THESIS

INVESTIGATING FACTORS INFLUENCING THE PROBABILITY OF SURVIVAL TO METAMORPHOSIS OF BOREAL TOADS AT MULTIPLE SCALES

Submitted by

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ABSTRACT

INVESTIGATING FACTORS INFLUENCING THE PROBABILITY OF SURVIVAL TO METAMORPHOSIS OF BOREAL TOADS AT MULTIPLE SCALES

Pond-breeding amphibians with biphasic life-histories tend to have highly variable recruitment rates, making their population dynamics complex. Populations of pond-breeding amphibians may decline in many or even most years, but remain relatively stable over the long term due to occasional "boom" years of high recruitment. Amphibian monitoring often focuses on the adult life stage, but especially in long-lived species, high adult survival can mask long-term declines due to low recruitment. Without reliable estimates of recruitment from long-term studies, it may be difficult to distinguish between a population with highly variable recruitment that is stable over the long term and a population in a slow decline due to low recruitment.

Many amphibian declines are due to emerging infectious diseases such as chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* [Bd]. In species susceptible to chytridiomycosis, such as the boreal toad (*Anaxyrus boreas boreas)*, this disease primarily impacts adult life stages, making recruitment even more important. Moreover, few effective strategies exist for increasing survival rates of adults in species such as the boreal toad that are declining in apparently pristine habitats. Many conservation strategies therefore focus on limiting the spread of Bd when possible and on earlier life stages, either through habitat manipulation or through translocations of tadpoles. Due to the high variation in early life stage survival, management interventions could have a much larger impact by focusing on these life stages rather than on adult survival, which may already be close to the upper bound. However, although life history theory suggests that survival in early life stages is highly variable, and there is evidence for variation in recruitment into the adult life stage, few studies have attempted to estimate survival probabilities of larval amphibians while accounting for imperfect detection. Accurate estimates of survival of early life stages would allow us to test the assumption of high variability and address hypotheses regarding the causes of variation.

This thesis explores several hypotheses related to early life-stage survival of boreal toads across different spatial and temporal scales. Bd infects tadpoles as well as adult toads, lowering their survival rates. Desiccation is known to cause the complete failure of reproduction at a breeding site, and tadpoles at ephemeral sites may be under stress associated with warming water temperatures and falling water levels even if the site does not dry out completely. Predation by aquatic insects and birds can be a major source of mortality and introduced trout could also attempt to ingest tadpoles despite their chemical defenses, lowering survival rates at sites with trout.

In the first chapter of this thesis, I use capture-recapture methods to test whether hydroperiod, trout presence, Bd presence, and reintroduction status effect the survival rates of boreal toad tadpoles. I censused egg masses laid at three sites in Rocky Mountain National Park as well as tadpoles head-started in hatcheries and introduced to a fourth site. When tadpoles at these sites began to metamorphose, I used two temporary removal samples to estimate the number of individuals that metamorphosed. I found that survival to metamorphosis varied among years and sites, and that this variation was not well explained by any of the factors I tested. Although I did not find a single factor that drove variation in survival, my results suggest that ongoing reintroductions of head-started tadpoles have survival to metamorphosis rates comparable to wild sites. By using robust methods that account for imperfect detection I was able to reliably estimate survival, providing evidence to support life-history theory that suggests survival in this stage would be highly variable.

Although I found no evidence that trout presence reduced tadpole survival, past work has suggested that trout could have an indirect effect on tadpoles through sublethal effects of trout attempting to ingest tadpoles or through tadpole avoidance of areas frequented by trout. In my second chapter, I test this hypothesis using multi-species occupancy modeling. I found that tadpoles use all available habitat, while trout are limited to deeper areas. Frequency of tadpole use is lower in areas also used by trout, but I observed no obvious behavioral or temporal avoidance. My results suggest that trout do not exclude tadpoles from habitats, which in combination with my results from chapter one and past work suggests that the impact of trout on toads in wild sites is negligible.

In the third chapter, I shift to a larger spatial and temporal scale to assess the causes of reproductive failure. While chapter one estimates the probability of survival for individual tadpoles at sites that supported metamorphosis, chapter three estimates the probability of at least some individuals metamorphosing at a site, given that breeding occurred at that site. I used multi-state occupancy models to test the impact of site elevation, permanence, snowpack, Bd status, and trout presence on the probability of breeding and the conditional probability of metamorphosis. I used two types of multi-state occupancy analysis to explore the impacts of these covariates as well as previous breeding state of the site. My results confirm past work showing that elevation and Bd status influence the probability of breeding. I found that the probability of metamorphosis, given breeding, is higher at permanent sites in years of low snowpack and at ephemeral sites in years of high snowpack. This suggests that long active seasons are an important determinant of whether metamorphosis occurs at a site. My use of two

types of analysis reveals the consequences of failing to meet model assumptions; my static analysis assumes that past state of a site does not influence breeding or metamorphosis probabilities, and the predictions of this model are qualitatively different than those made by my dynamic analysis.

Boreal toad conservation efforts often focus on early life-stages of toads as these are easily manipulated and may present a greater opportunity to make an impact. Reliable estimates of the causes and extent of variation in survival of these early life-stages will allow managers to make more informed decisions regarding where to reintroduce tadpoles. Tadpole reintroductions require a major investment of time and resources, but successful reintroductions can lead to robust populations that are self-sustaining and important to conservation of the species throughout the region, so appropriate criteria for site selection can be vital to the conservation effort.

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I would like to thank the members of the Bailey lab, many of whom also volunteered to look at toads, and all of whom endured various draft presentations and manuscripts from this thesis. Their comments helped immensely to improve the work, our discussions were very helpful in my understanding of the scientific concepts, and their friendship and commiseration, along with other members of the CSU community, helped me get through the difficulties on the way to this thesis.

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CHAPTER ONE: HIGHLY VARIABLE RATES OF SURVIVAL TO METAMORPHOSIS IN WILD BOREAL TOADS (*ANAXYRUS BOREAS BOREAS*)

Life history theory suggests that long-lived, pond-breeding amphibians should have low and highly variable early life-stage survival rates, but this theoretical expectation is often untested and the causes of variation are usually unknown. I evaluated the impact of hydroperiod, presence of a pathogen (*Batrachochytrium dendrobatidis*), presence of a potential predator (cutthroat trout *Oncorhychus clarki stomias)*, and whether animals had been reintroduced into a site on survival of early life stages of boreal toads (*Anaxyrus boreas boreas*). I used a multi-state mark-recapture framework to estimate survival of boreal toad embryos from egg to metamorphosis at four sites over five years. I found substantial spatial and temporal variation in survival to metamorphosis and documented some evidence that monthly tadpole survival was lower in sites with Bd, without trout, and at permanent sites. My results support theories of amphibian life history, aid in the management of this species of conservation concern, and contribute to my knowledge of the ecology of the species. Additionally, I present methodology that allows practitioners to account for different lengths of time between sampling periods when estimating survival probabilities and is especially applicable to organisms with distinct biological stages.

Introduction

Life history theory predicts that long-lived species should have high and stable adult survival probabilities but lower, more variable reproductive rates and juvenile survival rates (e.g., Péron et al., 2016). Population growth rates of long-lived species are most sensitive to changes in adult survival rates (Heppell et al., 2000), but the contribution of variation in survival rates to the variation in population growth rates may be small relative to the contribution of variation in reproductive rates (Mills et al., 1999; Saether & Bakke, 2000; Wisdom et al., 2000). Although these theories are well-established in mammalian literature (e.g., Gaillard et al., 1998), they are not well-documented in other taxa (but see e.g., Heppell, 1998; Wisdom & Mills, 1997; Saether & Bakke, 2000). Many species lack information on the causes and extent of variation in vital rates and the impact that this variation may have on population growth rates or viability (Biek et al., 2002).

Species of conservation concern often lack information on vital rates, and their populations tend to be small and isolated, making within-population processes particularly important. These combined factors are particularly concerning in pond-breeding amphibians. Amphibians are understudied relative to other vertebrate taxa (Lawler et al., 2006; Di Marco et al., 2017; de los Rios et al., 2018) and pond-breeding amphibians with biphasic life histories tend to have extremely high variation in recruitment (Berven, 1990; Pechmann et al., 1991; Semlitsch et al., 1996; Alford & Richards, 1999; Marsh & Trenham, 2001). Widespread declines in anurans due to the global threats, such as habitat loss and fragmentation and pathogens (e.g., the fungus *Batrachochytrium dendrobatidis* [Bd]), have reduced the size and number of many populations, reducing the capacity for immigration into remaining populations or recolonization of locally extirpated areas (Collins & Storfer, 2003; Daszak et al., 2000; Scheele et al., 2019).

A number of mechanisms can cause variation in early life-stage survival rates of pond-breeding amphibians in wild settings, including variation in hydroperiod, predation, and the presence of Bd, ranavirus, or other pathogens. Breeding sites may dry before larvae metamorphose, and even at sites where some individuals metamorphose successfully, the survival rate of larvae may be lower in ephemeral than in permanent breeding sites (Semlitsch & Wilbur, 1988; Wissinger & Whiteman, 1992; Sherman & Morton, 1993; Carey et al., 2005; Taylor et al., 2006; Amburgey et al., 2012). Both native and introduced predators can lead to major reductions in larval survival of pond-breeding amphibians, especially for palatable species where non-native salmonids have been introduced (Knapp, 2005; Knapp et al., 2005; Pilliod & Peterson, 2001). Pathogens that mainly affect adults, such as Bd, may be compensated for through higher recruitment (Pilliod et al., 2010), but Bd can also infect larval stages and may lower tadpole survival (Garner et al., 2009).

Population growth rates of long-lived, pond-breeding amphibians tend to be most sensitive to changes in adult survival rates (Biek et al., 2002), making these species particularly vulnerable to threats that affect terrestrial stages such as Bd (e.g., Scherer et al., 2005; Pilliod et al., 2010) or terrestrial habitat loss (Semlitsch & Wilbur, 1988; Goates et al., 2007; Harper et al., 2008). However, the natural range of variation in adult survival in such species is often quite low (e.g., Pilliod et al., 2010; Muths & Scherer, 2011; Muths et al., 2011), which limits the capacity of management actions to impact this life stage. Survival of earlier life stages is expected to be much lower, but a wider range of variation (Biek et al., 2002), potentially offers more opportunity to increase these vital rates through management actions (Blaustein et al., 2005; Garner et al., 2016; Gerber et al., 2018).

One example of a long-lived, pond-breeding amphibian challenged by a threat to adult survival is the boreal toad (*Anaxyrus boreas boreas*). Native to western North America, the boreal toad has declined in the Southern Rocky Mountains portion of its range over the last several decades (Carey, 1993; Muths et al., 2003; Mosher et al., 2018), largely due to the introduction of Bd. In the absence of Bd, adult survival rates of this species tend to be high and stable (Pilliod et al., 2010; Muths & Scherer, 2011), but populations in which the pathogen is prevalent have lower adult survival rates (Scherer et al., 2005; Pilliod et al., 2010) and may also have reduced tadpole survival (Blaustein et al., 2005; Lindauer & Voyles, 2019). Although the species, like all bufonids, is unpalatable to fish, there is some evidence that attempts by introduced greenback cutthroat trout (*Oncorhynchus clarki stomias*) to ingest tadpoles could reduce tadpole survival probabilities and extend time to metamorphosis (Lanier et al., 2017). Estimates of recruitment into the adult life stage are highly variable (e.g., Muths & Scherer, 2011; Muths et al., 2011) and this variation may be due to the toad's tendency to lay eggs in shallow wetland areas (Holland, 2002), leaving them vulnerable to desiccation or freezing (Carey et al., 2005). In addition to these sources of variation in survival in wild populations, toads have been introduced to several sites in Colorado (Muths et al., in press). Current introductions involve releasing head-started tadpoles raised to Gosner stage 25 (Gosner, 1960) in a hatchery from harvested wild eggs (Muths et al., in press). These tadpoles are expected to have higher survival rates than wild tadpoles due to this head-starting process.

I censused the number of eggs and sampled metamorphs at four breeding sites across five years and used these data in a capture-mark-recapture framework to test the impact of presence of Bd, wetland permanence, presence of trout, and head-starting on survival to metamorphosis of boreal toads. Evaluating the impact of each of these potential sources of variation on survival to metamorphosis will: (1) help guide management efforts for this species, especially by aiding in selection of reintroduction sites, (2) improve my understanding of the biology of this species, and (3) allow us to better evaluate aspects of life history theory that are rarely tested in amphibians.

Methods

Study Area Description

My study took place at three of the four remaining boreal toad breeding sites in Rocky Mountain National Park: Fay Lakes, Lost Lake, and Spruce Lake, and at one reintroduction site, Red Mountain (Figure 1.1). These locations vary in current Bd status, permanence, and presence of introduced trout. Spruce lake is Bd negative, while the other sites are Bd positive. The Fay Lakes breeding site is ephemeral, while the other sites are permanent. Lost and Spruce Lakes contain breeding populations of greenback cutthroat trout (*Oncorhynchus clarki stomias)*, while the other two sites do not contain fish. All breeding sites are located at high elevations in the park (between 2900m and 3300m) and surrounded by coniferous forest. All animal care approvals were obtained through Colorado State University's Animal Care and Use Committee (ACUC).

Data Collection

In 2013-2014 and 2017-2018 (four years), wild breeding sites were monitored in early summer for egg masses. Each egg mass was photographed by placing a white plastic tray behind it to serve as a background. A marker (e.g., colored flagging) was placed near each photographed egg mass to distinguish between multiple egg masses at a site. I used imageJ (Rasband, 2019) to census the number of eggs in each photograph (Table 1). For my reintroduction site, eggs were collected from other breeding sites in the region, counted, and reared in captivity until they were released as tadpoles (Gosner stage 25-42; see Muths et al., 2014 for captive rearing and release procedures). A known number of tadpoles were released at the reintroduction site (Red Mountain) in each of three years during my study (2014, 2015, and 2017; Table 1).

Following egg hatching or release of tadpoles, sites were monitored to track tadpole development. One week after late-stage tadpoles or metamorphs were observed at a site, I conducted a 2-3 pass temporary removal sample (Muths et al., 2014). Metamorphs were captured by hand and held until all passes were complete. The number of captured individuals were recorded for each pass, and all captured individuals were given a single batch mark of visible implant elastomer (Northwest Marine Technology, Inc.) on the left front leg (Swanson et al., 2013). Approximately one week later, after all individuals had metamorphosed, I conducted a second temporary removal sample and recorded the numbers of marked and unmarked individuals from each pass. This second removal sample was conducted at all sites except Fay Lakes in 2014, where all tadpoles had metamorphosed before the first removal sample, so I did not conduct a second sample.

All egg masses or released tadpoles for a given site and year were treated as independent "cohorts" of individuals with two exceptions: in 2014, at Red Mountain, two cohorts of tadpoles were released at different times (approximately two weeks apart) and tadpoles from the first cohort were metamorphosing when the second cohort was released, allowing us to distinguish between the two cohorts. At Lost Lake in 2017 egg masses were deposited in two different areas of the lake, with one egg mass in a small pool on the east side of the lake and two egg masses on the west side of the lake. The tadpoles hatching from different sides of the lake were separated by >100m of deep, cold water and were treated as independent cohorts.

Data Analysis

For each cohort, I generated capture histories consisting of three primary periods. The first primary period represented the release of the known number of eggs or tadpoles and the second and third periods corresponded to the captures from my two removal samples. Marked individuals captured during the final removal sample were distributed randomly among histories from the marked cohort obtained during the first removal sample to avoid bias (Converse et al., 2009; Muths et al., 2014).

I analyzed the capture histories using a multi-state robust design model (Pollock et al., 1990; Kendall & Nichols, 1995) implemented in Program MARK (White & Burnham, 1999). This model allows for estimation of three parameters: survival probability, transition probability, and detection probability. Survival probability, S_t^k, is the probability that an individual in state *k* at time *t* survives and remains in the study area between time *t* and *t+1*. Transition probability, ψ_t^{kl}, is the probability that an individual in state *k* at time *t* will be in state *l* at time *t+1*, given that it survives between the two primary periods. Detection probability, p_{tj}^k, is the probability that an individual will be captured during pass *j* of primary period *t*, given that it is alive and in state *k*. Several parameters were fixed to 0 to reflect biological and sampling realities. Transitioning from metamorph (M) to tadpole (T) is impossible and I did not capture tadpoles in the aquatic habitat, so ψ_t^{MT} , p_{2j}^T and p_{3j}^T were all fixed to 0. In addition, because tadpoles are not sampled (i.e., represent an unobservable state), two additional assumptions are required for the remaining parameters to be identifiable: I assumed that all tadpoles that survive and metamorphose have done so prior to the final removal sample ($\psi_2^{TM} = 1$) and that monthly survival probability of tadpoles is constant between primary periods ($S_{1\,monthly}^T = S_{2\,monthly}^T$, Muths et al., 2014).

I used a two-step process to analyze the data. In the first step, each cohort was analyzed independently to find the best model of detection probabilities using four model structures for each cohort (except for Fay Lakes in 2014). Detection could vary by: 1) sample and pass (additive relationship), 2) sample but not by pass, 3) pass but not by sample, and 4) neither sample or pass (i.e., constant detection probability). I did not consider interactions between pass and sample. I ranked models by AICc and selected the best detection structure for each cohort for use in the combined analysis (detailed below). Some models yielded unreasonable estimates (parameter estimates of either 0 or 1 with standard error estimates of 0); these models were excluded from further consideration (Table 2).

Next, I combined the data from all cohorts in a single analysis. Importantly, the intervals between primary periods were not equivalent across all cohorts. To account for this variation, I modeled tadpole survival using a log link function:

$$ln\left(S_{i,t}^T\right) = B_0 * \left(month_{i,t}\right) + B_1 * x_c * \left(month_{i,t}\right) \qquad \text{Eq. 1.1}$$

where $month_{i,t}$ represents the number of months between primary periods *t* and *t*+1 for cohort *i* and x_c is a variable of interest (e.g., Bd presence). Exponentiating both sides of Eq. 1.1 gives the cohort-specific tadpole survival estimate between primary periods:

$$S_{i,t}^T = e^{(B_0 + B_1 * x_c) * month_{i,t}} \qquad \text{Eq. 1.2}$$

Likewise, I compared monthly survival estimates for each cohort by setting the value of $month_{i,t}$=1, allowing the coefficient(s) associated with each variable to be interpreted as the effect(s) on monthly survival:

$$S_{i,t_{monthly}}^{T} = e^{B_0 + B_1 * x_c} \qquad \text{Eq. 1.3}$$

Both the probability of transitioning from tadpole to metamorph ($\psi_{i,1}^{TM}$) and the probability of metamorphs surviving and remaining in the study area ($S_{i,2}^{M}$) are largely a function of the timing of sampling relative to tadpole development and were estimated separately for each cohort. Detection probabilities were modeled based on the best-supported structure for each cohort from the previous analysis.

I fit 13 different models of tadpole survival to the combined capture histories (Table 3). The global model allowed monthly survival to vary among the 10 unique cohorts. I fit models where monthly survival varied: by site and year (but was constant across cohorts within a given site and year), by site only, by year only, and constant across all sites and years (Table 3). Likewise, to test my biological expectations, I fit models with spatial variation associated with my factors of interest (Bd presence, trout presence, permanence, and whether the site had reintroduced tadpoles). For each of my factors of interest, I fit one model that included annual variation (year) and one model with only spatial variation attributed to the chosen factor (Table 3).

Using estimates from best-supported model, I calculated survival from egg to metamorphosis for each cohort. This is given by the equation:

$$S^{EM} = S_1^T * (\psi_1^{TM} + (1 - \psi_1^{TM}) * S_2^T) \qquad \text{Eq. 1.4}$$

where S_1^T = the survival probability of tadpoles between hatching and the first removal sample, S_2^T = the survival probability of tadpoles between the first and second removal samples (Muths et al., 2014). For the cohorts at the reintroduction site (Red Mountain), this value was multiplied by captive rearing success (the proportion of harvested eggs that successfully hatched and survived to release) to insure values from the reintroduction site were comparable to wild sites.

Results

Metamorphosis occurred in 5 years across the 4 sites, with ten cohorts (Table 1). Egg masses were also detected at Fay Lakes in 2013 and at Spruce Lake in 2014 and 2018, but metamorphs were either not observed (Fay Lakes 2013, Spruce Lake 2014) or estimation failed due to lack of depletion (i.e., the number of individuals captured on successive passes did not decrease so abundance could not be estimated; Spruce Lake 2018; White et al., 1982, pg. 108).

Detection probabilities varied widely among cohorts and among samples and passes within cohorts (0.103 (SE 0.030) - 0.791 (SE 0.089)). All detection structures were supported by data from at least one cohort (Table 2), suggesting that metamorph detection probabilities varied among samples, passes, or both (Table 2). Detection structures that included variation among samples, or among samples and passes, occasionally yielded unreasonable parameter estimates (i.e., parameters being estimated at the boundary, 0 or 1) and were omitted from further consideration (Table 2).

Tadpole survival probabilities varied among cohorts (Table 3; model weight, $w = 1.0$). Although models containing my factors of interest had little support, the estimated effect sizes still gave us information about my *a priori* hypotheses. Consistent with my expectations, tadpole survival probabilities were lower at sites with Bd (Table 4). I found no evidence that trout negatively affect tadpole survival; monthly survival estimates are higher at sites with trout (Table 4). Head-started tadpoles had similar monthly survival rates to tadpoles at wild sites; reintroduction was the only factor that, when included in a model containing a year effect, did not improve the model fit (Table 5). Monthly survival was slightly lower in permanent sites (Table 4).

Survival to metamorphosis varied among sites and years as well as between cohorts within years (Table 5, Figure 1.2) and was affected by both monthly survival probability and time to metamorphosis. For wild sites, time to metamorphosis varied between 2.3-3 months (Table 5). The highest estimates of survival to metamorphosis occurred in those cohorts that had high monthly survival and a shorter time to metamorphosis (e.g., Spruce 2013); the lowest estimates of survival to metamorphosis occurred in cohorts with low monthly survival and a longer time to metamorphosis (e.g., Lost 2017(E)). In contrast, times to metamorphosis in cohorts of tadpoles released at the reintroduction site were approximately equal (0.5-0.8 months) by design (Table 5). At this site, variation in survival to metamorphosis was largely attributable to variation in monthly tadpole survival, and to a lesser degree hatching success (Table 5). The largest variation in survival to metamorphosis was observed at the reintroduction site (Table 5; Figure 1.2), but individuals metamorphosed from each released cohort (i.e., no reproductive failure), which was not the case for any of the wild sites. While the head-starting process leads to variable survival to metamorphosis, it is successful at producing metamorphs every year (this study, Muths et al. 2014).

Discussion

Pond-breeding amphibians have highly variable recruitment (Berven, 1990; Pechmann et al., 1991; Semlitsch et al., 1996; Alford & Richards, 1999; Marsh & Trenham, 2001), but most studies base this finding on counts of individuals, typically breeding adults and metamorphosing juveniles (e.g., Berven, 1990; Pechmann et al., 1991) or on the frequency of reproductive failure (Semlitsch et al., 1996). In these cases, variation in recruitment could be attributed to population size, age, sex or stage structure, breeding probability, aquatic survival rates, and detection probability. While Biek et al. (2002) were able to use theoretical population models to differentiate the relative contribution of each of these components to overall population growth rates, it is nearly impossible to obtain empirical estimates of recruitment-related vital rates from available count data because many of these vital rates and detection probabilities are confounded. By conditioning on a known number of eggs and accounting for variation in detection, my study provides robust estimates of one major component of recruitment (survival to metamorphosis) and quantifies the variation in this vital rate across space and time. Importantly, I explored whether this variation is related to factors hypothesized to impact survival in early life stages.

Previous work on boreal toads suggests that variation in early life-stage survival may contribute more to variation in overall population growth rates than variation in adult survival, despite the high elasticity of population growth rate to changes in adult survival (Biek et al., 2002). This idea is supported by recent analyses focused on the Southern Rocky Mountains populations of boreal toads (Hardy and Crockett, unpublished data). This study is the first to estimate survival to metamorphosis of wild toads and compare them to similar metrics from head-started reintroductions. Survival to metamorphosis varied widely among cohorts in my study, with no single factor explaining the observed spatial and temporal variation. All models that included annual variation outperformed models that included only spatial (or no) variation, suggesting that recruitment to the terrestrial life stage varies considerably over time within a population.

I found no evidence that trout negatively affect tadpoles, suggesting that trout predation is not a major factor in tadpole survival and trout presence should not be considered a deterrent when evaluating a potential site for reintroduction of toads. Additionally, I found evidence for a negative effect of Bd on tadpole survival, which supports laboratory evidence for an effect of Bd on tadpole growth and post-metamorphic survival (Garner et al., 2009). I found a negative effect of permanence on monthly tadpole survival, but the ephemeral site only supported metamorphosis in one year of my study, so ephemeral sites may not be better candidates for reintroductions than permanent sites.

I did not find evidence that monthly survival at the reintroduction site differed from wild sites, and estimates at the reintroduction site were highly variable. Released tadpoles metamorphosed quickly and survival to metamorphosis was comparable to estimates from wild sites. It is unclear what caused the difference in monthly survival between cohorts of tadpoles released at the reintroduction site; rearing conditions and source populations were similar for all cohorts. The cohorts released in 2014 are particularly difficult to explain since they experienced the same conditions during the time they overlapped in the water (approximately one week). Muths et al. (2014) reported a wide range of survival estimates across four years of tadpole releases at this site (2010-2013), but they suggested the higher survival probabilities seen in 2012 and 2013 were a consequence of releasing tadpoles that were more developed, leading to a short time to metamorphosis. Despite retaining the suggested rearing and release process, I saw a wide range of survival to metamorphosis estimates across the four cohorts of tadpoles released during my study. Still, the range of survival to metamorphosis in head-started, translocated tadpoles (in this study and Muths et al., 2014) is similar to the survival to metamorphosis of wild eggs in this region, with less reproductive failure. Accordingly, this method is likely an effective management strategy, particularly if reintroduction sites are Bd negative. In contrast to Bd, trout presence should not be a deterrent for future toad reintroduction efforts. In years in which metamorphosis occurred, Spruce Lake and Lost Lake had high survival to metamorphosis probabilities, suggesting that trout do not negatively affect larval toads (Crockett et al., in review).

Reproductive failure (i.e., no metamorphs emerging from a site where at least some eggs were laid) has been cited as a major cause of variation in survival to metamorphosis in the boreal toad (e.g., Carey et al., 2005) and is often attributed to desiccation at ephemeral breeding sites. I observed reproductive failure four times over the course of my study, twice at Fay Lakes (2013 and 2017) and twice at Spruce Lake (2014 and 2018). Both failures at Fay Lakes were attributed to desiccation, but the cause of failure at Spruce Lake is unclear. A recent analysis of breeding sites across the Southern Rocky Mountains found that in permanent sites, like Spruce Lake, the probability of reproductive failure is associated with years of high snowpack leading to delayed egg deposition and larval development (Crockett, 2019). There was no clear pattern between snowpack and reproductive failure in the wild sites in this study; 2013 and 2014 had high snowpack relative to 2017 and 2018, but reproductive failure occurred at some site(s) in all four years. Moreover, the range of monthly survival estimates and time to metamorphosis were similar for these two periods (2013-14, 2017-18), and cohorts with longer times to metamorphosis did not necessarily have lower survival to metamorphosis (Table 5). Collectively, this evidence suggests that snowpack alone is not responsible for my observed variation in survival to metamorphosis.

Capture-recapture methods are a powerful tool for estimating survival rates of organisms, but in situations where some life stages are inaccessible to sampling, additional constraints are necessary (Kendall, 2004**,** Bailey et al., 2010). In my case, I assumed that monthly tadpole survival was constant across the two primary periods. This is easily done when analyzing a single cohort (see Muths et al., 2014 for an example), but this necessary constraint presents a challenge when combining data from multiple sites with variable lengths of time between primary periods. Time to metamorphosis can have a large effect on survival to metamorphosis (Table 5, Figure 1.2), and using the log link function I were able to separate this time effect from the effects of different site-specific characteristics. A similar approach has been used to estimate the survival probabilities of both breeding (observed) and nonbreeding (unobservable) adults, where male and female breeders spend different amounts of time in the breeding habitat (e.g., Bailey et al., 2004; Church et al., 2007), but this is the first time the method has been used to estimate monthly tadpole survival and survival to metamorphosis. This method could be applied to other systems in which individuals enter and return from an unobservable state at variable times, especially when the individuals in different states are expected to have very different survival probabilities (e.g., individuals in different life stages, as in this study, or species that migrate between different habitats). Because the log link does not constrain survival estimates between 0-1, specifying initial values for the likelihood optimization may be necessary to obtain reasonable estimates of parameters and effect sizes.

My study provides one of few examples of estimating different components of recruitment while accounting for variation in detection probabilities. I found considerable variation in monthly survival probabilities, with higher monthly survival at ephemeral sites, sites with trout, and sites without Bd. Additionally, tadpole survival probabilities at my reintroduction site were comparable to those obtained at wild sites, but with fewer reproductive failures, suggesting that head-starting is a viable method to establish new populations, provided Bd is absent (Muths et al., in press). my results provide empirical support for the hypothesis that amphibians will have highly variable survival in early life-stages as well as providing information applicable to management of boreal toads.

Table 1.1. The known numbers of eggs or released tadpoles (Red Mountain) and the number of captured metamorphs from each cohort (*n* = 10).

Site	Year (cohort)	Number of eggs or tadpoles	Number of metamorphs captured
Spruce Lake	2013	5513	600
Spruce Lake	2017	7992	1242
Fay Lakes	2014	18065	712
Red Mountain	2014 (a)	351	187
Red Mountain	2014 (b)	7634	189
Red Mountain	2015	1298	333
Red Mountain	2017	1032	28
Lost Lake	2017 (E)	5873	133
Lost Lake	2017 (W)	3075	254
Lost Lake	2018	19957	2387

Table 1.2. Model selection results for four detection probability structures fit to capture histories from 10 cohorts. For one cohort, Fay Lakes in 2014, only one removal sample was conducted, so detection probabilities could not vary by sample. The best-supported model structure was selected using AICc. In all models, S^T, S^M, and ψ^{TM} were constrained to intercept-only structures. Models with parameters estimated at the boundary or with standard errors of zero were deemed unreasonable and were excluded from the selection process. The mean model weight was calculated across all cohorts, including only models that were deemed reasonable.

	Detection probability structures			
	sample+pass	sample	pass	constant
Number of cohorts tested	9	9	10	10
Freq. chosen as best model	3	3	3	1
Mean weight	0.450	0.438	0.377	0.123
Freq deemed unreasonable	4	3	1	0

Table 1.3. Model selection results for monthly tadpole survival probability models. In models that include an effect of a factor of interest (Bd, trout presence, permanence, or reintroduction sites), spatial variation among sites is modeled as a function of that factor. In all models, detection differed by cohort, using the top-ranked detection structure for that cohort from the previous model selection step. S^M and ψ^{TM} differed by cohort. The columns present the model notation, the number of parameters (K), the difference between the model's AICc value and that of the top model (ΔAICc), AICc weights (w), and the deviance (or -2 log likelihood) of the model.

Model	K	Δ AICc	w	-2log(L)
S^T (cohort)	49	0	1	54656.21
S^T (Trout+year)	45	25.08	0	54689.30
S^T (Permanent+year)	45	25.41	0	54689.63
S^T (Bd+year)	45	26.02	0	54690.24
S^T (site+year)	47	26.97	0	54687.18
S^T (year)	44	32.22	0	54698.44
S^T (Introduced+year)	45	32.62	0	54696.84
S^T (site)	43	63.82	0	54732.04
S^T (Bd)	41	133.80	0	54806.02
S^T (Introduced)	41	517.57	0	55189.80
S^T (.)	40	544.99	0	55219.22
S^T (Permanent)	41	552.45	0	55224.67
S^T (Trout)	41	553.30	0	55225.53

Table 1.4. Estimated effect sizes (beta coefficients) for factors of interest and the upper and lower bounds of the associated confidence intervals. These beta coefficients represents effects on monthly survival probability on the log scale (Eq. 3). Models are listed according to their AICc rankings (Table 3).

Model	Estimated Effect Size	Lower bound of confidence interval	Upper bound of confidence interval
S^T (Trout+year)	2.7870	2.3269	3.2471
S^T (Permanent+year)	-0.1848	-0.4803	0.1108
S^T (Bd+year)	-0.1143	-0.327	0.0983
S^T (Introduced+year)	-0.1873	-0.484	0.1095
S^T (Bd)	-0.2548	-0.2787	-0.231
S^T (Introduced)	-0.6729	-0.7842	-0.5615
S^T (Permanent}	-0.0683	-0.2224	0.0858
S^T (Trout)	0.0146	-0.0353	0.0645

Table 1.5. Tadpole monthly survival probability, hatching success, time to metamorphosis, and survival to metamorphosis (S^{EM}) for each cohort. All estimates are based on the best-supported model, in which tadpole survival was estimated separately for each cohort. Standard errors of S^{EM} given in parentheses.

Site	Year (cohort)	Monthly survival of tadpoles ($S^T{}_{monthly}$)	Hatching success (if applicable)	Time to metamorphosis (months)	Survival to metamorphosis (S^{EM})
Spruce Lake	2013	0.553	*N/A*	2.3	0.203 (0.036)
Fay Lakes	2014	0.487	*N/A*	3.0	0.119 (0.027)
Red Mountain	2014 (a)	0.437	0.53	0.5	0.342 (0.013)
Red Mountain	2014 (b)	0.229	0.89*	0.7	0.052 (0.012)
Red Mountain	2015	0.209	0.59	0.8	0.160 (0.0053)
Red Mountain	2017	0.033	0.61	0.7	0.021 (0.0042)
Spruce Lake	2017	0.527	*N/A*	2.8	0.163 (0.0045)
Lost Lake	2017 (E)	0.305	*N/A*	3.0	0.025 (0.0040)
Lost Lake	2017 (W)	0.470	*N/A*	2.5	0.119 (0.039)
Lost Lake	2018	0.393	*N/A*	2.3	0.108 (0.0051)

*Cohort b at Red Mountain in 2014 was a mix of tadpoles head-started at two different hatcheries, and hatching success was only reported from one of them; I assume the same hatchery success for all collected embryos.

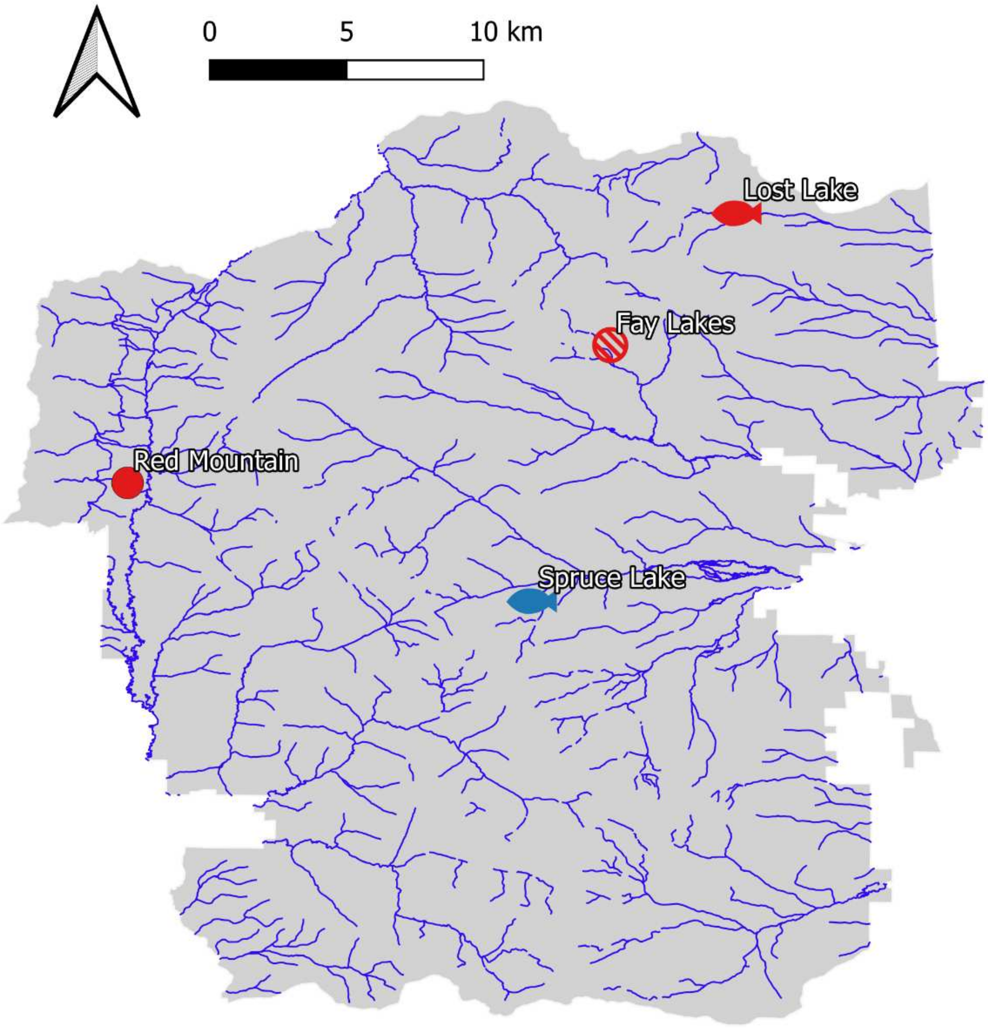

Figure 1.1. Location of study sites within Rocky Mountain National Park. Park lands are shaded in gray, and streams and rivers are shown in blue. Bd positive sites are shown in red and Bd negative sites in blue. Sites with trout are indicated by a fish symbol and sites without are indicated with a circle. The hashed symbol represents my single ephemeral site.

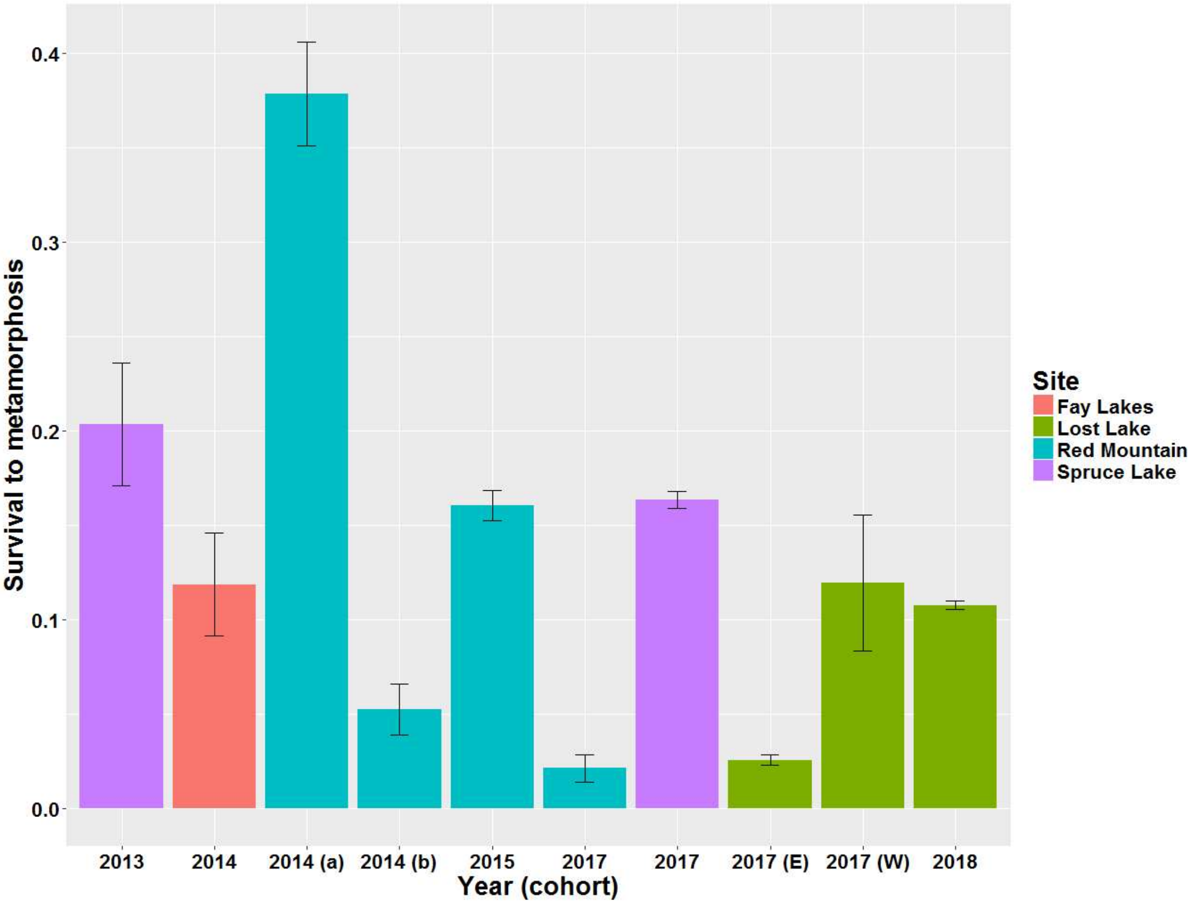

Figure 1.2. Estimates of survival from egg to metamorphosis for each cohort, based on the best-supported model, where estimated tadpole survival varied among cohorts. Note that monthly survival for the Fay Lakes cohort was high relative to Red Mountain or Lost Lake cohorts (Table 5), but overall survival to metamorphosis is low due to the long time to metamorphosis. Survival (both monthly and overall) of cohorts at Red Mountain varied widely.

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CHAPTER TWO: FEW IMPACTS OF INTRODUCED CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI*) ON AQUATIC STAGES OF BOREAL TOADS *(ANAXYRUS BOREAS BOREAS*)

Introduced salmonids have major impacts on aquatic ecosystems through both direct predation and indirect effects. I explored the effects of introduced cutthroat trout (*Onchoryncus clarki)* on boreal toad (*Anaxyrus boreas boreas)* survival and habitat use during two aquatic life stages of toads. I used robust methods to account for imperfect detection in estimating survival and habitat use during the embryo and tadpole stages at two toad breeding sites, one with trout and one without. I found no difference in embryo survival and higher tadpole survival at the site with trout. Trout are unlikely to use the shallow areas where toad eggs are deposited; however, during the tadpole stage, trout and tadpoles overlap broadly in near-shore (<15m) aquatic habitats. Frequency of tadpole habitat use is lower in trout-used areas, but I observed no obvious behavioral or temporal avoidance of trout by toads. my results suggest that trout do not have a negative effect on boreal toad embryo or tadpole survival in wild settings and that trout presence does not preclude tadpoles from using habitats.

This chapter is adapted from a manuscript for publication which combines the work described herein with work done by Wendy Lanier for her Master's thesis (Lanier 2015). All the analyses described in this section were performed by me, but data collected prior to 2017 were collected by Wendy Lanier. In addition, many of the same questions motivated both of our theses, and portions of the introduction to the first chapter of her thesis have been re-used here with permission.

Introduction

Human-facilitated introductions of non-native species pose a significant threat to endangered taxa around the globe (Clavero and Garcia-Berthou 2005; Wilcove et al. 1998). Although the classic examples of invasive species are those that have been moved from continent to continent, non-native species introduced over shorter distances may have similarly dramatic effects on native communities. This is particularly common in systems where natural colonization of a new area is unlikely to occur, as in high-elevation lakes that are not connected to other water bodies (Bahls 1992; Rahel 2007). In the last two centuries, humans have intentionally introduced many fishes, especially salmonids, into areas where they were not historically present, including 60% of all high-elevation (>800m) lakes in the western United States (Bahls 1992). Salmonids drastically alter trophic interactions when introduced to historically fishless lakes (Knapp et al. 2005; Pilliod and Peterson 2001). Such invasions have led to population declines or local extinction for amphibian species whose larval stages are vulnerable to fish predation (Knapp 2005; Pilliod and Peterson 2001; Pilliod et al. 2010; Amburgey et al. 2014).

To date, most trout-amphibian interaction studies report the common outcome of a reduction in amphibian distribution and abundance (e.g., Bradford et al. 1998; Knapp 2005; Welsh et al. 2006). Few studies have investigated the potential indirect mechanisms by which trout impact different amphibian life stages (but see Tyler et al. 1998; Kiesecker et al. 2001; Vredenburg 2004; Pearson and Goater 2009; Kenison et al. 2016). Given the many ways trout could impact amphibian populations, knowledge of the mechanisms by which trout negatively affect amphibian life stages may assist managers tasked with conserving declining populations.

The boreal toad (*Anaxyrus boreas boreas)*, currently considered a subspecies of the Western toad (*Anaxyrus boreas*; Dodd 2013), has declined throughout the Southern Rocky Mountains (Carey 1993; Muths et al. 2003; Mosher et al. 2018) and is listed as an endangered or Tier 1 species in Colorado, Wyoming, and New Mexico. Regional declines are primarily attributed to chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Muths et al. 2003; Scherer et al. 2005); however, some populations have declined despite low prevalence of the pathogen (Muths and Scherer 2011). One such population exists at Spruce Lake, a high alpine lake in Rocky Mountain National Park, where a small population of boreal toads is declining despite low pathogen prevalence and high adult survival (Muths and Scherer 2011). Although eggs are laid at the site in most years, recruitment into the adult breeding population is low (Muths and Scherer 2011), indicating low survival in either the aquatic or the subadult terrestrial life stages.

One hypothesis for the low recruitment is potential impacts of introduced greenback cutthroat trout (*Oncorhynchus clarki stomias)* on boreal toad aquatic life stages. This subspecies of cutthroat trout is listed as threatened under the Endangered Species Act and is the state fish of Colorado, making it a high priority for conservation efforts. In 1991, cutthroat trout believed to be greenbacks were introduced to Spruce Lake, which was historically fishless (USFWS 1998). Bufonid eggs and tadpoles contain bufotoxins, making them unpalatable to many vertebrate predators, including trout (Licht 1968; Kats et al. 1988; Crossland and Alford 1998). However, in a laboratory experiment, Lanier et al. (2017) found that, although trout rarely consumed tadpoles, they frequently attempted to, reducing tadpole survival probabilities. Trout may also lower tadpole survival by altering trophic interactions or by inducing behavioral changes that alter their use of resources (Knapp et al. 2005; Creel and Christianson 2008). For example, avoiding predation often involves additional energy expenditure and spending less time in resource-rich areas.

This paper tests findings from previous lab-based studies to explore potential interactions between these two species of concern in a natural setting. I determine the degree of overlap between trout and toad habitat use during the tadpole stage and estimate toad survival through metamorphosis at ponds with and without trout. My findings will inform recovery efforts for both species within their native ranges.

Methods

Study Areas

My study was conducted at two boreal toad breeding areas in Rocky Mountain National Park: Spruce Lake and a wetland complex near Fay Lakes (Figure 2.1). Both breeding sites are at high elevations (2900 m and 3300 m) surrounded by coniferous forest. Spruce Lake is a permanent lake with a robust, self-sustaining population of greenback cutthroat trout and a breeding population of boreal toads first documented in 1979 (Corn et al. 1997, Muths and Scherer 2011). Fay Lakes is a shallow, seasonal, fish-free wetland, where toad breeding was discovered in 2003. All animal care approvals were obtained through Colorado State University’s Animal Care and Use Committee (ACUC).

Habitat Use

At Spruce Lake, I explored potential overlap in habitat use between trout tadpoles. Boreal toads typically breed in a sheltered, shallow area on the eastern side of the lake, and tadpoles remain in or near the breeding area until they metamorphose (*personal observation*). I conducted my study after tadpoles had hatched and were large enough to be easily seen. My goal was to determine factors influencing both species’ use of aquatic habitat and to test if trout presence or intensity of use influence the spatial or temporal distribution of tadpoles.

Data Collection

I explored habitat use and potential interactions between trout and mobile tadpoles by collecting count data for both species August 3-12 2017 in the same area of Spruce Lake. To better differentiate the spatial and temporal features that influence habitat use by both trout and tadpoles, I overlaid a grid of 45 plots (2 x 2m) in the breeding area. Each morning and afternoon during the 10-day season I randomly selected 5 plots with replacement, allowing a plot to be visited multiple times during the 10-day season. I navigated to the selected plot and after a 10-minute waiting period conducted a 5-minute visual survey during which I recorded the highest number of tadpoles seen in the plot at one time, the total number of trout seen, water temperature, time of day, cloud cover, and wind conditions. Cloud cover and wind conditions were both binary variables, where a '1' indicated clouds that obscured the sun and wind ≥ 10 mph, respectively. I noted any interaction between the two species, such as a change in swimming direction towards or away from the other species, or any attempts by trout to ingest tadpoles. After the survey was completed, I moved to the next randomly chosen plot. Once all five plots were surveyed, I returned to the first plot and repeated the process. Successive surveys on the same plot were separated by at least 1 hour, but plots could be chosen on multiple days so that the time between surveys could be >24 hours. Over my 10-day season, 26 plots were surveyed twice (one morning or afternoon) and 15 plots were visited on multiple days (13 plots were surveyed 4 times and 2 plots were surveyed 6 times). I collected two habitat covariates at each of these plots based on findings from Lanier (2015): depth and vegetation density.

Data Analysis

I simplified the counts for each species to detection-nondetection data and used the two-species conditional occupancy model (Richmond et al. 2010; MacKenzie et al. 2018) to test whether the presence or detection of trout altered habitat use or frequency of use by boreal toad tadpoles. In this model, the probability that a plot is used by trout during the 10-day season (denoted as ψ^{A}) is assumed to be independent of tadpole presence (unconditional), while the probability of tadpole use is conditional on the plot being used or not used by the trout (denoted as ψ^{BA} and ψ^{Ba}, respectively). Species detection probabilities (or frequency of use) can differ among plots used by a single species or both species. For example, p^{A} and p^{B} denote the detection probabilities of trout and tadpoles, respectively, on plots only used by the designated species, while r^{A} and r^{B} denote species-specific detection probabilities on plots used by both species during the 10-day season. Finally, detection probability of tadpoles at plots used by both species can vary among surveys where trout were seen (r^{BA}) or not seen (r^{Ba}).

Using this model, I tested for potential trout and tadpole interactions at different spatial and temporal scales. If tadpoles are excluded from plots by trout during my 10-day season, I would expect $\psi^{BA} < \psi^{Ba}$. Alternatively, tadpoles may not completely avoid areas used by trout, but simply use these areas less frequently, resulting in a lower detection probability at plots used by trout (i.e. $r^{B} < p^{B\cdot}$). Finally, if tadpoles temporarily avoid plots when trout are physically present, I would expect $r^{BA} < r^{Ba}$, suggesting the probability of detecting tadpoles is lower when trout are also detected during the survey. I assumed trout frequency of use was independent of tadpole use of a plot during my 10-day season (i.e. I set $p^{A} = r^{A}$ in all models).

I evaluated the influence of habitat covariates (depth and/or vegetation density) on probability of use and frequency of use (or detection probability) for both species. Additionally, because successive surveys were $\geq$1 hour apart, I modeled detection probabilities as a function of time-specific variables (weather conditions and water temperature). Water temperature was not recorded on some surveys, so I predicted the missing temperature values using a simple regression model that included depth and time of day (R^2=0.82).

I used a sequential approach to model building and selection, and all models were fitted using program MARK (White and Burnham 1999) and ranked using Akaike's Information Criterion. I examined factors influencing model parameters in the following order: (1) trout detection probability (frequency of use), (2) tadpole detection probability, (3) trout probability of use, and (4) tadpole probability of use. Specifically, I fit 16 trout detection probability structures including all possible additive effects of four covariates (vegetation density, water temperature, cloud presence, and wind presence, Table 2.1).

Using the top model of trout detection probability, I tested all possible additive effects of four covariates (vegetation density, water temperature, cloud presence, and wind presence) on tadpole detection probability (16 total models; Table 2.2). In each of these models I included an additive effect of trout presence and trout detection on tadpole detection. I then tested a model in which tadpole detection depended on trout presence but not trout detection and one in which tadpole detection did not depend on trout presence (Table 2.3).

Using my top models of trout and tadpole detection probability, I tested all possible additive effects of depth and vegetation density on trout use, resulting in four possible models (Table 2.4). I then used the best-supported model of trout use and detection and tadpole detection and tested all possible additive effects of depth, vegetation density, and trout effects on tadpole habitat use, resulting in eight possible models (Table 2.5).

Toad Survival

I explored the potential effect of trout on survival probabilities for boreal toad tadpoles in 2013, 2014, and 2017. In 2013-14, a manipulative field experiment was conducted on wild egg masses at Fay Lakes (trout-free site, control) and at Spruce Lake (trout site) to estimate embryo survival and tadpole abundance (see Lanier 2015), followed by metamorph surveys to estimate tadpole survival. In 2017, I omitted the manipulative experiment and simply estimated survival from embryo through metamorphosis.

Data Collection

Embryo survival was estimated using temporary removal sampling of tadpoles just after hatching (see Lanier 2015). For 2013 and 2014, I used the resulting estimates of tadpole abundance as the initial release of tadpoles. In 2017, I did not estimate embryo survival. I photographed each egg mass against a white background and counted the number of eggs in each egg mass (As in Chapter 1). Tadpole development was monitored at both sites in all three years (2013, 2014, and 2017). One week after metamorphs were first detected, I performed a three-pass temporary removal sample (Muths et al. 2014). Metamorphs were captured by hand, held in small plastic containers with mesh lids, and counted after each pass. When all passes were complete, I batch marked each individual using a single Visible Implant Elastomer (VIE) mark and released them at their capture location. After all tadpoles had metamorphosed (1-2 weeks later), I performed a second removal sample, counting the number of previously marked and unmarked individuals on each removal pass. In 2014, all metamorphs had emerged from the Fay Lakes site by the first sample, so only one sample was conducted. In 2017, the Fay Lakes site dried before tadpoles could metamorphose.

Data Analysis

In 2013-2014, I combined the metamorph removal data and estimated tadpole abundances to generate capture histories for each site and year. Capture histories consisted of three primary periods: initial “release” of hatched tadpoles, followed by the two removal samples. Capture histories contained two states: tadpoles (T) (i.e., the estimated number of tadpoles hatched from my embryo survival study) and metamorphs (M) observed during the second and third periods (i.e., the temporary removal samples of metamorphs). Recaptured metamorphs were randomly distributed among individuals from the marked cohort to avoid bias (Converse et al. 2009). In 2017, embryo survival was not estimated so initial releases consisted of eggs counted from my photographs.

Due to time differences between primary periods and inconsistency in the stage at release, I analyzed data from each site and year separately using a closed robust-design multi-state model (Pollock et al. 1990; Kendall and Nichols 1995; Muths et al. 2014). This model allows for the estimation of three types of parameters: survival probability, transition probability, and detection probability. Survival probability, S_t^k, is the probability that an individual in state *k* at time *t* survives and remains in the study area between time *t* and time *t+1*. I accounted for unequal time intervals between primary periods, thus resulting estimates are weekly survival estimates. Transition probability, ψ_t^{kl}, is the probability that an individual in state *k* at time *t* will be in state *l* at time *t+1*, given that it survives between the two primary periods. Detection probability, $p_{t,j}^k$, is the probability that an individual will be captured during pass *j* of sample *t*, given that it is alive and in state *k*. I employed several constraints to address biological reality and statistical identifiability. Transitioning from metamorph to tadpole is impossible, and tadpoles are not captured in the metamorph removal samples, so ψ_t^{MT}, $p_{2,j}^T$, and $p_{3,j}^T$ were fixed to 0. Three additional assumptions are required for model identifiability (Muths et al. 2014): I assume that all surviving tadpoles have metamorphosed before the final removal sample ($\psi_2^{TM} = 1$), that weekly tadpole survival probability is constant during the study period ($S_{1\_wk}^{T} = S_{2\_wk}^{T}$), and that detection probability during the final pass was constrained (i.e., $p_{t,j}^{M} = p_{t,j-1}^{M}$).

For each site by year combination, I tested four detection structures: constant across all passes, varying by pass but not by removal sample, varying by removal sample but not by pass, and varying by both pass and removal sample. When only one removal sample was conducted (Fay Lakes 2014), I fit two detection structures: constant detection ($p_{1,1}^{M} = p_{1,2}^{M} = p_{1,3}^{M}$) or detection probability varied by pass ($p_{1,1}^{M}, p_{1,2}^{M} = p_{1,3,}^{M}$). Models were fit using program MARK (White and Burnham, 1999) and ranked using AICc.

Finally, I combined survival estimates of embryo survival and estimates from my best-supported model of tadpole survival to derive an estimate of survival through metamorphosis:

$$S^{EM} = S^{E} \times [S_1^{T}(\psi_1^{TM} + (1 - \psi_1^{TM})S_2^{T})] \qquad \text{Eq. 2.1}$$

where S_1^{T} is the survival probability of tadpoles between hatching and the first removal sample, S_2^{T} is the survival probability of tadpoles between the first and second removal samples, and $\psi_1^{TM}$is the probability of an individual transitioning from tadpole to metamorph before the first removal sample, given that it survived. In this equation, differences between $S_1^{T}$and S_2^{T} are solely a function of the time between periods, because weekly survival is constant ($S_{1\_wk}^{T} = S_{2\_wk}^{T}$). In 2017, embryo survival was not calculated separately and is included in the first survival term S_1^{T} and $S^{E}$is removed from the Eqn 2. I used the delta method to calculate associated variances.

Results

Habitat Use

I conducted 116 surveys of 41 plots during a 10-day period when trout and tadpoles are most likely to co-occur in near-shore habitat at Spruce Lake. I simultaneously observed trout and tadpoles in 15 plots during 20 surveys. I observed no predatory behavior between trout and tadpoles, though trout were seen ingesting numerous aquatic insects. I did not observe any change in tadpole or trout behavior when the two species encountered one another.

Consistent with findings during the embryo stage (Lanier 2015), I found that trout habitat use was influenced by depth and trout frequency of use was influenced by vegetation density and water temperature (cumulative AICc weight, $w_+ > 0.99$ for all covariates, Table 2.4; Figure 2.3). The probability that trout used a plot at least once during the 10-day season was high; estimated at 0.740 (SE=0.115), using an intercept-only model. Trout detection probability estimates ranged from 0.004 (SE=0.005) for surveys conducted at plots with high vegetation density during cold temperatures to 0.901 (SE=0.064) for surveys conducted at plots with no vegetation during warm temperatures.

I found that tadpole habitat use was also influenced by vegetation density (Table 2.5); tadpoles used nearly all plots with low vegetation density, but tadpole probability of use declined in plots with vegetation densities greater than 0.50. Only 7 of 41 plots had vegetation densities higher than 0.50, and average tadpole use probability across all plots was 0.977 (SE=0.075). Importantly, I found no evidence that trout influenced tadpole habitat use; while the second best-supported model does include a trout effect (Table 2.5), the inclusion of this effect does not improve model fit, indicating that this is a pretending variable (Arnold 2010). Tadpole frequency of use was lower at plots used by trout (r^B=0.712 SE=0.052) relative to plots not used by trout (p^B=0.969 SE=0.031), but tadpoles did not avoid plots when trout were seen during the same survey (Table 2.3).

Toad Survival

Despite egg deposition and variable embryo survival, metamorphs were only observed at the trout site in 2013 and the control site in 2014 (Table 2.6). Similarly, egg masses were laid at both sites in 2017 (7992 and 2371 eggs at the trout and control site, respectively), but metamorphs were only observed at the trout site (Table 2.6).

Metamorph detection probability varied among pass, removal sample, and year, ranging from 0.20-0.68. There was no consistent pattern to which sample or pass had higher detection probabilities. Tadpole survival varied across sites and years, ranging from 0.0-0.66 (Table 2.6). In years when metamorphs were detected, survival to metamorphosis was higher at the trout site than the control site (Table 2.6) but never exceeded ~0.20. Additionally, while there was only one year (2014) with complete reproductive failure at the trout site, reproductive failure occurred in two of the three years at the control site (2013 and 2017).

Discussion

I found no evidence that trout negatively affect boreal toad tadpoles, despite the two species sharing habitats. This finding agrees with numerous observational studies that show no negative impacts of trout on toad distributions (e.g., Knapp 2005; Welsh et al. 2006), but differs from lab experiments involving toads and trout (Grasso et al. 2010; Lanier et al. 2017). Those experimental studies suggest that naïve trout attempt to eat toad tadpoles before rejecting them (Grasso et al. 2010), and that these attempts reduce tadpole survival probabilities (Lanier et al. 2017). Lanier et al. (2017) found no evidence of trout learning to avoid toad tadpoles, but other studies have demonstrated a capacity for predatory fish to learn to avoid unpalatable prey (e.g., Kruse and Stone 1984; Nelson et al. 2010; Nelson et al. 2011; Szuroczki and Richardson 2011).

Boreal toads are the only amphibian species found at Spruce Lake, which may allow the trout to easily distinguish them as unpalatable. Moreover, if predatory behavior existed and persisted in natural environments, I would expect toad tadpoles would alter their behavior in the presence of trout and avoid areas frequented by trout. I found no evidence of avoidance in my study, corroborating similar findings in laboratory studies (e.g., Kiesecker et al. 1996), suggesting that unpalatability is a sufficient protection for boreal toads against trout predation.

Toad tadpoles and trout overlapped in the habitat they used, but trout were restricted to areas of deeper water while tadpoles used the entire study area. Tadpole frequency of use was lower in the trout-used areas, but there was no indication that this difference was due to negative interactions. At a finer temporal scale (i.e., during a given 5-minute survey), I found no evidence that tadpoles avoid areas that are simultaneously occupied by a trout. This is consistent with laboratory studies suggesting that toads do not show behavioral avoidance of fish (Kats et al. 1988; Kiesecker et al. 1996). Additionally, tadpole survival probability was higher at the trout site than the control site, although there was a large amount of variation among years at both sites.

Several hypotheses could explain higher tadpole survival at the trout site, but I am unable to distinguish between them in this study. Trout alter trophic interactions and could reduce the abundance of predatory aquatic insects such as *Dytiscus* or *Lethocerus* species (Knapp et al. 2001), which are known to prey on boreal toad tadpoles (Peterson and Blaustein 1992; Kiesecker et al. 1996). Alternatively, there could be differences in habitat quality unrelated to trout presence. The control site is a small, ephemeral pond at higher elevation than the trout site. Water bodies with larger perimeters are associated with toad presence (Welsh et al. 2006) and strictly ephemeral sites make larval toads more vulnerable to desiccation, as was observed at the control site in two of the three years in this study (Table 1.4). Additionally, the pathogen *Batrachochytrium dendrobatidis* is present at the control site but not at the trout site; although the pathogen primarily affects adult amphibians, it can also impact the survival probabilities of tadpoles (Garner et al. 2009; Chapter 1).

Introduced salmonids have drastically altered trophic interactions and limited amphibian distributions in high-elevation lakes in the western US (Knapp 2005; Pilliod and Peterson 2001; Pilliod et al. 2010). Bufonids are thought to be immune to this stressor, but laboratory studies suggest that naïve, hatchery-reared trout could lower survival and time to metamorphosis for resident toad populations immediately after release (Lanier et al. 2017). This study suggests that if this negative effect exists, it does not persist in native boreal toad systems. Boreal toads utilize a variety of ephemeral and permanent wetlands for reproduction (Holland 2002; Dodd 2013), but currently persist in high-elevation sites within their historic distribution in the southern Rocky Mountains (Mosher et al. 2018). These same wetlands and lakes are also targeted for restoration efforts for the threatened greenback cutthroat trout as barriers can be placed downstream to avoid colonization by non-native salmonids. These findings suggest that boreal toads and introduced cutthroat trout can co-exist, suggesting these habitats may serve as important refugia for multiple species of concern.

Table 2.1 Model selection results for trout detection ($p^A = r^A$) in a two species (greenback cutthroat trout and boreal toad tadpole) occupancy study at a boreal toad breeding area in Rocky Mountain National Park. All models had the global structure for tadpole detection (p^B/r^B; vegetation density +cloud +wind +temperature +trout use +trout detection), trout use (ψ^A; depth +vegetation density), and tadpole use (ψ^B; depth +vegetation density +trout use). "." indicates a model in which trout detection probability was constant across all plots and surveys. The columns present the model notation, Akaike's information criterion values adjusted for small sample size (AICc), the difference between the model's AICc value and that of the top model (ΔAICc), AICc weights (w), number of parameters (K), and the deviance of the model.

Trout Detection Model	AICc	ΔAICc	*w*	*K*	Deviance
vegetation density +temperature	238.11	0.00	0.73	17	177.50
vegetation density +cloud +temperature	241.25	3.14	0.15	18	174.16
vegetation density +cloud	243.00	4.89	0.06	17	182.39
vegetation density +wind +temperature	244.47	6.36	0.03	18	177.37
vegetation density	246.25	8.14	0.01	16	191.58
vegetation density +cloud +wind +temperature	248.20	10.09	0.00	19	174.01
temperature	248.64	10.54	0.00	16	193.98
vegetation density +cloud +wind	249.10	10.99	0.00	18	182.01
vegetation density +wind	250.73	12.62	0.00	17	190.12
.	252.65	14.54	0.00	15	203.45
cloud +temperature	253.04	14.93	0.00	17	192.43
cloud	253.12	15.01	0.00	16	198.46
wind +temperature	254.56	16.45	0.00	17	193.95
wind	256.56	18.45	0.00	16	201.89
cloud +wind	258.32	20.21	0.00	17	197.71
cloud +wind +temperature	259.52	21.41	0.00	18	192.42

Table 2.2 Model selection results for covariate models of tadpole detection (p^B/r^B) in a two species (greenback cutthroat trout and boreal toad tadpole) occupancy study at a boreal toad breeding area in Rocky Mountain National Park. All models had the best-supported structure for trout detection ($p^A = r^A$; vegetation density +temperature) and the global structure for trout use (ψ^A; depth +vegetation density), and tadpole use (ψ^B; depth +vegetation density +trout use). "." indicates a model in which tadpole detection probability was constant across all plots and surveys. All models included additive effects of trout use and detection on tadpole detection. The columns present the model notation, Akaike's information criterion values adjusted for sample size (AICc), the difference between the model's AICc value and that of the top model (ΔAICc), AICc weights (w), number of parameters (K), and the deviance of the model.

Tadpole Detection Model	AICc	ΔAICc	*w*	*K*	Deviance
.	219.99	0.00	0.74	13	180.51
wind	223.00	3.00	0.16	14	178.84
cloud +wind	226.97	6.97	0.02	15	177.77
wind +temperature	227.67	7.68	0.02	15	178.47
cloud +temperature	227.72	7.73	0.02	15	178.52
vegetation density +wind	227.97	7.98	0.01	15	178.77
vegetation density +temperature	228.40	8.41	0.01	15	179.20

cloud	229.26	9.27	0.01	14	185.11
temperature	229.66	9.66	0.01	14	185.50
vegetation density	230.40	10.40	0.00	14	186.24
cloud +wind +temperature	232.31	12.32	0.00	16	177.65
vegetation density +cloud +temperature	232.94	12.95	0.00	16	178.28
vegetation density +temperature	235.28	15.28	0.00	15	186.08
vegetation density +cloud +wind +temperature	238.11	18.11	0.00	17	177.50
vegetation density +cloud +wind	238.54	18.54	0.00	16	183.87
vegetation density +wind +temperature	239.63	19.64	0.00	16	184.97

Table 2.3 Model selection results for effects of trout use and detection on tadpole detection (frequency of use, p^B/r^B) in a two species (greenback cutthroat trout and tadpole) occupancy study at a boreal toad breeding area in Rocky Mountain National Park. All models had the best-supported structure for trout detection probability ($p^A= r^A$; vegetation density +temperature) and the global structure for trout use (ψ^A; depth +vegetation density), and tadpole use (ψ^B; depth +vegetation density +trout use). The columns present the model notation, Akaike‘s information criterion values adjusted for sample size (AICc), the difference between the model‘s AICc value and that of the top model (ΔAICc), AICc weights (w), number of parameters (K), and the deviance of the model.

Tadpole Detection Model	AICc	ΔAICc	*w*	*K*	Deviance
Effect of trout use but not trout detection $(p^B \neq r^{BA}=r^{Ba})$	215.75	0.00	0.71	12	180.60
No effect of trout use or detection $(p^B= r^{BA}=r^{Ba})$	217.53	1.78	0.29	11	186.42
Effect of trout use and detection $(p^B \neq r^{BA} \neq r^{Ba})$	226.4	10.65	0.00	13	186.91

Table 2.4 Model selection results for trout occupancy (ψ^A) in a two species (greenback cutthroat trout and tadpole) occupancy study at a boreal toad breeding area in Rocky Mountain National Park. All models had the best-supported structure for trout detection ($p^A= r^A$; vegetation density +temperature), tadpole detection probability (p^B/r^B; trout use), and tadpole use (ψ^B; depth +vegetation density +trout use). "." indicates a model in which trout use was constant across all plots. The columns present the model notation, Akaike‘s information criterion values adjusted for sample size (AICc), the difference between the model‘s AICc value and that of the top model (ΔAICc), AICc weights (w), number of parameters (K), and the deviance of the model.

Trout Habitat Use Model	AICc	ΔAICc	*w*	*K*	Deviance
Depth	215.60	0.00	0.52	11	184.49
Depth +vegetation density	215.75	0.15	0.48	12	180.60
.	225.95	10.36	0.00	10	198.62
vegetation density	230.78	15.18	0.00	11	199.67

Table 2.5. Model selection results for tadpole habitat use (ψ^B) in a two species (greenback cutthroat trout and tadpole) occupancy study at a boreal toad breeding area in Rocky Mountain National Park. All models had the same structure for trout detection ($p^A = r^A$; vegetation density + temperature), tadpole detection (p^B; varying by trout use), and trout use (ψ^A; varying by depth). "Trout use" indicates that tadpole use differs in plots predicted to be used by trout. "." indicates a model in which tadpole use was constant across all plots. The columns present the model notation, Akaike's information criterion values adjusted for sample size (AICc), the difference between the model's AICc value and that of the top model (ΔAICc), AICc weights (w), number of parameters (K), and the deviance of the model.

Tadpole Habitat Use Model	AICc	ΔAIC_c	*w*	*K*	Deviance
Vegetation density	208.77	0.00	0.60	9	184.96
Vegetation density +trout use	211.83	3.06	0.13	10	184.50
.	212.15	3.38	0.11	8	191.65
Depth +vegetation density	212.80	4.03	0.08	10	185.47
Trout use	214.18	5.41	0.04	9	190.37
Depth	215.55	6.78	0.02	9	191.75
Depth +vegetation density +trout use	215.60	6.83	0.02	11	184.49
Depth +trout use	217.59	8.82	0.01	10	190.26

Table 2.6. Estimated survival probability for embryos ($\hat{S}^E$), tadpoles ($\hat{S}^T$), from embryo through metamorphosis ($\hat{S}^{EM}$) and standard errors (in parentheses). $\hat{S}^T$ is tadpole survival through metamorphosis, given by the bracketed part of Eqn 2. Tadpoles were documented but no metamorphs found in 3 of the 6 site-year combinations, suggesting complete reproductive failure. Embryo and tadpole survival were not estimated separately in 2017, so I simply report the estimate of $\hat{S}^{EM}$.

Site	Year	Total eggs laid	$\hat{S}^E$	$\hat{S}^T$	$\hat{S}^{EM}$
Control Site (Fay Lakes)	2013	33876	0.06 (0.002)	0.00	0.00
	2014	18065	0.28 (0.004)	0.21 (0.048)	0.06 (0.013)
	2017	2371		0.00	0.00
Trout Site (Spruce Lake)	2013	5513	0.31 (0.008)	0.66 (0.106)	0.20 (0.033)
	2014	17097	0.32 (0.007)	0.00	0.00
	2017	7992		*N/A*	0.17 (0.005)

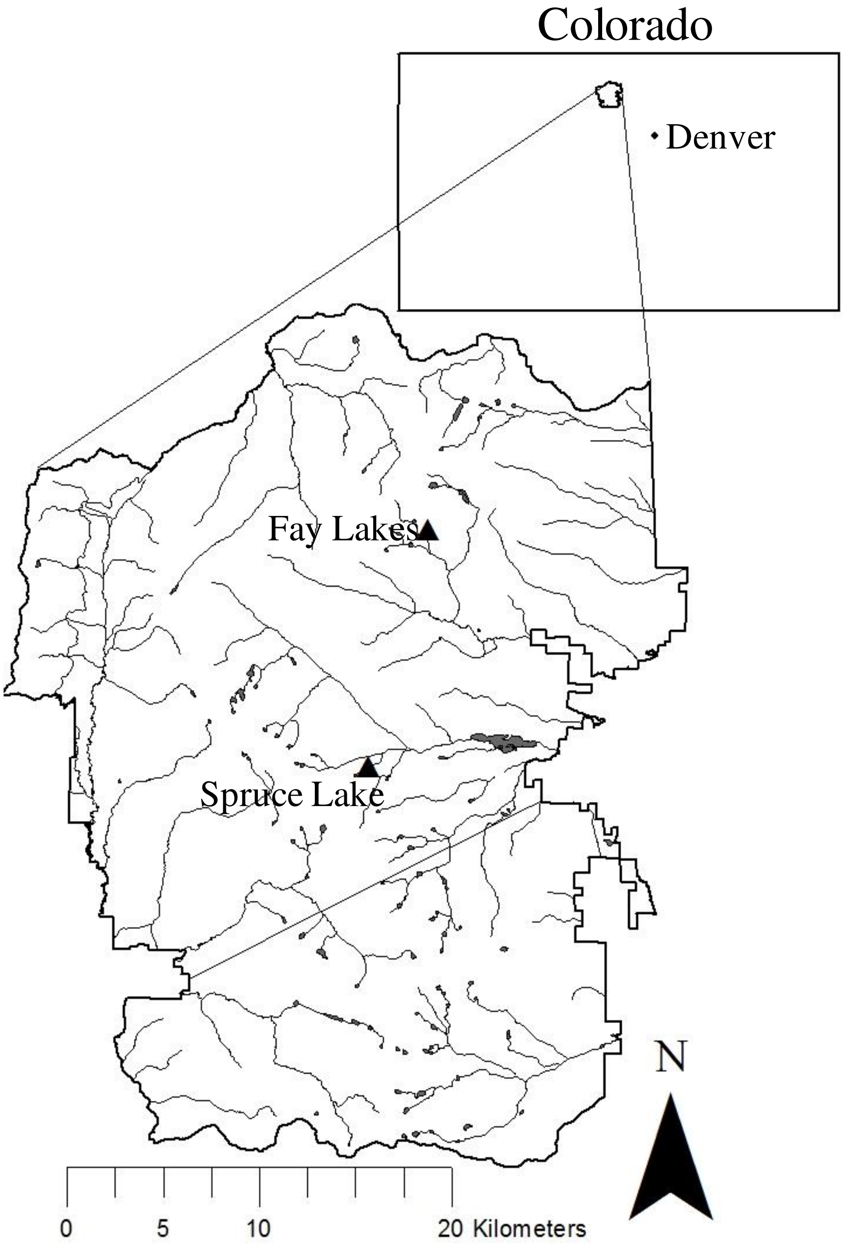

Figure 2.1. Map of Rocky Mountain National Park and its major water bodies. The two study sites, Spruce Lake and Fay Lakes, are denoted by black triangles. Reproduced with permission from Lanier (2015).

Figure 2.2. 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. Reproduced with permission from Lanier (2015).

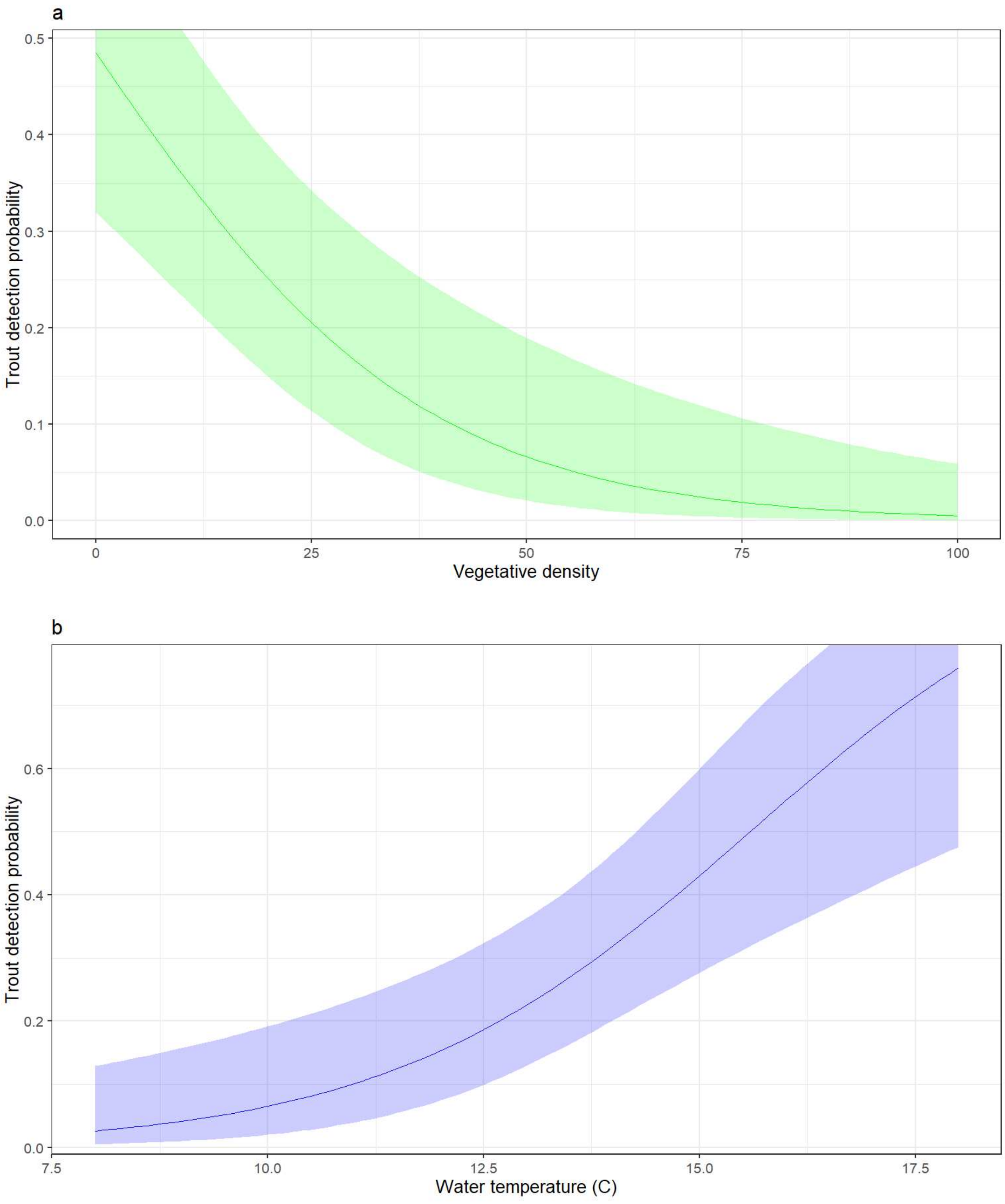

Figure 2.3. Estimated relationships between trout detection probability and vegetation density (a), and temperature (b) using the most parsimonious occupancy model, ψ(depth), p(depth+ vegetation), during the tadpole stage of toad development. Relationships with each covariate is plotted using the average value of the other covariates. Shaded areas represent 95% confidence intervals.

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CHAPTER THREE: HYDROPERIOD AND PREVIOUS BREEDING SUCCESS INFLUENCE RECRUITMENT DYNAMICS FOR A DECLINING AMPHIBIAN SPECIES

Populations of pond-breeding amphibians often have boom and bust patterns in recruitment, with large numbers of individuals metamorphosing in some years and few or none (i.e., reproductive failure) in other years. Environmental processes, such as pond freezing and drying, and biological factors (e.g., disease and predator community) can influence survival of early life stages and the probability of complete reproductive failure for local amphibian populations. I used multi-state occupancy models to estimate probability of breeding and successful metamorphosis (the complements of reproductive failure), and explored environmental and biological factors influencing these processes, in populations of boreal toads (*Anaxyrus boreas boreas*). I applied both static and dynamic multistate occupancy modeling techniques to ten years (2001-2010) of boreal toad data from 82 sites, and tested relationships between these probabilities and the presence of a pathogen (*Batrachochytrium dendrobatidis*), elevation, the hydrology of the site, relative snowpack in a given year, and the presence of trout (*Onchorhyncus spp.*, *Salvelinus fontinalis,* and *Salmo trutta*). I found that the probability of adults breeding at a site was influenced by elevation, with higher-elevation sites more likely to support breeding. The probability of metamorphosis, given breeding, was influenced by an interaction between hydrology of the site and relative annual snowpack. The probability of metamorphosis was higher at ephemeral sites in years of relatively high snowpack, while at permanent sites, years of low snowpack lead to higher probabilities of metamorphosis. Across all sites, the probability of metamorphosis was high for years with median snowpack levels (0.75) at sites with recent breeding attempts, but lower (0.25) at newly colonized sites. my results suggest that boreal toads are well-adapted to their current habitat, with both ephemeral and permanent sites likely to support breeding in years of median snowpack; shifts in precipitation patterns or temperatures may have negative impacts on this species.

Introduction

Pond-breeding amphibians present unique conservation challenges, in part because of their biphasic life history. These species, many of which are declining due to emerging infectious diseases (Scheele et al. 2019), tend to have highly variable recruitment (e.g., Berven 1990; Pechmann et al 1991; Semlitsch et al. 1996; Alford and Richards 1999; Marsh and Trenham 2001). This variation in recruitment makes short-term studies on population dynamics, particularly those that focus solely on adults, less informative than they may be in other taxa (Alford and Richards 1999, Semlitsch et al. 2017). There is some evidence that in stable pond-breeding amphibian populations, abundances of adults decline in most years, with a few "boom" years of high recruitment (Alford and Richards 1999). This pattern of many years of low recruitment with a few years of high recruitment could cause managers to infer that a decline was occurring when it was not, or vice versa (e.g., Muths and Scherer 2011; Semlitsch et al. 2017). For declining species especially, monitoring multiple populations and life stages, especially the reproductive success or failure of these sites, is likely to be more relevant than counts of adults (e.g., Alford and Richards 1999; Semlitsch et al. 2017).

Pond-breeding amphibians are best understood in a metapopulation context, where populations are linked by dispersal across a wider region (Marsh and Trenham 2001). Dispersal may facilitate rescue of declining populations as well as colonization or recolonization of unoccupied sites. Evidence is equivocal but many studies suggest that natal dispersal (i.e., dispersal of individuals from their natal pond) occurs at higher rates than adult dispersal (Bailey and Muths 2019). Metamorphosis at a single site can therefore impact both local and metapopulation dynamics (e.g., Griffiths et al. 2010).

A major contributor to variability in early life stage survival is reproductive failure (i.e., no individuals successfully metamorphosing despite egg deposition; Taylor et al. 2006). Reproductive failure can be caused by a variety of factors, including unfertilized eggs, freezing, desiccation, predation, or poor water quality (Carey et al. 2005). Desiccation is the most obvious cause because it affects entire ponds, but the relative importance of factors that can potentially contribute to reproductive failure is unknown. Information about the contributors to reproductive failure is useful because early life stages (those using aquatic habitats) are frequently the focus of management efforts (Gerber et al. 2018).

Reproductive success can be estimated across multiple sites using detection/non-detection data and multistate occupancy methods (Nichols et al. 2007, MacKenzie et al 2009). Although these methods do not estimate survival rate of individuals at a breeding site, they do estimate of how many breeding sites had at least some individuals successfully metamorphose (i.e., the rate of reproductive success), while accounting for imperfect detection of different life stages. This study addresses hypothesized causes of reproductive failure in a declining, pond-breeding amphibian, the boreal toad (*Anaxyrus boreas boreas*), using both static and dynamic multi-state occupancy models (Nichols et al. 2007; MacKenzie et al. 2009) fitted to data from long-term monitoring of historic breeding sites throughout the southern Rocky Mountains. Although reproductive failure is known to occur in this species, its frequency and common causes are unknown. This study will advance our biological knowledge and aid recovery efforts through selection of more suitable sites for reintroduction and inform management actions designed to address causes of reproductive failure (e.g., supplementing water levels in an ephemeral pond).

Methods

Study system

I analyzed data from 121 historic breeding sites of boreal toads in Colorado and Wyoming (Figure 3.1). All sites are wetlands or lakes at elevations between 2400 and 3700 m and are part of the Southern Rocky Mountains boreal toad population (Switzer et al. 2009). my study uses monitoring records from 1999 to 2010 and all sites were surveyed at least three times during at least one year. The species is abundant and widespread in many portions of its range, but has declined precipitously in Colorado and southern Wyoming in recent decades, primarily due to the introduced pathogen *Batrachochytrium dendrobatidis* [Bd], which infects amphibian skin and leads to reduced survival probabilities in adults (Carey 1993; Muths et al. 2003; Scherer et al. 2005).

Multistate Occupancy models

I tested hypotheses about factors influencing toad breeding and subsequent metamorphosis using a hierarchical, multistate occupancy framework. Under this framework, a site could be in one of 3 mutually exclusive states in any given year: (true states denoted as m): no breeding occurred ($m= 0$), breeding occurred but metamorphosis did not ($m=1$), or breeding and metamorphosis occurred ($m=2$). The probability that a site is in any of the 3 states can be described using two parameters: the probability of breeding (ϕ^1), and the probability of metamorphosis, given breeding (ϕ^2). Detection of these states is imperfect; the presence of eggs, tadpoles, or metamorphs can be missed so the observed data may not reflect the true state at the historic breeding site. This imperfect detection process is accounted for by detection and classification (δ) parameters. Detection probability (p) describes the probability of detecting breeding (e.g., eggs, tadpoles) given that breeding occurred at a site. The classification parameter (δ) describes the probability of detecting metamorphs at a site, given that breeding and metamorphosis occurred (i.e., the probability of correctly classifying a site as being in state 2 given that its true state is 2). Misclassification is assumed to occur in only one direction; if metamorphosis did not occur, metamorphs cannot be detected and if breeding did not occur, eggs, tadpoles, and metamorphs cannot be observed.

There are two types of multistate occupancy models: static (single-season, Nichols et al. 2007) and dynamic models that directly incorporate and estimate the processes that cause change in occupancy state from one season to the next (MacKenzie et al. 2009). Static models estimate only the parameters described above – the probability of breeding (ϕ^1), the probability of metamorphosis, given breeding (ϕ^2), and detection (p) and classification (δ) probabilities. Dynamic models estimate the occupancy probabilities for the first year of the study ($\varphi_{t=1}^{1}, \varphi_{t=1}^{2}$) and transition probabilities between years, namely the probability of breeding in year $t+1$, given that the site was in state m in year t (ψ^m), and the probability of metamorphosis in year $t+1$ given that breeding occurred in year $t+1$ and the site was in state m in year t (R^m).

When using a static model to estimate probabilities of breeding and of metamorphosis from multiple years of data, an implicit assumption is that the probabilities of breeding and of metamorphosis, given breeding, are independent of the state of the site in the previous year (MacKenzie et. al. 2018). Dynamic models can test this assumption directly, but typically require some consistency in sampling effort among sites and years and are less numerically stable when these criteria are not met, especially at the beginning of the study. Estimating transition probabilities require that all or a subset of be sampled in consecutive years and many of the sites (~40 sites) were surveyed in only 1-2 years at the beginning of the monitoring program. Applying the dynamic model to all the monitoring data yielded unreasonable estimates (i.e., estimates at the boundary with unreasonable confidence intervals), so I removed the first two years of data (1999-2000) and retained detection-nondetection information at 82 sites (2001-2010) for my dynamic model analysis .

Hypotheses

I expected that the probability of breeding would be influenced by elevation and the presence of Bd, as these factors are known to influence adult toad occurrence dynamics (Mosher et al. 2018b). Additionally, breeding probabilities may be influenced by site permanence as water availability at ephemeral ponds can vary among years leading to inconsistent breeding. Likewise, I expected that the conditional (on breeding) probability of metamorphosis would be affected by elevation, Bd status, the presence of introduced trout, permanence, snowpack in the previous winter, and an interaction between permanence and snowpack. Bd status can affect the probability of metamorphosis by lowering the abundance of adults (and therefore the abundance of eggs) or by lowering survival of tadpoles and/or lengthening time to metamorphosis (Blaustein et al. 2005; Garner et al. 2009, Chapter 1). Although other studies have shown that introduced trout do not influence the occurrence of toad species (Knapp 2005; Welsh et al. 2006; Chapter 2), a recent study suggested that naïve trout attempt to ingest boreal toad tadpoles, lowering their probabilities of survival (Lanier et al. 2017). Climatic factors may also influence conditional probability of metamorphosis. Most precipitation in the southern Rockies falls as snow, and snowpack is the major determinant of hydroperiod at ephemeral sites which may have lower probabilities of metamorphosis in years of low snowpack. Alternatively, years of heavy snowpack may mean that snow and ice conditions persist at toad breeding sites, delaying egg deposition and potentially shortening the active season. Similarly, high elevation sites are likely to thaw later and freeze earlier, shortening the active season during which larvae develop and grow.

The ability to detect breeding and correctly classify the true state of a site is influenced by larval developmental stage. Tadpoles are easier to detect than eggs and metamorphs, and metamorphs may leave the site soon after they emerge from the water limiting the time that they are observable. I therefore expected a quadratic relationship between the date of survey and the probability of detecting breeding at a site (i.e., any larval stage). I expected this relationship to be different for sites that supported metamorphosis, with a higher peak (as late-stage tadpoles are particularly conspicuous) and potentially a sharper decline at the end of the season (as metamorphs leave the area). I also expected that sites with Bd would have lower detection probabilities because these sites would have lower abundances of all life stages. I also expected the date of survey to affect the classification probability (δ), as metamorphs can only be detected after metamorphosis.

In addition to these hypotheses, I used my dynamic analysis to test the assumption of the static multistate model; namely, that all sites, regardless of previous state, have an equal probability of breeding, and of metamorphosis given breeding.

Data Collection

All 121 sites were surveyed at least three times during the active season in at least one year between 1999 and 2010. Most sites were visited in multiple years, but few sites were visited every year, yielding 571 site x year combinations. During each survey, observers recorded the date and detections of adult toads, eggs, tadpoles, and metamorphs. For each survey an observed state was assigned if: (1) metamorphs were detected (observed state “2”), (2) eggs or tadpoles were detected but not metamorphs (observed state “1), (3) no eggs, tadpoles, or metamorphs were detected (observed state “0”). I included two covariates that varied each year at each site: relative snowpack and Bd status. Using the nearest SNOTEL site to a given breeding site, I divided the maximum snowpack in a given year by the median maximum snowpack at that site (for most sites, this was for the period 1981-2010; some SNOTEL sites had been operating for fewer years, and for these sites the median was calculated using all years of data). The closest SNOTEL site was between 0.4 and 21.8 km from the toad breeding site, with an average distance of 8.4 and a median distance of 7.8. (Figure 3.1). A subset of captured adult toads were opportunistically swabbed for Bd during surveys conducted after 2003. Bd DNA was extracted from swabs and analyzed in triplicate using standard polymerase chain reaction (in years 2003-2005) or quantitative real-time PCR (2006-2010). Swabs were considered positive if Bd DNA was successfully amplified in two of three replicates. (Mosher et al. 2018b). Using raw, annual Bd detection/non-detection as a covariate can introduce bias in estimates of toad occurrence dynamics (Mosher et al. 2018a). Instead, I assumed that each site could transition only once, from Bd negative to Bd positive, and remains Bd positive thereafter (sensu Mosher et al. 2018b, c). For sites that tested positive the first year of swabbing, there is uncertainty associated with the year in which those sites transitioned from Bd negative to Bd positive, so I assigned a covariate value of 0.5 prior the first year they were tested. I also assigned a 0.5 to years between a site testing negative and testing positive, if there was a gap in testing, and to years at the end of the time series if a site was Bd negative in the last year it was tested.

Finally, a subset of site covariates were static during the entire study. Biologists familiar with each site were asked whether the site was permanent or ephemeral, whether it contained trout, and the elevation of the site. In the few cases in which the biologists were unsure of the site permanence or trout status, I assigned a “mean” value of 0.5 for the respective covariate. Thirty sites were assigned a value of 0.5 for permanence, and 31 for trout, but these sites represented only 38 and 44 of the 571 site by year combinations, respectively, indicating that these sites were less intensively sampled.

Static Analysis

I used a sequential process to fit a series of static multistate occupancy models to the multistate detection-nondetection data from 571 site x year combinations. my global model structure included: (1) effects of date (quadratic relationship), Bd status, occupied state, and an interaction between state and date on detection probability, (2) an effect of date on classification probability, (3) additive effects of permanence, Bd status, and elevation on the probability of breeding (ϕ^1), and (4) effects of permanence, snowpack, trout presence, elevation, Bd status, and an interaction between permanence and snowpack on the probability of metamorphosis, given breeding (ϕ^2).

I first fit models representing my hypotheses about factors likely to influence detection and classification probabilities, using the global structure for the probability of breeding and the probability of metamorphosis, given breeding. I fit 13 detection probability structures represented all possible additive effects of date of survey (linear and quadratic relationships), occupied state (m=1 or 2), Bd status, and an interaction between date of survey and state. Each detection structure was paired with either a constant classification probability structure or a structure that allowed classification probability to vary by date (26 total models, Table 3.1).

Using the best-supported detection and classification structures and my global structure for the probability of metamorphosis, I tested all possible additive combinations of effects of Bd, permanence, and elevation on the probability of breeding (8 models, Table 3.2). Finally, I tested all possible additive combinations of effects of permanence, snowpack, trout presence, elevation, Bd status, and an interaction between permanence and snowpack on the probability of metamorphosis, given breeding (40 models, Table 3.3). All models were fit in Program MARK (White and Burnham 1999) and I set the effective sample size equal to the number of independent sites (121).

Dynamic Analysis

I used the same global structure for detection and classification as the static analysis and because of the sparse data in the first year of my dynamic dataset (2001), I used a constant structure for the initial (2001) probability of breeding ($\phi^1(.)$) and the probability of metamorphosis, given breeding ($\phi^2(.)$). Additionally, my global model included: (1) additive effects of previous state, elevation, Bd status, and permanence on the dynamic probability of breeding in (ψ^m), and (2) additive effects of previous state, elevation, Bd status, permanence, trout presence, snowpack, and an interaction between snowpack and permanence on the conditional probability of metamorphosis, given breeding (R^m).

I verified the same detection and classification structures were supported by fitting the same set of 26 models as in the static analysis (Table 3.4). Next, I tested all possible combinations of additive effects on the state-specific probability of breeding (24 model structures, Table 3.5). When including an effect of previous state, I tested two structures, one including effects of breeding in the previous year and of metamorphosis in the previous year (ψ^m:

$\psi^0 \neq \psi^1 \neq \psi^2$) and one including only an effect of breeding in the previous year (ψ^m: $\psi^0 \neq (\psi^1 = \psi^2)$).

Using the best-supported structures of detection, classification, and the probability of breeding, I tested all possible additive combinations of my hypothesized factors on the probability of metamorphosis, given breeding. As before, I used two versions of an effect of state, one including effects of breeding and of metamorphosis in the previous year (R^m: $R^0 \neq R^1 \neq R^2$), and one including only an effect of breeding in the previous year (R^m: $R^0 \neq (R^1 = R^2)$). This resulted in 76 models of the probability of metamorphosis, given breeding (Table 3.6). Note that model structures that lack the effect of previous state represent the assumption of my static analysis; i.e., static models assume $\psi^0 = \psi^1 = \psi^2$ and $R^0 = R^1 = R^2$. Again, models were fit using Program MARK and model selection results were used to evaluate relative support for each of my competing hypotheses.

Results

Static Analysis

The best-supported detection structure included additive effects of date of survey (quadratic relationship), state of the site, Bd status, and an interaction between date and state; the correct classification probability also varied with date (Table 3.1). As expected, detection was lower at Bd positive sites and exhibited a different relationship with date of survey depending on the occupied state (Figure 3.2a). At sites with successful metamorphosis (state $m = 2$), toad detection peaked in late July and early August and then declined, while at sites without metamorphosis (state $m = 1$), the peak was lower and detection probability remained relatively high late into the season, indicating that tadpoles remained in the pond during this time (Figure

3.2a). I found strong evidence that the probability of breeding (ϕ^1) was influenced by Bd status and elevation (Table 3.2). Sites at higher elevations without Bd were more likely to support breeding (Figure 3.3a), a finding consistent with Mosher et al. (2018b). The conditional probability of metamorphosis was influenced by pond permanence, Bd status, snowpack, and elevation (Table 3.3). Permanent and high-elevation sites were less likely to support metamorphosis, and Bd presence and high snowpack also decreased the probability of metamorphosis (Figure 3.4). Still, the static analysis suggests that the conditional probability of metamorphosis was high (>0.70) in years where snowpack was less than 110% of the median (Figure 3.4), except at permanent, Bd positive sites.

Dynamic Analysis

Consistent with the static analysis, the best-supported detection structure was the global structure (Table 3.4), with similar estimated relationships between date of survey, occupied state, and Bd status (Figure 3.2b). I found strong evidence that the probability of breeding and conditional probability of metamorphosis differed based on the state of the site during the previous year (Tables 5 and 6), violating the assumption made in my static analysis that these probabilities were independent of the state of a site in the previous year. However, many of the biological findings were similar when I incorporated state-specific variation in modeling occupancy dynamics. Specifically, the probability of breeding (ψ^m) increased with increasing elevation and was higher at sites that supported breeding in the previous year (Table 3.5, Figure 3.3b). It was unlikely that a site without breeding in one year (state $m = 0$) would support breeding the following year ($\hat{\psi}^0 < 0.25$) regardless of elevation (Figure 3.3b). It is likely that the effect of Bd on the probability of breeding is not supported in the dynamic model because it is incorporated in the effect of state in the previous year.

Conditional probability of metamorphosis varied based on permanence, snowpack, breeding in the previous year, and an interaction between snowpack and permanence (Table 3.6). There was uncertainty regarding the effects of Bd status and of metamorphosis in the previous season (Table 3.6). Sites with breeding in the previous year had higher conditional probability of metamorphosis than sites without breeding (Figure 3.5). Similar to the static analysis, the conditional probability of metamorphosis declined with increasing snowpack at permanent sites, but the dynamic analysis suggests a different relationship for ephemeral sites where conditional probability of metamorphosis exhibited a positive relationship with snowpack (Figure 3.5). These relationships were similar for sites that did not support breeding in the previous season but the estimates of conditional probability of metamorphosis were much lower and imprecise (Figure 3.5b). The proportion of sites supporting successful metamorphosis in a year declined over the course of the study, from 0.73 in 2001 to 0.44 in 2010, with a corresponding increase in the proportion of sites without breeding (Figure 3.6).

Discussion

Addressing declines in species of conservation concern requires knowledge of the population dynamics and the causes of the decline. Boreal toads are challenging to conserve in part because many of the management options proposed for the species involve management of the aquatic habitat but the impacts of these actions on the life stages that use aquatic habitats are not well understood. Although authors (e.g. Carey et al 2005) have recognized that reproductive failure occurs in this species and have recorded the causes of failure in some instances, this thesis is the first attempt to quantify the frequency of reproductive failure (or its inverse, reproductive success) across many breeding sites in many years and to relate this to environmental factors hypothesized to affect reproductive success. I found that the probability that a site supported metamorphosis, given that it supported breeding, was high, but that the proportion of sites supporting metamorphosis in this region steadily declined over my study period.

The probability that boreal toads metamorphose at a site that supported breeding is dependent on hydrology; in years of high snowpack, ephemeral sites had higher probabilities of metamorphosis, while in years of low snowpack, permanent sites had higher probabilities of metamorphosis. Tadpole growth and development are strongly influenced by water temperature (Ruthsatz et al. 2018). Permanent sites, typically associated with perennial streams, likely warm slowly in years of heavy snowfall relative to ephemeral sites. However, if snowpack is low, ephemeral sites may dry out before tadpoles are able to metamorphose. Other amphibian studies in similar climates by McCaffery et al. (2014) and Gould et al. (2019) found a similar pattern in which amphibian metapopulations were buffered against climate variability by the presence of both permanent and ephemeral sites.

Notably, in years where snowpack is close to the median, probabilities of successful metamorphosis are relatively high for both permanent and ephemeral sites (>0.75 for sites that supported breeding in the previous year). This suggests that the boreal toad is well-adapted to the current climate; during a year of median snowpack, most sites that supported breeding in the previous season are likely to support breeding and metamorphosis in the following year, and within a normal range of climatic variation, at least one type of site (ephemeral or permanent) is likely to support metamorphosis given that breeding occurred. However, the proportion of sites that supported breeding the previous year declined from 0.8 to 0.6 (Figure 3.6), and sites that did not support breeding in the previous year were very unlikely to support metamorphosis, suggesting that declines are unlikely to be reversed. Although the impact of future climate change on winter snowpack is unclear, summer temperatures in Colorado are expected to be much warmer, potentially increasing the risk of desiccation for ephemeral sites (Lukas et al. 2014; Ryan et al. 2014). Although the response of toads or other amphibians to climate change likely depends on local conditions (Muths et al. 2017; Miller et al. 2018), more frequent reproductive failure due to desiccation of ephemeral sites could have significant negative impacts for permanent sites as well, as they would become increasingly isolated and would have lower immigration rates.

The static analysis supported effects of elevation and Bd on the probability of metamorphosis, give, breeding, while the dynamic analysis supported an effect of breeding in the previous season. The effect of breeding in the previous season likely combines several aspects of habitat quality; sites that supported breeding in the previous year have a breeding population of toads, are less likely to be Bd positive, and are likely higher in elevation than sites that did not support breeding in the previous season. Breeding in previous years is an important predictor of current breeding in other amphibian occupancy studies (e.g., Mosher et al. 2018b; Gould et al. 2019), but does not elucidate why the habitat that has historically supported breeding is so well-suited to the species. The static model helps to reveal the relationships between habitat and probabilities of breeding and metamorphosis that are included in the effect of previous state in the dynamic analysis.

The static and dynamic analyses yield similar results for the within-season detection parameters (detection probability and correct classification). Detection probability varied among sites based on the true occupancy state and peaked around late July and early August at sites with successful metamorphosis. Conversely, detection probability continued to increase throughout the season for sites with breeding but no metamorphosis. Collectively, my results suggest that late-stage tadpoles are more easily detected than other stages (especially eggs and metamorphs), and if metamorphosis occurs, most individuals metamorphose simultaneously, reducing the number of individuals available for detection late in the season. At sites where breeding occurred but metamorphosis did not, detection remained high late in the season, suggesting that tadpoles were still available for detection at these sites. Fewer surveys were conducted late in the season, especially at sites without metamorphosis, resulting in larger confidence intervals (Figure 3.2). Because the sampling was focused primarily on documenting breeding adults, it is possible that if few eggs or tadpole were seen in early visits, no effort was made to return to a site later in the season, leaving most late-season sampling at sites that had high abundances of tadpoles early in the season. Moreover, dry sites were not consistently recorded and I could not differentiate between visited sites that were dry and those that were wet but no toads were detected (observed state = 0 for both). If future monitoring included this information, models could utilize that information to simultaneously estimate the availability of aquatic habitat and species occurrence conditional on habitat availability (e.g., Falke et al. 2012; Gould et al. 2019).

Boreal toads, like many pond-breeding amphibians, have variable recruitment and reproductive success but high and stable adult survival (e.g., Biek et al. 2002; Pilliod et al. 2010; Muths and Scherer 2011; Muths et al. 2011). Although monitoring adult populations is important for detecting dramatic declines in breeding population size associated with pathogens like Bd (e.g., Carey 1993; Muths et al. 2003; Scherer et al. 2005), stable adult populations may mask long-term declines due to low or no recruitment (Muths and Scherer 2011; Semlitsch et al. 2017). Monitoring different components of reproduction allows us to better understand species occupancy, breeding, and recruitment dynamics, without substantial additional effort.

There are few effective strategies for slowing or reducing the declines of species challenged by disease (e.g., Garner et al. 2016), and my results reflect continued decline in boreal toad populations. Proposed management strategies to increase the number of active boreal toad breeding sites in the southern Rockies include manipulations of breeding ponds and water availability and the reintroduction of tadpoles into suitable unoccupied habitat (Gerber et al. 2018). Many of these strategies, notably reintroductions, are currently being implemented (Muths et al. in press). Reliable information on the causes of reproductive failure will help managers select sites that are well-suited to supporting metamorphosis. my results echo the importance of selecting sites that are Bd negative (Muths et al. in press; Chapter 1), as Bd can be detrimental to all life stages of susceptible species (e.g., Blaustein et al. 2005; Garner et al. 2009; Pilliod et al. 2010). Current introduction efforts incorporate monitoring for the pathogen before, during, and after tadpole releases (Muths et al. in press). In addition to selecting Bd negative sites, managers should consider reintroducing toads into a variety of potential breeding sites, including both permanent ponds and ephemeral wetlands, as this diversity of occupied sites increases the probability that at least some sites support metamorphosis despite yearly variations in snowpack, a finding supported by other work on pond-breeding amphibians (McCaffery et al. 2014; Ryan et al. 2014; Gould et al. 2019).

Table 3.1. Static analysis model selection results the best-supported structures [w > 0.001] for the probability of boreal toad detection (p) and correct classification (δ) at historic boreal toad breeding sites in the Southern Rocky Mountains, using a static multistate occupancy analysis. Interactions between breeding boreal toad states (m = 1 or 2) and date of survey are indicated with a star (*), additive effects are indicated with +, and quadratic relationships are indicated as (^{2}). All detection structures were fit using the global multistate occupancy structure, *φ^1(Perm+Bd+Elev) φ^2(Perm+Fish+Bd+Elev+Snow+Snow*Perm)*. AICc = Akaike's information criterion; ΔAICc = the difference between the model's AICc value and that of the top model; w = AICc weight; K = number of parameters), and the deviance of the model.

Detection and Classification Structure	AIC$_c$	ΔAIC$_c$	w	K	Deviance
p(State+Date^2+Bd+State*Date^2) δ(Date)	3999.62	0	0.994	20	3951.22
p(State+Date^2+Bd) δ(Date)	4010.48	10.86	0.004	18	3967.78
p(State+Date^2+ State*Date^2) δ(Date)	4012.40	12.77	0.002	19	3966.87

Table 3.2. Static analysis model selection results for the probability of breeding (φ^1). Probability of breeding model structure = model notation; "Bd" indicates a model that includes an effect of Bd status, "Elev" indicates and effect of elevation, and "Perm" indicates an effect of permanence. All breeding probability structures were fit using the best-supported structures for probability of detection and correct classification, *p(State+Date^2+Bd+State*Date^2) δ(Date)* and the global structure for the probability of metamorphosis, given that breeding occurred (*φ^2(Perm+Fish+Bd+Elev+Snow+Snow*Perm*). AICc = Akaike's information criterion, ΔAICc = the difference between the model's AICc value and that of the top model; w = AICc weight; K = number of parameters), and the deviance of the model.

Probability of Breeding Structure	AIC$_c$	ΔAIC$_c$	w	K	Deviance
φ^1 (Bd+Elev)	3997.29	0.00	0.49	19	3951.77
φ^1 (Perm+Bd+Elev)	3999.62	2.33	0.15	20	3951.22
φ^1 (Elev)	3999.77	2.48	0.14	18	3957.06
φ^1 (Bd)	4000.47	3.18	0.10	18	3957.77
φ^1 (Perm+Bd)	4001.18	3.89	0.07	19	3955.66
φ^1 (Perm+Elev)	4002.55	5.26	0.04	19	3957.02
φ^1 (.)	4008.35	11.06	0.00	17	3968.41
φ^1 (Perm)	4009.50	12.21	0.00	18	3966.79

Table 3.3. Static analysis model selection results for the probability of metamorphosis, given that breeding occurred (φ^2. I present the best-supported (w > 0.01) of 40 φ^2 structures. Interactions are indicated with a star (*), additive effects are indicated with a plus sign (+). Probability of metamorphosis structure notation; "Bd" indicates a model that includes an effect of Bd status, "Elev" indicates and effect of elevation, "trout" an effect of trout presence, "snow" an effect of the relative snowpack in the previous winter, and "Perm" an effect of permanence. All structures were fit using the best-supported structures for probability of detection and correct classification and breeding: *p(State+Date^2+Bd+State*Date^2) δ(Date) φ^1(Bd+Elev).* AICc = Akaike's information criterion, ΔAICc = the difference between the model's AICc value and that of the top model; *w* = AICc weight; K = number of parameters), and the deviance of the model.

Probability of metamorphosis, given breeding structure	AIC_c	ΔAIC_c	*w*	*K*	Deviance
φ^2 (perm+Bd+Elev+snow)	3992.29	0.00	0.23	17	3952.35
φ^2 (perm+Bd+snow)	3993.66	1.37	0.11	16	3956.43
φ^2 (perm+snow)	3994.22	1.93	0.09	15	3959.65
φ^2 (perm+Bd+Elev+snow+snow*perm)	3994.49	2.20	0.08	18	3951.79
φ^2 (perm+trout+Bd+Elev+snow)	3995.05	2.76	0.06	18	3952.34
φ^2 (perm+Elev+snow)	3995.08	2.79	0.06	16	3957.85
φ^2 (perm+Bd+snow+snow*perm)	3995.50	3.21	0.05	17	3955.56
φ^2 (perm+snow+snow*perm)	3995.72	3.43	0.04	16	3958.49
φ^2 (snow)	3995.78	3.49	0.04	14	3963.82
φ^2 (Bd+snow)	3995.95	3.66	0.04	15	3961.38
φ^2 (perm+trout+Bd+snow)	3996.35	4.06	0.03	17	3956.41
φ^2 (Bd+Elev+snow)	3996.57	4.28	0.03	16	3959.34
φ^2 (perm+Elev+snow+snow*perm)	3996.79	4.49	0.02	17	3956.84
φ^2 (perm+trout+snow)	3996.86	4.57	0.02	16	3959.63
φ^2 (perm+trout+Bd+Elev+snow+snow*perm)	3997.29	5.00	0.02	19	3951.77
φ^2 (Elev+snow)	3997.56	5.27	0.02	15	3962.99
φ^2 (perm+trout+Elev+snow)	3997.62	5.33	0.02	17	3957.68
φ^2 (trout+snow)	3998.10	5.81	0.01	15	3963.52
φ^2 (perm+trout+Bd+snow+snow*perm)	3998.25	5.96	0.01	18	3955.55
φ^2 (perm+trout+snow+snow*perm)	3998.39	6.10	0.01	17	3958.45
φ^2 (trout+Bd+snow)	3998.49	6.20	0.01	16	3961.26

Table 3.4. Dynamic analysis model selection results for the probability of boreal toad detection (*p*) and correct classification (δ) at historic boreal toad breeding sites in the SRM in the dynamic analysis. I present the best-supported (w > 0.001) of 26 detection structures. Interactions between boreal toad state (*m* = 1 or 2) and date of survey are indicated with a star (*), additive effects are indicated with a plus sign (+), and (^{2}) indicates a quadratic effect. All detection structures are fit using the global occupancy structure, $\varphi^1(.)\ \varphi^2(.)\ \psi^1(Perm+Bd+Elev)\ \psi^2(Perm+Fish+Bd+Elev+Snow+Snow*Perm)$. Probability of detection and classification model structure = model notation; AICc = Akaike's information criterion, ΔAICc = the difference between the model's AICc value and that of the top model; *w* = AICc weight; K = number of parameters), and the deviance of the model.

Detection and Classification Structure	AIC$_c$	ΔAIC$_c$	*w*	*K*	Deviance
p (State+Date+Bd+State*Date+ Date^2) δ(Date)	3261.98	0	0.92	26	3207.00
p (State+Date+ State*Date+ Date^2) δ(Date)	3266.81	4.83	0.08	25	3214.05

Table 3.5. Dynamic analysis model selection results for the probability of breeding in years other than the first year in the dynamic analysis (ψ^m). I present the best-supported (w > 0.01) of 24 ψ^m structures. In all models presented include the best-supported structure of detection and classification ($p(State+Date^2+Bd+State*Date^2)\ \delta(Date)$) and constant initial probabilities of breeding and metamorphosis, given breeding ($\phi^1(.)$ and $\phi^2(.)$). The probability of metamorphosis (R^m) was modeled using the global structure (R(*Perm+Fish+Bd+Elev+Snow+State+Snow*Perm*)). Probability of breeding model structure = model notation; "Breeding" indicates an effect of breeding in the previous year ($m_{t-1}=1$), "Meta" an effect of metamorphosis in the previous year ($m_{t-1}=2$), "Bd" an effect of Bd status, "Perm" an effect of permanence, and "Elev" an effect of elevation. AICc = Akaike's information criterion, ΔAICc = the difference between the model's AICc value and that of the top model; *w* = AICc weight; K = number of parameters), and the deviance of the model

Probability of breeding structure	AIC$_c$	ΔAIC$_c$	*w*	*K*	Deviance
ψ^m (Elev+Breeding+Meta)	3257.96	0.00	0.49	24	3207.42
ψ^m (Elev+Perm+Breeding+Meta)	3259.90	1.93	0.19	25	3207.14
ψ^m (Elev+Bd+Breeding+Meta)	3260.14	2.18	0.17	25	3207.38
ψ^m (Elev+Bd+Perm+Breeding+Meta)	3262.08	4.12	0.06	26	3207.09
ψ^m (Perm+Breeding+Meta)	3263.86	5.89	0.03	24	3213.31
ψ^m (Breeding+Meta)	3263.86	5.89	0.03	24	3213.31
ψ^m (Bd+Breeding+Meta)	3263.95	5.98	0.02	24	3213.40

Table 3.6. Dynamic analysis model selection table for the state-specific probability of metamorphosis, given that breeding occurred (R^m). I present the best-supported ($w > 0.01$) of 76 models structures that include effects of model notation; “Breeding” indicates an effect of breeding in the previous year ($m_{t-1}=1$), “Meta” an effect of metamorphosis in the previous year ($m_{t-1}=2$), “Bd” an effect of Bd status, “Perm” an effect of permanence, “Elev” an effect of elevation, “Snow” an effect of the relative snowpack in the previous winter, “Trout” an effect of trout presence, and “Snow*Perm” an interaction between snowpack and permanence. All models include the best-supported structure of breeding probability, detection and classification $((\psi^{m}(\mathit{Elev+Breeding+Meta})\ p(\mathit{State+Date^2+Bd+State{*}Date^2})\ \delta(\mathit{Date}$. The probabilities of breeding and metamorphosis in the first year were modeled as an intercept-only ($\phi^1(.)$ and $\phi^2(.)$). AICc = Akaike’s information criterion, ΔAICc = the difference between the model’s AICc value and that of the top model; *w* = AICc weight; K = number of parameters), and the deviance of the model

Probability of metamorphosis, given breeding structure	AIC$_c$	ΔAIC$_c$	*w*	*K*	Deviance
R (Perm+Snow+Snow*Perm+ Breeding)	3253.81	0.00	0.09	20	3212.05
R (Perm+Bd+Snow+Snow*Perm+ Breeding)	3253.90	0.09	0.09	21	3209.95
R (Perm+Snow+Snow*Perm+ Breeding+Meta)	3254.77	0.96	0.06	21	3210.82
R (Perm+Bd+Snow+Snow*Perm+ Breeding+Meta)	3255.05	1.24	0.05	22	3208.91
R (Perm+Bd+Elev+Snow+Snow*Perm+ Breeding)	3255.10	1.29	0.05	22	3208.96
R (Perm+Elev+Snow+Snow*Perm+ Breeding)	3255.32	1.51	0.04	21	3211.37
R (Perm+Trout+Snow+Snow*Perm+ Breeding)	3255.47	1.66	0.04	21	3211.52
R (Perm+Trout+Bd+Snow+Snow*Perm+ Breeding)	3255.54	1.73	0.04	22	3209.40
R (Perm+Bd+Elev+Snow+Snow*Perm+ Breeding+Meta)	3256.06	2.25	0.03	23	3207.73
R (Perm+Elev+Snow+Snow*Perm+ Breeding+Meta)	3256.13	2.32	0.03	22	3210.00
R (Perm+Bd+Snow+ Breeding)	3256.43	2.62	0.02	20	3214.67
R (Perm+Trout+Snow+Snow*Perm+ Breeding+Meta)	3256.49	2.68	0.02	22	3210.35
R (Snow+ Breeding+Meta)	3256.61	2.80	0.02	19	3217.02

R (Perm+Trout+Bd+Snow+Snow*Perm+ Breeding+Meta)	3256.70	2.89	0.02	23	3208.37
R (Bd+Snow+ Breeding+Meta)	3256.73	2.92	0.02	20	3214.97
R (Perm+Bd+Snow+ Breeding+Meta)	3256.80	2.99	0.02	21	3212.85
R (Perm+Snow+ Breeding)	3256.83	3.03	0.02	19	3217.24
R (Perm+Snow+ Breeding+Meta)	3256.87	3.06	0.02	20	3215.10
R (Bd+Snow+ Breeding)	3256.87	3.06	0.02	19	3217.28
R (Snow+ Breeding)	3256.91	3.10	0.02	18	3219.48
R (Perm+Trout+Bd+Elev+Snow+Snow*Perm+ Breeding)	3256.97	3.16	0.02	23	3208.63
R (Perm+Trout+Elev+Snow+Snow*Perm+ Breeding)	3257.16	3.35	0.02	22	3211.02
R (Perm+Bd+Elev+Snow+ Breeding)	3257.32	3.51	0.02	21	3213.38
R (Perm+Bd+Elev+Snow+ Breeding+Meta)	3257.49	3.68	0.01	22	3211.36
R (Bd+Elev+Snow+ Breeding+Meta)	3257.88	4.07	0.01	21	3213.93
R (Perm+Trout+Bd+Elev+Snow+Snow*Perm+ Breeding+Meta)	3257.96	4.15	0.01	24	3207.42
R (Perm+Trout+Elev+Snow+Snow*Perm+ Breeding+Meta)	3258.05	4.24	0.01	23	3209.72
R (Perm+Elev+Snow+ Breeding+Meta)	3258.06	4.25	0.01	21	3214.12
R (Elev+Snow+ Breeding+Meta)	3258.12	4.31	0.01	20	3216.36
R (Perm+Elev+Snow+ Breeding)	3258.18	4.37	0.01	20	3216.42
R (Perm+Trout+Bd+Snow+ Breeding)	3258.23	4.42	0.01	21	3214.28
R (Bd+Elev+Snow+ Breeding)	3258.26	4.45	0.01	20	3216.50

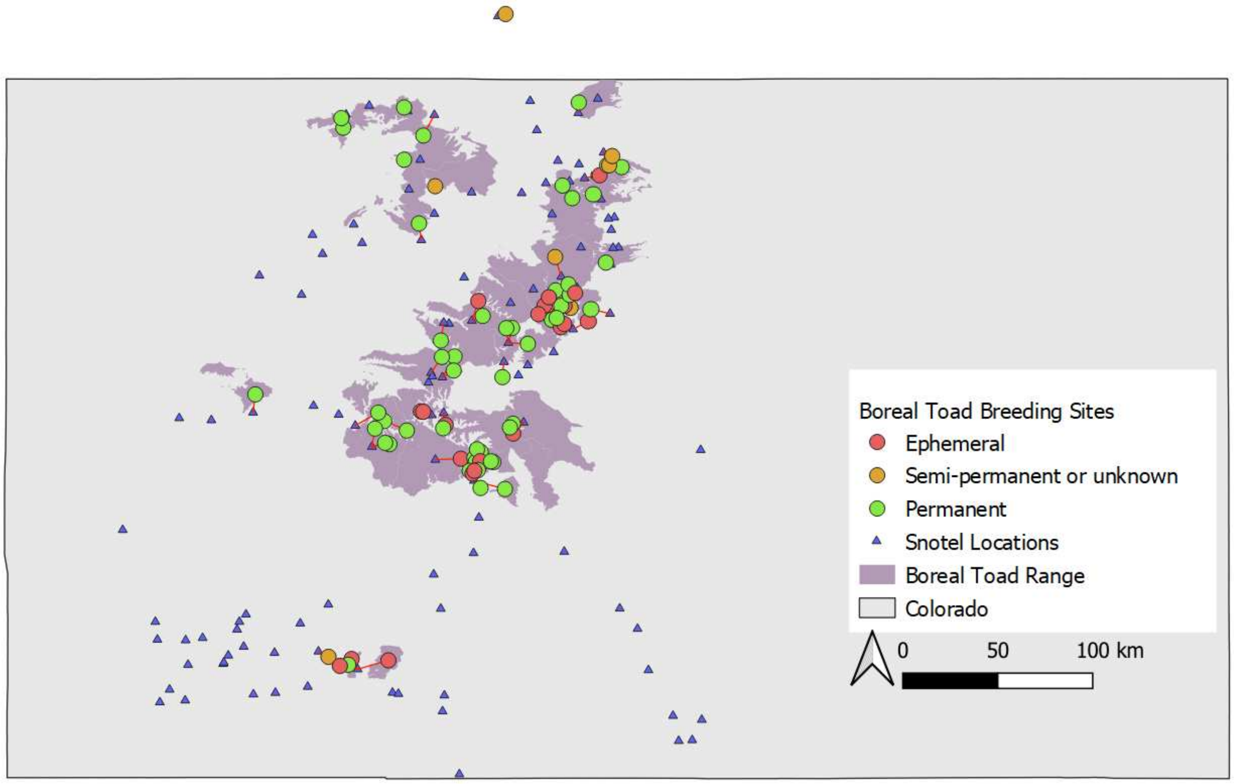

Figure 3.1. Map of the breeding sites included in the dynamic analysis. Breeding sites are indicated with circles; the color of the circle corresponds to the hydrology of the breeding site. Blue triangles indicate locations of SNOTEL sites. The (purple) shaded region is the historic range of boreal toads in Colorado, while the gray shaded area shows the extent of the state. Note that one of our sites is in Wyoming. Red lines show the path from each toad breeding site to the nearest SNOTEL site.

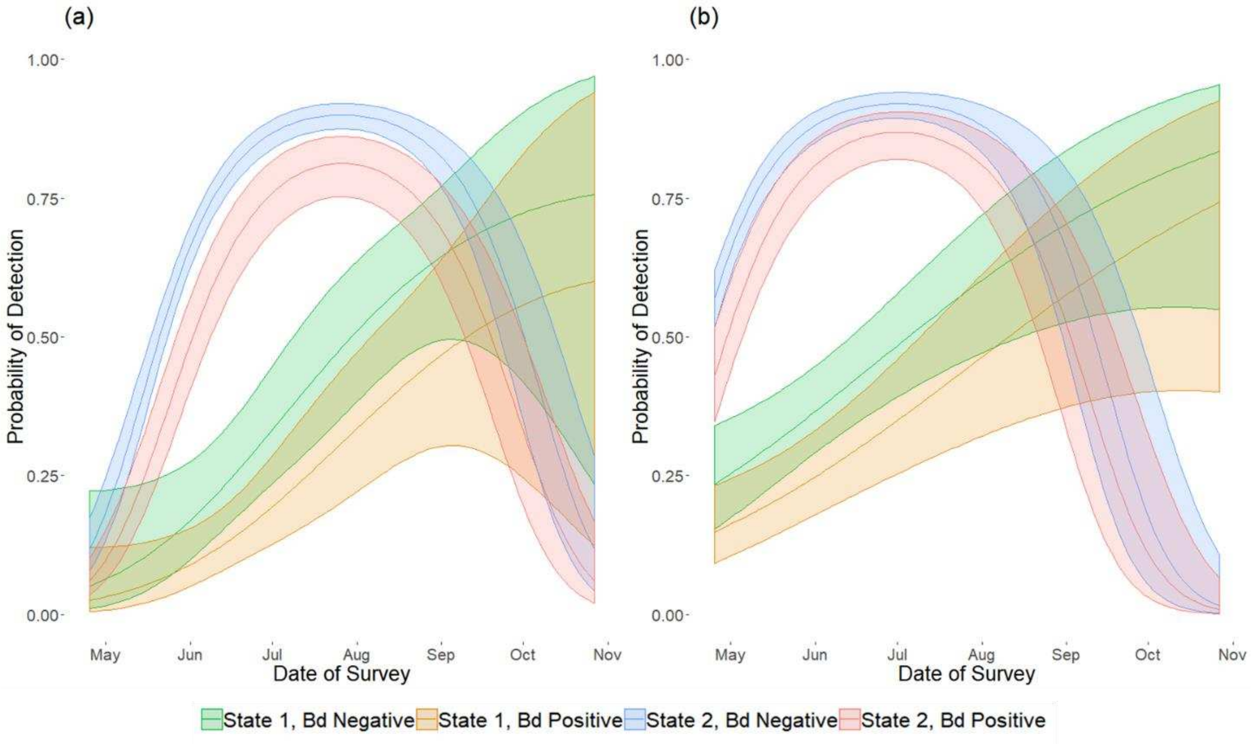

Figure 3.2. Relationship between the probability of detecting breeding at a site, given that it occurred, and the date of survey. Colors correspond to the state of the site (state 1: breeding but no metamorphosis, state 2: successful metamorphosis) and the Bd status of the site. Relationships are shown for the best-supported static (a) and dynamic (b) models. Shaded bands represent the 95% confidence interval for each estimate.

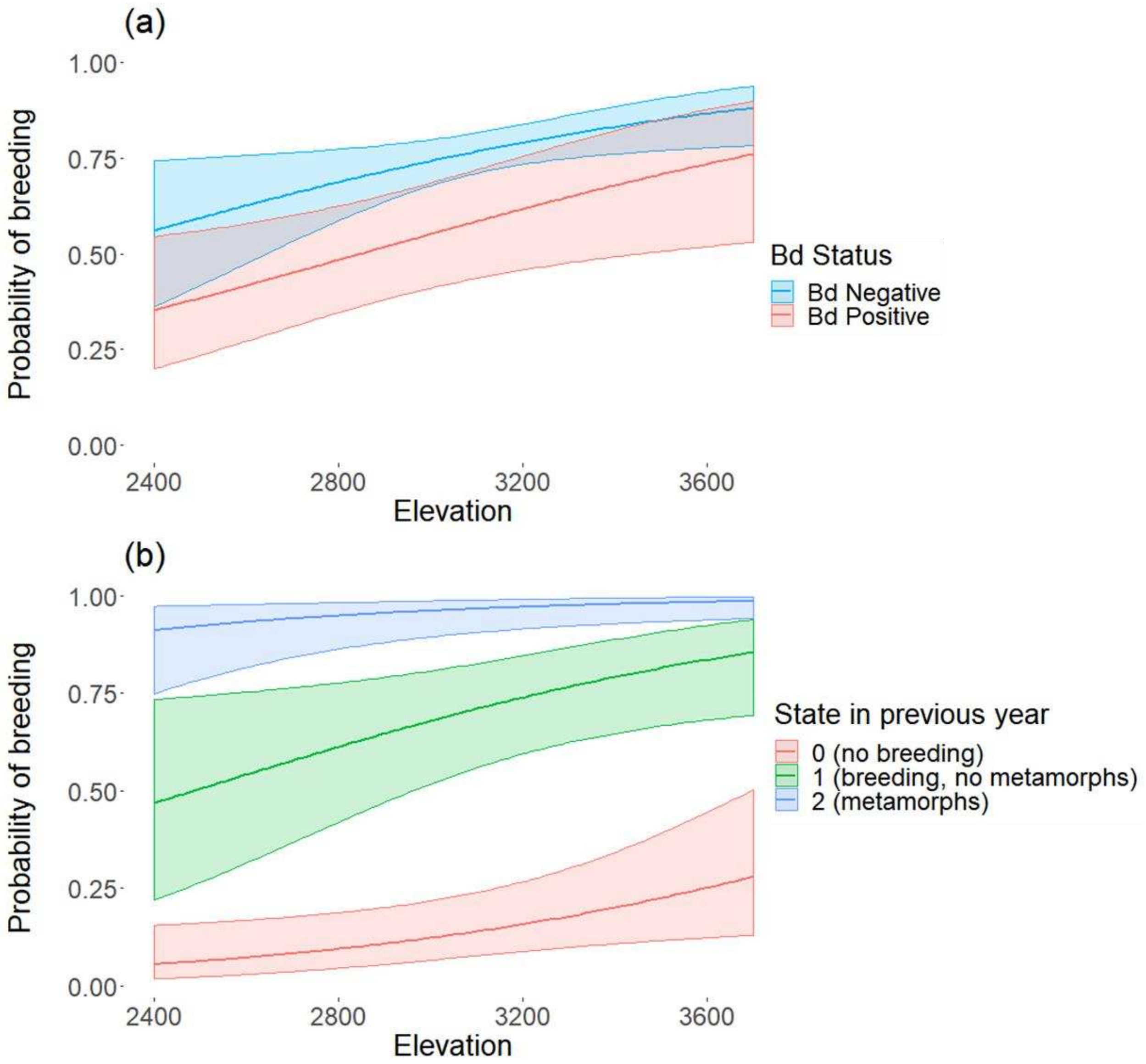

Figure 3.3. Relationship between the probability of breeding and the elevation of the site in the static model (a) and dynamic model (b). Colors in the static model correspond to the Bd status of the site in the given year, and in the dynamic model correspond to the state of the site in the previous year. Shaded areas represent the 95% confidence interval associated with each estimate.

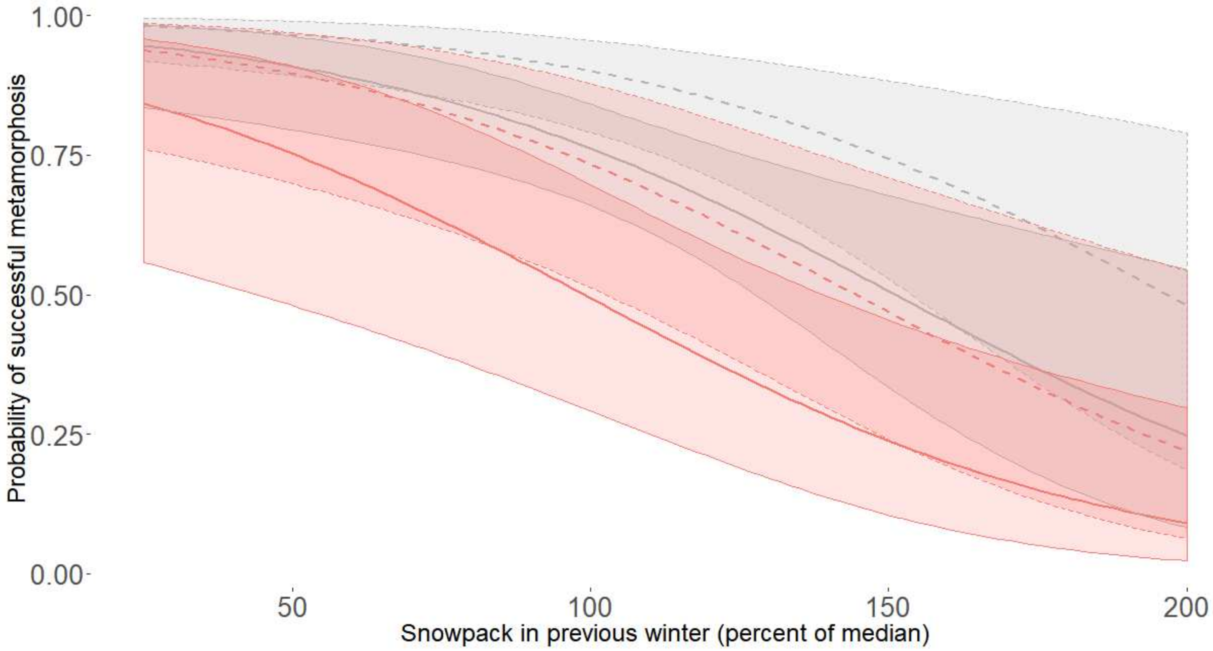

Figure 3.4. Relationship between the probability of metamorphosis, given that breeding occurred (φ^2) and winter snowpack in the static occupancy model. Colors correspond to the Bd status, where red indicates Bd positive sites and grey indicates Bd negative sites. Solid lines correspond to permanent sites and dashed lines represent ephemeral sites. The shaded bands represent 95% confidence intervals around each estimate. Snowpack values are the maximum snowpack in a given year at the nearest SNOTEL site to a toad breeding site, as a percent of the maximum snowpack in the median year at that site.

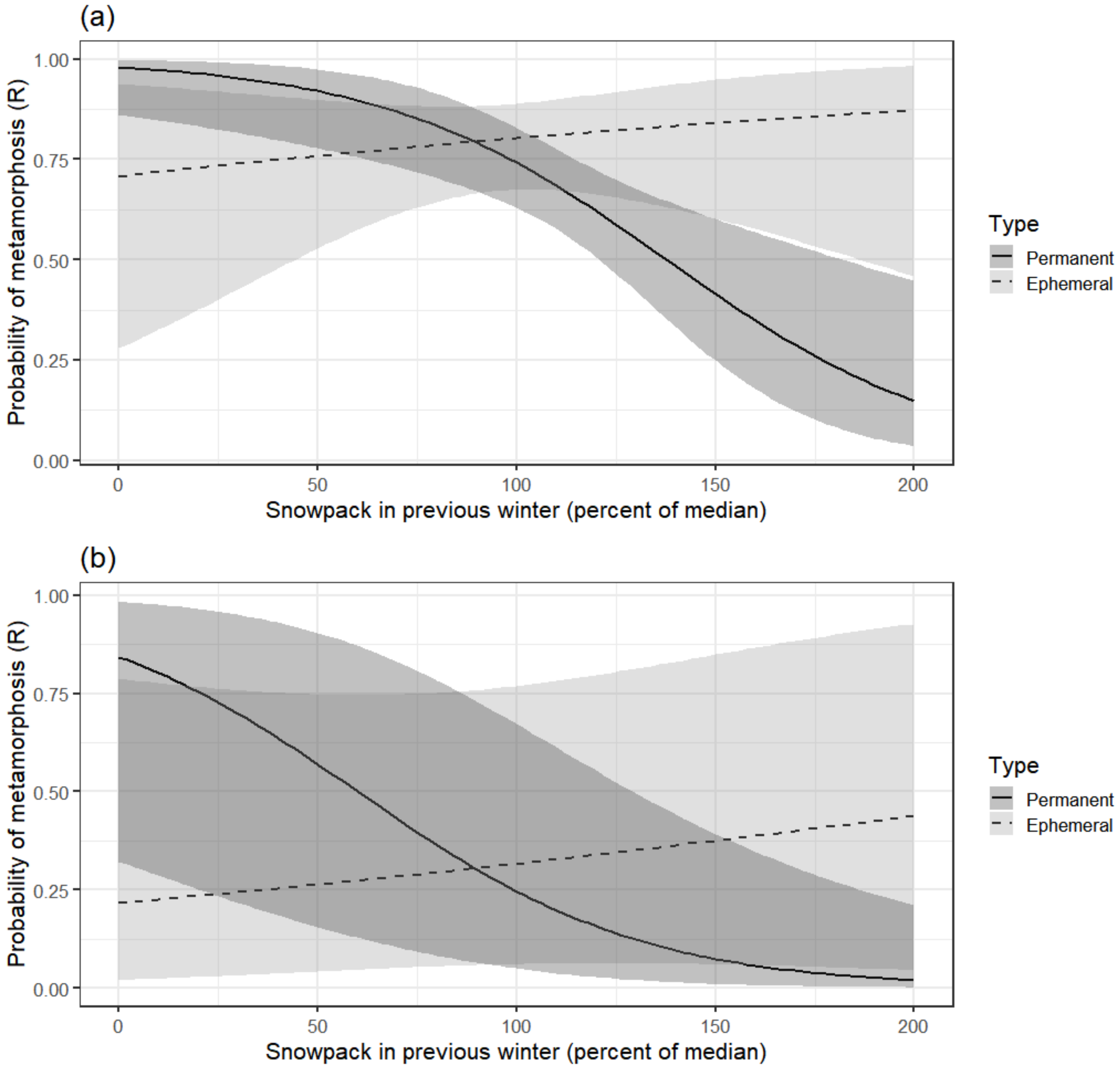

Figure 3.5. Estimates of the probability of metamorphosis, given that breeding occurred (R^{m}) from the best-supported dynamic model across a range of snowpack values, for sites that (a) supported breeding in the previous season, $R^{m=1=2}$, or (b) did not support breeding in the previous season $R^{m=0}$. Dashed lines indicate ephemeral sites while solid lines indicate permanent sites. Shaded areas represent 95% confidence intervals. Snowpack values are the maximum snowpack in a given year as a percent of the maximum snowpack in the median year at the nearest SNOTEL site to a toad breeding site.

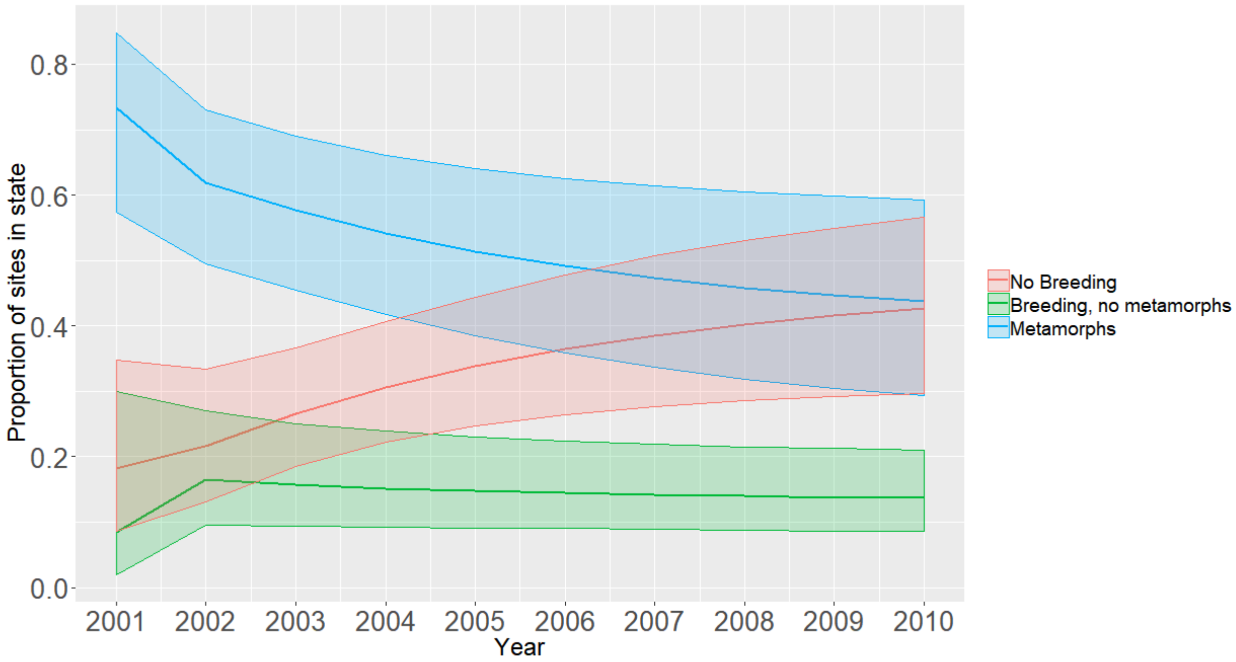

Figure 3.6. Derived estimates of the proportion of sites in each state from the best-supported dynamic model, assuming median snowpack levels. Colors correspond to states, and shaded areas represent 95% confidence intervals.

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