

THESIS

EVALUATING BOREAL TOAD (*BUFO BOREAS*) BREEDING
HABITAT SUITABILITY

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY A. ANDREW HOLLAND ENTITLED EVALUATING BOREAL TOAD (*BUFO BOREAS*) BREEDING HABITAT SUITABILITY BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

EVALUATING BOREAL TOAD (*BUFO BOREAS*) BREEDING HABITAT
SUITABILITY

The southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) is listed as endangered in the state of Colorado and is warranted but precluded for Federal listing as endangered under the Endangered Species Act. Translocation is probably necessary in order to meet recovery criteria. Breeding occurs in shallow margins of lakes and ponds and availability of suitable breeding habitat likely restricts distribution.

My objectives were to evaluate breeding habitat suitability by comparing habitat variables of breeding and adjacent nonbreeding sites, quantifying breeding habitat within breeding sites, and by evaluating factors that influence larval growth rates. In 1999, water temperature, variation in water temperature, bank slope, water level persistence, and surface area were compared between 10 breeding sites and 10 adjacent nonbreeding sites in Colorado. In 2000, breeding site physical characteristics were again evaluated by quantifying bank slopes, amount of shallows, and depths of deposited egg masses in 18 randomly selected breeding sites in Colorado. Differences between breeding and nonbreeding sites were difficult to detect but I

found that shallows ≤ 10 -cm deep were preferred for breeding. The response of larval growth rates to water temperature, variation in water temperature, breeding site water level persistence, breeding site surface area, and conductivity in the same 18 randomly selected breeding sites was also evaluated in 2000. Mixed effect and general linear models of absolute and linear larval growth rates provided evidence that water temperature, daily variation in water temperature, and breeding site persistence affect larval performance. Tadpoles experienced the most gain, in mg per day of development, in breeding sites that had the warmest and least variable water temperatures. Persistent water levels also positively influenced growth rate. Improved understanding of breeding habitat relationships can be used to select translocation sites, determine suitability of wetlands, and aid in development of wetlands for mitigation.

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CHAPTER 1

INTRODUCTION

Once considered common, the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) has declined (Corn et al. 1989, Carey 1993, Loeffler 1998). This population's decline has occurred despite its existence in relatively pristine environments, between 2,250 m and 3,600 m elevation in southern Wyoming, Colorado, and northern New Mexico, with the majority of breeding localities in Colorado (Loeffler 1998). The cause of the decline is unknown but chytrid fungus, *Batrachochytrium dendrobatidis* (Longcore and Pessier 1999), a mortality factor for boreal toads in Colorado (Jones 2000) has probably been a major contributing factor. As a result of this decline the southern Rocky Mountain population is listed as endangered in the state of Colorado and is regarded as warranted but precluded for Federal listing under the Endangered Species Act (Loeffler 1998).

These issues prompted the Boreal Toad Recovery Team to bring adults and eggs from several evolutionarily significant units into captivity in 2000. Offspring from these individuals will eventually be used to reestablish boreal toad populations in Colorado because translocation of boreal toad eggs, larvae, or adults is probably necessary to meet the recovery criteria set by the Boreal Toad Recovery Team (Muths et al. 2001). This emphasizes the importance of

quantifying boreal toad habitat relationships to determine what constitutes suitable habitat.

Historic areas will not always be the best candidates for translocation because sites change over time and may still harbor chytrid fungus. This project was developed to address issues outlined in sections 1.1, 3.5, 4.1, 5.0, and 5.1 of the Boreal Toad Conservation Plan and Agreement (Loeffler 1998) that deal primarily with identification of habitat requirements and research related to experimental translocation.

Breeding habitat is important because boreal toad distribution is limited to areas that contain suitable breeding habitat (Loeffler 1998). Boreal toads appear to be generalist in the types of terrestrial habitats that they occupy but their breeding habitat requirements are more specialized. There are undoubtedly many areas with suitable terrestrial habitat that do not support boreal toad populations because they lack a suitable breeding site. Breeding site quality also influences the number and quality of metamorphosing larvae. In fact, amphibian species' success at the population level is determined primarily by the number and quality of metamorphosing larvae leaving a breeding site (Semlitsch 2000).

Several researchers have modeled an amphibian species' presence or absence in relation to environmental variables (Sjogren-Gulve 1994, Corn et al. 1997, Demaynadier and Hunter 1998, Munger et al. 1998, Vos and Chardon 1998). Others have related density, reproduction, or survival to environmental variables (Wilbur 1987, Berven 1990, Bury et al. 1991, Block and Morrison 1998, Vos and Chardon 1998). The objectives of modeling these relationships are to formalize current understanding, learn which environmental factors affect

distribution and abundance of a species, generate hypotheses, and ultimately make predictions (Morrison et al. 1992).

The objective of this study is to use models to provide the Colorado Division of Wildlife with appropriate habitat variables, and their associated levels, that can be used to evaluate breeding habitat of potential translocation sites. Boreal toad breeding habitat suitability was evaluated in several ways. In Chapter 2, habitat characteristics of breeding and adjacent nonbreeding sites were compared in 1999 to evaluate breeding site selection by breeding adults. Breeding site selection was evaluated differently in 2000 by quantifying characteristics of egg deposition areas within breeding sites. Next, habitat suitability for larval boreal toads was evaluated. Factors influencing larval growth, development, and metamorphosis were modeled for both wild and laboratory reared tadpoles. More specifically, the main focus in 2000 was measuring and modeling conditions within breeding sites that, given successful reproduction, allow tadpoles to metamorphose at a large size in a short amount of time. Information from field efforts on wild sites is presented in Chapter 3 and a comparison of wild and captive reared tadpoles is presented in Appendix II. Chapters 2 and 3 were written so they could be used independently for publication. Appendix II is also written to be used independently and is intended to be a report for the Colorado Division of Wildlife captive rearing program.

This project is compatible with other boreal toad research on adult habitat use (Jones 2000), larval predator prey relationships (Livo 1999), evaluating methods for reintroduction (Scherff-Norris 1999, Muths et al. 2001) and ongoing disease and population viability research.

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CHAPTER 2

DESCRIBING BOREAL TOAD (*BUFO BOREAS*) BREEDING HABITAT

Abstract. The southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) is listed as endangered in the state of Colorado and is warranted but precluded for Federal listing as endangered under the Endangered Species Act. Translocations are probably necessary in order to meet recovery criteria. This species breeds in shallow margins of lakes and ponds and availability of suitable breeding habitat likely restricts distribution. Breeding habitat suitability was evaluated by comparing habitat variables of breeding and adjacent nonbreeding sites and by quantifying breeding habitat within breeding sites. Differences between breeding and nonbreeding sites were difficult to detect but evaluation of breeding areas revealed that shallows ≤ 10 -cm deep were preferred for breeding. Improved understanding of breeding habitat relationships can be used to select translocation sites, determine suitability of wetlands, and aid in development of wetlands for mitigation.

Despite once being considered common, the distribution and abundance of the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) have declined (Corn et al. 1989, Carey 1993, Loeffler 1998). It is currently listed as endangered in the state of Colorado and is regarded as warranted but precluded for Federal listing under the Endangered Species Act (Loeffler 1998).

In southern Wyoming, Colorado, and northern New Mexico, this species appears to be a generalist in terms of occupied terrestrial habitat with breeding localities in lodgepole pine (*Pinus contorta*), spruce (*Picea* sp.), subalpine fir (*Abies lasiocarpa*), and alpine meadows at elevations between 2,250 m and 3,600 m. Distribution is restricted to areas with suitable breeding habitat (Loeffler 1998). There are 40 known breeding localities for this population (Loeffler 1998)(Figure 2.1), and in 2000, the 33 that had breeding activity occurred in Colorado (Loeffler 2000). One breeding locality may consist of several breeding sites which are simply bodies of water where breeding occurs (Loeffler 1998). Boreal toads breed in the margins of ponds and lakes as soon as ice melts which typically occurs in May and June. Common types of breeding sites in Colorado are American beaver (*Castor canadensis*) ponds, high elevation lakes and ponds, glacial kettle ponds, and human excavated wetlands. Breeding site types are diverse but not necessarily equally preferred. Ideal boreal toad breeding sites presumably contain still water, very shallow margins, and persistent water levels. Egg masses are typically deposited communally in the shallowest available areas of the breeding site.

Amphibian egg and larval development are directly related to water temperature (Herreid and Kinney 1967), so the temperature regime of breeding sites influence successful hatching and metamorphosis. Once hatched, tadpoles behaviorally thermoregulate to control body temperature and metabolic rate (Brattsrom 1962, Beiswenger 1977, 1978). Because water temperature is dependent on depth (Barandun and Reyer 1997), boreal toad tadpoles migrate to deeper water at night and on sunny days aggregate in very shallow water (Beiswenger

1977, Livo 1999). Metamorphosis must be achieved before breeding site dessication and by the end of summer when breeding sites typically start to freeze.

This project was developed to address issues outlined in sections 1.1, 3.5, and 4.1 of the Boreal Toad Conservation Plan and Agreement (Loeffler 1998) which focus primarily on identification of habitat requirements and research related to experimental translocation. The overall objective was to characterize the habitat of boreal toad breeding sites. The first year, 1999, breeding and adjacent nonbreeding sites were compared. In the second year, 2000, the focus was on quantifying characteristics of egg deposition areas within breeding sites.

METHODS

Breeding Versus Non-breeding Site Comparisons 1999

In 1999, 22 breeding sites were chosen to assess the entire range of egg mass numbers and elevations for boreal toads in the Rocky Mountains of Colorado. Upon visitation, 10 breeding sites had 1 or more adjacent ponds with no breeding activity (see Appendix II). These 10 breeding sites and adjacent non-breeding sites (if several nonbreeding sites accompanied a breeding site, then a nonbreeding site was randomly selected for study) were compared. Study sites ranged in elevation from 2,900 m to 3,480 m and were located in Chaffee, Summit, Clear Creek, and Larimer Counties, Colorado. Sites were primarily in wet meadows within upper montane and subalpine communities dominated by spruce-fir forests. Several sites were also associated with lodgepole pine communities. *Salix* spp. and *Carex* spp. were usually found in riparian areas surrounding sites. All study sites were American beaver ponds except for 1 high elevation lake and 1 site of anthropogenic origin. Study sites were

typically visited monthly from breeding to metamorphosis, which was typically late May to late August, and mean water temperature, mean daily variance in water temperature, breeding site persistence, bank slope, and breeding site surface area were measured.

Water temperature variables were measured hourly (± 0.2 °C) by placing Onset Computer Corporation Optic Stowaway[®] temperature loggers (Onset Computer Corporation, Pocasset Massachusetts) at 30-cm depth as close to the egg masses as possible because amphibians select the warmest areas of the breeding site for oviposition (Seale 1982). Temperature loggers were placed in similar shallow areas in nonbreeding sites at 30-cm depth.

Thirty-cm depth was selected to avoid dessication as water levels fluctuate naturally and depth of each temperature logger was recorded at each visit. Pond surface area (Pearman 1995) and depth affect water temperatures (Beiswenger 1977, Pearman 1995), therefore, temperature loggers were placed at 10 cm, 30 cm, and 60 cm in a small, medium, and large breeding site to estimate the slope of the effect of depth on temperature. These slope parameters and the depths of the temperature loggers were used to adjust water temperatures as if they were recorded at a constant 30-cm depth. This adjustment allowed comparison of temperatures between sites that were recorded at different depths. These adjusted temperatures were used to calculate July mean temperature and July daily temperature variation within each site. Temperature logger depth measurements were used to estimate breeding site persistence by calculating the rate of change in water level in cm per day over the larval period.

Water depth measurements were taken at 0.30 m, 1 m, and 5 m from shore on each side of 3 transects established across the width of sites, with 1 positioned to record bank slope

of the egg deposition area. An average bank slope for each site was estimated by averaging each depth divided by the distance from shore that it was taken. Breeding site surface area was estimated using a maximum length and 3 width measurements and the equation for the area of an ellipse.

Variable means and 95% confidence intervals were estimated and a paired t-test was performed with PROC TTEST to compare means of breeding and adjacent nonbreeding sites (SAS Institute Inc. 2000). A paired analysis was necessary to avoid comparing the temperatures from sites at different elevations where snow melt and breeding occurred on different dates.

Breeding Site and Ovipositioning Area Characteristics 2000

Breeding sites that had at least 1 egg mass for 4 consecutive years (1997 to 2000) were identified. In 2000, 33 breeding sites from 25 breeding localities, met this criterion. Eighteen of these sites were randomly selected for study. Only 14 breeding localities were represented because multiple breeding sites were selected from some localities.

Study sites were in Chaffee, Clear Creek, Summit, Eagle, Routt, and Gunnison Counties, Colorado (see Appendix II) and ranged in elevation from 2,542 m to 3,487 m. Sites were primarily in wet meadows in subalpine communities dominated by spruce-fir, but several sites were also associated with lodgepole pine communities. *Salix* spp. and *Carex* spp. were usually found in riparian areas surrounding sites. Study sites were visited during breeding and were comprised of 9 beaver ponds, 1 high elevation lake, 3 high elevation meadow ponds, and 5 sites of anthropogenic origin.

Habitat variables measured in 2000 focused on the egg deposition area within each breeding site. Water depth of every identifiable egg mass was measured to estimate depths selected for oviposition. Depths were not measured unless toads were still breeding at the site to ensure that water levels did not fluctuate between breeding and measurement. Mean depth of egg masses was calculated at each site. Amount of shallows present, and consequently potential breeding habitat, was quantified by measuring the length of shoreline that had water ≤ 10 -cm deep, measured 30 cm from the margin. Ten centimeters was chosen because this was the maximum egg deposition depth observed in 1999 (A. Holland, unpublished data). Finally, shoreline characteristics of breeding areas were evaluated by estimating bank slope by measuring depths at 0.30 m, 1 m, and 5 m from the margin on 10 transects in the egg deposition area. For each site, linear regression was used to model depth as a function of distance from shore.

RESULTS

Breeding Versus Non-breeding Site Comparisons 1999

Number of egg masses deposited ranged from 1 to 17 per site. Eggs hatched in 9 sites but metamorphosis was only achieved at 3. Temperatures were missed as a result of logistics of finding and visiting all sites when breeding was occurring and because of logger dessication at 1 breeding site and 3 nonbreeding sites. Thus, only July temperatures are reported here because this was the only period when temperatures were recorded at all sites. Mean depth of temperature loggers through the field season was 16.5 cm. The slope estimate for effect of depth on temperature was -0.036 (se = 0.009) with an intercept of 15.36. In July, breeding site

water temperatures tended to be warmer than nonbreeding sites (paired t-test, difference = 2.27°C , n = 7, p = 0.10) (Table 2.1).

Breeding sites tended to have greater daily temperature variation, less breeding site persistence, and steeper bank slopes, but significance levels were large for these characteristics (Table 2.1). Mean breeding site surface area was 3,411 m² (range = 28 - 18,452) and was greater than the nonbreeding mean surface area of 2,190 m² (range = 25 - 13,134) (paired t-test, difference = 1,222 m², n = 10, p = 0.052).

Breeding Site and Ovipositioning Area Characteristics 2000

Breeding was initiated between 5 May 2000 and 9 June 2000. Mean number of egg masses deposited was 5.4 (se = 1.1, n = 14) and ranged from 1 to 13 per site. Depths of 38 egg masses were measured at 10 sites. Mean depth of egg masses was 6.1 cm (se = 0.6, n = 10) and ranged from 3.0 cm to 9.8 cm. Metamorphosis was achieved at all 14 sites and was initiated between 21 July 2000 and 29 August 2000.

Mean length of shallow shoreline was 52.1 m (se = 9.9, n = 18) and ranged from 10 m to 190 m. Mean bank slope of the egg mass ovipositioning area was 0.07 (se = 0.007, n = 17) or a 7-cm drop in water level for every 1-m increase in distance from shore. Bank slopes ranged from 0.02 to 0.12.

DISCUSSION

The finding of warmer breeding sites compared to nonbreeding sites in 1999 (see Table 2.1) is similar to that of Livo (1999) who measured a breeding site mean temperature of 17.1°C (se = 0.6, n = 19) versus 14.8°C (se = 0.8, n = 6) for nonbreeding sites for boreal toads during

two 24-hour periods between 25 June and 31 August 1998 in Colorado. Selection for warmer breeding sites has also been shown in the natterjack toad, *Bufo calamita*, (Banks and Beebee 1987), pool frog, *Rana lessonae*, (Sjogren-Gulve 1994), and yellow-bellied toad, *Bombina variegata*, (Barandun and Reyer 1997). Selection for warmer breeding sites would be expected because anuran egg development (Herreid and Kinney 1967), tadpole growth (Herreid and Kinney 1967, Beebee 1983, Buchholz and Hayes 2000) and successful metamorphosis (Wilbur 1987) are directly related to water temperature. The observed breeding site mean temperature of 13.4°C is much lower than the 28°C to 34°C preferred body temperature for boreal toad larvae (Beiswenger 1978) which provides additional evidence that higher water temperatures should be preferred. Due to missing data, the results were limited to one month (July) of the development period, and future studies should strive to compare temperatures from breeding to metamorphosis.

Mean daily variance in water temperature was slightly higher in breeding sites. Warmer temperatures observed in breeding sites may contribute to increased daily variation. In a related study, it was found that daily variation in water temperature negatively affects larval growth rate almost as much as increased mean water temperature positively affects growth rates (see Chapter 3).

Breeding sites were less persistent than nonbreeding sites. These results were not expected because boreal toads require persistent water levels to ensure that eggs do not desiccate. Livo (1999) cited desiccation as the cause of egg and embryo mortality for 28 of 77 egg masses, in 7 boreal toad breeding sites, in Clear Creek County, Colorado. Egg mass

desiccation was observed in this study but to a much lesser degree. Breeding adults likely select shallow ovipositioning areas without the ability to predict which sites will be more persistent.

Breeding sites tended to be larger than nonbreeding sites, however, breeding sites ranged in size from 28 m² to 18,452 m² indicating that large wetlands are not required. Banks and Beebee (1987) found that natterjack toad use was correlated with breeding site surface area at one study area and not at another. Large breeding sites have the benefits of increased persistence and lower daily temperature variation, however, increased persistence results in increased larval predator abundance and size (Perman 1995, Skelly 1996).

Maximum observed egg mass depth in 2000 was 9.8 cm. This depth is consistent with those observed in 1999 and might be considered the maximum depth that Colorado boreal toads deposit their eggs. In contrast, at lower elevations of 1,220 m to 1,950 m in Oregon with presumably greater water temperatures, Olsen (1989) reported depths of oviposition for *Bufo boreas* between 5 and 25 cm, and on vegetation in up to 2-m of water at 1 site. Eggs are deposited in shallows despite the increased potential for egg mortality that exists as a result of desiccation and freezing (Livo 1999). Shallows that allow elevated water temperatures on sunny days (Barandun and Reyer 1997) could be especially important to boreal toads because of cold water temperatures at high elevation.

Gradually sloping banks ensure that suitable breeding habitat exists at a variety of water levels. Gradually sloping breeding site banks were found to be an important factor in predicting use by natterjack toads (Banks and Beebee 1987). The mean bank slope of 7% observed in 2000 is steeper than would be expected given the fact that boreal toads appear unwilling to

deposit eggs deeper than 10 cm. The steep bank slopes of study sites limit egg deposition areas to shallows that appear fairly limited in Rocky Mountain wetlands.

Mean length of shoreline in breeding sites with ≤ 10 -cm deep water was 52.1 m. Size and suitability of ovipositioning areas may be more attractive to amphibians than actual breeding site area (Reading et al. 1991). Loman (1988) found that common frog (*R. temporaria*) breeding sites were best predicted by the amount of shallows. The communal breeding behavior of boreal toads does not require large breeding sites or large egg deposition areas. Even though breeding sites were larger than adjacent nonbreeding sites, my observations suggest that oviposition site selection is linked more closely to the presence of scarce shallows.

Based on 2 summers of data, it is speculated that the presence of very shallow water is the cue that breeding adults use when selecting ovipositioning areas. For a wetland to be considered suitable it should contain at least 1 gradually sloping bank with water ≤ 10 -cm deep during the breeding season. Potential sites should also be examined in August to ensure that breeding site persistence is sufficient to allow completion of the larval period. In addition, a deeper area of water may be necessary to provide tadpoles with a night refuge of warmer water (Beiswenger 1977, Bradford 1984). An old, but active, American beaver pond complex seems an ideal model for a breeding locality because shallow, eutrophicated ponds exist in concert with water level maintenance by beaver.

The boreal toad breeding habitat characteristics quantified in this study will be useful in selecting translocation sites and when creating suitable wetlands. Evaluating how novel translocation sites and human excavated wetlands with different shoreline characteristics are

used by breeding adults will provide additional information on how boreal toads balance the tradeoffs of desiccation and embryonic growth and development when depositing eggs.

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Table 2.1. Means and 95% CI (in parentheses) for characteristics of Colorado breeding and adjacent nonbreeding sites in 1999. Water temperatures are adjusted to 30-cm depth based on the slope of temperature as a function of depth to allow comparison of sites where temperature loggers were at different depths.

Characteristic	n	Breeding Sites	n	Nonbreeding Site	p value
Mean July water temperature (°C)	9	13.4 (11.6, 15.1)	7	11.0 (8.5, 13.5)	0.10
Daily July water temperature variance (°C)	9	8.5 (3.2, 13.8)	7	5.6 (-2.8, 13.9)	0.43
Change in water level (cm/day)	9	-0.13 (-0.26, 0.01)	9	-0.08 (-0.12, -0.04)	0.48
Bank slope (cm)	9	0.22 (0.09, 0.35)	9	0.15 (0.08, 0.22)	0.24
Breeding site surface area (m ²)	10	3411 (-533, 7356)	10	2189 (-681, 5060)	0.05

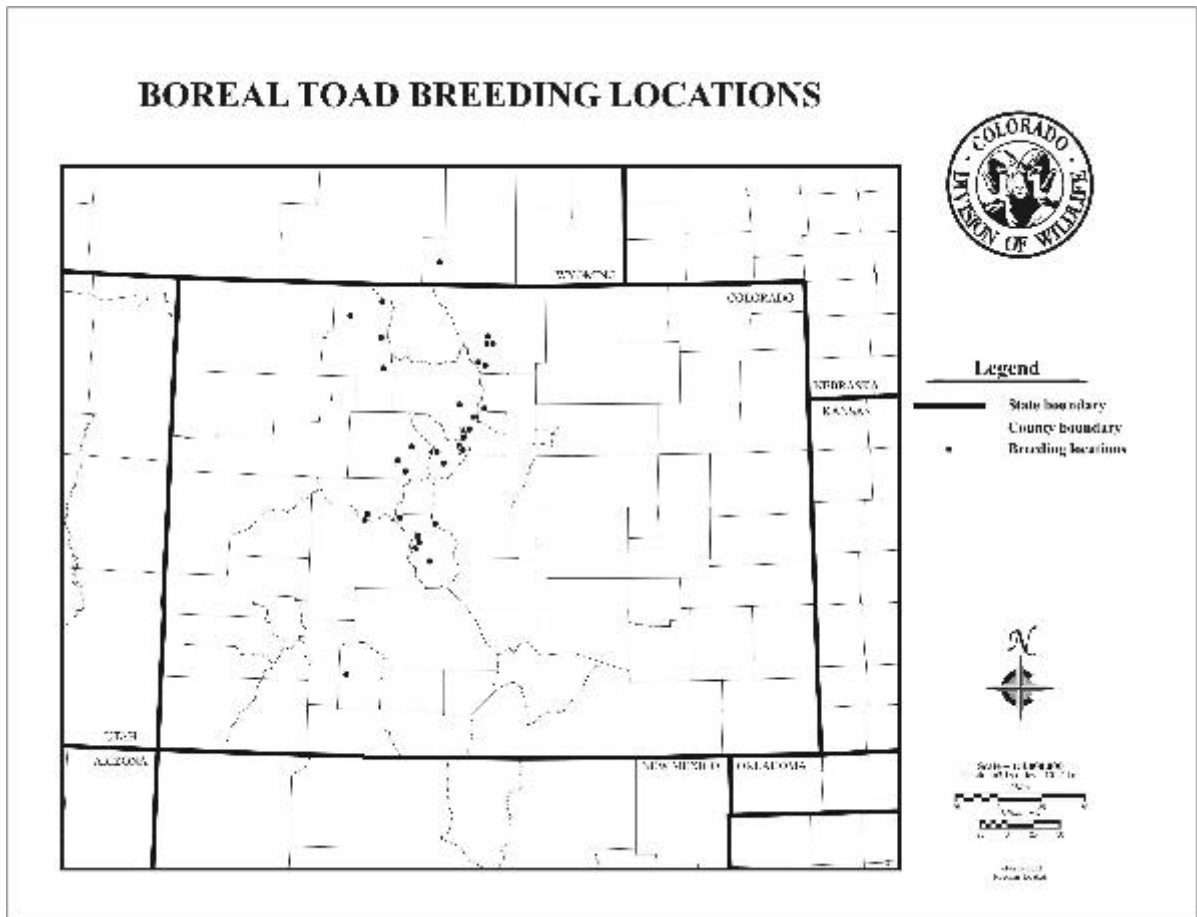


Figure 2.1. Known breeding localities for the Southern Rocky Mountain population of boreal toad, *Bufo boreas boreas*, (from Loeffler 1998).

CHAPTER 3

EVALUATING BOREAL TOAD (*BUFO BOREAS*) BREEDING SITE QUALITY BY MODELING FACTORS THAT INFLUENCE LARVAL PERFORMANCE

Abstract. The distribution and abundance of the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) has declined to the point that reintroduction is probably necessary for recovery. This population relies on the shallow margins of lakes and ponds for breeding. In 2000, 18 of 33 active breeding sites in Colorado were studied to develop methods to identify suitable breeding sites for boreal toad translocation by modeling indicators of larval habitat quality. Mass at metamorphosis, as a function of length of larval period, and larval growth rates were used as indicators of breeding site quality. Model selection and strength of evidence for parameter estimates in selected models were used to identify factors that influence larval growth rates in the wild. Mixed effect and general linear models provided evidence that water temperature, daily variation in water temperature, and breeding site persistence affect larval growth rates. Tadpoles experienced the most gain, in mg per day of development, in breeding sites that had the warmest and least variable water temperatures. Persistent water levels also positively influenced growth rate. Improved understanding of breeding habitat relationships can be used to select translocation sites, determine suitability of wetlands, and aid in development of wetlands for mitigation.

Many amphibian larvae show plasticity in the timing of and size at metamorphosis. This plasticity is the result of physiological responses to larval conditions. Larval performance is often evaluated using growth rates, length of larval period, and mass at metamorphosis. Travis (1983: 496) summarized the relationship between these responses when he stated that “growth rate, length of larval period, and size at metamorphosis are phenotypically variable, often intercorrelated, and play critical roles in determining larval and juvenile survival.”

Larval growth rate influences larval development rate and the timing and size at metamorphosis (Wilbur and Collins 1973, Alford and Harris 1988, Hensley 1993) and growth rate has been correlated with metamorphic success (Banks and Beebee 1988). The Wilbur and Collins (1973) model predicts that larvae have a minimum size that must be achieved before metamorphosis can be initiated and a maximum larval size at which metamorphosis is obligatory. Between these sizes larval growth rates influence the timing and size at which metamorphosis will actually occur. Growth rate is influenced by the biotic and abiotic conditions of the breeding site. If larval conditions are poor or deteriorating, then many amphibian larvae have the ability to metamorphose quickly, but at a smaller size, chancing that the terrestrial environment will be more favorable.

Mass at metamorphosis is positively correlated with juvenile survival (Berven 1990, Morey and Reznick 2001). Larger metamorphs are better foragers and able to consume larger prey (Newman 1999), are faster (Newman 1999, Beck and Congdon 2000), have more endurance (Beck and Congdon 2000), mature more quickly (Smith 1987), and are ultimately larger as adults (Smith 1987, Berven 1990). Metamorphic size can be indicative of larval

conditions and breeding site suitability. Ideal breeding sites produce larvae that metamorphose at a large size while minimizing larval period length.

Factors including water temperature, larval density, food availability, breeding site persistence, and predation affect larval growth, development, and size at metamorphosis. Rate of anuran egg development (Herreid and Kinney 1967), tadpole growth rates (Herreid and Kinney 1967, Beebee 1983, Buchholz and Hayes 2000) and successful metamorphosis (Wilbur 1987) are directly related to water temperature. Typically, as water temperatures increase larval periods are shortened (Marian and Pandian 1985, Newman 1998, Beck and Congdon 2000, Buchholz and Hayes 2000) and mass at metamorphosis decreases (Marian and Pandian 1985, Newman 1998, Beck and Congdon 2000).

Larval density, through intraspecific competition, may influence growth rate, which can then determine the time to obtain the minimum size required for metamorphosis (Wilbur and Collins 1973). For example, higher densities result in longer larval periods (Wilbur 1987, Newman 1998) and lower mass at metamorphosis (Travis 1984, Wilbur 1987, Goater 1994, Newman 1998, Buchholz and Hayes 2000, Morey and Reznick 2001).

As food availability decreases so do larval growth rates (Pandian and Marian 1985, Buchholz and Hayes 2000, Morey and Reznick 2000) and mass at metamorphosis (Travis 1984, Alford and Harris 1988, Newman 1998, Morey and Reznick 2000). Different anuran species are affected differently by changes in food quantity and quality (Steinwascher and Travis 1983). Anuran larvae are suspension feeders of phytoplankton and detritus and growth of algae

is related to light intensity, temperature, organic nutrients, and inorganic nutrients (Wetzel 1975).

Breeding site persistence is critical for both successful hatching (Livo 1999) and metamorphosis. Larvae respond to breeding site dessication by reducing length of larval period and size at metamorphosis (Wilbur 1987, Denver et al. 1998). The influence of breeding site drying on metamorphic plasticity is difficult to demonstrate because as water levels recede larval density, water temperature, and variation in water temperature all increase (Leips et al. 2000). Breeding site duration is not always beneficial to amphibians. Most amphibians balance tradeoffs between predation and breeding site duration when selecting breeding sites. At one extreme, aquatic predators are small and scarce, but breeding site drying is likely to occur before larval development is complete. At the opposite extreme, breeding sites are more persistent, but predators are more numerous and larger (Skelly 1996).

Despite being once considered common, the distribution and abundance of the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) have declined (Corn et al. 1989, Carey 1993, Loeffler 1998). It is currently listed as endangered in the state of Colorado and is regarded as warranted but precluded for Federal listing under the Endangered Species Act (Loeffler 1998). As a terrestrial adult, this species appears to be a generalist. Distribution of boreal toads is restricted to areas with suitable breeding habitat between the elevations of 2,250 m and 3,600 m (Loeffler 1998) with most breeding sites located in lodgepole pine (*Pinus contorta*), spruce (*Picea* spp.), subalpine fir (*Abies lasiocarpa*), and alpine meadows. One breeding locality may consist of several breeding sites which are bodies of water in which

breeding occurs (Loeffler 1998). Boreal toads typically breed in May and June along the margins of ponds and lakes as soon as ice melts. Ideal boreal toad breeding sites presumably contain still water, very shallow margins, and stable water levels. Egg masses typically are deposited communally in the shallowest available areas of the breeding site and tadpoles typically begin metamorphose in August and can vary greatly in size (Livo 1999). Because of the high elevations that they occupy, boreal toad metamorphs have a much better chance of attaining food and surviving if they metamorphose in early August than September.

This project was developed to address issues outlined in sections 1.1, 4.1, and 5.0 of the Boreal Toad Conservation Plan and Agreement (Loeffler 1998) that focus on identification of habitat requirements and research related to experimental translocation. Moving egg masses is probably the most cost-effective method of boreal toad reintroduction and may have the best chance of success (Muths et al. 2001).

The objective of this study was to develop methods to identify characteristics of suitable breeding sites by modeling indicators of larval habitat quality. Mass at metamorphosis, as an absolute growth rate, and linear larval growth rates were modeled as a function of habitat variables. It was hypothesized that water temperature is the primary factor influencing larval growth and development because the high elevations occupied by boreal toads result in colder water temperatures. It was thus predicted that mass at metamorphosis and growth of boreal toad larvae would be positively correlated with water temperature. Variance in water temperature was hypothesized to have a negative affect on growth and mass at metamorphosis based on observations in 1999. It was further hypothesized that mass at metamorphosis and

growth would be positively correlated with breeding site persistence and negatively correlated with density.

METHODS

There are 40 known breeding localities for this population (Figure 3.1) (Loeffler 1998) and in 2000, 33 of these were active (Loeffler 2000). All breeding sites that had at least 1 egg mass for 4 consecutive years, 1997 to 2000, were identified. Thirty-three breeding sites, from 25 breeding localities, met this criterion and all were located in Colorado. Study sites were chosen by randomly selecting 18 of the 33 breeding sites. Fourteen breeding localities were represented because multiple breeding sites were selected from several breeding localities (Figure 3.2).

Study sites were in Chaffee, Clear Creek, Summit, Eagle, Routt, and Gunnison counties of Colorado (see Appendix II). They ranged from 2,542-m to 3,487-m elevation and were primarily in wet meadows within alpine and subalpine communities dominated by spruce-fir forests. Several sites were also associated with lodgepole pine communities. *Salix* spp. and *Carex* spp. were usually found in riparian areas surrounding sites. Study sites consisted of 9 American beaver (*Castor canadensis*) ponds, 1 high elevation lake, 3 high elevation meadow ponds, and 5 human excavated wetlands.

Sites were visited about every third week from breeding until metamorphosis. Water temperature was measured (± 0.2 °C) hourly by placing Onset Computer Corporation Optic Stowaway® temperature loggers (Onset Computer Corporation, Pocasset Massachusetts) near adults in amplexus or next to egg masses during breeding in all sites. Temperature loggers were

later moved near tadpole aggregations during larval development to mimic daytime temperatures experienced by tadpoles because tadpoles select the warmest temperatures (Beiswenger 1977). Depth of temperature loggers was measured at each visit.

Depth and water temperature at one tadpole aggregation was also measured at each site visit. These measurements estimated differences in temperatures recorded by loggers and temperatures actually experienced by tadpoles on a given day and time.

Water temperature is dependent on depth (Pearman 1995, Barandun and Reyer 1997) and tadpoles behaviorally thermoregulate by moving to and from shallows to control body temperature and metabolic rate (Brattstrom 1962, Beiswenger 1977, 1978). Boreal toad tadpoles aggregate in very shallow water on sunny days (Beiswenger 1977, Livo 1999), therefore, water temperatures recorded by temperature loggers were adjusted to approximate temperatures experienced by tadpoles. Adjustment was also necessary to allow water temperature comparisons among sites where loggers were at different depths. Measured temperatures were adjusted up to approximate temperatures in shallows on sunny days and to approximate warmer, deep water on cool days and at night. Breeding sites were visually stratified by size (small, medium, large) to reduce effects of breeding site size on temperature (Pearman 1995) resulting in breeding sites $\leq 350 \text{ m}^2$ being classified as small and breeding sites $\geq 5,000 \text{ m}^2$ classified as large. Three breeding sites from each stratum were randomly selected to receive 3 temperature loggers at increasing depths up to 30 cm. Remaining loggers were placed opportunistically, resulting in 4 sites with 1 temperature logger, 5 sites with 2 temperature loggers, and 9 sites with 3 temperature loggers.

After combining data from breeding to metamorphosis, temperature as a function of depth was regressed for every 1 hour period in each of the 14 sites with multiple loggers. Slope parameters from the 9 randomly selected sites that received 3 loggers were used to adjust the depth of the 4 sites with only 1 logger. Breeding site size was accounted for by using the correct size strata.

Temperatures recorded at the shallowest logger in each site were adjusted based on 3 criteria. If the slope estimate for temperature as a function of depth was negative, i.e., water was warmest in the shallows and the logger was less than 4.5-cm deep (4.5 cm being the average observed daytime depth of measured tadpole aggregations, $n = 35$), then the recorded temperature was used and was not adjusted. If the slope estimate was negative, and the logger was greater than 4.5-cm deep, the temperature was adjusted up as if the logger had been at a depth of 4.5 cm using the regression equation. If the slope estimate was positive (deeper water is warmer) then temperatures were adjusted to 30-cm depth which was approximately the depth of the deepest loggers. Differences between adjusted temperature logger data and temperatures measured at tadpole aggregations for the same date and time were evaluated by regression and a paired t-test (PROC GLM, PROC TTEST, SAS Institute Inc. 2000).

Independent Variables

Independent variables related to adjusted water temperature were calculated at each site from the period of egg deposition to metamorphosis. These included: mean water temperature (MEANTEMP), mean daily variance of water temperature (DAILYVAR), mean daily temperature range (DAILYRANGE), and degree days (DEGREEDAYS). Degree days is a

measure of the aquatic growing season calculated by summing daily mean temperatures above 0°C (Allan 1995). In a few cases temperature information was lost when temperature loggers were moved by American beavers or water levels dropped and exposed the logger. In most cases there were multiple loggers in the breeding site which still provided temperatures. When this occurred in the 4 sites without backup loggers, means of the 3 previous and 3 following days were used to replace missing temperatures. The greatest number of missing days of temperatures used in the analysis was 5 days, which occurred during 1 period in 1 site.

Tadpole density (DENSITY1) was visually estimated as low, medium, or high. Breeding site persistence was estimated from the change in measured temperature logger depths as the absolute change (CHANGE) in water level over the egg and larval period. Water samples were collected at each site visit to measure specific conductance (CONDUCTIVITY) which was used as an index of water chemical composition. Finally, breeding site surface area (AREA) was estimated using a maximum length and 3 width measurements and the equation for the area of an ellipse. An AREA by CHANGE interaction was included in models with AREA because a given drop in water level may affect a small breeding site more than a large one.

Response Variables

Tadpole mass at metamorphosis and growth rate were response variables for evaluating breeding site quality. Mass at metamorphosis was defined as the mass of Gosner (1960) stage 45 metamorphs (Figure 3.3). Only Gosner (1960) stage 45 tadpoles were used in this analysis because this is the final aquatic stage. Once Gosner (1960) stage 46 is reached it is difficult to determine how long metamorphs at that stage. This insured that individuals had not lost or

gained mass after leaving the larval environment. Only the first individuals that metamorphosed from a site were used to estimate mass at metamorphosis and length of larval period. This restriction was necessary because late egg masses were sometimes deposited in sites. This measurement was also easily related to first date of egg deposition which avoided the need to estimate means, such as mean date of egg deposition and mean date of metamorphosis. Metamorph mass was measured by individually weighing randomly selected metamorphs to the nearest 0.01-g wet weight with an electronic balance.

Mass at metamorphosis and length of larval period, defined in this study as the number of days from first egg deposition to the earliest date when metamorphosis was completed, are often correlated (Denver et al. 1998). In this study, mass at metamorphosis increased 3.65 mg (95% CI = -1.29-8.59, $p = 0.13$) per day of development. Therefore the variable, MASSRT, computed as mass of Gosner (1960) stage 45 metamorphs divided by length of larval period, was used to account for mass at metamorphosis and length of larval period. MASSRT is an absolute growth rate, in mg gained per day of development, calculated from mass at metamorphosis. An optimal breeding site produces large metamorphs in a short amount of time and would thus have a large value of MASSRT. Egg size doesn't vary much, mean egg diameter 1.6 mm (SD = 0.07, $n = 13$ clutches) (Livo 1999) so metamorphs have a similar initial size.

Additionally, at each site visit between 30 and 100 tadpoles were randomly collected with an aquarium net. Each tadpole was blotted on a paper towel to remove excess water before mass was measured to the nearest 0.01 g with an electronic balance. Growth rates

(GROWTHRATE) for the population of tadpoles within each site were estimated from the slope of the linear regression of larval mass as a function of days of development. Growth rate estimates apply to tadpole populations within each site because tadpoles were not individually marked and measured on subsequent occasions. Analysis was restricted to Gosner (1960) stages 25 to 40 because tadpoles begin active feeding and growth at Gosner (1960) stage 25 (Pandian and Marian 1985) and metamorphosis is initiated at Gosner (1960) stage 40 (Figure 3.4). In this analysis, independent variable measurements were restricted to this same period resulting in slightly different independent variable values for modeling each response variable.

Modeling and Model Selection

A priori candidate models were developed for each response variable based on independent variables. Mean values of independent and response variables for each site were used in each analysis. Absolute growth rate (MASSRT) was modeled as a function of independent variables with mixed effect models (PROC MIXED, SAS Institute Inc. 2000) where site specific independent variables were fixed effects and the influence of each site on MASSRT was a random effect (SITE). Inclusion of a random effect was necessary to account for both within and between site variation in MASSRT. Maximum likelihood methods were used for model selection and restricted maximum likelihood was used to obtain parameter estimates from competing models (Wolfinger 1993, Franklin et al. 2000). Linear growth rates (GROWTHRATE) were modeled with general linear models (Proc GLM, SAS Institute Inc. 2000).

Traditional R^2 values cannot be calculated for mixed models because they have more than one random effect. Percent of total process variation explained by a model was computed using the variance components analysis procedures of Franklin et al. (2000). Total process variation can be expressed as $\hat{\sigma}_{\text{total}}^2 = \hat{\sigma}_{\text{model}}^2 + \hat{\sigma}_{\text{residual}}^2$. The $\hat{\sigma}_{\text{residual}}^2$ is the amount of total variation not explained by fixed effect parameters in a given model. The amount of variation accounted for by fixed effects parameters in the model $\hat{\sigma}_{\text{model}}^2$ can then be estimated as $\hat{\sigma}_{\text{model}}^2 = \hat{\sigma}_{\text{total}}^2 - \hat{\sigma}_{\text{residual}}^2$. Percent of total variation explained by a model can then be estimated as $\hat{\sigma}_{\text{model}}^2 / \hat{\sigma}_{\text{total}}^2$. No evidence of violation of assumptions was found when the random site effect, SITE, predicted values were plotted against studentized residuals using PROC GLM (SAS Institute Inc. 2000).

Best approximating models were selected with likelihood-based methods using the small sample bias adjustment to Akaike's Information Criteria (AICc) (Akaike 1973, Burnham and Anderson 1998). AIC balances model bias and precision by minimizing deviance and number of parameters. Models were ranked by comparing ΔAICc values (Burnham and Anderson 1998) computed as

$$\Delta\text{AICc}_i = \text{AICc}_i - \text{AICc}_{\text{min}}$$

where AICc_{min} is the model with the lowest AICc value and i indicates other competing models. Models with ΔAICc values less than 2 are generally considered to have substantial support based on the data when making inference (Burnham and Anderson 1998). Akaike weights (w_i) for a given model, which are considered as weight of evidence in favor of a given model and are useful for evaluating model selection uncertainty, were calculated (Burnham and

Anderson 1998). The relative likelihood of model i vs. model j was computed as w_i/w_j , and this ratio provides a measure of strength of evidence between alternative models. Model averaging was used, to estimate the weighted average parameter estimates for models with $\leq 2 \Delta AICc$, to account for model selection uncertainty (Burnham and Anderson 1998).

The change in deviance associated with the inclusion of a parameter, i , can be used to further evaluate if that parameter's inclusion is warranted. Deviance here is defined as $-2 \log$ likelihood for $\text{Model}_{\text{saturated}} + -2 \log$ likelihood Model_i . $\text{Model}_{\text{saturated}}$ here is a model which includes a parameter for every site. A decrease in deviance would not be expected from the inclusion of a nuisance parameter.

Observed site influence on MASSRT was investigated by estimating empirical best linear unbiased predictors (EBLUPs) for each site's random site effect. EBLUPs can be used to compare random effects from each site (SAS Institute Inc. 2000). This allows ranking of sites based on achieving large values of MASSRT. EBLUPs were also estimated with and without fixed effect parameters in the model. This method was used to compare how each site's fixed effects influence MASSRT relative to other sites. For example, a site may be ranked relatively high without including fixed effects and low when accounting for fixed effects such as temperature. This would suggest that the site has a suitable temperature regime but has or lacks other factors which negatively influence MASSRT.

A variable for elevation was added post hoc to top competing models for MASSRT and GROWTHRATE. Intuitively elevation could play an important role on larval conditions in these

high elevation breeding sites. However, elevation was not included in a priori models because it did not appear to be an important factor in 1999.

RESULTS

Breeding was initiated between 10 May 2000 and 12 June 2000. Mean number of egg masses deposited per site was 5.4 (se = 1.1, n = 14) and ranged from 1 to 13. Four sites, from 4 breeding localities, were censored because: eggs failed to hatch in 2, the temperature logger was lost in 1, and in 1 site ≥ 5 days of temperature information were lost because human-regulated water levels dropped and rose dramatically. All censored sites, except the missing logger, were of anthropogenic origin. This resulted in the monitoring of 14 breeding sites, at 12 breeding localities. Metamorphosis was achieved at all 14 sites and was initiated between 21 July 2000 and 29 August 2000. MASSRT was only estimated for 13 sites, at 12 localities, because Gosner (1960) stage 45 metamorphs were not found at 1 site. GROWTHRATE was only estimated for 12 sites, at 10 breeding localities, because tadpoles were not measured below Gosner (1960) stage 30 at 2 sites resulting in no representation for stages 25-29.

Average mean daily adjusted temperatures were 1.31°C warmer than those temperatures actually recorded by temperature loggers (adjusted mean = 15.28°C, 95% CI = 14.04-16.5, n = 14) versus actual mean of 13.97°C (95% CI = 12.79-15.16, n = 14). Mean temperature at tadpole aggregations was 20.28°C (95% CI = 18.69-21.87, n = 35), adjusted mean water temperature at the same time was 18.47°C (95% CI = 17.09-19.85, n = 35), and unadjusted mean water temperature was 17.61°C (95% CI = 16.08-19.13, n = 35).

Temperatures of thermoregulating tadpoles were fairly well approximated by temperature logger

readings from the shallow temperature loggers ($R^2 = 0.60$ for adjusted temperatures and $R^2 = 0.70$ for unadjusted temperatures). Mean temperatures measured at tadpole aggregations was 1.80°C warmer (95% CI = 0.79-2.8; t-test, $p = 0.0009$, $n = 35$) than adjusted means and 2.67°C warmer (95% CI = 2.11-3.56; t-test, $p = 0.0001$, $n = 35$) than actual means. Adjusted temperatures were not altered to account for this bias, because, in addition to effect sizes being small, there was no evidence for differential bias between sites because all confidence intervals for parameter estimates of site effect on bias overlapped zero.

Modeling MASSRT

Mean length of larval period was 75 days (se = 10.48, $n = 14$) and ranged from 62 to 98 days. A total of 170, Gosner (1960) stage 45, individuals were weighed at 13 sites. Mass was highly variable, ranging from 152 mg to 491 mg with a mean of 257 mg (se = 24.99, $n = 13$). Mean MASSRT was 3.45-mg gained per day (se = 0.28, $n = 13$) and ranged from 1.97 to 5.71. Fifteen *a priori* models were evaluated to determine which habitat variables best explained MASSRT. Three competing models had ΔAICc values ≤ 2 (Table 3.1). These models all contained the water temperature variable, MEANTEMP, and contained either DAILYVAR or DAILYRANGE. The third ranked model also contained the variable CHANGE. Based on the Akaike weights, Model {MEANTEMP, DAILYVAR} is 1.26 times as likely as Model {MEANTEMP, DAILYRANGE} and 2.6 times as likely as Model {MEANTEMP, DAILYRANGE, CHANGE}. The 95% confidence interval for MEANTEMP slope parameter does not overlap zero in any of the top 3 models (Table 3.2). With the effects of other variables held constant, for every 1°C increase in MEANTEMP, MASSRT increases

0.249, 0.235, or 0.276 mg per development day respectively for each competing model (Table 3.2).

Using model averaging, the average parameter estimate for MEANTEMP (MEANTEMP_a) based on the top 3 models (Table 3.2) was 0.249 mg per day of development (95% CI (MEANTEMP_a) = 0.042, 0.456; unconditional VAR(MEANTEMP_a) = 0.0088). The unconditional variance is very similar to the conditional variances for MEANTEMP of each model (0.0088, 0.0089, and 0.0076) indicating that there is little uncertainty associated with selecting any one top model for inference with respect to MEANTEMP.

There is also evidence that variation in daily water temperature negatively influences MASSRT. Parameter estimates for DAILYVAR and DAILYRANGE were negative and their associated 95% confidence intervals did not overlap zero in the top 3 models (Table 3.2). There is also weak evidence for an effect due to breeding site persistence. With the inclusion of CHANGE deviance decreases 4.10 from Model {MEANTEMP, DAILYRANGE}, indicating that CHANGE does help explain some variation.

Holding MEANTEMP constant and increasing DAILYVAR 1°C decreases MASSRT 0.069 mg per development day (Table 3.2). DAILYRANGE has a similar influence on MASSRT in both models in which it was included. When holding MEANTEMP constant in Model {MEANTEMP, DAILYVAR} and MEANTEMP and CHANGE constant in Model {MEANTEMP, DAILYRANGE, CHANGE}, MASSRT decreases 0.223 and 0.206, respectively, for every 1°C increase in DAILYRANGE (Table 3.2). Positive effects of high

daily means are only slightly greater than negative effects associated with a highly variable daily temperature regime.

Model {MEANTEMP, DAILYVAR} explained 53.6% of the variation in MASSRT.

Model {MEANTEMP, DAILYRANGE} explained 52.3 % of the variation and the inclusion of CHANGE in the model increased the amount of variation explained to 62.2%.

Modeling GROWTHRATE

Fifteen *a priori* models were examined to evaluate the possible influences on tadpole growth rates (Table 3.3). Linear growth rate estimates ranged from 9.44- to 32.83-mg gained per day with a mean of 16.58 (95% CI = 11.35-21.8, n = 12). Only Model {MEANTEMP, CHANGE} had a $\Delta AICc$ value less than 2.0 and was therefore the best approximating model (Table 3.3). Parameter estimates for this model were MEANTEMP = 4.28 (95% CI = 2.16-6.39) and CHANGE = -0.99 (95% CI = -1.64--0.36) indicating that tadpole growth rate was positively affected by increasing water temperature and negatively affected by dropping water level ($R^2 = 72.8\%$).

Empirical Best Linear Unbiased Predictors

Breeding site suitability can also be ranked using the EBLUPs, or random site effects, for each site (Table 3.4). Factors contributing to a site's suitability can be evaluated, relative to other sites, by including and excluding fixed effects (e.g., MEANTEMP and DAILYVAR) for a given model (Table 3.4). Comparing rankings that include and exclude fixed effects can be useful to help understand why a site ranks where it does. For example, without fixed effects included the Donut site ranks 10th with a EBLUP of -0.59 (Table 3.4), but it ranks last with an

EBLUP of -1.3 with fixed effects, MEANTEMP and DAILYVAR, included. This is a measure of the amount of variation left after the fixed effects are accounted for and shows that Donut site has a favorable temperature regime but lacks other variables that favorably influence MASSRT. Therefore, the growth of tadpoles at Donut site is worse than would be predicted based on only temperature covariates. Conversely, Mount Bethel site ranks last without temperature in the model but its ranking improves with temperature covariates included (Table 3.4).

Post Hoc Analysis of Elevation

Adding elevation as a parameter to the top AIC_c models for MASSRT and GROWTHRATE always resulted in a poorer fitting model, indicating no evidence for an elevational influence on MASSRT or GROWTHRATE in these data. Additionally, the regression of average adjusted water temperature as a function of elevation resulted in an $R^2 = 0.04$ and a parameter estimate of -0.0004 (95% CI = -0.0018-0.0008).

DISCUSSION

These results support the hypotheses that water temperature, daily variation in water temperature, and breeding site persistence affect boreal toad larval growth rate. Tadpoles experienced the most gain in mg per day of development in breeding sites that have the highest, and least variable, water temperatures. Persistent water levels also positively influenced growth rate.

Tadpoles selected water temperatures that were 1.80 °C warmer than adjusted temperatures. This difference is not surprising considering that tadpoles are expected to seek the warmest possible localities (Brattstrom 1962, Beiswenger 1977, 1978) while temperature

loggers are stationary. The importance of water temperature is underscored by the fact that boreal toad tadpoles prefer water temperatures between 28°C and 34°C in laboratory thermal gradients (Beiswenger 1978). This preferred body temperature is much greater than the observed mean of 15.28°C. Even the observed mean daily maximum temperature, 22.05°C was below preferred body temperature and far below lethal temperatures of 37-40°C (Beiswenger 1978). Moreover, Hayes et al. (1993) showed that *B. boreas* tadpoles raised at 27°C grew faster and metamorphosed sooner, but at a smaller size, than tadpoles raised at 22°C. My studies' results suggest that increasing water temperature increases both growth rate and mass at metamorphosis in wild boreal toad larvae. This discrepancy is likely the result of much lower temperatures in the wild than reported by Hayes et al. (1993). Future laboratory experiments conducted with boreal toads tadpoles can gain biological realism by using temperatures and daily variance in temperatures observed in field studies.

For MASSRT models, positive effects of high daily mean temperatures on growth rate are only slightly greater than negative effects associated with a highly variable daily temperature regime. The most suitable sites not only reach high daytime temperatures but do not cool excessively at night. Such sites would not only have less variation in water temperature but also higher means because of less nightly cooling.

The inclusion of a parameter for breeding site persistence (CHANGE) in the models increased the amount of total process variation explained by 10% to 62.2% for MASSRT models and by 37.8% to 72.8% for GROWTHRATE models. There was weak evidence that CHANGE negatively affected growth rate when modeling MASSRT and stronger evidence that

CHANGE affects growth rate when modeling GROWTHRATE. Reduction in growth rate in drying breeding sites is likely the result of energy being allocated to increased development in an attempt to metamorphose more quickly from a site with declining suitability (Wilbur and Collins 1973, Alford and Harris 1988, Hensley 1993).

Evaluating EBLUPSs can help determine the relative influence of fixed effects included and not included in a model. This process helps focus on variables that are favorably or adversely affecting growth rates, allows ranking of study sites, and allows comparison of new sites to study sites provided that metamorphosis occurs at the new site. Unfortunately, this approach can only indicate that additional factors beyond the modeled fixed effects influence growth. Variation not explained by temperature variables and breeding site persistence may be attributed to between site differences such as larval density and water chemical composition that were not included in the models selected for inference. Other factors that could be important include genetic variation, food quality and quantity, and disease. For example, costs associated with insect predator avoidance have been shown to cause boreal toad larvae to grow more slowly and metamorphose smaller (Livo 1999). Obtaining numerical estimates of larval density in breeding sites could improve models but would require considerable effort as breeding sites are typically structurally complex. DENSITY1 may not have been selected as a competing model because boreal toad larval densities are not large enough to induce density dependent growth limitation in many sites as was the case with natterjack toads (Banks and Beebee 1988). Estimating phosphorous levels or periphyton abundance may increase the amount of variation explained by models because food availability and quantity can be used to predict amphibian

growth rates (Pandian and Marian 1985). However, temperature may explain some of the variation in food quantity because algal production is positively correlated with temperature (Wetzel 1975). Finally, increasing the number of sites and years of study would increase understanding of important model parameters. Based on Western Regional Climate Center monitoring, mean temperatures for June-August in 2000 were an average of 2.5°C warmer than the long-term average in Buena Vista, Dillon, and Kremmling, Colorado; three areas near study sites. These warm and dry conditions were favorable for boreal toad metamorphosis with hatching and metamorphosis occurring in 16 of the 18 randomly selected sites. In contrast, tadpoles in only 3 of 10 study sites achieved metamorphosis in 1999; a year when June-August temperatures were 1.9°C cooler than in 2000, based on the same 3 Western Regional Climate Center sites. However, Harig and Fausch (2002) studying translocation sites for cutthroat trout (*Oncorhynchus clarki*), found no evidence of a year effect on water temperatures in high elevation streams in Colorado and New Mexico during 1997, 1998, and 1999.

Management Implications

Methods and models presented here could be used to evaluate suitability of potential translocation sites. The suitability of breeding sites and wetlands that may be impacted by human disturbance could also be evaluated. This would be accomplished by collecting temperature and breeding site persistence data with methods similar to those used in this study.

Potential translocation sites could be evaluated in several ways. Predicted values of MASSRT for a site, that lacks boreal toads, can be estimated by incorporating the environmental covariates measured at that site into the top competing models presented here

(see Tables 3.1-3.3). The new sites' predicted MASSRT and 95% prediction intervals could then be compared to predicted MASSRT values from this study (Table 3.5). Using, PROC MIXED estimate statements included in top models (SAS Institute Inc. 2000), prediction intervals for a new site can be estimated with the standard errors of predicted values using the following equation:

$$\text{Predicted estimate} \pm t_{\alpha/2} \sqrt{[(\text{se}(\text{predicted estimate}))^2 + \hat{\sigma}^2 \text{residual}]}$$

This interval incorporates the random site variation ($\hat{\sigma}^2$ residual) that would be associated with selecting a new site. Prediction interval estimates for a new individual from an existing site presented in Table 3.5 do not incorporate random site effects and were estimated as follows.

$$\text{Estimate} \pm t_{\alpha/2} \text{se}(\text{estimate})$$

This comparison would be especially useful if a known successful site was selected for comparison that was physically similar to the new site to be evaluated.

Another method is to compare predicted values from a new site with observed values from existing sites (Table 3.5). This too would allow ranking of new sites relative to existing sites with known larval performance. If a new site has a predicted value of MASSRT similar to a site that had a high MASSRT in 2000 and is a consistent producer in other years, managers could be fairly confident it is suitable for translocation efforts. Finally, new sites can be evaluated independently. If a prediction interval overlaps zero, the site is less likely to be suitable for growth, development, and metamorphosis. This less sensitive method may only be able to identify extremely poor sites.

As an example, the Hesbo site was censored from analysis because more than 5 days of water temperature information were missing as a result of logger dessication. This allows it to be evaluated as a new site. When Hesbo's temperature covariates for MEANTEMP and DAILYVAR are incorporated into Model {MEANTEMP, DAILYVAR} the resulting predicted value of MASSRT is 2.69 mg (95% prediction interval = 1.00, 4.37). This prediction interval overlaps the observed 95% confidence interval (1.78, 2.46) and fails to overlap zero, indicating that the site has a suitable temperature regime. Incidentally, observed MASSRT for Hesbo in 2000 was 2.12 mg.

The Boreal Toad Recovery Team coordinates the monitoring of all known breeding sites by state and federal wildlife agencies. Temperature loggers could be deployed in all monitored breeding sites and mass at metamorphosis data could be obtained. These efforts would allow a relatively inexpensive way to evaluate and improve these models. To validate these models, it's suggested that "experimental" testing occur by selecting translocation sites based on model predictions that range from less to more suitable.

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Table 3.1. Hypothesized models ranked by AICc, small-sample correction of Akaike's information criterion (Burnham and Anderson 1998), and ΔAICc , difference between a given model and the model with the lowest AICc, for dependent variable MASSRT (absolute growth rate of Colorado boreal toad larvae in mg gained per day of development) as a function of independent variables (see methods) at 13 breeding sites. Competing models are also ranked by AICc Akaike weights (w_i), considered as weight of evidence in favor of a given model, and are included for competing models with $\text{AICc} \leq 2$.

Hypothesized model parameters	K ¹	AICc	ΔAICc	w_i
MEANTEMP DAILYVAR	4	221.05	0	0.461
MEANTEMP DAILYRANGE	4	221.52	0.467	0.365
MEANTEMP DAILYRANGE CHANGE	5	222.98	1.93	0.176
DAILYRANGE	3	223.46	2.41	
DAILYVAR	3	223.65	2.59	
DEGREEDAY DAILYRANGE	4	225.44	4.38	
DEGREEDAY	3	226.15	5.09	
CONDUCTIVITY	3	226.65	5.59	
MEANTEMP	3	226.88	5.82	
CHANGE	3	227.39	6.33	
MEANTEMP CHANGE	4	227.99	6.94	
MEANTEMP CONDUCTIVITY	4	229.23	8.18	
DENSITY1	4	230.98	9.93	
MEANTEMP DENSITY1	5	234.21	13.15	
CHANGE AREA AREA*CHANGE	5	236.28	15.22	

¹ Number of model parameters

Table 3.2. Estimated slope parameters and 95% CI for competing models (see Table 3.1) of MASSRT (absolute growth rate of Colorado boreal toad larvae reported in mg gained per day of development) as a function of independent variables (see methods) at 13 breeding sites. β_1 = intercept and β_2 through β_4 are slope parameter estimates for corresponding model parameters.

Model parameters	Estimated slope parameters (95% CI)
MEANTEMP DAILYVAR	$\beta_1 = 0.951$ (-2.160, 4.062) $\beta_2 = 0.249$ (0.041, 0.459) $\beta_3 = -0.069$ (-0.115, -0.025)
MEANTEMP DAILYRANGE	$\beta_1 = 2.529$ (-0.807, 5.865) $\beta_2 = 0.235$ (0.024, 0.445) $\beta_3 = -0.223$ (-0.373, -0.074)
MEANTEMP DAILYRANGE CHANGE	$\beta_1 = 1.191$ (-2.266, 4.647) $\beta_2 = 0.276$ (0.078, 0.474) $\beta_3 = -0.206$ (-0.344, -0.068) $\beta_4 = -0.082$ (-0.183, 0.018)

Table 3.3. Hypothesized models ranked by AICc, small-sample correction of Akaike's information criterion (Burnham and Anderson 1998), and $\Delta AICc$, difference between a given model and the model with the lowest AICc, for GROWTHRATE (linear growth rate of Colorado boreal toad tadpoles reported in mg gained per day of development) as a function of independent variables (see methods) for boreal toad larvae at 12 breeding sites.

Hypothesized model parameters	K ¹	AICc	$\Delta AICc$
MEANTEMP CHANGE	3	77.00	0
MEANTEMP DAILYRANGE CHANGE	4	80.78	3.77
MEANTEMP	2	83.74	6.74
DAILYRANGE	2	85.05	8.05
MEANTEMP CONDUCTIVITY	3	85.49	8.48
MEANTEMP DAILYRANGE	3	85.73	8.73
CONDUCTIVITY	2	85.78	8.78
MEANTEMP DAILYVAR	3	85.87	8.87
DAILYVAR	2	85.91	8.9
MEANTEMP CHANGE AREA AREA*CHANGE	5	86.95	9.94
MEANTEMP CHANGE DENSITY1	5	87.08	10.07
CHANGE	2	87.74	10.73
DENSITY1	3	90.96	13.95
MEANTEMP DENSITY1	4	91.73	14.73
CHANGE AREA AREA*CHANGE	4	95.31	18.30

¹ Number of model parameters

Table 3.4. Rankings by site of empirical best linear unbiased predictors (see methods) for random site effects of MASSRT (absolute growth rate of Colorado boreal toad larvae reported in mg gained per day of development) for top linear model {MEANTEMP DAILYVAR} (see Table 3.2) including and excluding fixed effects (MEANTEMP DAILYVAR).

Site	Random site effect with fixed effects	Site	Random site effect without fixed effects
S. Cottonwood Creek	0.85	S. Cottonwood Creek	2.23
East Lake Creek	0.72	East Vail	1.13
S. Cottonwood West	0.49	Upper N. Fk of Snake R.	0.74
East Vail	0.43	S. Cottonwood West	0.27
Collegiate Peaks West	0.17	Hartenstein Lake	0.24
Hartenstein Lake	0.14	Hartenstein Lk outlet	0.02
Upper N. Fk. of Snake R.	0.07	Morrison Creek	-0.21
Mount Bethel	0.02	Collegiate Peaks West	-0.22
Hartenstein Lake outlet	-0.21	East Lake Creek	-0.26
Morrison Creek	-0.32	Donut	-0.59
Lower North Tenmile Cr.	-0.47	Lower North Tenmile Cr.	-0.93
Triangle Pass	-0.59	Triangle Pass	-0.97
Donut	-1.30	Mt. Bethel	-1

Table 3.5. Observed versus predicted MASSRT (absolute growth rate of Colorado boreal toad larvae reported in mg gained per day of development) for 13 sites. Predicted MASSRT was estimated from Model 1 {MEANTEMP, DAILYVAR} (see Table 3.2). The number of Gosner (1960) stage 45 Colorado boreal toads metamorphs measured at each site (n) and the observed versus predicted 95% CI (in parentheses) are listed.

Site	n	MASSRT	
		Observed	Predicted
Collegiate Peaks West	1	3.19	2.96 (0.98, 2.92)
Donut	52	2.86 (2.76, 2.97)	4.17 (3.57, 4.78)
East Lake Creek	15	3.19 (2.98, 3.40)	2.46 (1.44, 3.48)
East Vail	1	4.76	4.18 (3.55, 4.81)
Hartenstein Lake outlet	1	3.48	3.76 (3.15, 4.37)
Hartenstein Lake	1	3.73	3.55 (3.09, 3.99)
Lower North Tenmile Creek	9	2.51 (2.35, 2.66)	2.99 (2.40, 3.59)
Morrison Creek	2	3.23 (-11.12, 17.57)	3.59 (2.75, 4.44)
Mount Bethel	17	1.97 (1.87, 2.08)	1.95 (0.98, 2.92)
South Cottonwood West	20	3.72 (3.61, 3.83)	3.22 (2.21, 4.23)
South Cottonwood Creek	16	5.71 (5.36, 6.05)	4.83 (3.93, 5.75)
Triangle Pass	24	2.48 (2.32, 2.64)	3.08 (2.59, 3.57)
Upper North Fork of Snake River	11	4.20 (3.90, 4.50)	4.12 (3.39, 4.86)

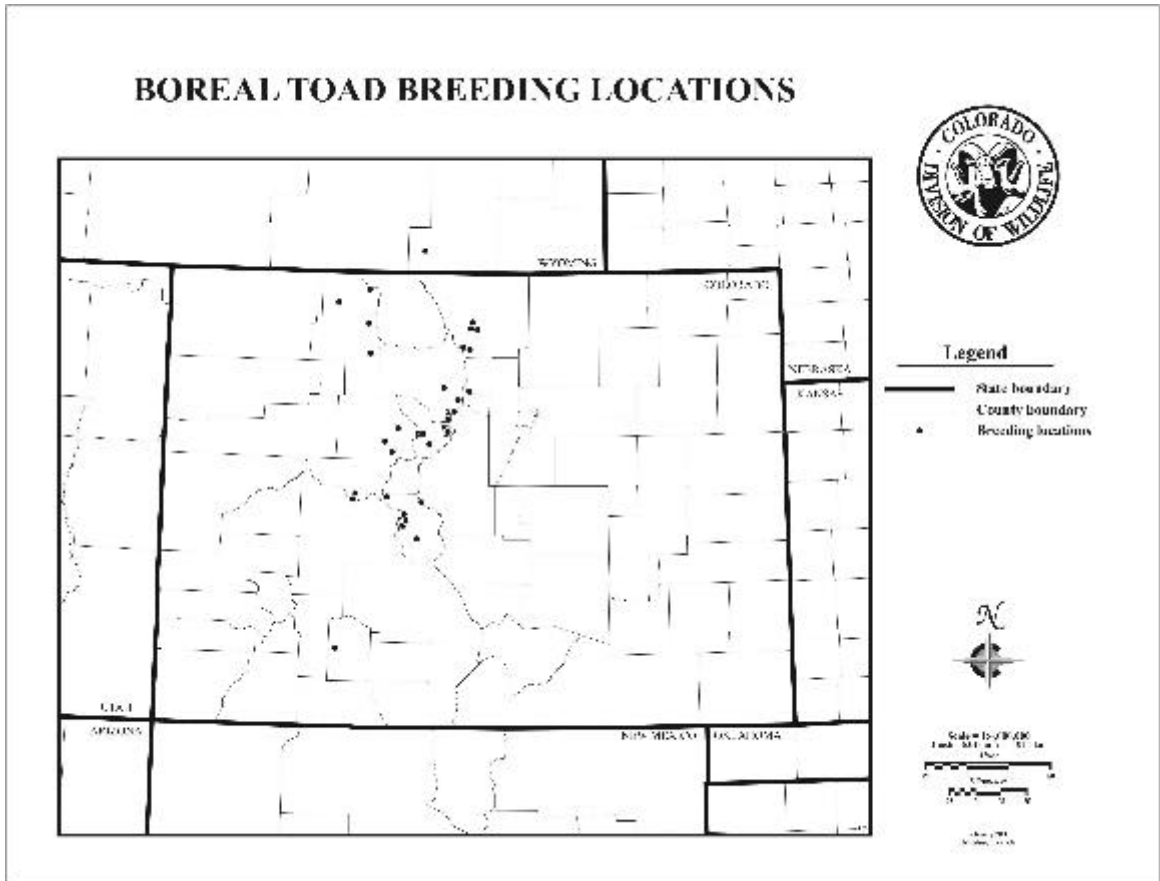


Figure 3.1. Known breeding localities for the Southern Rocky Mountain population of boreal toad, *Bufo boreas boreas*, (from Loeffler 1998).

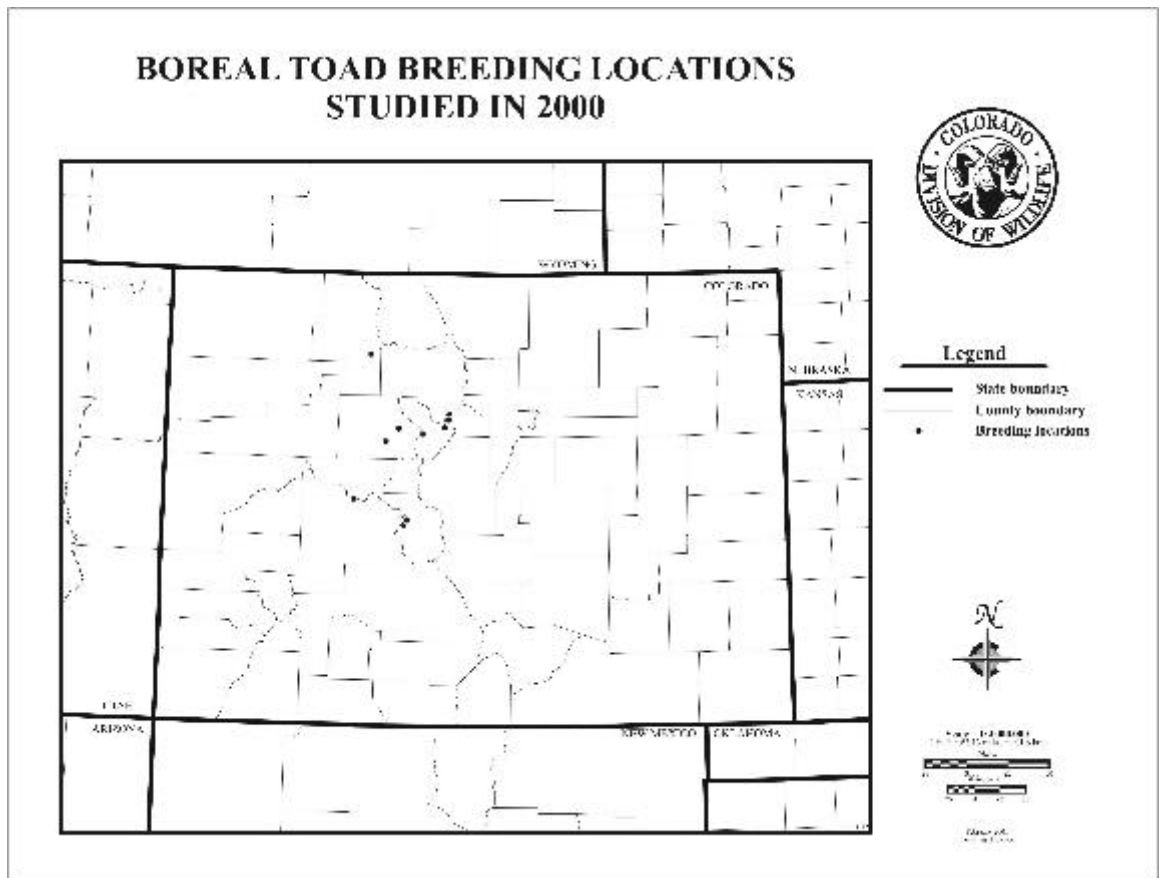


Figure 3.2. Twelve breeding localities for the Southern Rocky Mountain population of boreal toad, *Bufo boreas boreas*, used in the analysis in 2000. Sites were identified by randomly selecting 18 of the 33 consistent breeding sites for the population. Only 12 localities were used because 2 sites from the same locality were selected in two instances and 4 sites, from 4 localities, were censored due to missing data (see methods). See Appendix I for specific site names.

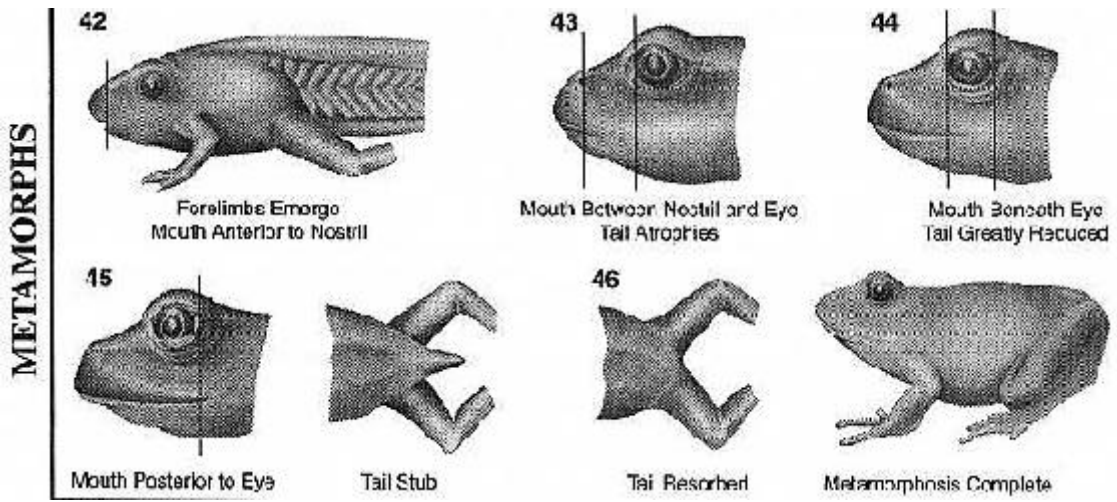


Figure 3.3. Gosner (1960) stages 42-46 (from McDiarmid and Altig 1999). Mass of Gosner (1960) stage 45 metamorphs were measured to estimate individual absolute growth rate in mg gained per day of development. Only Gosner (1960) stage 45 metamorphs were used because larvae are independent of larval environment and little mass has been gained or lost since leaving the larval site.

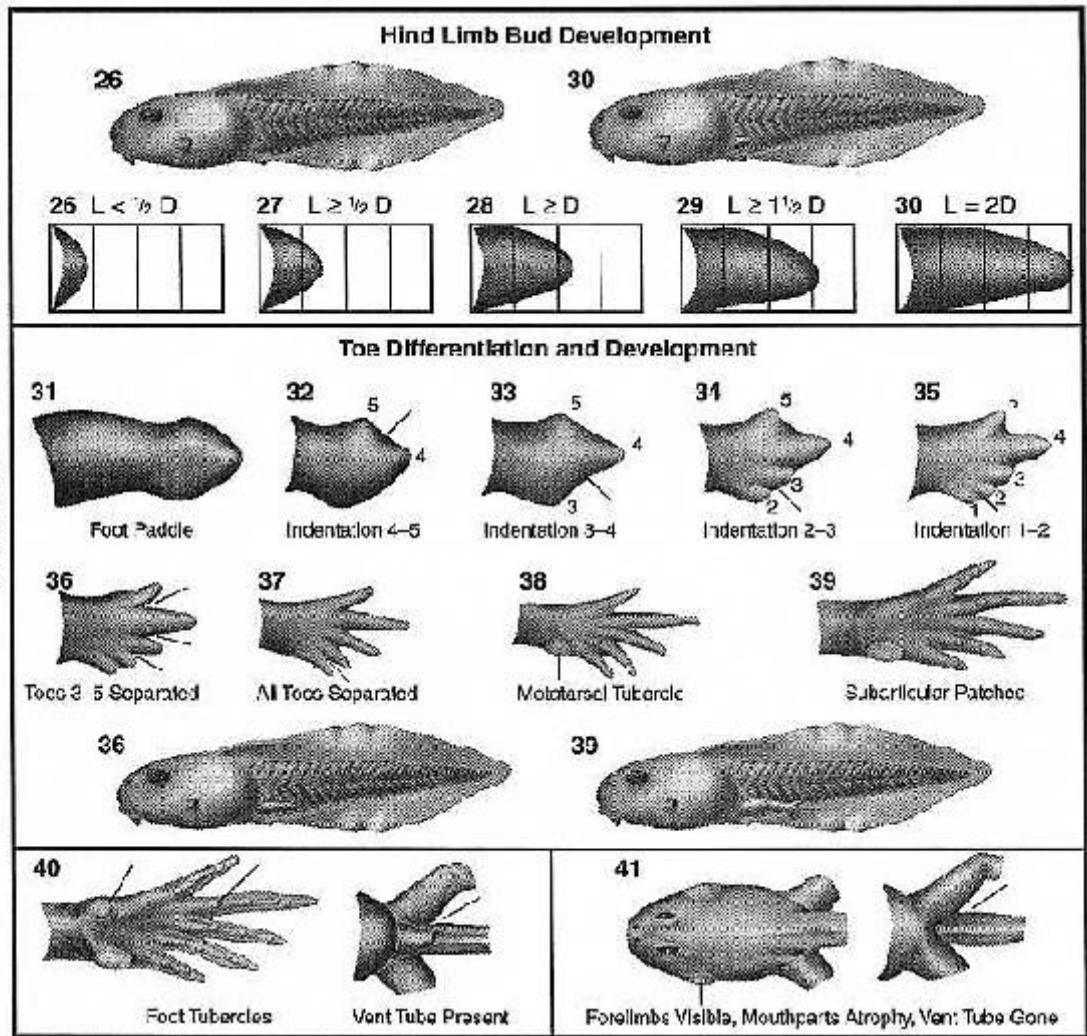


Figure 3.4. Gosner (1960) stages 26-41 (from McDiarmid and Altig 1999). These stages were used to estimate larval growth rates for each site with linear regression. Active feeding and growth begins at Gosner (1960) stage 25 and ends at approximately Gosner (1960) stage 40 at which time metamorphosis is initiated.

APPENDIX I

**COMPARING GROWTH RATES OF WILD AND LABORATORY-RAISED
BOREAL TOAD (*BUFO BOREAS*) LARVAE**

Abstract. Individuals from the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) were brought into captivity to preserve evolutionarily significant units and supply individuals for reintroduction efforts. Captive larval growth rates, mass at metamorphosis, and length of larval period were modeled and compared to wild larval performance to provide better understanding of factors that influence wild larval growth. General linear models of absolute and linear larval growth rates corroborated findings in the wild and provided evidence that water temperature and daily variation in water temperature are important factors in larval growth. Tadpoles experienced the most gain, in mg per day of development, in breeding sites and captive tanks that had the warmest and least variable water temperatures. In addition to validating wild growth models, these findings could be used to improve captive tadpole rearing methods.

Amphibian larval growth rates, length of larval period, and mass at metamorphosis are measures of larval performance that can be used to evaluate breeding site quality. Ideal

breeding sites produce larvae that metamorphose early and at a large size, because larger metamorphs are able to consume larger prey (Newman 1999), are faster (Newman 1999, Beck and Congdon 2000), have more endurance (Beck and Congdon 2000), mature more quickly (Smith 1987), and are ultimately larger as adults (Smith 1987, Berven 1990). Further, juvenile survival is positively correlated with mass at metamorphosis (Berven 1990, Morey and Reznick 2001).

Water temperature is directly related to anuran egg development (Herreid and Kinney 1967), tadpole growth (Herreid and Kinney 1967, Beebee 1983, Buchholz and Hayes 2000) and successful metamorphosis (Wilbur 1987). Typically, as water temperatures increase larval periods are shortened (Marian and Pandian 1985, Newman 1998, Beck and Congdon 2000, Buchholz and Hayes 2000) and mass at metamorphosis decreases (Marian and Pandian 1985, Newman 1998, Beck and Congdon 2000).

As density increases, larval periods are longer (Wilbur 1987, Newman 1998) and metamorphosis occurs at a lower mass (Travis 1984, Wilbur 1987, Goater 1994, Newman 1998, Buchholz and Hayes 2000, Morey and Reznick 2001). Density effects are likely the result of food limitation because as food availability decreases so do larval growth rates (Pandian and Marian 1985, Buchholz and Hayes 2000, Morey and Reznick 2000) and mass at metamorphosis (Travis 1984, Alford and Harris 1988, Newman 1998, Morey and Reznick 2000).

The southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) has declined in distribution and abundance, is now listed as endangered in the state of Colorado, and

is regarded as warranted but precluded for Federal listing under the Endangered Species Act (Corn et al. 1989, Carey 1993, Loeffler 1998). Distribution of boreal toads is restricted to areas with suitable breeding habitat between the elevations of 2,250 m and 3,600 m (Loeffler 1998). By definition, a breeding locality may consist of several breeding sites which are simply bodies of water in which breeding occurs (Loeffler 1998).

This project was developed to address issues outlined in sections 1.1, 4.1, and 5.0 of the Boreal Toad Conservation Plan and Agreement (Loeffler 1998) which focus on identification of habitat requirements and research related to experimental translocation. Translocation of boreal toad eggs, larvae, or adults is probably necessary to meet the recovery criteria set by the Boreal Toad Recovery Team (Muths et al. 2001). Moving egg masses is the most cost-effective method of boreal toad reintroduction and may have the best chance of success (Muths et al. 2001). The objective of this study was to compare growth rates of laboratory-raised (captive) larvae with those observed in the wild to improve understanding of factors influencing larval growth. It was hypothesized that water temperature was the primary factor influencing larval growth and development and that growth and mass at metamorphosis were positively correlated with water temperature. Based on observations in 1999, variance in water temperature was hypothesized to have a negative effect on growth and mass at metamorphosis in the laboratory.

METHODS

Breeding sites for the southern Rocky Mountain population of boreal toad that had at least 1 egg mass for 4 consecutive years, 1997 to 2000, were identified. Study sites were chosen by randomly selected 18 breeding sites from the 33 breeding sites that met this criterion.

The Colorado Division of Wildlife (CDOW) brought egg masses from 5 of these 18 sites into captivity to conserve genetic stock from evolutionarily significant units throughout Colorado. Growth rates of these captive stocks were compared to wild growth rates of the 18 random sites. When several egg masses from 1 site were collected and maintained in separate tanks, 1 tank (1 egg mass) was randomly selected for study per site. In a single case, 3 tanks (3 egg masses) from 1 wild site were randomly selected because a technician was available to help monitor larvae. Thus, this study consisted of 7 egg masses from 5 wild sites. Eggs were hatched and tadpoles reared to metamorphosis in several CDOW labs and hatcheries. These included the John Mumma Native Aquatic Species Restoration Facility, CDOW Aquatic Research Laboratory, and CDOW Fish Research Hatchery. Tank size and water levels varied by facility with volumes of 2,903 cm³, 2,188 cm³, and 2,545 cm³, respectively. Density effects on captive growth of tadpoles were controlled by providing food ad libitum and thus reducing competition for food. The John Mumma Native Aquatic Species Restoration Facility fed Missouri Feed® and Spirulina Chips while the other facilities fed frozen Romain lettuce and #0 fish feed.

Independent Variables

Water temperatures were measured hourly (± 0.2 °C) by placing Onset Computer Corporation Optic Stowaway® temperature loggers (Onset Computer Corporation, Pocasset Massachusetts) in hatchery tanks and near adults in amplexus or next to the egg masses during breeding in the wild. In the wild they were later moved near tadpole aggregations during larval development to mimic daytime temperatures experienced by tadpoles because tadpoles select the warmest temperatures (Beiswenger 1977, Livo 1999). Depth of temperature loggers in the

wild were measured at each visit and temperatures were adjusted depending on depth and the relationship between temperature and depth for specific times and sites (Chapter 3).

Multiple water temperature variables were calculated from the period of egg deposition to metamorphosis. These included mean temperature (MEANTEMP), mean daily variance of temperature (DAILYVAR), and mean daily temperature range (DAILYRANGE). Wild tadpole density (DENSITY1) was visually estimated as low, medium, or high and captive tank densities were obtained by counting all tadpoles in each tank (DENSITY2). Breeding site persistence (CHANGE), surface area, and specific conductance were measured in the wild but not in captivity. Similar models were evaluated explaining captive and wild response variables.

Response Variables

Length of larval period was defined as the number of days from first egg deposition to the earliest date when metamorphosis was completed. Mass at metamorphosis is defined as the mass of Gosner (1960) stage 45 metamorphs (Figure A.1). Only Gosner (1960) stage 45 tadpoles were used in this analysis because Gosner (1960) stage 46 is the final stage and once this stage is reached it is difficult to determine how long metamorphs have been there. This insured that individuals had not lost or gained mass after leaving the larval environment. Only the first individuals that metamorphosed were used to estimate mass at metamorphosis and length of larval period. This was necessary because late egg masses were sometimes deposited in sites. This measurement was also easily related to first date of egg deposition which avoided the need to estimate means, such as mean date of egg deposition and mean date of metamorphosis.

Metamorph mass was measured by individually weighing randomly selected metamorphs to the nearest 0.01-g wet weight with an electronic balance.

We used a composite variable (MASSRT) to evaluate absolute growth rate and account for larval period length, because growth rate and length of larval period are often dependent (Travis 1984) and correlated (Denver et al. 1998). In this study, mass at metamorphosis in the wild increased 3.65-mg (95% CI = -1.29-8.59) per day of development. MASSRT was calculated by dividing the mass of Gosner (1960) stage 45 metamorphs by larval period length. MASSRT is an absolute growth rate, in mg gained per day of development, calculated from mass at metamorphosis.

Linear growth rates (GROWTHRATE) for the population of tadpoles within each tank and site were estimated from the slope of the linear regression of larval mass as a function of days of development. Captive tadpoles were measured approximately weekly and wild tadpoles were measured approximately every third week. At each visit between 30 and 100 tadpoles were randomly netted with an aquarium net. Each tadpole was blotted on a paper towel to remove excess water before mass was recorded to the nearest 0.01 g with an electronic balance. Growth rate estimates apply to tadpole populations within each site because tadpoles were not individually marked and measured on subsequent occasions. The analysis was restricted to Gosner (1960) stages 25 to 40 larvae because tadpoles begin active feeding and growth at Gosner (1960) stage 25 (Pandian and Marian 1985) and metamorphosis is initiated at Gosner (1960) stage 40 (Figure A.2). In this analysis, independent variable

measurements were restricted to this same period resulting in slightly different independent variable values for modeling the absolute and linear growth rates.

Modeling and Model Selection

A priori candidate models were developed for each response variable based on independent variables. Mean values of independent and response variables for each site were used in each analysis. MASSRT in the wild was modeled as a function of independent variables with mixed effect models (PROC MIXED, SAS Institute Inc. 2000) where site specific independent variables were fixed effects and the influence of each site on MASSRT was a random effect (SITE). Inclusion of a random effect for wild models was necessary to account for both within and between site variation in MASSRT. MASSRT for captive larvae and GROWTHRATE were modeled with general linear models (Proc GLM, SAS Institute Inc. 2000).

Best approximating models were selected with likelihood-based methods using the small sample bias adjustment to Akaike's Information Criteria (AICc) (Akaike 1973, Burnham and Anderson 1998). AIC balances model bias and precision by minimizing deviance and number of parameters. Models were ranked by comparing $\Delta AICc$ values (Burnham and Anderson 1998) computed as

$$\Delta AICc_i = AICc_i - AICc_{\min}$$

where $AICc_{\min}$ is the model with the lowest AICc value and i indicates competing models.

Models with $\Delta AICc$ values less than 2 are generally considered to have substantial support based on the data when making inference (Burnham and Anderson 1998). For captive data,

plotting studentized residuals versus predicted values revealed increasing variance for the models with MASSRT. Therefore, these MASSRT values were natural log transformed (LnMASSRT) to satisfy model assumptions.

RESULTS

Metamorphosis was achieved at 14 of the 18 sites; the 4 sites without metamorphosis were censored because the eggs didn't hatch or data was missing (see Chapter 3). Average mean daily water temperatures from egg deposition to start of metamorphosis were 17.91°C (95% CI = 15.80-20.01, n = 7) and 15.28°C (95% CI = 14.04-16.5, n = 14) for captive tanks and wild sites, respectively.

Modeling MASSRT

MASSRT from 7 tanks, representing 5 sites, were compared to 13 wild breeding sites because Gosner (1960) stage 45 metamorphs were not measured at 1 site. Mean length of larval period was longer and more variable in captivity at 92 days (95% CI = 54-131, n = 7) versus 74 days (95% CI = 68-81, n = 14) in the wild. Number of days to achieve metamorphosis in captivity ranged from 51 to 139. Mean mass at metamorphosis for captively reared tadpoles, 528 mg (95% CI = 455-602, n = 7) was more than twice the wild mean of 258 mg (95% CI = 203-312, n = 13). Mean captive MASSRT was 7.1 mg per day (95% CI = 3.6-10.5, n = 7), versus 3.5 mg per day (95% CI = 2.8-4.1, n = 13) in the wild.

Seven a priori models were evaluated to see which independent variables best explained LnMASSRT for captive toads. Model {MEANTEMP, DAILYRANGE} and model {MEANTEMP, DAILYVAR} had $\Delta\text{AICc} \leq 2.0$ (Table A.1), suggesting that MEANTEMP,

DAILYRANGE, and DAILYVAR best explained the variation in LnMASSRT . Parameter estimates for MEANTEMP were 0.196 and 0.205 for the top models (Table 4.2). Parameter estimates were -0.096 for DAILYRANGE and -0.093 for DAILYVAR. Model {MEANTEMP, DAILYRANGE} and model {MEANTEMP, DAILYVAR} both resulted in R^2 values of 0.78 (Table A.2) and in all cases, confidence intervals did not overlap zero. Although the model containing only MEANTEMP was not selected as a competing model by $\Delta AICc$ (Table A.1), it is interesting to note that this variable alone resulted in an R^2 of 0.69.

Parameter estimates in model {MEANTEMP, DAILYRANGE} were back transformed to allow easier comparison with parameter estimates from the same model of MASSRT in the wild. Observed means for independent variables were used. For captive toads, with DAILYRANGE held constant, captive MASSRT increases 1.03 mg per day for every 1°C increase in MEANTEMP. Holding MEANTEMP constant, MASSRT decreased 0.53 mg per day for every 1°C increase in DAILYRANGE. With the same model, wild MASSRT increased 0.24 mg per day for every 1°C increase in MEANTEMP and decreased 0.22 mg per day for every 1°C increase in DAILYRANGE (Chapter 3).

Modeling GROWTHRATE

Two captive tanks (from 2 wild sites) and 1 wild site were censored from GROWTHRATE analysis because of missing data. Thus, only 5 tanks, from 3 sites, were compared to 12 wild sites. Laboratory conditions resulted in a average linear growth rate of 14.7 mg per day (95% CI = -1.4-30.8, n = 5) compared to 16.6 mg gained per day (95% CI = 11.4-21.8, n = 12) in the wild.

Eight models were evaluated to look at the influence of laboratory independent variables on GROWTHRATE (Table 4.3). Only model {MEANTEMP DAILYVAR} had a ΔAIC_c value less than 2.0 (Table 4.3). Parameter estimates for MEANTEMP and DAILYVAR were 5.74 (95% CI = 4.93-6.55) and -5.27 (95% CI = -6.45 - -4.09) respectively. This model explained 99.8% of the variation in larval growth rate observed in the lab environment. The parameter estimate for MEANTEMP was similar to the 4.28 mg per day (95% CI = 2.16-6.39) observed in the wild model {MEANTEMP, CHANGE}.

DISCUSSION

Raising larvae under laboratory conditions allowed the estimation of growth rates with respect to known temperature and density while assuming unlimited food availability. Although an experiment would be required to show cause and effect, these data and selected models support the hypotheses that mean water temperature and daily variation in water temperature influence boreal toad larval growth rates. These findings corroborated observations at wild breeding sites where captive stocks arose (Chapter 3). Models {MEANTEMP, DAILYRANGE} and {MEANTEMP, DAILYVAR} were the best approximating models for MASSRT in both laboratory and wild conditions. Moreover, model {MEANTEMP, DAILYVAR} was the best model for explaining GROWTHRATE in the laboratory. LnMASSRT models containing only water temperature variables explained approximately 78% of the variation in LnMASSRT. This was more than the 52.3% to 53.6% explained by the same models in the wild (Chapter 3). Modeling GROWTHRATE provided further evidence for the effects of water temperature and variation in water temperature as model {MEANTEMP,

DAILYVAR} was the best model (Table 4.3) and explained 99.8% of the variation in GROWTHRATE. Even though mean DAILYRANGE was much smaller in the laboratory at 2.21°C than in the wild at 11.97°C, there was still evidence for the influence of DAILYRANGE on LnMASSRT (Table 4.2). Observed laboratory water temperatures were cold relative to other studies but the range of laboratory temperatures (MEANTEMP) did overlap those observed in the wild. The importance of water temperature is underscored by the fact that boreal toad tadpoles prefer water temperatures between 28°C and 34°C in laboratory thermal gradients (Beiswenger 1978). This temperature is much greater than temperatures observed in the laboratory (17.91°C) and the wild (15.28°C). *B. boreas* tadpoles raised at 27°C grew faster and metamorphosed sooner, but at a smaller size, than tadpoles raised at 22°C (Hayes et al. 1993). Although, laboratory and wild temperatures were substantially lower in this study, the relationship between growth rate and water temperature was the same. The direct relationship between water temperature and mass at metamorphosis was consistent with my hypotheses.

Changes in MASSRT were greater in the laboratory than the wild. For example, using model {MEANTEMP, DAILYRANGE}, with DAILYRANGE held constant, MASSRT in the laboratory increased 1.03 mg per day for every 1°C increase in MEANTEMP compared to 0.24 mg per day in the wild. With MEANTEMP held constant, laboratory MASSRT decreased 0.53 mg per day for every 1°C increase in DAILYRANGE compared to a decrease of 0.22 mg per day in the wild. Similarly, growth rates achieved by natterjack toad larvae in the lab were much larger than those observed in the wild for a given temperature (Banks and Beebee 1988). Larger effect sizes, and a greater amount of variation explained, would be

expected in the laboratory because of less variation in laboratory conditions and fewer confounding factors. For example in the wild, additional factors such as food quality (Buchholz and Hayes 2000), food quantity (Hensley 1993, Buchholz and Hayes 2000, Morey and Reznick 2000), costs of predator avoidance (Livo 1999), and sibship (Travis 1983) can influence larval growth rates.

Certainly, there are limitations with using laboratory data. In this study, many limitations are a result of gathering information from a project that was designed for other purposes, i.e., protection of an endangered species. The laboratory tank, water temperature, and food at a facility are confounded with the wild site of origin. Ideally, tadpoles from each wild site would have been randomly assigned to each rearing facility, allowing rearing at a variety of temperatures and foods. Or as many factors should be held constant as possible which could eliminate confounding due to most factors. Increased sample sizes could also improve future studies. Tadpoles in the 3 tanks from the single wild site were reared at the coldest temperatures, grew the slowest, and had the longest larval period. Evidence for the influence of water temperature on growth rate was strengthened by the fact that wild tadpoles from this site performed comparatively well indicating slow growth in the laboratory was not the result of inferior genetics at that site. Finally, density was measured and modeled, and density dependent growth limitation due to food limitation would not be expected because food was unlimited. Although not an option in this study, manipulating density on a fixed or varied food budget could greatly increase understanding of its influence on growth rates (Wilbur 1977).

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Table A.1. Hypothesized models ranked by AICc, small-sample correction of Akaike's information criterion (Burnham and Anderson 1998), and ΔAICc , difference between a given model and the model with the lowest AICc, for dependent variable LnMASSRT (Ln absolute growth rate reported in mg gained per day of development) as a function of independent variables (see methods) for captive stocks of boreal toad (*Bufo boreas*) larvae.

Hypothesized Model	K ¹	AICc	ΔAICc
MEANTEMP DAILYRANGE	3	-3.00	0
MEANTEMP DAILYVAR	3	-1.49	1.51
MEANTEMP	2	9.08	12.08
MEANTEMP DENSITY2	4	37.69	40.69
MEANTEMP DAILYRANGE DENSITY2	5	57.34	60.35
DAILYRANGE	2	109.79	112.80
DENSITY2	3	159.72	162.73

¹ Number of model parameters

Table A.2. Estimated slope parameters and 95% CI for competing models (see Table A.1) of LnMASSRT (Ln(absolute growth rate) reported in mg gained per day of development) as a function of independent variables (see methods) for laboratory raised boreal toad (*Bufo boreas*) larvae. β_1 = intercept and β_2 through β_3 are parameter estimates for corresponding model parameters. Shown are competing models with $\Delta\text{AICc} \leq 2$, ΔAICc is the difference between a given model and the model with the lowest AICc.

Hypothesized Model	K ¹	ΔAICc	Estimated slope parameters (95% CI)	R ²
MEANTEMP DAILYRANGE	3	0	$\beta_1 = -1.478$ (-1.976, -0.979) $\beta_2 = 0.196$ (0.172, 0.219) $\beta_3 = -0.096$ (-0.131, -0.060)	0.78
MEANTEMP DAILYVAR	3	1.51	$\beta_1 = -1.767$ (-2.212, -1.319) $\beta_2 = 0.205$ (0.183, 0.228) $\beta_3 = -0.093$ (-0.129, -0.057)	0.78

¹ Number of model parameters

Table A.3. Hypothesized models ranked by AICc, small-sample correction of Akaike's information criterion (Burnham and Anderson 1998), and ΔAICc , which is the difference between a given model and the model with the lowest AICc, for GROWTHRATE (linear growth rate of Colorado boreal toad tadpoles reported in mg gained per day of development) as a function of independent variables (see methods) for larvae raised in captivity.

Hypothesized Model	K ¹	AICc	ΔAICc
MEANTEMP DAILYVAR	3	37.37	0
MEANTEMP DAILYRANGE	3	42.51	5.14
DENSITY2	2	42.65	5.28
MEANTEMP	2	43.46	6.08
DAILYRANGE	2	46.81	9.44
DAILYVAR	2	48.09	10.72
MEANTEMP DENSITY2	3	61.57	24.20
MEANTEMP DAILYRANGE DENSITY2	4		

¹ Number of model parameters

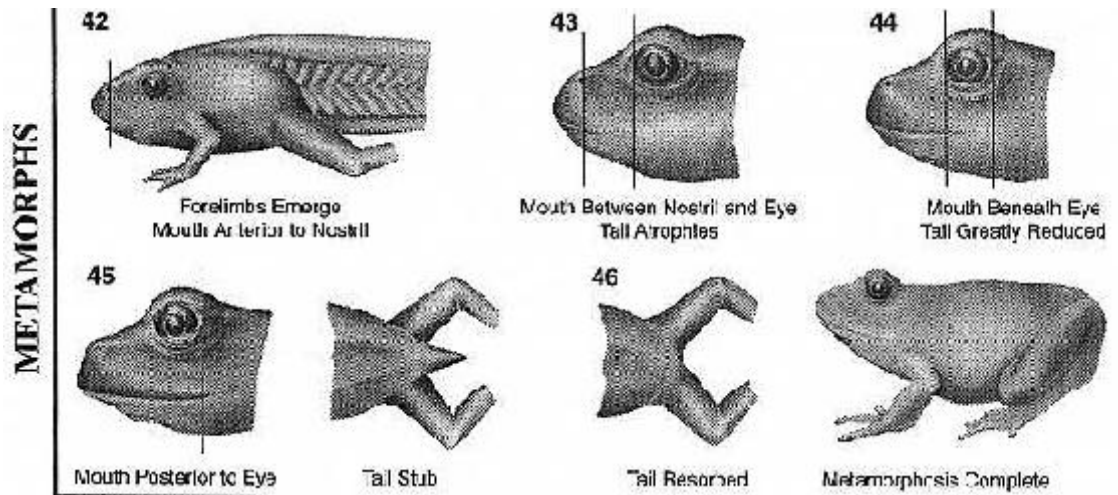


Figure A.1. Gosner (1960) stages 42-46 (from McDiarmid and Altig 1999). Mass of Gosner (1960) stage 45 metamorphs were measured to estimate individual absolute growth rate in mg gained per day of development. Only Gosner (1960) stage 45 metamorphs were used because larvae are independent of larval environment and little mass has been gained or lost since leaving the larval breeding site.

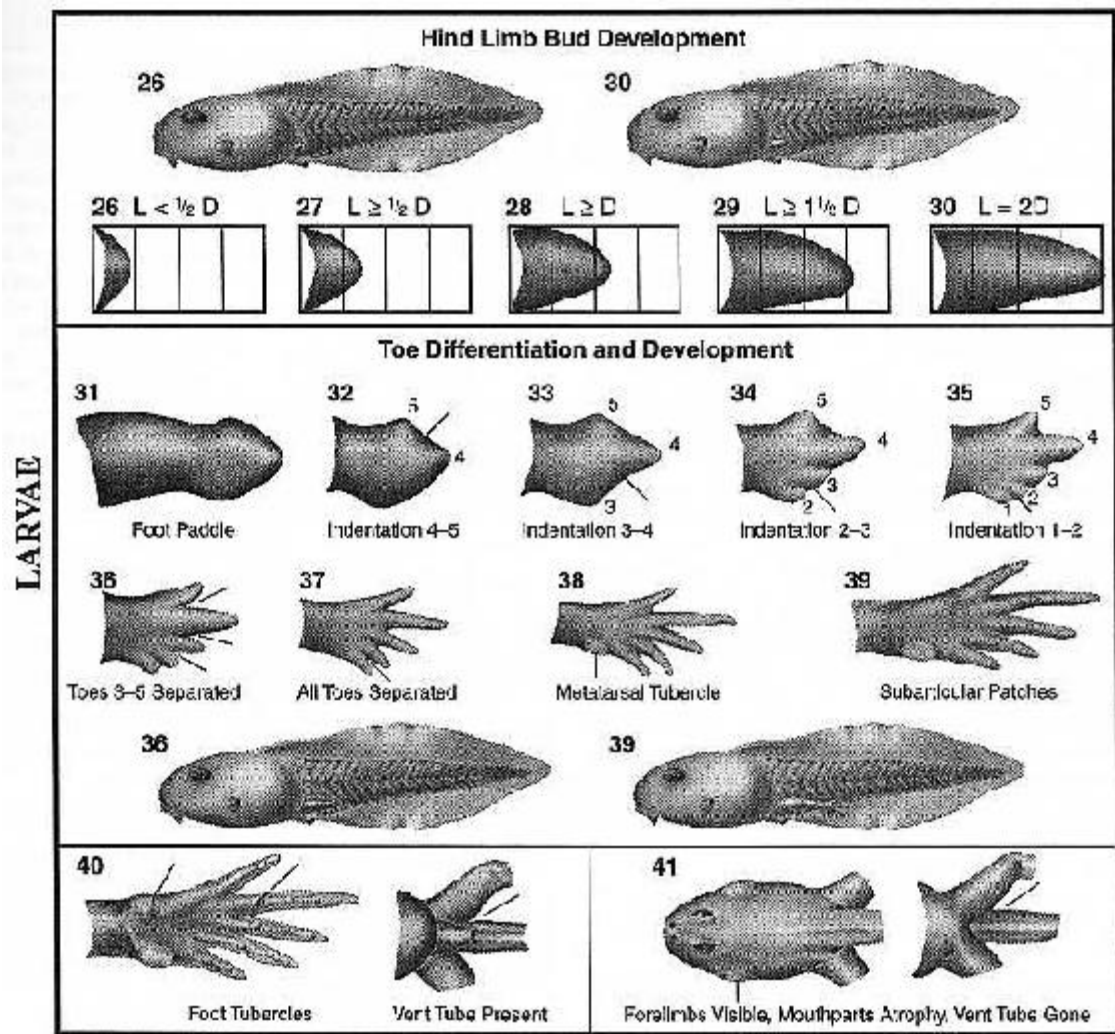


Figure A.2. Gosner (1960) stages 26-41 (from McDiarmid and Altig 1999). Stages 25 to 40 were used to estimate larval growth rates for each site with linear regression. Active feeding and

growth begins at Gosner (1960) stage 25 and ends at approximately Gosner (1960) stage 40 at which time metamorphosis is initiated.

APPENDIX II

STUDY SITES

Breeding sites, and their associated non-breeding sites, studied in 1999 listed by county. Specific breeding site locations can be obtained from the Colorado Division of Wildlife, Fort Collins, CO.

Chaffee County

Four Mile Creek
Hartenstein Lake (outlet)
Morgan's Gulch
South Cottonwood Creek
South Cottonwood Creek West

Clear Creek County

Donut
Upper Urad Reservoir

Larimer County

Lost Lake

Summit County

Lower North Tenmile Creek
Peru Creek

Breeding sites studied in 2000 were randomly selected from all breeding sites for the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) that had at least 1 egg mass for 4 consecutive years (1997 to 2000). Eggs did not hatch in Upper Urad Reservoir and Herman Gulch. Hesbo and Denny Creek were censored because of dessication and missing temperature loggers respectively. Specific breeding site locations can be obtained from the Colorado Division of Wildlife, Fort Collins, CO.

Chaffee County

Collegiate Peaks East
Collegiate Peaks West
Denny Creek (breeding site number 3)
Hartenstein Lake
Hartenstein Lake (beaver breeding site downstream of lake)
South Cottonwood Creek

South Cottonwood Creek West (breeding site 6)

Clear Creek County

Mount Bethel

Donut

Hesbo

Herman Gulch

Upper Urad Reservoir

Eagle County

East Lake Creek

East Vail

Gunnison County

Triangle Pass

Routt County

Morrison Creek

Summit County

Lower North Tenmile Creek

Upper North Fork of the Snake River