data. The top model for the second time period, age 24 days to metamorphosis, performed much better. Again, survival probabilities were higher for the control tadpoles ($\hat{S}_{T2,control}$= 0.98, SE = 0.02) compared to the trout-exposed individuals ($\hat{S}_{T2,trout}$= 0.89, SE = 0.13). Both the second and third most supported models for survival in this second period contained the factor of *Source* and had high weights (*w* = 0.33, *w* =

0.29, respectively). In these fixed-effect models, the coefficients for *Source* were both positive indicating that wild-bred tadpole had a higher survival probability than captive-bred tadpoles ($\hat{\beta}$ = 15.93, SE = 1087.11; $\hat{\beta}$ = 0.59, SE = 0.46, respectively). The overall survival for both periods for the control individuals was 0.98 versus 0.78 for trout-exposed tadpoles. Despite the lower survival among the tadpoles exposed to trout, only one of the 138 tadpoles in the trout treatment groups was consumed by a trout (the trout showed no ill effects from the consumption). The other tadpoles died during or following the four hour exposure periods as a result of stress or injury from trout gustation. Visible injuries were observed frequently and included hematomas (n = 2), broken tails (n > 25), and eviscerations (n = 16).

The analysis of survival as a function of strike rate per tadpole showed little evidence of a relationship between the two variables. The top model contained only the *Source* factor (*w* = 0.53) and was about 3 times more likely than any other competing model (Appendix 2.iii.). This model indicated that captive-bred tadpoles had lower survival probability than wild-bred tadpoles ($\hat{S}_{captive}$= 0.60, SE = 0.13; $\hat{S}_{wild}$= 0.94, SE = 0.07). The next highest ranked model (*w* = 0.18) did include strike rate, and the estimated effect of strike rate was negative ($\hat{\varphi}$ = -2.41, SE($\hat{\varphi}$) = 4.53) as one would expect survival probability to decrease as strike rate increased. Still, this estimate was imprecise, likely due to small sample size (6 groups of tadpoles, 3 for each source type).

Tadpole Growth

The covariance structure that best fit the data had autoregressive covariances and heterogeneous variances. Using this covariance structure with the additive model, I found that both *Treatment* and *Source* influenced daily growth rate (Table 2.2). Trout exposure reduced daily growth rate as expected; however, *Source* had a stronger influence on daily growth rate

based on the magnitude of the coefficients. Wild-bred control groups had the highest daily growth rates and captive-bred trout-exposed groups showed the smallest daily growth rates (Table 2.2, Figure 2.2).

Body condition at emergence was influenced more by the source of the tadpoles than by trout exposure (Appendix 2.iv). The top model only contained the *Source* factor (w = 0.59) and was twice as likely as any other model. Captive-bred individuals had a slightly higher body condition than the wild-bred individuals ($\widehat{BC}_{captive}$ = 0.052 g/mm, SE = 0.00091; $\widehat{BC}_{wild}$ = 0.049 g/mm, SE = 0.0015, Table 2.1).

Tadpole Development

The top model explaining time to metamorphosis contained an interaction of *Source* and *Treatment* (w = 0.66, Appendix 2.v.). Trout exposure delayed time to metamorphosis by 0.86 and 2.55 days for wild-bred and captive-bred tadpoles, respectively (Table 2.1, Figure 2.3). In addition, tadpoles that were wild-bred showed slower development than those that were captive-bred (Table 2.1, Figure 2.3).

Post Metamorphic Survival

Both *Source* and *Treatment* influenced survival during the post-metamorphosis periods (Appendix 2.vi. and 2.vii.). During the period from metamorphosis to emergence, which typically lasted four days, estimates of survival were high for the wild-bred individuals ($\hat{S}_{M1,wild,control}$= 0.97, SE = 0.03; $\hat{S}_{M1,wild,trout}$= 0.98, SE = 0.01), but markedly lower for captive-bred individuals ($\hat{S}_{M1,captive,control}$= 0.62, SE = 0.12; $\hat{S}_{M1,captive,trout}$= 0.79, SE = 0.17). Only one individual died during the time from emergence to four weeks post-metamorphosis (captive-bred, control treatment), yielding a survival probability of $\hat{S}_{M2,captive,control}$= 0.94, SE

= 0.06. Survival probabilities for all other groups were 1.0. There was no evidence that trout exposure negatively influenced post-metamorphic survival probabilities.

Post Metamorphic Growth

The top model for the change in body condition data contained only *Source* (w = 0.68, Appendix 2.viii.). Individuals from both sources experienced reduction in body condition over the four weeks following metamorphosis, but the loss was greater for captive-bred individuals ($\widehat{\Delta BC}_{captive}$ = -0.015 g/mm, SE = 8.21 x 10^{-4} ; $\widehat{\Delta BC}_{wild}$ = -0.0071 g/mm, SE = 1.33 x 10^{-3}) (Table 2.1).

Discussion

In my study, trout-exposure decreased the survival probability of the tadpoles despite the fact that only one tadpole was consumed. While previous literature suggests that bufotoxins should protect early life stages of toad tadpoles from direct predation (Knapp 2005, Welsh et al. 2006, Hartman et al. 2014), my video footage showed tadpoles being repeatedly engulfed and estimates of tadpole survival were clearly lower in trout-exposed groups. It is likely that the non-consumptive gustation of tadpoles resulted in lower survival probabilities that I observed.

Reduced tadpole survival in this experiment is contrary to effects of nonnative trout on western toads in other studies (Knapp 2005, Welsh et al. 2006, Hartman et al. 2014). These authors concluded that toad occupancy was not negatively impacted by trout presence because the toad tadpoles are unpalatable. This apparent disagreement between my work and these studies may be because the physical complexity of the natural aquatic systems could offer the toad tadpoles refuges whereas in my experimental setting tadpoles had no cover. Another explanation is that these studies examined a different state variable, occupancy, which requires the detection of only one individual, while my study investigated factors that influence fitness,

namely survival, growth, and development. For long-lived species, like the boreal toad, populations (e.g., Spruce Lake) may persist for a long time with reduced recruitment. Lastly, none of the previous studies involved greenback cutthroat trout.

Trout exposure also led to slower development and increased time to metamorphosis of boreal toad tadpoles. While trout exposure delayed time to metamorphosis in both wild-bred and captive-bred tadpoles, the effect of trout exposure was greater in the captive-bred tadpoles. This may be because captive-bred individuals entered the experiment at a younger age and, therefore, were exposed to trout for a longer time period. My results are contrary to previous literature that found expedited metamorphosis in the presence of a predator (Devito et al. 1998, Chivers et al. 1999). The delay in metamorphosis I observed in both captive-bred and wild-bred tadpoles may be detrimental to both wild and reintroduced populations of boreal toads. Boreal toads are an alpine species that must complete their aquatic life stages in only a few months. Tadpoles must grow, develop, metamorphose, and gain condition during this time. If stochastic weather events further shorten the breeding season, a delay in metamorphosis due to trout presence might result in individuals that have insufficient time to prepare for their first winter. Thus, trout exposure in the larval stage has the potential to decrease first winter survival.

We did not observe any lingering effects of trout exposure post-metamorphosis. In the analysis of survival from metamorphosis to emergence, the top model predicted higher survival in the trout-exposed individuals, but the magnitude of this effect was small and was dismissed as unimportant. Survival from emergence to four weeks was high for all individuals of both treatment groups. I also did not see any impact of trout exposure on change in body condition following metamorphosis. my results suggest that the impacts of trout presence in the larval stage do not carry over to the early terrestrial stage, which is consistent with recent literature that

suggests that carry-over effects among anurans is slight and early life history traits (i.e. size at metamorphosis) have little impact on juvenile survival (Schmidt et al. 2012, Green and Bailey *in press*).

Additional studies are needed to refine the understanding of the effects of greenback cutthroat trout on boreal toad recruitment. Experiments such as this one can be replicated in field enclosures to see if trout have the same effect on toad tadpoles when the habitat is complex. Secondly, I did not observe any evidence of learning among the trout (multiple tadpole gustation events within a short time period by the same individuals). Research should be done to determine if trout are able to learn to recognize unpalatable prey in a long-term setting, which could result in an attenuation of the negative effects of introduced trout. Research could also be conducted to investigate potential competition for the food resource of emerging aquatic insects between introduced greenback cutthroat trout and post-metamorphosis boreal toads. Lastly, more mark-recapture studies could be conducted to compare recruitment at boreal toad breeding populations with and without introduced trout.

The source of the tadpoles had a greater impact than I expected. Survival from metamorphosis to emergence was much lower in the captive-bred individuals. Many captive-bred individuals had reduced limb development, and when they did appear, limbs often appeared small and weak. I surmised that limb weakness made it difficult for these individuals to emerge, causing them to drown after their lungs developed. Captive-bred tadpoles also experienced reduced daily growth rates and following metamorphosis, surviving captive-bred individuals experienced a greater loss in body condition than their wild-bred counterparts.

Slow or incomplete development in toad early life stages may be the result of captive breeding (Araki et al. 2007). In the wild, processes such as natural selection and mate choice

work to increase the vitality and fitness of individuals. However, it is difficult in a captive breeding facility to replicate the natural processes that might otherwise remove an unfit breeder from a wild population (Lynch and O'Hely 2001). Furthermore, captive facilities might promote fixation of alleles that benefit individuals in captivity but are detrimental in the wild (Lynch and O'Hely 2001, Ford 2002). Thus, progeny produced via captive breeding may be less robust and fit than their wild bred counterparts (de Mestral and Herbinger 2013, Milot et al. 2013, Rollinson et al. 2014). Captive breeding has been successful in bolstering some imperiled populations, but my data suggest there are still some unresolved issues with breeding boreal toads in captivity. More research needs to be done to understand the cause of these issues and then breeding facilities should work to fix them if captive breeding of boreal toads is to continue. The current issues with captive-bred boreal toads further highlight the need to preserve existing breeding boreal toad populations, both for their own persistence but also as a source of eggs for reintroductions.

The only response variable that seemed to favor captive-bred individuals was time to metamorphosis and body condition at emergence. However, this was likely because these individuals entered the experiment at a younger age. Captive-bred individuals were exposed to the environmental conditions in the lab for about three weeks longer than wild-bred tadpoles that spent their first three weeks in the hatchery. I followed many of the same husbandry methods (i.e. type, amount, and timing of food) as the hatchery, but the temperature of the water is different between the lab and the hatchery. Temperature influences the rate of development for many larval amphibians and could explain the observed differences in development (Newman 1998, Mitchell and Seymour 2000, O'Regan et al. 2014). Further, higher body condition at emergence for captive-bred tadpoles may be an artifact of the high mortality of poorly developed

captive-bred individuals prior to emergence. In this scenario, only robust individuals emerge and would bias high their apparent condition at emergence. There was no difference in survival probabilities post-emergence for individuals of either source.

We have demonstrated that hatchery-reared greenback cutthroat trout have the ability to substantially reduce larval survival which could impact recruitment to the breeding adult stage in boreal toad populations. Since both of these species are imperiled throughout their range, my results have implications for the future conservation of both greenback cutthroat trout and boreal toads. Most of the historic range of greenback cutthroat trout is now inhabited by nonnative brook, brown, and rainbow trout which can outcompete, or in the case of rainbow trout, hybridize with the native cutthroat trout (Behnke 2002, Dunham et al. 2002, Metcalf et al. 2008). As such, it is no longer feasible to conserve the greenback cutthroat trout in much of its native range. An effective strategy for cutthroat trout conservation has been to introduce them into water bodies free of the nonnative trout, often alpine reservoirs and lakes (US Fish and Wildlife Service 1998). Currently, researchers are similarly implementing and optimizing methods for reintroducing boreal toads into suitable breeding ponds within their range (Muths et al. 2001, Muths et al. 2014). My results suggest that toad conservation efforts are likely compromised by the presence of trout. Given this new knowledge, toad conservation areas and trout conservation areas should be isolated from one another. In areas where the two species exist together, such as Spruce Lake, my results indicate that the presence of the trout could negatively influence recruitment in the boreal toad population. Managing this difficult situation requires detailed knowledge of the particular dynamics of the system and careful application of that knowledge. In such situations, managers could employ a structured decision making framework to determine their conservation objectives and evaluate the effectiveness of various potential management

actions (Gregory et al. 2012). Such a process would yield a management strategy grounded in the best available science and balanced among the values of various stakeholders regarding each species.

Conservation of two species is difficult when the current conservation strategies of one species contribute to the decline of the other. Interactions between two species can often be more complex than simple predator-prey relationships. Previous understanding led managers to expect little interactions between boreal toads and greenback cutthroat trout due to the toxicity of the toads at all life stages. However, I have demonstrated that greenback cutthroat trout do have the ability to exert negative direct and indirect effects on boreal toad tadpoles. While both greenback cutthroat trout and boreal toads are important native species that deserve conservation attention, future management decisions should recognize the negative effects that greenback cutthroat trout can exert on this unpalatable toad.

Table 2.1. The most parsimonious model with appropriate random effects structure for different response variables of boreal toads. *Source* refers to whether the toads were wild- or captive-bred and *Treatment* refers to whether the toads were exposed to greenback cutthroat trout or not. Estimates and standard errors (in parentheses) are provided for each fixed-effect parameter in the given model. Parameters include: μ, the intercept that represents captive-bred individuals in the control (no trout exposure) treatment; α, the tadpole source (wild); β, the trout treatment; αβ, the interaction term for the combination of *Source* and *Treatment*; and γ, the random effect of container or tank (see text).

Response Variable	Top Model	Intercept $\hat{\mu}$	*Source* (wild) $\hat{\alpha}$	*Treatment* (trout exp.) $\hat{\beta}$	Interaction $\widehat{\alpha\beta}$
Tadpole Survival (3 days to 24 days)	$Logit\ (S_{T1}) = \mu + \beta(Treatment)$	19.57 (1963.41)	--	-17.62 (1963.41)	--
Tadpole Survival (24 days to Metamorphosis)	$Logit\ (S_{T2}) = \mu + \beta(Treatment) + \gamma_{Ta(Tr)}$	4.09 (0.94)	--	-1.98 (0.94)	--
Tadpole Survival (Strike Rate Analysis)	$Logit\ (S_T) = \mu + \alpha(Source) + \gamma_{Ta(Tr)}$	0.41 (0.53)	2.43 (1.16)	--	--
Body Condition at Emergence	$BC = \mu + \alpha(Source) + \gamma_{Co(Ta(Tr))}$	0.052 (0.001)	-0.003 (0.001)	--	--
Time to Metamorphosis	$Days = \mu + \alpha(Source) + \beta(Treatment) + \alpha\beta(Source * Treatment) + \gamma_{Ta(Tr)}$	63.75 (0.54)	7.05 (0.73)	2.55 (0.68)	-1.69 (0.91)
Post Metamorphosis Survival (Metamorphosis to Emergence)	$Logit\ (S_{M1}) = \mu + \alpha(Source) + \beta(Treatment) + \gamma_{Co(Ta(Tr))}$	0.48 (0.50)	2.84 (0.75)	0.85 (0.60)	--
Post Metamorphosis Survival (Emergence to 4 Weeks)	$Logit\ (S_{M2}) = \mu + \alpha(Source) + \beta(Treatment)$	2.77 (1.03)	19.55 (7426.27)	19.65 (7292.40)	--
Change in Body Condition	$\Delta BC = \mu + \alpha(Source) + \gamma_{Co(Ta(Tr))}$	-0.015 (0.001)	0.008 (0.001)	--	--

Table 2.2. Parameter estimates and standard errors (in parentheses) for the quadratic regression model for daily boreal toad tadpole growth rate. Parameters include: μ , the intercept; θ_1, the coefficient for age (in days); θ_2, the coefficient for age squared; α, the fixed effect for tadpole source (wild-bred); and β, the fixed effect for trout exposure treatment.

Model	Fixed Effects				
	$\hat{\mu}$	$\hat{\theta}_1$	$\hat{\theta}_2$	$\hat{\alpha}$	$\hat{\beta}$
$Growth = \mu + \theta_1(age) + \theta_2(age^2) + \alpha(Source) + \beta(Treatment)$	0.18 (0.04)	0.09 (0.003)	-0.002 (0.0001)	0.25 (0.03)	-0.06 (0.02)

Figure 2.1. Photographs of the tanks and containers used in the boreal toad tadpole-greenback cutthroat trout experiment. Photo "a" shows the tadpole holding tanks within the troughs. Photo "b" shows the experimental tanks where the trout exposure treatments occurred. Photo "c" shows the transition tanks where the tadpoles were place once they reached Gosner stage 42. Photo "d" shows terrestrial tanks where the individuals were housed for the post-metamorphosis monitoring phase.

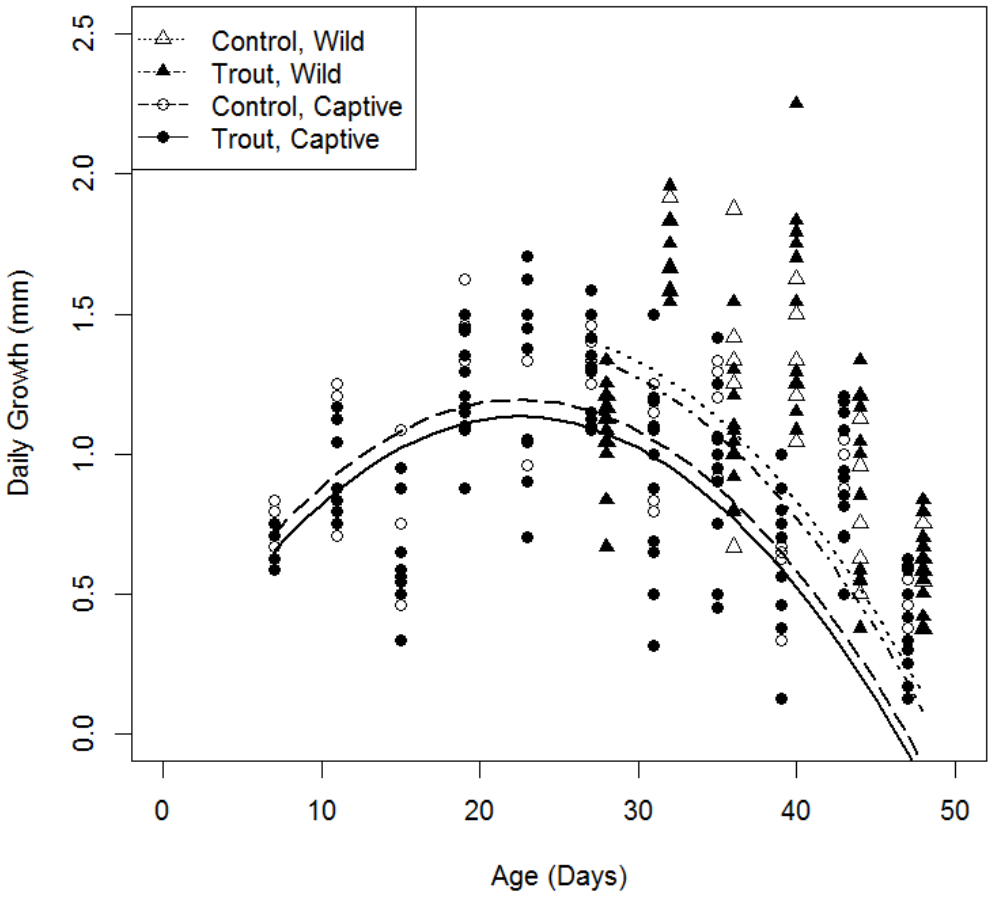

Figure 2.2. Quadratic relationship between daily boreal toad tadpole growth rate (mm) and age (days) Lines represent predicted values for the four combinations of fixed effects: trout-exposed, captive-bred groups (solid); control, captive-bred groups (dashed); trout-exposed, wild-bred groups (dot-dashed); and control, wild-bred groups (dotted). No data points or predicted values are given for the wild-bred groups before age 28 because these individuals were obtained when they were 24 days old. Each dot represents a group measurement of daily growth rate.

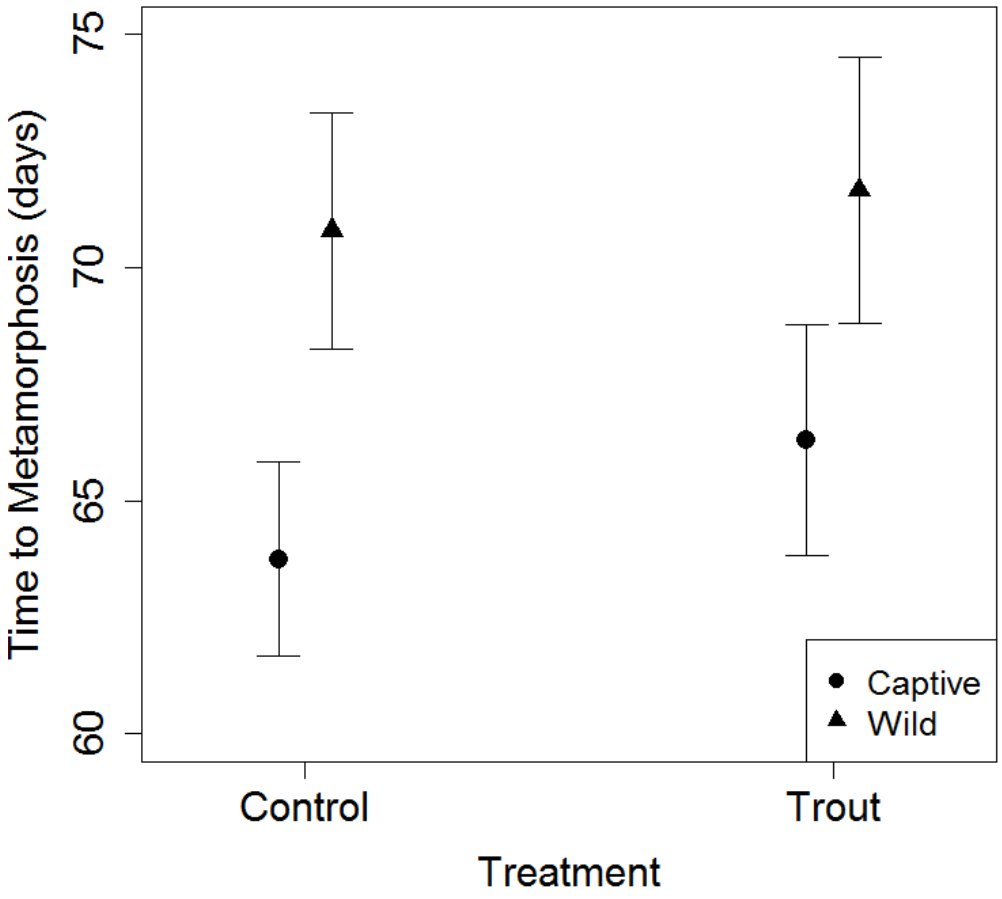

Figure 2.3. Mean estimates and 95% confidence intervals of time to metamorphosis of boreal toads tadpoles of different sources (wild- or captive-bred) and treatments (exposed to greenback cutthroat trout or not). Time to metamorphosis is defined as the number of days between when the tadpole hatched and when it developed a forelimb (Gosner stage 42). Estimates are from the top ranking model, $\boldsymbol{Days = \mu + \alpha(Source) + \beta(Treatment) + \alpha\beta(Source * Treatment) + \gamma_{Ta(Tr)}}$. These estimates represent the mean time to metamorphosis of the tadpoles in each treatment-source combination, thus the random effect of tadpole tank is not represented in these estimates and confidence intervals.

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CHAPTER THREE:

INTEGRATING BIOLOGY AND FIELD LOGISTICS TO OPTIMIZE PARAMETER ESTIMATION FOR IMPERILED SPECIES

Conservation of imperiled species often requires knowledge of vital rates and population dynamics. However, these can be difficult to estimate for rare species and small populations. This problem is further exacerbated when sites are difficult to access and individuals are not available for detection during some surveys. Here I explore this issue with a simulation study of two separate boreal toad (*Anaxyrus boreas boreas*) populations, one easily accessible and one not. I examine the bias and precision of survival and breeding probability estimates generated by survey designs that differ in effort and timing of surveys for these populations. My findings indicate that the logistics of accessing a site can greatly limit the ability to estimate survival and breeding probabilities. Simulations similar to what I have performed can be useful for researchers to determine the optimal survey designs for their system before initiating their sampling efforts.

Introduction

Conservation of imperiled populations often requires knowledge of the vital rates that drive population dynamics (Biek et al. 2002, Oostermeijer et al. 2003). Well-designed studies and appropriate analysis of mark-recapture data can be useful to estimate these vital rates and explore relative changes in population dynamics under various environmental and management scenarios (White and Burnham 1999, Caswell 2000). Projection matrix models, parameterized with these vital rates, can be used to identify vulnerable life stages, determine vital rates whose changes pose the greatest threat to the population, and prioritize conservation and monitoring

programs that target these critical life stages and the associated vital rates (Wisdom and Mills 1997, Heppell et al. 2000, Biek et al. 2002).

Projection matrix models usually require female-specific parameter estimates, such as breeding probability and fecundity (Caswell 2000). However, in some species, females are only detectable during the breeding season and are cryptic for the rest of the year, making it difficult to obtain reliable parameter estimates. This problem is exacerbated when females skip breeding opportunities and are only available for detection every few years. Skipping breeding opportunities and the associated non-detection issues are common in taxa such as albatross (Kendall et al. 2009), sea turtles (Kendall and Bjorkland 2001, Dutton et al. 2005, Rivalan et al. 2005), marine mammals (Fujiwara and Caswell 2002), and some amphibians (Bailey et al. 2004, Muths et al. 2010). This can result in datasets that are too sparse to estimate the parameters necessary to model the female component of the population. The problem is further exacerbated for species of conservation concern, because populations are often small, such that the low abundance of females is compounded by their inconsistent availability for capture due to skipped breeding. Thus, conservation managers are often forced to make decisions without the benefit of accurate female vital rates in population models, which is troubling given that conservation can often benefit from matrix-models (Boor 2014).

In addition, the process of skipped breeding opportunities is considered temporary emigration, which violates the assumptions of many mark-recapture models. Failing to address temporary emigration can bias estimates of survival and abundance, especially if the temporary emigration is nonrandom (i.e. Markovian; Kendall et al. 1997).

Researchers can combat these issues with model-based solutions as well as design solutions. Open robust design models allow for the incorporation of temporary emigration which

can improve estimates of other parameters (Schwarz and Stobo 1997, Kendall and Bjorkland 2001). Improvements to survey designs may also maximize the opportunity to detect females by conducting surveys when females are present, though this is sometimes difficult. It is also difficult to predict when females will be present and logistics and funding can limit the number and duration of surveys during the predicted window of opportunity. In addition, excessive numbers of surveys might disturb sensitive habitats or disrupt the natural behaviors (Altwegg et al. 2014). Therefore, it is important to determine the optimal number and timing of surveys needed to collect enough data to estimate parameters adequately for the female portion of the population (Legg and Nagy 2006).

To address this issue, I conducted a simulation study aimed at optimizing female parameter estimation in two scenarios that researchers might encounter: easily accessible populations where surveys are relatively unrestricted and populations where access is difficult and survey opportunities are restricted. I based the simulations on two such boreal toad (*Anaxyrus boreas boreas*) populations, and evaluate the ability of different survey designs to estimate female parameters using a multi-state open robust design framework. My objective was to determine the study design that minimizes bias and maximizes precision of estimates for survival and breeding probabilities.

Methods

Boreal Toads

The boreal toad is an alpine species that ranges across Utah, Wyoming, Colorado, Idaho, and Nevada, US. Their breeding ponds, summer range, and overwinter refugia, all occur in higher elevation lodgepole pine or spruce-fir forests from 2,100 to 3,600 meters (Campbell 1970). While males occasionally skip breeding opportunities, they usually return to the breeding

area every year (Muths et al 2006). In contrast, females almost always skip one or more breeding opportunities after breeding (Muths et al. 2010). Non-breeding females do not migrate to breeding areas, making their detection difficult with current mark-recapture methods.

This species is also a relevant example because the Southern Rocky Mountain (SRM) population of boreal toads is declining (Carey 1993, Scherer et al. 2005, Muths and Scherer 2011). The population is currently "warranted but precluded" from listing under the Endangered Species Act (U.S. Fish and Wildlife Service 2012) and a listing decision by the Fish and Wildlife Service is slated for 2017. While much as been learned about the ecology of SRM boreal toads (Goebel 1996, Carey et al. 2006) most of that knowledge derives from male-only data (Muths et al. 2003, Scherer et al. 2005, Muths et al. 2011). Detailed assessments of female demographics have been precluded by aforementioned issues of female availability and detection. The challenge is thus to gather data sufficient to create accurate and precise models that use female parameter estimates.

Multi-State Open Robust Design Mark-Recapture Model

Our simulations were conducted using a multi-state open robust design (MSORD) mark-recapture model (Schwarz and Stobo 1997, Kendall and Bjorkland 2001). The MSORD model is particularly useful for several reasons. First, it allows for multiple states including breeders and non-breeders. In addition, MSORD relaxes the assumption that the population is closed, which allows individuals to enter and leave the breeding (or study) area at different times. Parameters in the MSORD model that describe these dynamics can be classified into two categories: within-season and between-season parameters.

Within-season Parameters

The robust design aspect of MSORD divides the breeding season into individual survey occasions. The parameter $pent_{ij}^{s}$ is the probability that an individual in state *s* (i.e. breeder or non-breeder) enters the breeding area before survey *j* and is therefore available for detection in the season *i*. Once an individual is in the breeding area, φ_{ij}^{s} describes the probability that the individual in state *s* during season *i* will remain in the survey area between surveys *j* and *j* + 1. This model allows for one entry and exit for each individual during the course of the breeding season. The probability of detection, p_{ij}^{s}, describes the probability that an individual in state *s*, in season *i*, is detected during survey *j* (Kendall and Bjorkland 2001). In my example, detection probability is zero for individuals that are not in the breeding area (e.g., non-breeders, and breeders that have not yet arrived or those that have already left the breeding area).

Between-season Parameters

In addition to incorporating multiple states, the MSORD model allows for transitions between those states. These transitions are assumed to occur between seasons, thus an individual's state does not change during a season. Female boreal toads are obligate non-breeders following a season in which they breed (Muths et al. 2010). Thus, the transition probability between breeding and non-breeding states, $\psi_i^{B,NB}$, is fixed at one, or conversely the probability of remaining in the breeding state for two successive seasons, $\psi_i^{B,B}$, is zero (1- $\psi_i^{B,NB}$). The probability of transitioning from the non-breeding state to the breeding state, $\psi_i^{NB,B}$, is considered the breeding probability. Similarly, the probability of staying in the non-breeding state $\psi_i^{NB,NB}$, is one minus the breeding probability. The other between-season dynamic parameter is survival. S_i^{s} describes the probability that an individual in state *s* will survive between seasons *i* and *i* + 1. Like the state transitions, survival or mortality (1- S_i^{s}) only occurs between seasons.

Simulated Populations

We simulated the female portion of two different boreal toad populations, one inaccessible population and one easily accessible (Table 3.1). I based the inaccessible population on parameters from a boreal toad population at Denny Creek, Colorado (Muths et al. 2010). Denny Creek is a high elevation, remote site that is difficult to access prior to breeding due to deep snowpack and safety concerns. The first survey of the breeding area occurs later in the breeding season, often after the first eggs are laid. Accordingly, many females have already arrived in the breeding area, suggesting that the probability of entry prior to the first survey, $pent_1^S$, is high (i.e., near or equal to 1, Table 3.1). In contrast, my accessible population parameters are based on a population in Wyoming (E. Muths, B.R. Hossack, P.S. Corn, and D.S. Pilliod, unpublished data). Here, first surveys are often conducted early in the breeding season before most females arrive. Surveys can be conducted multiple times during the breeding season enabling females to be captured as they arrive, resulting in a more constant probability of entry across the sampling occasions within each season (Table 3.1).

A first requirement of simulation models is to define the duration of a breeding season. Muths et al. (2010) did not report survey-specific estimates of within-season parameters, but the time period between surveys varied between 1-21 days. I based my simulations on daily surveys and assumed that the breeding season lasted 21 days (j = 21), a realistic length of a boreal toad breeding season and is long enough to account for late breeders (Muths et al. 2006, Muths and Scherer 2011). The within-season parameters, $pent_j^B$ and φ_j^B, were different between the simulated populations to represent the access logistics of the two different breeding sites. The within-season parameters used to simulate data for the inaccessible population were obtained from Muths et al. (2010). They did not report survey-specific estimates of $pent_j^B$, but instead

reported most females (88%) were already present at the first survey. I used this estimate as the first probability of entry, $pent_1^B$, and then gradually reduced the values for subsequent probabilities of entry until the total summed to one. All other values for probability of entry after that were zero. My simulations were based upon daily surveys, therefore I needed to convert the estimate of probability of remaining, φ_j^B, from Muths et al. (2010) to reflect the shortened interval. To calculate the new estimate of φ_j^B I used the average number of days between the surveys from the Muths et al. (2010) data, *d*, and took the d^{th} root of their estimate. I then treated φ_j^B as constant for all surveys in all years.

The within season parameters used to simulate the accessible population were estimated directly from data collected at the Wyoming site from 2003-2012. Due to the sparseness of these data, I pooled the years so that capture histories for individuals across different years were treated as though they were all captured during the same survey season. Since varying climatic conditions might delay or expedite the start of the breeding season each year, I standardized the survey dates by the date of the first survey. With these data, I fit a model with survey-specific $pent_j^B$ and constant $\varphi_\cdot^B$. The estimates were used to simulate the accessible population.

The between season parameters, S_i^s and $\psi_i^{NB,B}$, and p_{ij}^B were the same for both simulated populations and were obtained from Muths et al. (2010; Table 3.1). These parameters were assumed to be constant for all years and surveys, for detection probabilities ($p_{\cdot\cdot}^B$). Since non-breeding females are not detectable in this system, p_{ij}^{NB} and all other within season parameters for the non-breeding state were fixed to zero for both simulated populations. Survival probability was assumed the same for breeding and non-breeding individuals in both populations.

All simulations were run with a total population size of 20 individuals, which is realistic for populations of conservation concern, over the course of 10 years. Since survival is < 1in these

simulations, additional individuals were recruited to the simulated population to maintain a constant population size of 20 for each year.

Simulated Survey Designs

We considered sampling designs that varied in the number of surveys conducted during the breeding season as well as the timing of those surveys (Table 3.2). I attempted to create survey designs that were representative of what is feasible for boreal toad researchers. The first set of designs assumed that sampling was concentrated in the first 3-5 days of the 21-day breeding season (Table 3.2). I also considered "random" survey designs in which the surveys were conducted on 3-5 days distributed randomly throughout the breeding season. The next set of designs represented surveys that were conducted at weekly intervals. These simulations all contained four surveys but due to the constraint of having a 21-day season, one of the intervals between surveys was forced to be six days instead of seven. I simulated all three possible placements of this six day interval (a, b, and c). The final set of designs simulated four surveys with weekly spacing but with an added element of coupling. In these designs, two of the four surveys were conducted on consecutive days. The three possible couplings were simulated (Table 3.2). For comparison, I included a survey design with surveys on all 21 days. This is impractical in practice but for this exercise it serves as a useful reference to compare the other simulations. I fixed p to zero for days when a survey was not conducted.

All 13 survey designs were simulated with the inaccessible and accessible population parameter values and 500 iterations were performed for each survey design (13,000 total simulations: 2 populations x 13 survey designs x 500 iterations). The simulated data from each iteration were fit to models with constant survival and breeding probabilities across the ten years. I calculated bias and precision for resulting survival and breeding probability estimates for each

survey design. Bias was calculated by subtracting the true value of the parameter used in the simulation from the mean of the parameter estimates. Precision was determined by examining the variance of the estimates from the 500 iterations. All simulations were run in Program MARK (White and Burnham 1999).

Results

Survival

The survival estimates from the simulated inaccessible population were all negatively biased (proportional bias range: -32% to -15%) and imprecise (variance range: 0.088-0.15) regardless of the survey design (Figure 3.1). However, designs that clustered the surveys at the beginning of the season ("First 3", "4", and "5") were the least biased and most precise estimates, especially the designs with four or five surveys (Figure 3.1). In contrast, the survival estimates from the accessible population simulations were all relatively unbiased (proportional bias range: -2.3% to 4.6%), with only the random surveys showing a slight positive bias (Figure 3.1). These estimates were also all fairly precise (variance range: 0.0069-0.014; Figure 3.1).

Breeding Probability

The estimates of breeding probability from the inaccessible population simulations showed similar, positive bias among survey designs with four or more surveys (proportional bias range: 25% to 72%; Figure 3.2). The "First 3" design was extremely biased (proportional bias = 125%; Figure 3.2). All survey designs had relatively imprecise estimates of breeding probability (variance range: 0.13-0.16), except the "First 3" design (variance = 0.050; Figure 3.2). The 'First 3' design produced highly biased, but relatively precise estimates, which could be misleading if used in projection models. Aside from this survey design, the estimates from the "random"

survey designs had the most precise estimates of all the survey designs using the simulated inaccessible population parameters (variance range: 0.12-0.13; Figure 3.2).

The breeding probability estimates from the accessible population simulation were also positively biased (proportional bias range: 21% to 58%), with the "random" survey designs showing the most bias (Figure 3.2). Similar to the inaccessible population simulations, the "random" survey designs, were also the most precise of all the survey designs in the accessible population simulations (Figure 3.2). In general, the variances in breeding probability estimates were much lower for the accessible population simulations compared to the inaccessible population simulations, for all survey designs (variance range: 0.035-0.077; Figure 3.2).

Discussion

The disparate results for the two breeding populations demonstrate the importance of considering not only the breeding biology and behavior of the species, but also the logistics of accessing the survey area. The ability to access the survey area can affect the number of females available for detection, thereby affecting the quality and quantity of data collected. As these results show, the difference in bias and precision between the accessible and inaccessible populations was greater than the difference between the survey designs employed on a given population. The underlying difference between these two simulated populations is that in the accessible population, surveys began before many of the females arrived. In contrast, in the inaccessible population, surveys began after most of the females had already arrived. Thus, the female availability (sample size) was greater in the accessible populations, thereby reducing bias and increasing precision.

If researchers are fortunate enough to control the timing of their surveys with respect to the arrival of animals in the area, my simulations suggest that it is beneficial to begin surveys as

individuals are beginning to arrive. In these situations any survey design that is nonrandom (i.e. the "first" and "weekly" survey designs) with at least four surveys performed well in my simulations. The coupling aspect of the second set of "weekly" designs did not improve estimation.

We acknowledge that accessing the study site at the beginning of the season is not always possible. In such cases, researchers should be careful in selecting their survey design. My simulations showed that the different survey designs under the inaccessible population scenario performed very differently. Survey designs that clustered the surveys at the beginning (as soon as researchers are able to access the site) performed the best. This makes intuitive sense because the individuals that have already entered the breeding area prior to the first survey have a limited time that they will remain in the breeding area. Concentrating the survey effort once researchers are able to access the site will maximize the probability of finding these individuals before they leave. Similar to the accessible population, estimates for inaccessible populations were greatly improved by survey designs that included at least four surveys.

As stated before, MSORD models are particularly useful in systems like the one I have simulated here. If the model assumptions are met, parameter estimates are generally unbiased for large sample sizes (Kendall and Nichols 2002). However, when sample sizes are small, as is the case for my simulations (only 20 females), even unrealistic designs where surveys were conducted every day still showed bias. This should not deter researchers from using this model in systems where it is applicable as simpler models will yield similar or greater bias (Kendall and Nichols 2002). However, researchers should be aware of data limitations if their populations of interest are small.

Our simulation exercise focused on the female portion of the population because information is often lacking for females. Although females may be the most important subset of the population for modeling future population dynamics, researchers may not want to ignore the male portion of the population entirely. While not specifically addressed in this paper, I believe that designs that are optimal for obtaining female vital rates will also perform well for obtaining estimates for males that are relatively unbiased with good precision. This is because males remain in breeding areas for longer periods in an attempt to find females, thus males are likely to be present during surveys targeted at detecting females.

Detection probability was modeled as constant within and between the survey seasons in my simulations, which is a fair assumption for boreal toads (Muths et al. 2010, Muths and Scherer 2011). However, this might not be true in all populations. The optimal survey design in populations where detection probability is lower or changes throughout the season will likely be different from what I have presented here. This issue along with other differences between my simulated populations and other populations illustrate the need for researchers to conduct their own simulations specific to their population of interest.

We recommend researchers employ simulations similar to those I demonstrate to determine optimal sample designs for their system before initiating major sampling effort (Devineau et al. 2006, Reynolds et al. 2011). Simulations can be set up easily in programs such as MARK and then used to explore the efficacy of different survey designs while targeting parameters that researchers deem the most important for monitoring. The most difficult hurdle is often obtaining realistic parameter information to set up the simulations. Initial parameter estimates can come from existing data, estimates reported in the literature for the same or similar species, or, if necessary, via experience and intuition. This practice can then be repeated in an

adaptive or iterative framework as data become more abundant (Lindenmayer and Likens 2009, Guillera-Arroita and Lahoz-Monfort 2012). One caveat to this adaptive and iterative design paradigm is that it is necessary to consider how changes to the study design might affect the analysis. Too much disparity in design over the course of data collection may preclude the use of models with constant parameters across seasons and this may be especially important for long-term ecological datasets.

Knowledge of population dynamics is critical in the conservation of imperiled populations and adult survival and breeding probability play a large role in driving vertebrate dynamics. Populations most in need of conservation are often small, rare, or difficult to access such that gathering data to estimate vital rate is challenging. Careful and deliberate planning, such as simulating different survey designs, can help to maximize the opportunities to collect enough data to estimate important vital rates.

Table 3.1. Initial parameter estimates used to simulate boreal toad populations with different accessibility logistics. Survival, transition, and detection probabilities were the same for both simulated populations. $Pent_1^B$, the probability of entering the breeding area before survey 1, for the inaccessible population simulations is very large, indicating that 88% of breeding females had arrived at the breeding area prior to the first survey. Comparatively, the probabilities of entry for the accessible population are more evenly distributed. The probability of survival, *S*, was constant for both states: breeders (B) and non-breeders (NB). The probability of remaining in the breeding area between consecutive surveys, φ^B, is constant but the probability of remaining for multiple surveys decreases as the number of surveys in which the individual remains increase. For example, the probability of staying in the breeding area of the inaccessible population for two surveys is 0.906 but the probability of staying for three surveys is $0.906^2 = 0.821$.

	Inaccessible	**Accessible**
$\boldsymbol{S}$	0.87	
$\boldsymbol{\psi^{B,\,NB}}$	1.00 (fixed)	
$\boldsymbol{\psi^{NB,\,B}}$	0.36	
$\boldsymbol{p^B}$	0.22	
$\boldsymbol{p^{NB}}$	0.00 (fixed)	
$\boldsymbol{pent_1^B}$	0.88	0.12
$\boldsymbol{pent_2^B}$	0.03	0.10
$\boldsymbol{pent_3^B}$	0.02	0.09
$\boldsymbol{pent_4^B}$	0.01	0.08
$\boldsymbol{pent_5^B}$	0.01	0.07
$\boldsymbol{pent_6^B}$	0.01	0.06
$\boldsymbol{pent_7^B}$	0.01	0.06
$\boldsymbol{pent_8^B}$	0.01	0.05
$\boldsymbol{pent_9^B}$	0.01	0.05
$\boldsymbol{pent_{10}^B}$	0.01	0.04
$\boldsymbol{pent_{11}^B}$	0	0.04
$\boldsymbol{pent_{12}^B}$	0	0.03
$\boldsymbol{pent_{13}^B}$	0	0.03
$\boldsymbol{pent_{14}^B}$	0	0.03
$\boldsymbol{pent_{15}^B}$	0	0.03
$\boldsymbol{pent_{16}^B}$	0	0.02
$\boldsymbol{pent_{17}^B}$	0	0.02
$\boldsymbol{pent_{18}^B}$	0	0.02
$\boldsymbol{pent_{19}^B}$	0	0.02
$\boldsymbol{pent_{20}^B}$	0	0.02
$\boldsymbol{pent_{21}^B}$	0	0.02
$\boldsymbol{\varphi^B}$	0.906	0.909

Table 3.2. Graphical representation of the simulated boreal toad survey designs, where "X" indicates a day in which a survey is conducted within the simulated 21-day breeding season. The days represented in the random designs are not indicative of the actual days used in the simulations; they merely demonstrate that the random survey designs had no consistent pattern.

Survey Scenario	**Day within the Breeding Season**																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
First 3	X	X	X																		
First 4	X	X	X	X																	
First 5	X	X	X	X	X																
Random 3			X							X				X							
Random 4	X				X		X												X		
Random 5				X				X	X					X			X				
Weekly 4, no coupling A	X						X							X							X
Weekly 4, no coupling B	X							X						X							X
Weekly 4, no coupling C	X							X							X						X
Weekly 4, first coupled	X	X							X							X					
Weekly 4, second coupled	X							X	X							X					
Weekly 4, last coupled	X							X							X	X					
Full 21	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

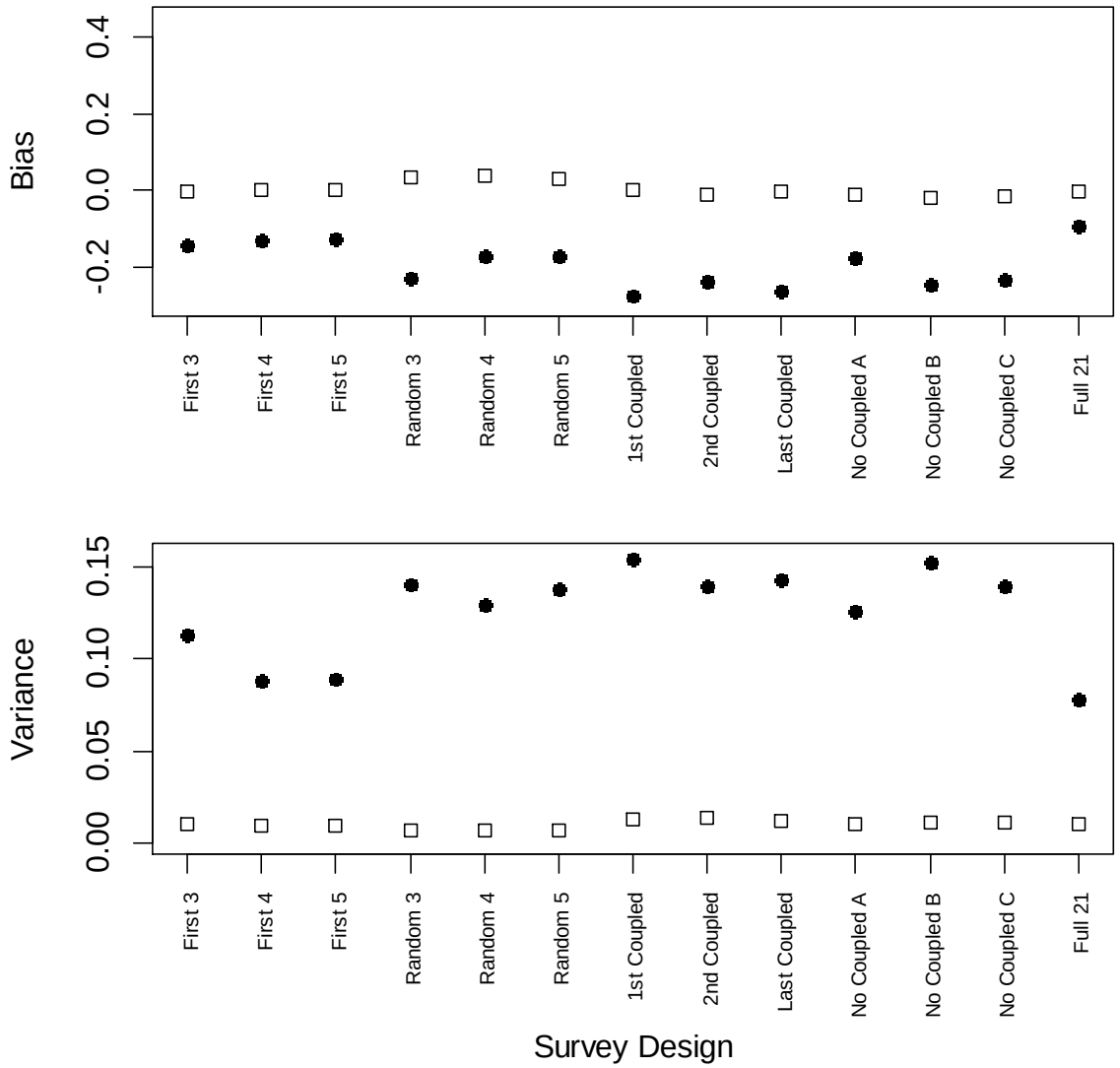

Figure 3.1. Bias and precision of boreal toad survival probability, S_i, estimates under 13 different survey designs (described in the text) for two different simulated populations: easily accessible and inaccessible. Simulations using the inaccessible population parameters are indicated with the opaque circles and accessible population simulations are indicated with the open squares. The true value for survival used in these simulations was 0.87.

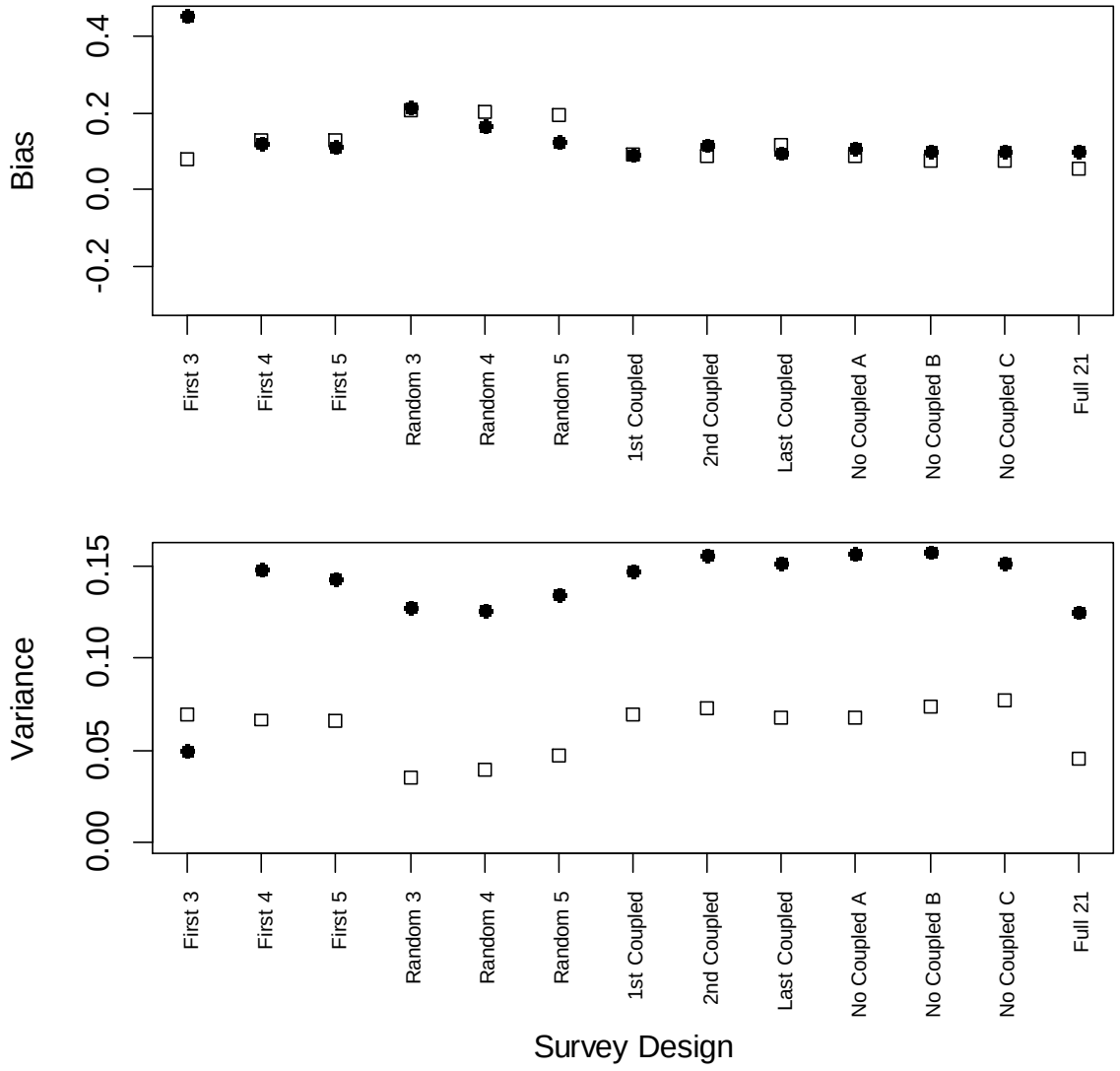

Figure 3.2. Bias and precision of boreal toad breeding probability, $\psi_i^{NB,B}$, estimates under 13 different survey designs (described in the text) for two different simulated populations: easily accessible and inaccessible. Simulations using the inaccessible population parameters are indicated with the opaque circles and accessible population simulations are indicated with the open squares. The true value for breeding probability used in these simulations was 0.36

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APPENDIX 1

An example of the data input file for the closed robust design model for one boreal toad egg mass half. Each row represents a possible encounter history. The dummy variable column is necessary because Program MARK requires at least two secondary periods for each primary period. The dummy variable overrides this and does not change the analysis if the recapture probability (*c*) for that associated secondary period is fixed to 1. Because the egg count was a census, the initial capture probability for the first period (*p*) is also fixed to 1. The second primary period consists of each removal sweep (secondary period). A "1" in these columns indicates that an individual was caught during that sweep. The counts column is the number of individuals with the encounter history described in the associated row. For example, row 2 shows that 241 tadpoles caught on the second removal sweep. The last row describes the encounter history for individuals that were counted as eggs but never recaptured as tadpoles.

Primary Period 1		Primary Period 2											
Egg Stage	Dummy Variable	Sweep 1	Sweep 2	Sweep 3	Sweep 4	Sweep 5	Sweep 6	Sweep 7	Sweep 8	Sweep 9	Sweep 10	Sweep 11	Counts
1	1	1	0	0	0	0	0	0	0	0	0	0	382
1	1	0	1	0	0	0	0	0	0	0	0	0	241
1	1	0	0	1	0	0	0	0	0	0	0	0	291
1	1	0	0	0	1	0	0	0	0	0	0	0	110
1	1	0	0	0	0	1	0	0	0	0	0	0	62
1	1	0	0	0	0	0	1	0	0	0	0	0	33
1	1	0	0	0	0	0	0	1	0	0	0	0	34
1	1	0	0	0	0	0	0	0	1	0	0	0	12
1	1	0	0	0	0	0	0	0	0	1	0	0	16
1	1	0	0	0	0	0	0	0	0	0	1	0	7
1	1	0	0	0	0	0	0	0	0	0	0	1	5
1	1	0	0	0	0	0	0	0	0	0	0	0	2523

APPENDIX 2

Model selection results for models with fixed effects only. "Treatment" refers to the trout exposure treatment: exposed or control. "Source" refers to the source of the tadpoles: captive-bred or wild-bred. Akaike's information criterion adjusted for sample size, AIC_c, is a model selection tool which is calculated as: $AIC_c = -2 \log (L) + 2 K * (n / (n - K - 1))$, where L is the likelihood of the model, K is the number of parameters in the model, and n is the sample size. ΔAIC_c is the difference between a given model AIC_c and that of the best model (the model with the lowest AIC_c). Model weight, *w*, can be considered as the probability that the model is the best of the candidate models, given the data.

i. Tadpole Survival 3-24 Days

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Treatment	58.30	0.00	54.26	2	0.91
Intercept Only	62.88	4.59	60.88	1	0.09

ii. Tadpole Survival 24 Days to Metamorphosis

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Treatment	136.57	0.00	132.55	2	0.36
Source * Treatment	136.75	0.18	128.62	4	0.33
Source + Treatment	136.95	0.38	130.89	3	0.29
Intercept Only	143.50	6.93	141.50	1	0.01
Source	143.70	7.13	139.68	2	0.01

iii. Tadpole Survival vs. Per Tadpole Strike Rate

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Source	32.31	0.00	27.91	2	0.53
Source + Strike Rate	34.45	2.14	27.62	3	0.18
Source * Strike Rate	34.95	2.63	25.52	4	0.14
Intercept Only	36.24	3.92	34.11	1	0.07
Strike Rate	36.39	4.07	31.99	2	0.07

iv. Body Condition at Emergence

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Source	-1436.19	0.00	-1440.28	2	0.59
Source + Treatment	-1434.51	1.68	-1440.68	3	0.26
Source * Treatment	-1432.96	3.23	-1441.25	4	0.12

Intercept Only	-1429.53	6.66	-1431.56	1	0.02
Treatment	-1428.11	8.08	-1432.20	2	0.01

v. Time to Metamorphosis

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Source * Treatment	366.07	0.00	357.83	4	0.66
Source + Treatment	367.36	1.29	361.22	3	0.34
Source	377.60	11.54	373.53	2	0.00
Treatment	490.26	124.19	486.19	2	0.00
Intercept Only	493.10	127.03	491.08	1	0.00

vi. Survival from Metamorphosis to Emergence

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Source + Treatment	127.16	0.00	121.02	3	0.45
Source	127.53	0.37	123.46	2	0.37
Source * Treatment	129.06	1.90	120.82	4	0.17
Intercept Only	151.19	24.03	149.17	1	0.00
Treatment	151.52	24.36	147.45	2	0.00

vii. Survival from Emergence to 4 Weeks

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Source + Treatment	13.78	0.00	7.61	3	0.25
Treatment	13.89	0.11	9.80	2	0.23
Intercept Only	13.96	0.18	11.93	1	0.22
Source	14.08	0.30	10.00	2	0.21
Source * Treatment	15.89	2.12	7.61	4	0.09

viii. Change in Body Condition Post Metamorphosis

Model	AIC_c	ΔAIC_c	-2log(L)	K	*w*
Source	-1457.71	0.00	-1461.79	2	0.68
Source + Treatment	-1455.64	2.07	-1461.81	3	0.24
Source * Treatment	-1453.52	4.18	-1461.81	4	0.08
Intercept Only	-1412.60	45.10	-1414.63	1	0.00
Treatment	-1410.90	46.80	-1414.99	2	0.00