Colorado Division of Wildlife

Boreal Toad Research Progress Report

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STATE OF COLORADO

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INTRODUCTION

Boreal toads (*Bufo boreas*) previously were considered common amphibians in the mountains of Colorado (see, for example, Burnett, 1926; Burt, 1933; Burger and Bragg, 1947; Blair, 1951; Campbell, 1970). Colorado's boreal toad populations mysteriously declined beginning in the 1970s (Corn et al., 1989; Carey, 1993). The Boreal Toad Recovery Team was formed in 1994, in response to reports of significant declines in boreal toad distributions in the Southern Rocky Mountains. These apparent declines resulted in an "Endangered" listing by Colorado and New Mexico and a "Status 2" species designation in Wyoming. The boreal toad is currently considered "warranted but precluded" for federal listing under the Endangered Species Act. The first Boreal Toad Recovery Plan was completed in 1994 (revised Jan. 1997) under the direction of John Goettl; the Recovery Plan and Conservation Agreement have now been combined into one working document titled the Conservation Plan and Agreement (Loeffler [ed.] 1998). This document is currently under revision to keep content applicable to ongoing activities and to document progress. The Boreal Toad Recovery Team is coordinated by Chuck Loeffler, the Colorado Division of Wildlife (CDOW) Wildlife Manager for Reptiles, Amphibians, Mollusks, and Crustaceans.

Since 1995, a broad range of research has been completed, by numerous members of the Recovery Team, as outlined in the Conservation Plan and Agreement. Highlights of this research include UV- radiation impacts, statewide genetic analyses, heavy metal toxicology, habitat use and movement, early life history ecology, predators, long term population monitoring, immunology, pathology, and propagation. This research constitutes great progress toward our understanding of boreal toad biology and the circumstances which resulted in population declines. The first section of this progress report summarizes breeding site monitoring activities in the Henderson/Urad valley in 2000, statewide chytrid sampling efforts, and other ongoing activities related to boreal toad recovery efforts. The second section summarizes the boreal toad breeding habitat modeling effort by A. Andrew Holland (Andy), which comprises the bulk of his graduate research. The tools developed through Andy's work will be extremely useful in evaluating future translocation sites and when making recommendations for wetland mitigation in boreal toad habitats.

In the late 1990s, researchers discovered a chytrid fungus (*Batrachochytrium dendrobatidis*) infecting frogs at areas experiencing amphibian population declines in Central America and Australia (Berger et al., 1998; Berger et al., 1999; Longcore et al., 1999). In 1999, a decline in the Henderson/Urad boreal toad population was attributed to this "frog chytrid" (Jones, 2000; Livo, 2000; Milius, 1999). Subsequent pathological work by Dr. Allan Pessier has shown that chytrid fungus was present at this locality as early as 1995. Chytrid fungus has now been identified in boreal toads from at least three populations and evidence exists that this pathogen was in Colorado during the declines in the late 1970's and early 1980's (Carey et al. 1999).

The chytrid life cycle begins with a motile zoospore, which is the infective stage of this pathogen. During the course of infection, chytrid zoospores enter skin cells on the amphibian.

The fungus grows and develops asexually within the skin cells. Eventually, discharge tubes form that extend to the surface of the cells. Mature zoospores emerge from the discharge tube and begin the life cycle again (Longcore et al., 1999). Infections are restricted to the skin of the amphibian. Infected amphibians often slough the skin more frequently than healthy amphibians. Future research must be aimed at mitigating the adverse impacts of this pathogen on the boreal toad, both in the wild and in captivity.

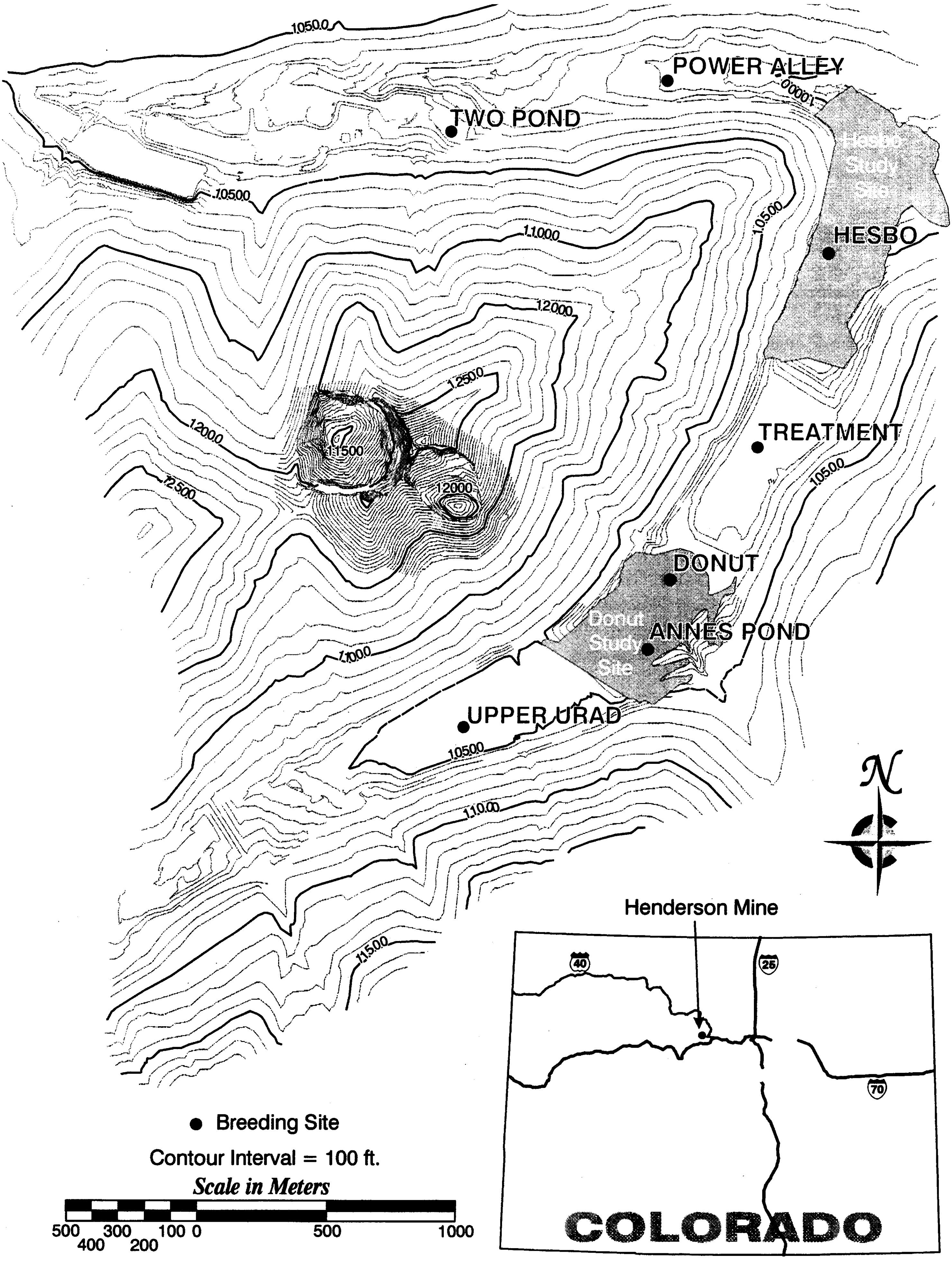
HENDERSON/URAD BOREAL TOAD STUDIES

SITE DESCRIPTION AND BACKGROUND

Research on population size, stability, movement, and habitat use has been conducted at the Henderson/Urad Mine since 1995. The Henderson Mine breeding locality consists of numerous ponds and wetlands in an area which is heavily disturbed due to molybdenum mining by the Climax Molybdenum Company. The mine is located west of Empire, Colorado at an elevational range of 10,000 to 10,500 feet. The specific breeding sites have been designated as follows: Power Alley, Hesbo, 2-Pond, Treatment Pond, Donut, Anne's Pond, and Upper Urad (Figure 1).

Hesbo and 2-Pond were the main breeding locations in 1995 and 1996. Hesbo and Donut were the primary breeding sites from 1997 to 2000. In 1995 and 1996, Hesbo and 2-Pond were influenced by pre-treated mine effluent running through them at an elevated temperature of 19-21°C. Climax finished a new water treatment facility on the Urad side of the facility in 1997. As a result, 2-Pond is no longer an active breeding site and Hesbo has reduced water temperatures in the spring and no long term source of water. As a result of the changes in water supply to Hesbo, we had to pump water to the site once each week from July to September during the 1998, 1999, and 2000 seasons. In an attempt to remedy this situation, the Mine provided a backhoe to install a dam and water control structure and increase the depth of the channel in October 1998. Structural modifications were also made to Anne's Pond in 1998. The improvements seemed to function well at Hesbo in 1999 and 2000 but Anne's Pond still went dry quickly. Even though Hesbo has the largest population of adult toads during breeding, this site did not recruit from 1995 to 1997. From 1998 to 2000, Dyticid beetle larvae were removed which resulted in substantial survival to metamorphosis in these years.

Power Alley is a beaver pond complex along the West Fork of Clear Creek and is the most natural breeding site in the area. It is not directly influenced by mine effluent and therefore the water temperature is colder than the previously mentioned sites and breeding occurs one to two weeks later. This site, however, has dried up during the last four years and desiccated the egg masses present. There were very few breeding adults at the Power Alley breeding site in 2000.


POWER ALLEY
TWO POND
Hesbo Study Site
HESBO
TREATMENT
DONUT
Donut Study Site
ANNES POND
UPPER URAD
N
Henderson Mine
40
25
70
COLORADO
Breeding Site
Contour Interval = 100 ft.
Scale in Meters
500 400 300 200 100 0 500 1000

Treatment is a man-made wetland complex which is dissected by the Urad Mill Road located north of the water treatment facility. Breeding activity is restricted to the pond(s) on the west side of the road. It does not have a large number of adults during breeding but produced 10,000-15,000 toadlets in each year from 1996 to 2000. Recruitment at this site is low as there is minimal overwinter refuge for toadlets.

Donut is a newer pond above the water treatment facility which serves as a catch basin for some of the upstream runoff. This site typically has 5-6 egg masses but because it is higher in elevation than the other sites, breeding occurs later making weather conditions post metamorphosis critical to toadlet survival and dispersal. In addition, there are few suitable hibernaculum close to this site. All toadlets froze in 1995 and 1996. We believe that some toadlets survived in 1997. Survival of toadlets was good in 1998 and 1999, presumably a result of increased vegetation and small mammal burrows on the islands. In 2000, metamorphosis was good at this site but survival is unknown.

Anne's Pond is a small wetland area south of Donut which is fed by ground water and runoff. Because the average depth is less than 10 cm, the water temperature stays warm and tadpoles grow quickly. In 1996 this pond had several thousand tadpoles but dried up in July. At our request, the Henderson Mine personnel put in a water supply pipe to keep the water level constant, which resulted in successful recruitment in 1997 and 1998. In October, 1998 we used a backhoe to increase the main channel depth and added a side channel; these drain to a deep water thermal refuge. All egg masses desiccated in 1999 and 2000 because we were unsuccessful in our attempts to supplement water levels by pumping.

Upper Urad is a large, man-made wetland area at the west end of the valley at an elevation of 10,500 ft. Due to the elevation, this is the last site for breeding activity each year. It produced toadlets in 1995 and 1996 but they froze in 1995 and were eaten by sand pipers in 1996. No successful reproduction occurred at this site from 1997 to 2000.

MATERIALS AND METHODS

Starting in 1996, all breeding sites have been surveyed one time during daylight hours and one time at night each week during the period when toads were actively breeding. Each site was surveyed according to the protocols outlined in the Conservation Plan and Agreement (Loeffler [ed.] 1998). Each toad captured during the night surveys was sexed, weighed (±0.1g), and measured (snout to urostyle length, ± 0.01mm). Each individual was then scanned for a PIT tag and if one was not found, a tag (AVID ITI-125S) was inserted dorsally. The tags were inserted by pinching the skin on the toads back (slightly off center and anterior), making a small incision using sterile scissors, inserting the sterile tag in a posterior direction using forceps, and closing the incision using surgical adhesive. All PIT tag numbers were recorded along with the other pertinent data on individuals and site. All sites were monitored until no new individuals were encountered. All PIT tag numbers from 1995 to 2000 were entered into a database with other pertinent information on each toad. Water quality samples were taken at each breeding site a minimum of three times per year. Once in May, one time while tadpoles were present, and again during metamorphosis.

Capture-recapture methods were used to estimate population numbers of males at each breeding site from 1995 to 2000. Only male boreal toads could be estimated as there was never a recapture of a female in the same year, indicating females breed and immediately leave the breeding site. The computer program Capture (White et al. 1978) was used for the analyses and White et al. 1978 should be referenced for a full description of procedures and model selection.

In January 2000, a contract was initiated with Dr. Seanna Annis and Dr. Joyce Longcore at the University of Maine at Orono to develop a polymerase chain reaction (PCR) test for *Batrachochytrium dendrobatidis*. At this time, it is not possible to sample the environment (water, soil, and so on) for the chytrid fungus. However, because the chytrid fungus infects the skin of an amphibian, it is possible to obtain samples from the amphibians themselves that can be tested for chytrid. The amphibians can be released after collection of the sample. Samples for PCR testing for chytrid fungus were collected from approximately 150 adult toads from 26 boreal toad breeding sites in 2000. Three samples were taken from each individual as decided below.

PCR Sample Collection

Solutions: Buffer solutions were provided by Seanna Annis, based on Seutin et al. (1991). Buffer A was comprised of 0.25M EDTA at pH8 saturated with NaCl. For each sample, 1 ml of Buffer A was placed in a 2 ml screw cap plastic cryogenic tube. Buffer B was comprised of 0.1M Tris, 0.1M NaCl, 0.1M EDTA, 10% lauryl sarcosine at pH7.5. For each sample, 1 ml of Buffer B was placed in a 15 ml screw top plastic test tube.

Sample collection: Boreal toad (*Bufo boreas*) breeding sites were visited during the 2000 field season. Each site was searched for adult and juvenile toads. When a toad was encountered, it was placed in an individual round ZipLoc® container (236 ml size) with a perforated lid. Each toad soaked for two hours in 10 ml distilled water. After two hours, 10 ml of water was decanted into a test tube containing buffer B; if the toad had absorbed water, a few ml of additional distilled water was poured over toad and decanted to bring the level of liquid in the test tube to 11 ml (10 ml water plus 1 ml Buffer B). The purpose of this sample was to obtain any zoospores that may have been released while the toad was soaking.

The pointed end of a wooden stick (2-ml diameter) was scraped along the toad's ventral surface and the webbing of the rear feet. With some toads, this procedure dislodged skin that was in the process of sloughing, while with other toads no detectable material was dislodged. The end of the stick was cut off and placed, pointed end down, in a tube containing Buffer A.

Fine scissors were used to remove a toe from a rear foot (usually the right rear foot); the toe clip was stored in a tube containing Buffer A. The wound was sealed with a drop of 3M®

Vetbond before the toad was released. Both the skin scrape and toe clip were collected to yield samples that might contain chytrid fungus within skin cells.

In addition to collecting samples from boreal toads, a limited number of samples from other amphibian species were collected. The same procedures were used for these animals as for the boreal toad, except for larval tiger salamanders (*Ambystoma tigrinum*). Because these animals are aquatic, they were soaked in 25 ml distilled water, 10 ml of which was decanted into the test tube with Buffer B.

All samples are being held at room temperatures pending completion and validation of the PCR test. To minimize the chance of introducing the chytrid fungus to amphibian populations, we adhered to Declining Amphibian Populations Task Force guidelines regarding disinfecting boots, nets, and other equipment between sites (guidelines are posted at http://www.mpm.edu/collect/vertzo/herp/Daptf/fcode_e.html). Disposable gloves were used and scissors and other sample collection equipment were disinfected before use with each toad.

Henderson Mine Radio Tracking

Nineteen toads (seventeen males and two females) were radio tagged in May and June 1999 at Hesbo, Donut, and Anne's Pond with Holohil BD-2G radio transmitters weighing 2g each, with an expected battery life of six months. The radios were fixed to the toads using a waist harness constructed of plastic coated fishing leader material fastened with crimp collars inside 2mm vinyl tubing. An additional seven toads (four males and three females) were tagged during the summer as replacements for individuals killed by various predators (Jones et al 1999, Jones and Stiles 2000), disease, or which lost their transmitters (Table 1). The primary objective of following radio tagged individuals in 2000 was to monitor mortality associated with chytridiomycosis.

Each radioed toad was located one time per week from May until they went into hibernation or were lost for various reasons. Toad locations were recorded in Universal Transverse Mercator (UTM) coordinates using a Trimble Pathfinder Basic Plus global positioning system (GPS) with an external antenna. Location files were downloaded to a computer, differentially corrected, and imported into ARC/INFO (ESRI 1997) for spatial analysis.

Pathology work in 2000 was performed by Dr. Allan Pessier, Zoo and Wildlife Pathologist with the University of Illinois Zoological Pathology Program. Specimens were either sent live on ice packs, preserved in formalin, or frozen with dry ice depending on their condition and the anticipated tests/procedures to be done.

Table 1. Contact statistics for radio-tagged boreal toads in the Henderson study area in 2000.

Toad Number	Date Tagged	Days Monitored	Sex	Comments
582	5/11/00	42	M	Dead 6/22/00, Path. No. 14, chytrid pos.
576	5/11/00	68	F	Radio quit 7/18/00
773	7/6/00	148	M	Hib. In willows above flume
575	5/15/00	33	M	Radio fell off 6/16/00
602	5/24/00	112	F	Dead 9/13/00, Path. No.21, chytrid pos.
47	5/31/00	20	M	Hawk kill
58	5/24/00	51	F	Lost signal at Berthoud Falls
88	5/24/00	13	M	Dead 6/6/00, Path. No. 5, chytrid pos.
98	5/23/00	141	M	Hib. By concrete flume
99	8/1/00	28	F	Predator kill, radio up tree by Lower Urad Res.
130	5/24/00	55	M	Dead in Lower Urad Res., not recovered
316	7/14/00	89	F	Hib. in 2nd berm below Hesbo
169	5/11/00	43	M	Dead 6/22/00, Path. No. 13, chytrid pos.
481	8/8/00	115	F	Hib. below Lower Urad dam by outlet
523	5/24/00	7	M	Radio fell off
580	5/31/00	29	M	Dead 6/29/00, Path. No.17, chytrid pos.
621	5/24/00	28	M	Dead 6/20/00, Path #12, chytrid pos.
642	5/15/00	150	M	Hib. below Hesbo by spring
711	5/31/00	29	M	Dead 6/29/00, Path. No. 16, chytrid neg.
730	9/13/00	28	M	Hib. below Hesbo
820	5/24/00	119	M	In berm below Hesbo, may be dead
839	5/31/00	14	M	Dead, Path. No. 19, autolysis, not definitive
867	8/1/00	71	M	Hib. top of concrete flume
940	5/31/00	20	M	Radio quit
918	5/11/00	5	M	Dead 5/15/00, Path. No. 1, chytrid neg.
920	7/21/00	82	F	Hib. below Hesbo by spring

RESULTS and DISCUSSION

Breeding Site Monitoring: 2000

Hesbo-Hesbo was monitored at night weekly from May 11 to May 30, 2000. The peak of breeding activity occurred on May 11 with 23 adults observed (16 male, 7 female). Ten egg masses were deposited, resulting in approximately 15,000 tadpoles. Metamorphs were observed.

Power Alley-Power Alley was monitored at night weekly from May 11 to May 23, 2000. Additional daylight surveys were conducted throughout the summer. No adult toads were seen during monitoring. No egg masses were found. There were a few tadpoles in the upper pond and 10 to 12 metamorphs were observed.

Upper Urad-Upper Urad was night monitored weekly from June 6 to June 14, 2000. Additional daylight surveys were conducted throughout the summer. Two adult toads were observed (1 male, 1 female). Five egg masses were deposited at this site, all fungused and died. No successful reproduction at this site in 2000.

Donut-Donut was monitored at night from May 15 to June 6, 2000. Additional daylight surveys were conducted throughout the summer. The peak of breeding activity occurred on May 23 with 11 adults (4 male, 7 female). Twelve egg masses were observed, resulting in approximately 15,000 tadpoles. Approximately 500 metamorphs observed.

Treatment-Treatment was night monitored from May 11 to May 23, 2000. Additional daylight surveys were conducted throughout the summer. Only one male was observed at this site. Two egg masses were deposited and approximately 100 to 200 metamorphs were observed.

Anne's Pond-Anne's Pond was night monitored weekly from May 15 to June 6, 2000. Additional daylight surveys were conducted throughout the summer. Five adult toads (2 male, 3 female) were observed. Three egg masses were deposited, all desiccated. No successful reproduction at this site for 2000.

Other Sites

Hassel Lake- This site is a small lake located just below timberline at the headwaters of the Woods Creek drainage. A survey was conducted on July 21, 2000, one female was observed.

Lower Urad Lake-Lower Urad Lake was surveyed on June 14, 2000. Three male toads and one egg mass were observed. No recruitment at this site in 2000.

Breeding Site Population Estimates

Boreal toads at the Urad/Henderson breeding sites were PIT tagged during 1995 to 2000 breeding site monitoring activities. Monitoring begins in mid-May and continues until no new individuals are found at each site. Males typically persist at the breeding site for several weeks after breeding activity ceases. As stated in methods, the program Capture (White et al. 1982) was used to estimate the number of males at each site for each year monitored. In 2000, the population levels dropped dramatically due to chytrid and we took actual counts at some sites.

Listed below is a brief description of each possible model selection, see White et al. 1982 for complete descriptions.

Model M_o : Population estimation with constant probability of capture.

Model M_h : Population estimation with variable probability of capture by animal.

Model M_b : Population estimation with behavioral response to capture.

Model M_{bh} : Population estimation with behavioral response and heterogeneity.

Model M_t : Population estimation with time specific changes in probability of capture.

Model M_{th} : Population estimate under time variation and individual heterogeneity in capture probabilities.

Model M_{tb} : Population estimation under time variation and behavioral response to capture.

Model M_{tbh} : Population estimate under time variation, behavioral response, and heterogeneity.

Table 2. Population estimates for male boreal toads at the breeding sites in the Urad/Henderson area from 1995 to 2000.

Site	Year	Model	Estimate	SE	95% CI
Hesbo	1995	M_{bh}	141	1.57	141 to 148
Hesbo	1996	M_b	119	4.79	114 to 134
Hesbo	1997	M_t	120	2.52	117 to 127
Hesbo	1998	M_t	120	2.73	117 to 128
Hesbo	1999	M_t	94	3.55	90 to 104
Hesbo	2000	M_t	17	<0.00	17 to 17
2 Pond	1995	M_t	32	0.95	32 to 36
2 Pond	1996	M_o	6	0.91	4 to 8
Power Alley	1996	M_{th}	61	6.72	54 to 82
Power Alley	1997	M_{tb}	80	5.10	80 to 113
Power Alley	1998	M_{tb}	80	0.66	80 to 80
Power Alley	1999	M_t	53	4.22	49 to 66
Power Alley	2000		1[a]		
Upper Urad	1996	M_{tb}	41	0.26	40 to 41
Upper Urad	1997	M_o	34	7.59	27 to 59
Upper Urad	1998	M_h	29	5.27	23 to 44
Upper Urad	1999		9[a]		
Upper Urad	2000		5[a]		
Donut	1997	M_{th}	19	4.32	16 to 37
Donut	1998	M_t	44	6.29	37 to 63
Donut	1999	M_t	15	2.19	14 to 24
Donut	2000	M_o	12	8.42	7 to 51
Anne's Pond	1998	M_b	33	0.44	33 to 33
Anne's Pond	1999	M_t	26	1.79	25 to 33
Anne's Pond	2000		3[a]		

[a] Actual count

In all cases, the estimate derived from the Capture model (Table 2.) was nearly the same as the total number handled at each site indicating we had PIT tagged and handled close to the entire breeding population of males each year at each site. Based on the 1996 estimates, the male breeding population in the Henderson/Urad metapopulation was approximately 227, 233 in 1997, 306 in 1998, 197 in 1999 and approximately 38 in 2000. Assuming a 50:50 sex ratio, the number of breeding age adults in the population was reduced 36% in 1999 and 88% in 2000 (compared with 1998). It is probable based on pathology results that these reductions are primarily due to chytridiomycosis. In 2000, out of nine toads sent to Dr. Allan Pessier for necropsy, six were confirmed positive for chytrid fungus, two were negative, and one was suspect but inconclusive. We will continue to monitor the Henderson population in 2001 to document the outcome of this disease event.

PCR Sampling

Twenty-nine *Bufo boreas* breeding sites were visited at least once and often two or more times during the 2000 field season (Figure 2). Four of these sites did not yield any toads, so are not represented by samples. A total of 150 boreal toads were sampled at the 26 sites that produced one or more toads.

Three of the sample areas are known to be positive for chytrid fungus. That is, researchers have submitted one or more toads for pathological examination and the toads were determined to be infected. These areas are the Woods Creek drainage (Clear Creek County), North Fork drainage in Rocky Mountain National Park (Larimer County), and Conundrum Creek (Pitkin County). We obtained one or more samples from each of these areas. In addition to presence or absence of disease, it also is important to determine the prevalence of disease. Ultimately, effective management of boreal toads will require an understanding of the other factors, both biotic and abiotic, that may affect the fate of a population affected by chytrid fungus.

Sites were also sampled that are not known to be positive for chytrid fungus, but are geographically proximate to positive areas. Results from these sites may provide information on chytrid dispersal. Of particular interest is information on what geographic or landscape features may constitute effective barriers to chytrid dispersal.

In addition to sampling boreal toad sites, 64 samples were collected from seven other amphibian species (Figures 3 and 4). Of these species, the northern leopard frog (*Rana pipiens*) has also experienced population declines that likely are attributable to chytrid fungus infection (Milius, 2000). Samples were collected from this species in two areas on City of Boulder Open Space.

In 1999, Woodhouse's toads (*Bufo woodhousii*) in No Thoroughfare Canyon, Colorado National Monument (Mesa County) experienced a die-off (Livo, pers. obs.). Because of the possibility that chytrid fungus might be involved in this mortality event, it was important to try to collect samples from this species. Conditions were very dry when No Thoroughfare Canyon was visited, and only one amphibian (a canyon treefrog, *Hyla arenicolor*) was observed. However, samples were obtained from juvenile Woodhouse's toads from an adjacent canyon.

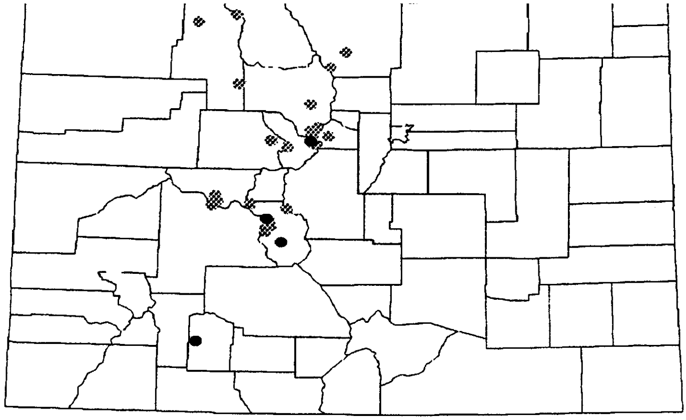


Figure 2. *Bufo boreas* breeding sites visited during 2000 (gray circles: samples collected for PCR test; black circle: site visited but no samples obtained).

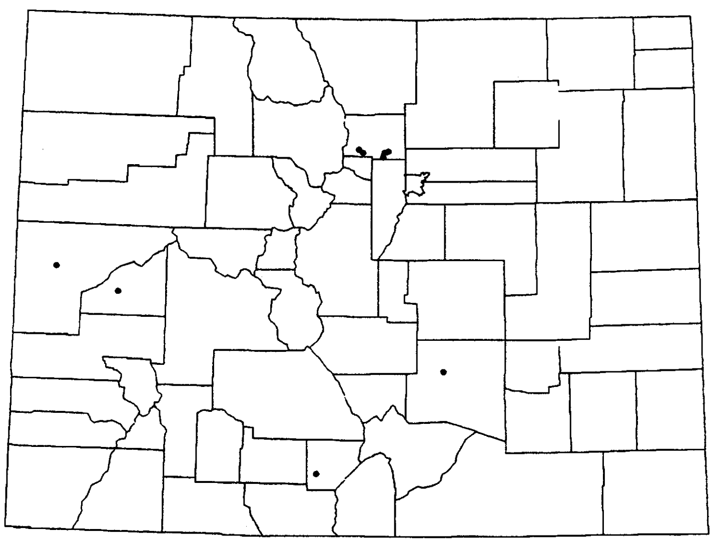


Figure 3. Non-*Bufo boreas* amphibian sites from which samples were collected for the PCR test.

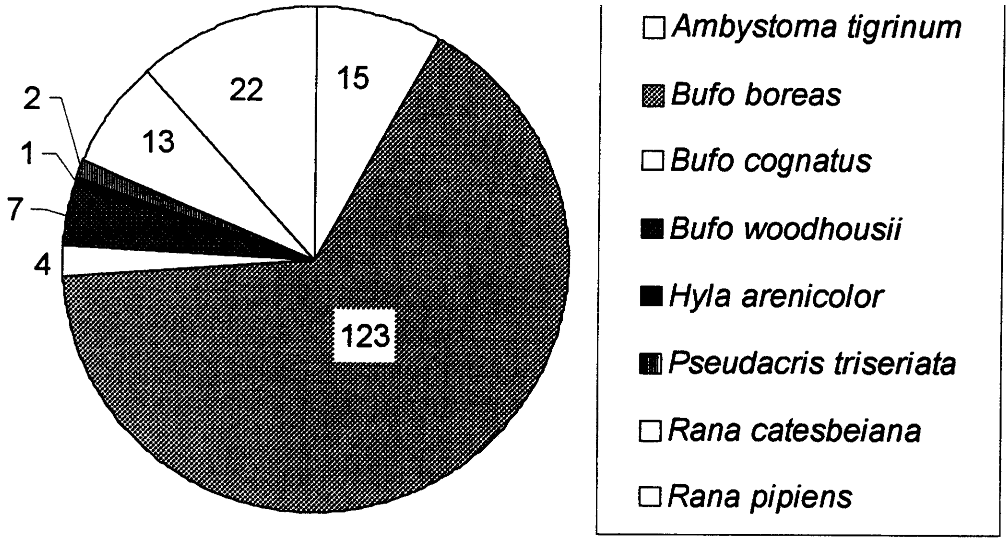


Figure 4. Species from which samples were obtained.

Other amphibian species have not experienced obvious population declines. Whether these species act as reservoirs for chytrid fungus or simply are less susceptible to infection is not known. At low elevations, non-native bullfrogs (*Rana catesbeiana*) have replaced native leopard frogs in many areas. Tiger salamanders (*Ambystoma tigrinum*) and chorus frogs (*Pseudacris triseriata*) occur in the mountains of Colorado, but unlike the boreal toad, have not experienced detectable contractions in geographic distribution. Most of the tiger salamanders were sampled from the Grand Mesa, an area proposed as a potential restoration site for boreal toads.

Limited samples of local amphibians were also collected in the vicinity of the John W. Mumma Native Aquatic Species Restoration Facility in Alamosa. Because this hatchery is the site for rearing of boreal toads, it is important to learn whether chytrid fungus is present in the area.

The sampling efforts described here cannot be analyzed until the completion of the PCR test. However, when complete, the results will provide important information on the distribution of chytrid fungus in Colorado. Future management efforts for the boreal toad will require a better understanding of the chytrid fungus, its ecology, and its effects on amphibian populations.

Technical Assistance and Other Activities

Prepare Publications- publications submitted during this reporting period.

Carey, C., P. S. Corn, M. S. Jones, L. J. Livo, E. Muths, and C. W. Loeffler. In press. Environmental and life history factors that limit recovery in Southern Rocky Mountain populations of boreal toads (*Bufo boreas*). *In:* Status and Conservation of US Amphibians. M. J. Lannoo (ed.).

Dastoor, F. P., P. Daszak, E. Muths, M. S. Jones, J. Longcore, S. Annis. PCR assay to detect Batrachochytrium on amphibians and in the environment. To be presented at the Mycological Society of America Conference, August 2001.

Jones, M. S. and L. J. Livo. Submitted. Improved Waistband Attachment of Radio Transmitters for Toads. Herpetological Review.

Jones, M. S. and L. J. Livo. Submitted. Boreal Toad (Bufo boreas) Overwintering Site Selection in Colorado. Journal of Herpetology.

Livo, L. J. and M. S. Jones. 2000. Amphibian death kits. FrogLog. 39:3-4.

Death Kits

To enhance the chances of collecting at least some evidence of amphibian mortality events, we developed a self-contain "amphibian death kit" intended for use by field personnel associated with the Boreal Toad Recovery Team. The kit contains the necessary materials to make an initial collection of at least a small number of specimens (plastic storage bag for kit, plastic jar sized for specimens likely to be encountered, glass jar with formalin, clear plastic and paper stick-on labels, disposable gloves, whirl paks to freeze samples, pencils, sanitizing towellettes, and plastic laminated instruction cards. The kits were distributed to all agencies associated with amphibian monitoring and the concept was well received. All pathology specimens received in 2000 were properly preserved and labeled using the kits. Appropriate buffer(s) and containers will be included in 2001 for collection of samples for PCR.

Consultations

1. Henderson Mine Candidate Conservation Agreement with Assurances

On June 17, 1999, the U.S. Fish and Wildlife Service (Service) and National Marine Fisheries Service jointly issued a Final Policy for Candidate Conservation Agreements with Assurances (CCAA's or Agreements) under the Endangered Species Act of 1973, as amended (64 FR 32726). The Agreements are intended to provide landowners incentives to promote implementation of voluntary conservation actions for proposed and candidate species, or those species that are likely to become candidates or proposed species. Ultimately, these Agreements will preclude or remove the need to federally list a species should all necessary property owners enter into Agreements.

During this reporting period we have been assisting Henderson Operations to write and implement a CCAA between the Climax Molybdenum Company, Colorado Division of Wildlife and U. S. Fish and Wildlife Service (USFWS). At this point the agreement is still in draft format and still under review by the USFWS. A copy of the draft is included in Appendix B. to serve as a model for other members of the Recovery Team or private landowners interested in this concept to 1) see what a CCAA is, and 2) offer a working structure for future agreements. It should be remembered that this agreement is still in negotiation.

2. Henderson Mine 404 Mitigation

In cooperation with the Army Corps of Engineers and Henderson Operations, modifications were recommended to a proposed 404 mitigation plan to enhance the resultant wetlands for boreal toad breeding. Part of the original plan was discarded as the proposed activities disturbed active breeding sites. Additional features were added to provide more breeding habitat or enhance areas which currently have limiting factors.

3. Colorado's Ocean Journey

We continue to assist Colorado's Ocean Journey (COJ) in maintaining a boreal toad display at their facility. During this reporting period we worked with COJ, CSU Veterinary Teaching Hospital, and Dr. Allan Pessier to determine the causative agent involved in the illness of several of their toads. We are still working to resolve this situation.

4. Powder Horn Ski Area Expansion

During this reporting period, the principal investigator reviewed and commented on plans for future expansion of the ski area. Although no boreal toads are known to still exist on the Grand Mesa, suitable habitats within the project area were identified and recommendations to reduce conflicts with possible future translocations were discussed.

5. Black Hawk Tunnel Project

Plans for a proposed tunnel from I-25 to Hwy 119 near Black Hawk were reviewed for any potential conflicts with known boreal toad breeding locations. Several wetlands in the project area were surveyed and comments forwarded to the consulting firm conducting the study.

6. Proposed I-70 Corridor Expansion

Information was forwarded to a Consulting firm working for CDOT (J. F. SADO & Assoc.) on several potential conflicts which may impact the project as proposed. The Recovery Team actively monitors three breeding sites within the project boundaries. These consultations will be ongoing if the project is funded.

Factors Influencing Boreal Toad (*Bufo boreas*) Breeding Habitat Suitability

INTRODUCTION

The distribution and abundance of the southern Rocky Mountain population of boreal toad (*Bufo boreas boreas*) has declined (Corn et al. 1989, Carey 1993, Loeffler 1998). This decline has occurred despite its existence in relatively pristine environments. The cause of the decline is unknown but chytrid fungus, *Batrachochytrium dendrobatidis* (Longcore and Pessier 1999), has been identified as a mortality factor for boreal toads in Colorado and was probably a contributing factor in the decline of this population. These issues prompted the Boreal Toad Recovery Team to bring adults and eggs from several evolutionarilly significant units into captivity. Offspring from these individuals could eventually be used for reestablishing boreal toad populations in Colorado. Translocation of boreal toad eggs, larvae, or adults is probably necessary to meet the recovery criteria (Muths 2001). Reintroduction and translocation efforts involving boreal toads have thus far been unsuccessful. This emphasizes the importance of quantifying boreal toad habitat relationships to determine what constitutes suitable habitat. Quantifying breeding habitat requirements is important because historic areas will not always be the best candidates for translocation because sites change over time and they 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). These sections deal primarily with the identification of habitat requirements and research related to experimental translocation.

Boreal toad distribution is limited to areas that contain suitable breeding habitat. 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. Amphibian species' success at the population level is determined primarily by the number and quality of metamorphosing larvae leaving a pond (Semlitsch 2000).

Boreal toad breeding habitat suitability was evaluated in several ways. First, eggs were moved from breeding to adjacent nonbreeding ponds to see if ponds that were not selected by breeding adults are in fact unsuitable. Secondly, the characteristics of the breeding habitat within breeding sites was evaluated. Lastly, the factors influencing larval growth, development, and metamorphosis were modeled for both wild and captively reared tadpoles.

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). Still others have related density, reproduction, or survival with 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 underlying objective of this study is to provide the Colorado Division of Wildlife (CDOW) with appropriate habitat variables, and their associated levels, that can be used to evaluate potential breeding and translocation sites. Breeding site selection, both between and within site, was the focus of field efforts in 1999. These data will be useful for identifying habitat attributes of suitable boreal toad breeding habitat. In 2000, within site breeding habitat characteristics were evaluated. However, the main focus in 2000 was measuring and modeling conditions within breeding sites that, given successful reproduction, allow tadpoles metamorphose at a large size in a short time.

EGG MASS TRANSLOCATION

Boreal toads often breed communally in one pond leaving adjacent ponds vacant. This may be a result of habitat selection, returning to a natal pond, or for social or behavioral reasons. Breeding site choice may not be solely based on physical factors. This may be explained by the fact that many amphibians exhibit high site fidelity to breeding sites. Toads reproducing for the first time may simply return to their natal pond. Berven and Grudzien (1990) found adult wood frogs to be 100% faithful to the breeding site in which they first bred and documented that only 18% of juveniles dispersed to breed in ponds other than their natal pond. Eighty-three percent of common toad (*Bufo bufo*) males and 100% of females returned to their natal pond to breed (Reading et al. 1991). Ninety-four percent of male boreal toads return to the same breeding site every year in Clear Creek County, Colorado (Jones 2000). Possible explanations for the adaption of communal egg deposition are breeding behavior, predator swamping, egg and tadpole modification of water temperatures, and larval densities in the wild that are not severe enough to invoke density dependence.

Eggs were moved from breeding ponds to adjacent, unselected, ponds in order to evaluate if the unselected ponds were indeed unsuitable. Quality of breeding and nonbreeding ponds could be addressed by comparing larval growth rates, length of larval period, metamorphic success, and mass at metamorphosis.

Breeding locations had to meet several criteria before eggs could be moved. These included that the nonbreeding site be within 100 m of the breeding site and thus available. The nonbreeding site must also contain habitat suitable for breeding and larval rearing. Also, the breeding site was required to have at least six egg masses deposited in it prior to being considered as a "donor" site. Upon meeting these criteria, approximately 500 eggs from each of two to three of the egg masses would be moved from the breeding site to the adjacent nonbreeding site. In 1999, approximately eight breeding locations in the state were identified that would meet these criteria. In 2000, however, only four met the criteria. Several did not have the required six egg masses deposited in them. In several other cases boreal toads bred in the "nonbreeding" sites that were identified as potential translocation sites.

Eggs were moved at three breeding locations: Hartenstein Lake, South Cottonwood, and Denny Creek. The eggs being moved were photographed to estimate their number, a sample preserved to evaluate development stage and viability, and tempered to avoid temperature shock before being placed in the nonbreeding pond.

At Denny Creek natural breeding occurred in the "nonbreeding" pond shortly after eggs were moved into it. The Hartenstein Lake nonbreeding pond's water level receded presumably before the eggs hatched. This pond was very stable in 1999. The translocated eggs hatched at South Cottonwood. Tadpoles were measured once but could not be located again because there were only a couple hundred tadpoles in a very large pond with a lot of vegetation. They were last seen by Brad Lambert (CNHP) on 22 June, 2000. Three newly metamorphosed toadlets were seen in the margin of the South Cottonwood transplant pond which is approximately 65 m from the breeding pond. These metamorphs likely came from the nonbreeding site but it is possible that they could have dispersed from the breeding pond after metamorphosing there.

This type of study shows promise at evaluating whether breeding sites are selected for habitat, sociality, or simply the reuse of natal ponds. To be successful it would have to be conducted on a much larger scale however. In most cases it was possible to understand why they bred where they did and suggests that one site does actually contain the best habitat in a given year.

CHARACTERISTICS OF BREEDING AREAS WITHIN BREEDING SITES

Boreal toads breed in the margins of ponds and lakes. The most common types of breeding sites in Colorado are American beaver (*Castor canadensis*) ponds, high elevation lakes, glacial kettle ponds, and human excavated wetlands. Ideal boreal toad breeding sites contain still water, very shallow margins, and stable water levels. Females deposit one egg mass averaging 6,661 ± 294 eggs (Carey et al. in press) every two to possibly six years (M. S. Jones, Colo. Div. of Wildl., pers. commun.). These eggs are typically deposited communally in the shallowest available areas of the breeding site. Very shallow water is probably the primary cue that breeding adults use when selecting ovipositioning areas within breeding sites.

The size and suitability of ovipositioning areas may be more attractive to amphibians than actual pond area (Reading et. al 1991). The presence and amount of shallows are often used to evaluate amphibian breeding habitats. Loman (1988) found that common frog, *Rana temporaria*, breeding ponds were best predicted by the amount of shallows they contained. Similarly, gradually sloping pond banks are an important factor in predicting use by naterjack toads, *Bufo calamita* (Banks and Beebee 1987). Shallows that allow elevated water temperatures on warm days could be especially important for the boreal toad because of the cold water temperatures and short growing season at high elevation.

METHODS

Several habitat variables were measured to quantify the amount of shallows present and the depth characteristics of the actual breeding area within each site. The amount of shallows present in breeding sites was quantified by measuring the length of shoreline that had water less than 10 cm deep, 30 cm from the margin. This measurement estimates the amount of potential breeding habitat within a site. The shoreline characteristics in breeding areas were evaluated by estimating bank slope and measuring the depths of all identifiable egg masses. Bank slope of the breeding area was estimated in each site by measuring the depths at 0.30 m, 1 m, and 5 m out from the margin on 10 transects. A linear regression was then performed for each site modeling depth measurements as a function of the distance from shore that they were taken. This results in the mean bank slope for each breeding area within each site.

RESULTS

Total length of shallow shoreline measurements were obtained from all 18 randomly selected study sites. These sites had a mean of 52.1 m (SE = 9.9) of shoreline that was less than 10 cm deep, 30 cm from the shore. The minimum amount of shallow shoreline was 10.0 m and the maximum was 190.0 m.

Bank slopes of the breeding areas were calculated for 17 of the randomly selected sites. The mean of these slopes is 7% or a 7 cm drop in water level for every 1 m increase in distance from shore. The 95% confidence interval for this mean is 0.056 to 0.086 and bank slopes ranged from a minimum of 0.02 to a maximum of 0.12.

The depths for a total of 38 egg masses were measured in 10 sites. Overall descriptive statistics were calculated using the mean depth from each site. The mean depth at which egg masses were deposited in those 10 sites was 6.1 cm. The 95% confidence interval for this mean ranges from 4.7 cm to 7.5 cm. The shallowest egg mass observed was deposited at a depth of 3.00 cm and the deepest observed was 9.8 cm.

DISCUSSION

The maximum observed egg mass depth in 2000 was 9.8 cm. This depth is consistent with those observed in 1999 and should be considered as a guideline to the maximum depth that boreal toads in Colorado are willing to deposit their eggs. For a potential breeding site to be considered suitable it must contain water less than 10 cm deep.

The mean bank slope of 7 cm per m increase in distance from shore is steeper than would be expected given that 10 cm seems to be as deep as boreal toads are willing to deposit eggs. Steep bank slopes restrict the area available for egg deposition and require that eggs be deposited very close to shore. Steep banks and egg masses close to shore increase the chance that an egg mass will dessicate if the water level drops. At least one very shallow sloping bank should be designed when constructing wetlands in the range of the boreal toad. This will ensure suitable shallows for breeding at a variety of water levels. This effect will be enhanced if a large portion of the shoreline has these shallow margins. Boreal toads in this study were willing, however, to breed in ponds with as little as 10 m of shallow shoreline. An area of deeper water should also be created to provide tadpoles with a night refuge of warmer water. Although not evaluated in this study, deeper areas should probably be at least 2 m deep. This will also reduce the daily variation in water temperature within a pond.

These guidelines are applicable when surveying for new breeding sites and when selecting translocation sites. They can also be used to evaluate habitat suitability for boreal toads in areas proposed for development or other disturbances. Finally, guidelines of what constitutes suitable boreal toad breeding habitat will also be useful when developing wetlands in the range of the boreal toad. It should be cautioned that when using these guidelines keep in mind wetland attributes can change. Change can be slow in the form of eutrophication or in a matter of days when water levels fluctuate as a result of pond drying or beaver activity.

An ideal breeding location contains many potential breeding sites of various forms and ages. An old, but active, beaver pond complex exemplifies an ideal breeding location because of the shallow eutrophicated ponds that exist in concert with water level maintenance by beaver.

Modeling Factors Influencing Larval Growth, Development, and Mass at Metamorphosis in the Wild

The amphibian literature is rich with experiments evaluating the factors affecting larval performance. Larval performance is often evaluated by looking at growth rates, length of larval period, and mass at metamorphosis. Travis (1983) summarized the relationship between these responses when he stated that “tadpole 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”.

Boreal toad metamorphs have a much better chance of attaining food and surviving if they metamorphose in early August than in late September. Juveniles that metamorphose early and at a large size have higher survival (Berven 1990). Large metamorphs also mature more quickly (Smith 1987) and are larger as adults (Smith 1987, Berven 1990). Boreal toad mass at metamorphosis and length of larval period can vary widely between sites because of the extreme environments in which they live. This results in large differences in the number of successful metamorphs. Metamorphic size can be indicative of larval conditions and adult fitness.

Many amphibians show plasticity in the timing of metamorphosis and their size at metamorphosis. This plasticity is the result of physiological responses to larval conditions. If larval conditions are favorable, tadpoles may put more energy into growth and remain in the aquatic environment. If, however, larval conditions are poor or deteriorating many amphibian larvae have the ability to metamorphose quickly, but at a smaller size, in the hopes that the terrestrial environment will be more favorable. Factors such as water temperature, larval density, food availability, water level consistency, and predation have been shown experimentally to affect larval growth, development, and metamorphosis.

Anuran tadpole growth is directly related to water temperature (Buchholz and Hayes 2000) and successful metamorphosis has been shown to depend on warm water temperatures (Wilbur 1987). Boreal toad tadpoles prefer water temperatures between 28° C and 34° C in laboratory thermal gradients (Beiswenger 1978). Hayes et al. (1993) showed a water temperature of 27°C caused *B. boreas* tadpoles to grow faster and metamorphosed sooner, but at a smaller size, than tadpoles raised at 22 ° C. Many experiments have reached similar conclusions with other species of Anurans. Generally as water temperatures increase, mass at metamorphosis decreases (Marian and Pandian 1985, Newman 1998). Likewise larval periods are typically shortened by increasing temperature (Marian and Pandian 1985, Newman 1998, Buchholz and Hayes 2000)

Tadpole growth rates may also be affected by the availability of food. Anuran larvae are suspension feeders of phytoplankton and detritus. Boreal toad larvae also have keritinized mouth parts which allow them to ingest periphyton. Growth of algae is related to light intensity, temperature, organic nutrients, and inorganic nutrients (Wetzel 1975). Nutrients such as phosphorous often limit primary production (McQueen et al. 1986, Allan 1995,). Total dissolved solids are correlated with total phosphorous and nitrogen (Chow-Fraser 1991). Further, measurements of specific conductance in cold, low ionic-strength waters can provide good estimates of total dissolved solids (Thomas 1986). Conductivity, through correlation, can then be useful as a estimator of productivity (Lind 1979).

Amphibian larval growth, development, and metamorphosis can be density dependent. Density dependence, through intraspecific competition, may determine growth rate, which can then determine how long it takes larvae to obtain the minimum size required for metamorphosis (Wilbur and Collins 1973). As expected, larval growth rates decrease as food availability decreases (Buchholz and Hayes 2000, Morey and Reznick 2000). Higher densities also result in longer larval periods (Wilbur 1987, Newman 1998) and lower mass at metamorphosis (Wilbur

1987, Newman 1998, Buchholz and Hayes 2000). Similarly, mass at metamorphosis is smaller at lower food availability (Newman 1998, Morey and Reznick 2000). *Bufo americanus* tadpoles reared at high density metamorphosed 56 days later and at 58% of the body mass of low density populations (Wilbur 1987).

Pond persistence is another factor that has been shown to affect growth rates, length of larval period, and mass at metamorphosis. Water level stability is critical for successful egg development. Dessication because of receding water level is a common cause of boreal toad egg mortality (Livo 1999). Rising water levels from spring snow melt and American beaver activity can also decrease hatching success. Greater depth reduces water temperature (Pearman 1995) which in turn increases egg mortality (Herreid and Kinney 1967).

Surprisingly, pond duration is not always beneficial to amphibians. Most amphibians balance tradeoffs between predation and pond duration when selecting breeding sites. At one extreme, aquatic predators are small and scarce, but pond drying is likely to occur before larval development is complete. At the opposite extreme, ponds are more persistent, but predators are more numerous and larger (Skelly 1996).

Predaceous insect larvae are a mortality factor for boreal toad larvae (Livo 1999). Larger, more stable ponds may benefit boreal toad tadpoles in that they are not only more persistent but are more likely to sustain trout populations. Trout do not prey on boreal toad tadpoles but they do consume predaceous insect larvae.

All of the above factors affect boreal toad larvae but the underlying hypothesis for this study was that water temperature is the primary factor influencing larval growth and development. The high elevation environment in which boreal toads live may promote temperature to a higher status among possible limiting factors.

The working hypotheses regarding water temperature variables in this study are as follows: growth and mass at metamorphosis are positively correlated with water temperature, degree days will be positively correlated with mass at metamorphosis. A final hypothesis that variance in water temperature will have a negative influence on growth and mass at metamorphosis was generated from the 1999 data.

Larval density, specific conductance, and pond persistence were estimated in addition to water temperature variables. Hypotheses regarding how these variables influence larval performance are consistent with the literature. Growth and mass at metamorphosis will be positively correlated with pond persistence. Growth and mass at metamorphosis will be negatively correlated with density. Growth and mass at metamorphosis will be positively correlated with conductivity.

A priori candidate models were developed from the above variables and hypothesized effects. The majority of the *a priori* models contain parameters involving water temperature variables. Breeding site area was included with models containing pond persistence because of the interaction that likely exists between them. A given drop in water level is much more important in a small shallow pond than a large lake.

METHODS

The southern Rocky Mountain population has 33 active breeding locations.. A location may consist of several breeding sites which are simply bodies of water in which breeding occurs. Onset Computer Corporation Optic Stowaway® temperature loggers were placed, at the time of breeding, in almost all of the known, active breeding sites. Study sites were selected by first identifying all the breeding sites that had at least one egg mass deposited in them for four consecutive years, including 2000. Thirty three breeding sites in the State of Colorado met this criterion. Study sites for 2000 were identified by randomly selecting 18 sites from those 33 sites.

The randomly selected study sites included: Mount Bethel, Hesbo, Upper Urad Reservoir, Herman Gulch, and Donut in Clear Creek County; Lower North Tenmile Cr. and Upper North Fork of the Snake River in Summit County; East Vail in Eagle County; Morrison Cr. in Routt County; East Lake Creek in Eagle County; Triangle Pass in Gunnison County; and Hartenstein Lake, Hartenstein Lake outlet pond, Denny Cr. (number 3), South Cottonwood, South Cottonwood West (pond 6), Collegiate Peaks East, and Collegiate Peaks West in Chaffee County. The eggs did not hatch in Upper Urad Reservoir or Herman Gulch. The remaining sites were visited about every third week from breeding until metamorphosis.

Egg masses are typically deposited communally in the shallowest area of the breeding pond. Loggers were placed near adults in amplexus or next to the egg masses during breeding in all 18 sites. These temperature loggers were programmed to record the water temperature every hour, 24 hours a day. They were moved near tadpole aggregations during larval development to approximate daytime temperatures experienced by tadpoles. The depth of all the temperature loggers was measured at each visit. This measurement was necessary because the temperature and variance in temperature recorded by a logger are dependent on the loggers depth. This measurement was also used to calculate pond persistence for the larval period.

Tadpoles behaviorally thermoregulate to maximize body temperature. On sunny days they often aggregate in as little as 2 cm of water and then move to deeper water at night when the shallows cool off. The depth and water temperature of two tadpole aggregations were also measured at each site visit. These measurements allow the estimation of bias between temperatures recorded by loggers and temperature actually experienced by tadpoles on a given day and time.

A method was developed to adjust the water temperatures recorded by loggers. This adjustment was necessary to mimic temperatures experienced by tadpoles and to allow water temperature comparisons between sites where loggers were at different depths. Recorded temperatures were always adjusted to a higher temperature. Temperatures were adjusted up to approximate the temperatures in the shallows on sunny days and up to approximate warmer, deep water on cool days and at night.

Part of the adjustment involved stratifying breeding sites by size (small, medium, large). Three ponds from each strata randomly selected to receive three temperature loggers at increasing depths up to 30 cm. Enough loggers remained to put at least two in all but four of the remaining sites. The slope of temperature as a function of depth was estimated for different times of day at every site except four. The slope parameters from the randomly selected sites were used for the four sites that did not receive multiple loggers. These were assigned depending on which size strata the ponds belonged to.

Every temperature recorded by the shallowest logger in each site was adjusted with three criteria. If the slope parameter estimate for temperature as a function of depth was negative (the water is warmest in the shallows) and the logger was less than 4.5 cm deep, the recorded temperature was used and was not adjusted (4.5 being the average depth of measured tadpole aggregations) If the slope parameter estimate for temperature as a function of depth is negative and the logger was greater than 4.5 cm deep, the temperature was adjusted up as if the logger had been 4.5 cm deep. If the slope parameter estimate for temperature as a function of depth is positive (deeper water is warmer) then all the recorded temperatures were adjusted as if the logger was positioned 30 cm deep. Every temperature recorded at every site was adjusted according to these criteria.

Independent Variables

Several water temperature variables were measured in this study from the period of egg deposition to metamorphosis. These included mean temperature (MEANTEMP), mean daily variance of 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).

Tadpole growth and development rates were estimated by measuring the individual Gosner (1960) stage, length to nearest .5 mm, and mass to nearest 0.01 g (wet weight) for between 30 and 100 random tadpoles. This is an estimate of the growth rate of the tadpoles within the site and not an individual growth rate because the same individuals were not measured on successive visits.

Tadpole density (DENSITY1) was evaluated at each visit with a visual, categorical, estimate of low, medium, or high. Pond persistence was estimated by measuring the depth of the temperature loggers at each visit. The absolute change (CHANGE) in water level over the egg and larval period was calculated from these measurements. Water samples are routinely taken at breeding sites to monitor water quality. Water samples were collected at each site visit to measure pH and specific conductance (CONDUCTIVITY). Dissolved oxygen was also measured with a Hach titration kit at each visit.

A laser rangefinder was used to estimate pond surface area (AREA). A maximum length and three width measurements were taken. AREA was estimated using the equation for the area of an ellipse which is equal to pi times the product of one half of the major and one half of the minor axes.

Response Variables

Tadpole growth rates, length of larval period, and mass at metamorphosis were all estimated and used as response variables to evaluate breeding site quality. Linear larval growth rates for each site were estimated from tadpole lengths and masses between Gosner (1960) stages 25 and 40. Gosner stages used were limited to between 25 and 40 in an attempt to capture the most linear portion of tadpole development. This was necessary because some sites were not visited often enough to fit nonlinear models. Growth rates for each site then, were estimated by the slope of a linear regression line of length as a function of days of development and mass as a function of days of development.

Length of larval period is the number of days from first egg deposition to the first date when metamorphosis was completed. Mass at metamorphosis and length of larval period were modeled separately and together. A composite variable (MASSRT) was created because mass at metamorphosis is somewhat correlated with length of larval period. Tadpoles that develop longer typically achieve a larger size. Using MASSRT as a response variable also allows mass at metamorphosis to be modeled without including a parameter for length of larval period. This variable is created by dividing the mass of individual metamorphs by the length of their 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 a large metamorph in a short amount of time and would thus have a large value of MASSRT.

Modeling and Model Selection

Tadpole growth rates, length of larval period, and mass at metamorphosis, as a function of the independent variables, were modeled with multiple linear regression. MASSRT was modeled with mixed effect models and represents the only results reported here. Mixed models contain both fixed and random effects. Site specific independent variables were modeled as fixed effects which are the equivalent to standard regression parameters. The influence of site on MASSRT was included as a random effect using SAS Proc Mixed.

Random effects models were used because there were two sources of variation to be modeled, the within site variation in mass at metamorphosis and the between site variation in mass at metamorphosis. Fixed effects models only account for one source of random variation, the random error term, and assume within site variation to be zero. This is important because unequal numbers of metamorphs were weighed between sites in this study. Random effects models shift the inference from the specific study sites to the population from which these sites were randomly selected. In this case, all the consistent breeding sites for the southern Rocky

Mountain population. Looking at it differently, mixed effect models allow a look at what would be expected if different sites were selected.

Traditional R^2 values cannot be calculated for mixed models because they have more than one random effect. Analysis of variance components is necessary to evaluate the amount of the total process variation explained by the model, by the random site effect, and by the random sampling variation related to within site differences in MASSRT. Total process variation ($\hat{\sigma}^2_{total}$) can be estimated with an intercept only model which contains no fixed effects except the intercept. Total process variation can be expressed as

$$\hat{\sigma}^2_{total} = \hat{\sigma}^2_{model} + \hat{\sigma}^2_{residual}$$

The $\hat{\sigma}^2_{residual}$ is the amount of the total variation not explained by the fixed effect parameters in a given model. The amount of variation accounted for by the fixed effects parameters in the model σ^2_{model} can then be estimated from

$$\hat{\sigma}^2_{model} = \hat{\sigma}^2_{total} - \hat{\sigma}^2_{residual}$$

The percent of total variation explained by a model can be estimated by the following equation:

$$\text{Percent variation explained by model} = \hat{\sigma}^2_{model} / \hat{\sigma}^2_{total}$$

The full maximum likelihood option was used in SAS Proc Mixed for comparing models with different fixed effects. Once the competing models were identified they were re-analyzed using the restricted maximum likelihood. This approach is preferred for models used for inference because it's known to be unbiased when the data are balanced and its properties are known and thought to be good with unbalanced data as is the case here.

To evaluate mixed model assumptions site was modeled as a fixed effect and as a function of MASSRT with SAS Proc Glm. Studentized residuals were homoscedastic, distributed equally about zero, when plotted against predicted values. No evidence was seen to indicate violations of assumptions or the necessity for transforming these data.

Akaike's Information Criteria (Akaike 1973) was used for model selection. A small sample bias adjustment, AICc, was used to select best approximating models from the lists of candidate models. AIC consists of two components, one being how well the model approximates the data and the other is a penalty for the number of estimable parameters in the model. AICc was used for model selection to attempt a balance between bias and precision with respect to the number of parameters included in the model (Burnham and Anderson 1998). This prevents over-fitting the data which results in modeling those data and not the system from which they came. Given the same data, AICc values from competing models are relative and can be evaluated by calculating the AICc differences (ΔAICc) between each model and the best approximating model. This simply ranks competing models by their AICc values relative to the model with the lowest AICc. This makes the best approximating model's ΔAICc value zero. Models with ΔAICc values less than two are generally considered when making inference.

RESULTS

Length of larval period and mass at metamorphosis can be highly variable. The mean length of larval period for the randomly selected sites in this study was 75 days with a standard deviation of 10 days. Larval period ranged from 62 to 98 days in these study sites. Mass at metamorphosis was highly variable with a mean of 250.6 mg (std = 90.3). Mass varied from a low of 151.8 mg to a high of 490.6 mg.

Seventeen models were evaluated to see which models best approximated the data (Table 3). Three competing models ended up with ΔAICc values less than 2.0 (Table 4). The best approximating models all contained the water temperature variable MEANTEMP and contained either DAILYVAR or DAILYRANGE (Table 4). The 3rd ranked model contains MEANTEMP, DAILYRANGE, and CHANGE (Table 4).

Table 3. Hypothesized models ranked by AICc for MASSRT as a function of independent variables for wild sites

Hypothesized Model	K	AICc	ΔAICc
MASSRT = MEANTEMP DAILYVAR	4	221.05	0
MASSRT = MEANTEMP DAILYRANGE	4	221.52	0.467
MASSRT = MEANTEMP DAILYRANGE CHANGE	5	222.98	1.93
MASSRT = DAILYRANGE	3	223.46	2.41
MASSRT = DAILYVAR	3	223.65	2.59
MASSRT = DEGREEDAY DAILYRANGE	4	225.44	4.38
*MASSRT = CHANGE SQRCHANGE	4	225.88	4.82
MASSRT =DEGREEDAY	3	226.15	5.09
MASSRT =CONDUCTIVITY	3	226.65	5.59
MASSRT =MEANTEMP	3	226.88	5.82
MASSRT =CHANGE	3	227.39	6.33
MASSRT =MEANTEMP CHANGE	4	227.99	6.94
MASSRT =MEANTEMP CONDUCTIVITY	4	229.23	8.18
*MASSRT =MEANTEMP CHANGE SQRCHANGE	5	230.74	9.69
MASSRT =DENSITY1	4	230.98	9.93
MASSRT =MEANTEMP DENSITY1	5	234.21	13.15
MASSRT =CHANGE AREA AREA*CHANGE	5	236.28	15.22

* Not an *a priori* model

Table 4. Competing models ranked by AICc for MASSRT as a function of independent variables for wild sites

Hypothesized Model	K	AICc	ΔAICc
MASSRT = MEANTEMP DAILYVAR	4	221.05	0
MASSRT = MEANTEMP DAILYRANGE	4	221.52	0.467
MASSRT = MEANTEMP DAILYRANGE CHANGE	5	222.98	1.93

There is evidence for a MEANTEMP effect in all three of the competing models because none of the 95% confidence intervals for the slope parameter estimates overlap zero (Table 5). There is also evidence that large fluctuations in daily water temperature negatively influences MASSRT. The parameter estimates for DAILYVAR and DAILYRANGE are negative and their associated 95% confidence interval fail to overlap zero in all 3 competing model. It is clear that of the models examined in this study, those that contain mean water temperature and a variable explaining variation in daily water temperature explain these data the best.

With other variable's effects 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 5). Holding MEANTEMP constant, increasing DAILYVAR 1° C decreases MASSRT 0.069 mg per development day (Table 5). DAILYRANGE has a similar impact on MASSRT, only with a larger effect size, in both of the models in which it is included. Holding MEANTEMP and CHANGE constant, MASSRT decreases 0.223 and 0.206 or every 1°C increase in DAILYRANGE (Table 5). The negative effect of a site having highly variable daily temperature regime is nearly as great as the benefit of achieving a high daily mean. MEANTEMP and the variation in daily temperature variables are likely correlated to some degree but including DAILYVAR and DAILYRANGE enhances the models enough to warrant the inclusion of , and penalty for, the extra parameter. As expected, CHANGE has a negative influence on MASSRT. The evidence for this effect is weak, however, because the 95% confidence interval for the CHANGE parameter estimate slightly overlaps zero (Table 5).

Table 5. Estimated slope parameters for competing models ranked by AICc for MASSRT as a function of independent variables

Hypothesized Model	K	ΔAICc	Estimated slope parameters (95% CI)
MASSRT = MEANTEMP DAILYVAR	4	0	β_1 = 0.951 (-2.160, 4.062) β_2 = 0.249 (0.041, 0.459) β_3 = -0.069 (-0.115, -0.025)
MASSRT = MEANTEMP DAILYRANGE	4	0.467	β_1 = 2.529 (-0.807, 5.865) β_2 = 0.235 (0.024, 0.445) β_3 = -0.223 (-0.373, -0.074)
MASSRT = MEANTEMP DAILYRANGE CHANGE	5	1.93	β_1 = 1.191 (-2.266, 4.647) β_2 = 0.276 (0.078, 0.474) β_3 = -0.206 (-0.344, -0.068) β_4 = -0.082 (-0.183, 0.018)

The model including MEANTEMP and DAILYVAR left 46.6% ($\hat{\sigma}^2_{residual}$) of the variation in MASSRT unexplained (Table 6). This model explains an estimated 53.6 percent of the total variation in MASSRT ($\hat{\sigma}^2_{model} / \hat{\sigma}^2_{total}$ in Table 6). A model with MEANTEMP and DAILYRANGE left 47.8 % ($\hat{\sigma}^2_{residual}$) of the variation in MASSRT unexplained and explained 52.3 % of the variation (Table 6). The inclusion of CHANGE in the model increases the amount of variation explained to 62.2% (Table 4). Within site variation in MASSRT was 0.1616 for these sites.

Table 6. Sources of process variation explained by competing models for MASSRT in wild Colorado boreal toads.

Model	Type of variation $\hat{\sigma}^2_{total}$	$\hat{\sigma}^2_{residual}$	$\hat{\sigma}^2_{model}$	Percentage explained by model ($\hat{\sigma}^2_{model} / \hat{\sigma}^2_{total}$)
MASSRT=	1.0028			100.0
MASSRT=MEANTEMP DAILYVAR		0.4657	0.5371	53.6
MASSRT=MEANTEMP DAILYRANGE		0.4784	0.5236	52.3
MASSRT=MEANTEMP DAILYRANGE CHANGE		0.3787	0.6241	62.2

DISCUSSION

There are great differences in mass at metamorphosis and length of larval period. Issues of interest in this study were determining what pond level variables influence larval performance, to what degree these variables affect MASSRT, the amount of variation in MASSRT they can explain, and finally if these factors can be measured with sufficient precision in the wild to allow their use in evaluating breeding site suitability and selection of potential translocation sites.

These data and selected models support the hypotheses that water temperature and daily variation in water temperature have an important influence on boreal toad larval growth rate, length of larval period, and mass at metamorphosis. Tadpoles experience the most gain in mg per day of development in breeding sites that have the highest, and least variable, water temperatures. There are likely other important factors that were not measured or modeled in this study. From the models examined here (Table 3), there is evidence that water temperature is more important than density, pond persistence, or pond conductivity for achieving large mass at metamorphosis in a short amount of time. Models containing only water temperature variables explain approximately 53.6% and 52.3% of the variation in mg gained by metamorphs per day of development. The inclusion of a nearly significant parameter for pond persistence increased the amount of total process variation explained by the model to 62.2%.

These models demonstrate that some sites offer a much more favorable environment for boreal toad larvae than others. The estimates for $\hat{\sigma}^2_{residual}$ show that nearly half of the variation in MASSRT is not explained by the fixed effects parameters. Variation not explained by temperature variables and pond persistence can be attributed to between site differences that were not included as fixed effects. These factors include larval density and pond productivity because, although measured in this study, they were not included in the models used for inference. Other factors that make up $\hat{\sigma}^2_{residual}$ include things such as genetic variation, food quality, costs of predator avoidance, and just about anything else that may vary between sites.

All of the variation cannot be explained while still maintaining model parsimony. The inclusion of more parameters in the model would have increased $\hat{\sigma}^2_{model}$. This could not occur, however, without risking over-fitting these data. This model selection approach was taken to identify models that were most likely given the data and yet did not explain the data to such a degree that they did not adequately model the system from which the data came. Obtaining numerical estimates of larval density and estimating phosphorous levels in the breeding sites may result in these factors being selected as competing models. However, these factors, especially density, cannot be estimated without considerable effort. Boreal toad larval densities in the wild may not be great enough to induce density dependent growth limitations. Another way to increase $\hat{\sigma}^2_{model}$ would be to increase the number of sites studied. Increasing the amount of data would increase the number of parameters that could be included in a model and still maintain parsimony.

Management Implications

Methods and models presented here can be used to evaluate the suitability of potential translocation sites. The suitability of other potential breeding sites and wetlands that may be impacted by human disturbance could likewise be evaluated. This would be accomplished by collecting temperature and pond persistence data with similar methods to those used in this study. These measurements could then be input into the top competing models (Table 5). Predicted values of MASSRT for potential sites should fall within the 95% confidence intervals from this study or at least be within the range seen here.

The fact that these models represent only one year of temporal variation represents this study's largest weakness given that large temporal variation exists. In 1999 , tadpoles in only 8 of 22 study sites achieved metamorphosis. In 2000, this number jumped to 16 out 18 which included all sites in which the eggs hatched.

All known boreal toad breeding sites are monitored throughout the breeding and larval development period. Temperature loggers could be deployed in all monitored breeding sites and mass at metamorphosis data could be gathered opportunistically. These efforts would allow a relatively inexpensive way to validate these models.

Future Steps

The next steps in this project are to continue summarizing modeling efforts that have been completed using the response variables length of larval period and mass at metamorphosis modeled individually. Tadpole growth rates that were estimated between the Gosner (1960) stages of 25 and 40 were also used as response variables for a series of models and must be summarized. Model averaging will also be conducted on all models to incorporate model selection uncertainty and to get a single parameter estimate for variables that were present in multiple models. Predicted values and their associated 95% confidence intervals also should be estimated for all top models. Comparing the deviance in models with and without the parameter CHANGE, relative to a global model, will also be done to evaluate usefulness of including this parameters.

Modeling Factors Influencing Larval Growth, Development, and Metamorphosis in the Lab

In 2000, boreal toad eggs were brought in from the field and raised in captivity to conserve genetic stock from evolutionary significant units throughout Colorado. The CDOW brought eggs into captivity from 5 of the 18 randomly selected study sites used in this study. Tadpoles were reared to metamorphosis in several labs and hatcheries. When wild clutches were split into several tanks, one study tank was randomly selected for use here. Temperature loggers were used to measure the water temperature associated with these tanks. A departure from the techniques used in the wild was that numerical estimates of larval density were obtained. This was accomplished by censussing the number of tadpoles in each tank. The same methods were used to estimate growth rate, length of larval period, and mass at metamorphosis as were employed in the wild sites. Although not an experiment, this allowed the estimation of larval growth rate, length of larval period, and mass at metamorphosis with respect to known temperature and density while assuming unlimited food availability. A major limitation of these data is that tank and temperature are confounded with the site that the tank's tadpoles came from. This occurs because tadpoles from each site were not reared at a variety of temperatures. Another weakness is that only six tanks were monitored.

Data from captive tadpoles were analyzed differently than those collected in the wild. Multiple linear regression was used instead of mixed effects models because rearing locations were not selected randomly and all the units in Colorado that would likely be used to raise boreal toads were represented. Variation in mass at metamorphosis was assumed to be the same between tanks. Ranking by respective ΔAICc values was again employed as the model selection method.

Plotting studentized residuals by their predicted values revealed increasing variance and therefore violation of the model's assumptions. MASSRT values were natural log transformed which produced homoscedastic residuals and met model assumptions.

RESULTS

Mean length of larval period was longer and more variable for captive tadpoles than in the wild. Mean length of larval period was 92 days. Length of larval period was much more variable in captivity than in the wild with a standard deviation of 42 (std wild = 10). The minimum number of days to achieve metamorphosis in captivity was 51 and the maximum was

139. The mean mass at metamorphosis for captively reared tadpoles (528.4 mg) was more than twice as large as the wild mean (250.6 mg). Indoor masses ranged from 446.0 mg to 638.7 mg. The variation in mass at metamorphosis was 79 for captive sites is similar to that measured in the wild. The confidence intervals for mean mass at metamorphosis in the wild and captivity did not overlap.

Seven *a priori* models were evaluated for their ability to describe the captive tadpole data. Only two models had ΔAICc less than 2.0 (Table 7). These models both contained the parameter MEANTEMP (Table 1). DAILYRANGE was included in the top model and the 2nd model contained DAILYVAR (Table 1).

Table 7. Hypothesized models ranked by AICc for LnMASSRT as a function of independent variables for captive stocks.

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

Examination of the 95% confidence intervals associated with these parameters reveals that there is evidence for the influence of MEANTEMP, DAILYRANGE, and DAILYVAR on LnMASSRT with these data (Table 8). None of the parameter estimates' associated 95% confidence intervals overlap zero. Parameter signs are also in line with their hypothesized effect on LnMASSRT. MEANTEMP's parameter estimates were 0.196 and 0.205 respectively (Table 8). Also as hypothesized, variation in daily temperature had a negative influence on LnMASSRT. The regression resulted in a parameter estimate of -0.096 for DAILYRANGE and a parameter estimate of -0.093 for DAILYVAR. Models LnMASSRT = MEANTEMP DAILYRANGE and LnMASSRT = MEANTEMP DAILYVAR resulted in an R^2 values of 0.783 and 0.784 respectively (Table 8). Although the model containing only MEANTEMP was not selected as a competing model by ΔAICc (Table 7), it is interesting to note that this variable alone resulted in an R^2 of 0.685.

Table 8. Estimated slope parameters for competing models ranked by AICc for LnMASSRT as a function of independent variables for captive stocks

Hypothesized Model	K	ΔAICc	Estimated slope parameters (95% CI)	R^2
LnMASSRT = MEANTEMP DAILYRANGE	3	0	β_1 = -1.478 (-1.976, -0.979) β_2 = 0.196 (0.172, 0.219) β_3 = -0.096 (-0.131, -0.060)	0.7833
LnMASSRT = MEANTEMP DAILYVAR	3	1.51	β_1 =-1.767 (-2.212, -1.319) β_2 = 0.205 (0.183, 0.228) β_3 = -0.093 (-0.129, -0.057)	0.7837

DISCUSSION

Model selection with the captive tadpoles validated the wild site analysis in that the same competing models were selected by AICc. Even though the captive information must be used with several caveats because only six tanks were monitored, it is encouraging that the same models were selected. Again, ideal larval conditions have high water temperatures with low daily variation in temperature. These models were selected over models containing numerical density estimates. It should be noted that all the indoor tadpole densities were fairly high and a large range in density was not modeled.

The mean temperature that indoor tadpoles experienced was 17.7 ° C. Indoor temperature means ranged from 14.7° C to 19.8° C. These temperatures are on the low end of temperatures in which boreal toad tadpoles could be reared and probably approximate natural water temperatures fairly well. The confidence intervals for wild mean temperature and captive mean temperature overlap indicating the means are not different. If it was desirable to raise larger metamorphs in a shorter amount of time these temperatures should be increased. The lab temperatures reported here are far below the 28-34° C preferred body temperature for boreal toad tadpoles reported by Beiswenger (1978). It should be noted that tadpoles reared at 27° C metamorphosed at smaller sizes than when raised at 22 ° C (Hayes et al. 1993). Facilities raising boreal toads should recognize the tradeoffs in terms of length of larval period versus mass at metamorphosis when setting temperatures beyond the range of temperatures evaluated in this study. Any experiments conducted with boreal toads tadpoles can gain biological realism by using temperatures, and daily variance in temperature, similar to those recorded in wild sites during this study.

SUMMARY

Gaining a better understanding of boreal toad breeding habitat requirements will provide information that is necessary for its effective management and preservation. Information gained from this project will also be useful for selecting translocation sites, determining the suitability of wetlands that face human disturbance, and when developing wetlands for mitigation. This research will become even more valuable if additional boreal toad populations are impacted by chytrid fungus and are no longer available for habitat relationships studies in the wild.

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APPENDIX A

Breeding site water quality results for 1997 to 2000.

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
ABOVE TRICKLE PARK RES 215GM1	08/03/98	12.3	50.5	7.82	28.0	132	<10	<0.20	4.2	1710	26.2	<5.0	<5.0	<5.0
ANN'S POND	05/16/00		114	6.36	4.8	1576	<15	3.93	10.7	148	4764	7.6	<2.0	1064
BEAVER POND 215 GM2	08/03/98	12.4	127.5	8.04	80.0	223	<10	<0.20	4.5	1096	287	<5.0	<5.0	5.2
BROWN'S CREEK	08/22/97	10.1	41	7.45	21.0	32	<10	<0.20	2.3	448	56.7	<5.0	<5.0	<5.0
BROWN'S CREEK	06/29/99		044	7.69	20.6	365	<15	<0.15	<1.0	227	<10	<2.0	<2.0	<10
BROWN'S CREEK	08/29/99					34	<15	<0.15	<1.0	54	<10	<2.0	<2.0	<10
BROWN'S CREEK	05/11/00		59	7.28	23.2	229	<15	<0.15	<1.0	133	<10	<2.0	<2.0	<10
BRUSH CREEK	06/14/00		203	7.56	132.0	15	<15	<0.15	<1.0	132	14	<2.0	<2.0	<10
BUCK MTN POND #2	06/19/00		26	6.52	12.6	117	<15	<0.15	2.2	1128	21	<2.0	<2.0	<10
CALIFORNIA PARK	08/05/00		176	7.26	101.0	645	<15	0.23	15.8	1475	389	<2.0	<2.0	99
CALIFORNIA PARK ELKHEAD CREEK	06/15/00		207	7.56	86.6	1202	<15	<0.15	4.3	2168	159	<2.0	<2.0	<10
CALIFORNIA PARK ROUTT N. FOREST	05/26/99		065	5.5	41.2	943	<15	<0.15	1.4	2330	96	<2.0	<2.0	<10
COLL. PEAKS CAMPGROUND	07/28/99		091	7.50	58.0	67	<15	<0.15	1.0	929	17	<2.0	<2.0	<10
COLLEGIATE PEAKS	08/23/97	9.2	67	7.63	37.8	80	<10	<0.20	1.4	369	17.8	<5.0	<5.0	5.6
COLLEGIATE PEAKS	05/22/99					1300	<15	<0.15	18.7	23474	93	2.4	<2.0	11
COLLEGIATE PEAKS CHAFFEE CO	08/28/99		097	7.55	60.8	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS EAST	05/10/00		69	7.23	33.0	32	<15	<0.15	<1.0	213	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS EAST	06/21/00		76	7.15	36.8	31	<15	<0.15	1.5	287	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS EAST	07/19/00		79	7.07	38.0	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS LOWER POND	05/28/98		83.8		46.0	137	<10	<0.20	1.1	760	21.5	<5.0	<5.0	<5.0
COLLEGIATE PEAKS MIDDLE POND	05/28/98		71.6		34.8	66	<10	0.21	1.5	395	17.7	<5.0	<5.0	<5.0
COLLEGIATE PEAKS WEST	05/10/00		83	7.02	48.6	201	<15	<0.15	1.5	876	14	<2.0	<2.0	<10
COLLEGIATE PEAKS WEST	06/22/00		105	7.20	59.6	112	<15	<0.15	1.7	1372	30	<2.0	<2.0	<10
COLLEGIATE PEAKS WEST	07/19/00		108	6.92	59.2	41	<15	<0.15	1.2	1258	24	<2.0	<2.0	<10
CONUNDRUM	05/28/00		62	6.76	8.0	83	<15	<0.15	<1.0	593	24	<2.0	<2.0	<10
CONUNDRUM SILVER DOLLAR SITE	07/23/00		242	8.59	43.8	40	<15	<0.15	4.1	1830	26	<2.0	<2.0	<10
CRAIG'S POND FETKAVICH	06/08/99					54	<15	<0.15	1.1	196	<10	<2.0	<2.0	<10
CUCUMBER GULCH	06/18/97	21.8	44	7.08	14.2	83	<10	<0.20	1.4	368	17.7	<5.0	<5.0	5.5
CUCUMBER GULCH	06/24/98					59	<10	<0.20	1.6	192	12.5	<5.0	<5.0	<5.0
CUCUMBER GULCH	05/25/00		174	7.52	35.2	81	<15	<0.15	3.5	2395	22	<2.0	<2.0	<10
DENNY #1	06/23/00		42	7.17	19.0	32	<15	<0.15	1.0	165	11	<2.0	<2.0	<10
DENNY CREEK	08/23/97	8.8	38	7.51	23.0	224	<10	0.33	8.4	2280	45.1	5.7	<5.0	26.7
DENNY CREEK	05/28/98					104	<10	<0.20	<1.0	262	13.1	<5.0	<5.0	<5.0
DENNY CREEK	05/27/99					236	<15	<0.15	<1.0	604	28	<2.0	<2.0	<10
DENNY CREEK	09/04/99		045	7.72	29.0	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
DENNY CREEK	09/04/99		034	7.71	18.8	55	<15	<0.15	<1.0	316	14	3	<2.0	<10
DENNY CREEK POND 2	05/16/00		37	7.28	16.8	125	<15	<0.15	<1.0	380	17	<2.0	<2.0	<10
DENNY CREEK POND 4	05/16/00		38	7.24	17.6	142	<15	<0.15	<1.0	582	36	<2.0	<2.0	<10
DENNY CREEK WEST	06/23/00		42	6.93	17.8	44	<15	<0.15	1.5	2172	127	<2.0	<2.0	<10
DENNY CREEK WEST	07/01/00		62	7.23	31.6	90	<15	<0.15	2.9	3433	140	<2.0	<2.0	<10
DENNY EAST	07/06/00		44	6.85	21.8	114	<15	<0.15	1.7	224	19	<2.0	<2.0	<10
DIAMOND PARK	06/27/97	24.0	73	7.53	30.6	184	<10	<0.20	3.3	886	82.4	<5.0	<5.0	8
DIAMOND PARK	06/02/99		057		34.8	95	<15	<0.15	2.3	309	10	<2.0	<2.0	<10
DIAMOND PARK	07/07/99					126	<15	<0.15	3.6	267	<10	<2.0	<2.0	<10
E. LAKE CREEK LOWER	06/30/00		20	6.54	6.8	53	<15	<0.15	49.4	164	<10	<2.0	<2.0	<10
E. LAKE CREEK UPPER	05/30/00		53	5.77	2.0	103	<15	<0.15	1.4	61	15	<2.0	<2.0	14
E. MAROON CREEK	05/29/00		1144	7.12	90.4	363	<15	1.48	20.4	1384	37	47.6	<2.0	151
E. MAROON CREEK	06/08/00		1112	7.79	52.2	<15	<15	<0.15	2.7	113	17	<2.0	<2.0	<10
EAST LAKE CR	07/12/00		66	6.30	1.8	112	<15	<0.15	<1.0	30	24	<2.0	<2.0	25
EAST MAROON UPPER BREEDING	07/24/00		1360	7.44	98.6	259	<15	1.50	7.2	1474	98	29.4	<2.0	51
FOUR MILE CREEK CHAFFEE, CO	08/19/97	9.6	47	8.12	21.5	442	<10	<0.20	1.6	1558	106	<5.0	<5.0	<5.0
FOUR MILE CREEK CHAFFEE, CO	08/04/98		53.2		22.6	145	<10	<0.20	83.3	1302	110.6	<5.0	<5.0	10.1
FOUR MILE CREEK CHAFFEE, CO	09/19/98		63.8		27.6	254	<10	<0.20	1.3	2188	122.3	<5.0	<5.0	<5.0
FOUR MILE CREEK CHAFFEE, CO	06/09/99					381	<15	<0.15	<1.0	754	64	<2.0	<2.0	<10
FOURMILE	05/16/00		40	7.27	14.4	252	<15	<0.15	<1.0	658	41	<2.0	<2.0	<10
GORE CREEK SOUTH SIDE	07/22/99		166	6.65	91.2	52	<15	<0.15	<1.0	239	20	<2.0	<2.0	<10
GUNNISON TRIANGLE PASS	06/19/98	14.4	11.9	6.5	8.6	176	<10	<0.20	1.2	406	15.9	<5.0	<5.0	5.4
HARTENSTEIN LAKE	08/30/97	10.1	18	6.93	10.6	72	<10	<0.20	<1.0	162	3.7	<5.0	<5.0	<5.0
HARTENSTEIN LAKE	09/07/98		27.4		13.8	74	<10	<0.20	1.8	470	16.5	<5.0	<5.0	6.2
HARTENSTEIN LAKE	06/24/99					56	<15	<0.15	<1.0	87	<10	<2.0	<2.0	<10
HARTENSTEIN LAKE	07/13/99		15.1		8.2	66	<10	<0.20	1.2	95	3.4	<5.0	<5.0	<5.0
HARTENSTEIN LAKE	06/01/00		25	6.46	2.8	302	<15	<0.15	2.8	971	14	<2.0	<2.0	<10
HARTENSTEIN LAKE OUTLET POND	07/06/00		20	6.84	10.8	33	<15	<0.15	<1.0	142	26	<2.0	<2.0	<10
HARTENSTEIN LAKE PROPER	07/06/00		17	7.02	7.6	34	<15	<0.15	<1.0	78	<10	<2.0	<2.0	<10
HATRENSTEIN LAKE NON-BREEDING	07/06/00		26	6.74	12.2	63	<15	<0.15	1.6	1754	201	<2.0	<2.0	<10
HERMAN GULCH	05/28/97					2471	<10	<0.20	7.1	1441	38.2	<5.0	<5.0	21.2
HERMAN GULCH	06/24/97		92	7.21	19.8	181	<10	0.26	3.1	665	264.5	<5.0	<5.0	<5.0
HERMAN GULCH	05/20/98	12.1	100.0	7.23	34.2	159	<10	0.26	1.9	1047	286.4	<5.0	<5.0	<5.0
HERMAN GULCH	05/20/98	12.1	100.0	7.23	34.2	179	<10	0.30	2.4	1415	621.8	<5.0	<5.0	<5.0
HERMAN GULCH	07/23/98	12.5	100.0	8.10	48.6	175	<10	<0.20	2.2	102	4.7	<5.0	<5.0	5.6
HERMAN GULCH	08/22/98		589		59.4	103	<10	<0.20	<1.0	265	13.2	<5.0	<5.0	<5.0

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HERMAN GULCH	07/11/00		725	7.36	47.6	25	<15	<0.15	1.3	777	78	<2.0	<2.0	<10
HERMAN GULCH (RUT)	06/24/97		711	7.30	61.6	419	<10	0.33	4.3	1400	225.4	<5.0	<5.0	7.2
HOLY CROSS S UPPER POOL EAGLE	07/31/98		16.1		8.2	127	<10	<0.20	1.1	26	2.1	<5.0	<5.0	6.4
JL2 SUMMIT CO	06/29/98					36	<10	<0.20	2.4	33	5.8	<5.0	<5.0	<5.0
JUMPER CREEK	05/21/98	12.5	51.6	6.75	29.8	611	<10	<0.20	1.5	323	8.2	<5.0	<5.0	<5.0
JUMPER CREEK	08/18/98		81.2		42.6	21	<10	<0.20	1.0	121	2.4	<5.0	<5.0	<5.0
JUMPER CREEK	05/31/99					491	<15	<0.15	<1.0	259	<10	<2.0	3.7	<10
JUMPER CREEK	07/05/99					527	<15	<0.15	3.0	440	45	<2.0	<2.0	<10
KETTLE TARN II	05/01/00		20	7.03	6.4	66	<15	<0.15	2.1	1323	39	<2.0	<2.0	<10
KETTLE TARN RMNP	05/05/00		23	6.52	10.0	112	<15	<0.15	1.7	1372	30	<2.0	<2.0	<10
KETTLE TARN RMNP	07/12/00		34	6.04	9.0	57	<15	<0.15	1.2	230	10	<2.0	<2.0	18
KROENKE LAKE	08/29/97	10.5	22	6.93	10.4	150	<10	0.22	4.0	861	14.3	<5.0	<5.0	8.2
KROENKE LAKE	06/30/98					63	<10	<0.20	1.6	142	10.1	<5.0	<5.0	<5.0
KROENKE LAKE	06/30/99					48	<15	<0.15	1.5	561	18	<2.0	<2.0	<10
LAKE OWEN BEAVER POND	08/20/99					70	<15	<0.15	2.5	837	68	<2.0	<2.0	12
LILY PAD LAKES TRAIL	07/01/98					87	<10	<0.20	1.7	1355	94.4	<5.0	<5.0	7.3
LOST LAKE	06/05/00		44	6.98	16.4									
LOST LAKE BOULDER, CO	08/22/97	20.8	46	6.98	17.4	290	<10	<0.20	3.6	437	17.9	<5.0	<5.0	11
LOST LAKE BOULDER, CO	08/18/98		48.3		24.2	38	<10	<0.20	1.2	253	12.2	<5.0	<5.0	6.9
LOST LAKE RMNP	05/16/00		18	6.61	6.0	41	<15	<0.15	2.0	778	83	<2.0	<2.0	<10
LOST LAKE RMNP	08/08/00		15	6.13	5.6	20	<15	<0.15	1.2	243	12	<2.0	<2.0	<10
LOWER N. FORK SNAKE	05/23/00		154	7.02	54.6	562	<15	<0.15	4.7	579	16	<2.0	<2.0	<10
LOWER N. TENMILE	05/23/00		61	6.74	33.0	39	<15	<0.15	1.8	77	<10	<2.0	<2.0	<10
LOWER N. TENMILE	07/11/00		77	7.15	37.8	26	<15	<0.15	<1.0	161	<10	<2.0	<2.0	<10
MAGDALENE GULCH	09/01/99		038	7.67	22.4	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
MIDDLE COTTONWOOD	05/10/00		87	7.24	44.0	<15	<15	<0.15	1.4	1129	16	<2.0	<2.0	<10
MIDDLE COTTONWOOD CHAFFEE	07/28/99		042	7.60	25.8	19	<15	<0.15	<1.0	66	<10	<2.0	<2.0	<10
MIDDLE COTTONWOOD CHAFFEE CO	09/11/99		049	7.90	28.4	<15	<15	<0.15	<1.0	77	<10	<2.0	<2.0	<10
MORGAN'S GULCH	05/31/00		40	7.08	20.4	74	<15	<0.15	<1.0	510	<10	<2.0	<2.0	<10
MORGAN'S GULCH CHAFFEE CO	07/12/99		045	7.39	26.0	104	<15	<0.15	1.3	1215	<10	<2.0	<2.0	<10
MORGAN'S GULCH CHAFFEE CO	09/11/99		040	7.62	21.2	208	<15	<0.15	3.2	2058	14	<2.0	<2.0	<10
MORGANS GULCH CHAFFEE, CO	09/06/97	12.1	52	6.98	28.2	52	<10	<0.20	2.7	2442	243.7	<5.0	<5.0	6
MORGANS GULCH CHAFFEE, CO	06/15/98		32.9		17.4	73	<10	<0.20	1.0	204	2.4	<5.0	<5.0	<5.0
MOUNT BETHEL	06/02/97					343	<10	<0.20	2.8	208	6.3	<5.0	<5.0	<5.0
MOUNT BETHEL	06/16/97					204	<10	<0.20	7.2	131	10	17.5	<5.0	5
MOUNT BETHEL	05/20/98	11.8	39.7	7.51	22.8	200	<10	<0.20	1.5	151	3.8	<5.0	<5.0	<5.0

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MOUNT BETHEL	07/23/98	14.4	67.4	8.66	38.4	20	<10	<0.20	<1.0	37	4.1	<5.0	<5.0	<5.0
MOUNT BETHEL	08/22/98		83.5		44.8	24	<10	<0.20	<1.0	67	6.4	<5.0	<5.0	<5.0
MOUNT BETHEL	06/09/00		49	6.93	25.4	89	<15	<0.15	1.9	103	<10	<2.0	<2.0	<10
MOUNT BETHEL	07/13/00		82	7.87	39.8	77	<15	<0.15	<1.0	138	11	<2.0	<2.0	<10
MOUNT BETHEL	07/31/00		95	7.75	46.6	39	<15	<0.15	1.0	112	15	<2.0	<2.0	<10
N. FORK ELK RIVER UPPER SITE	06/08/00		32	6.87	16.4	37	<15	<0.15	3.1	225	<10	<2.0	<2.0	<10
N. TEN MILE CREEK NT6	06/22/98					27	<10	<0.20	2.7	115	13.3	<5.0	<5.0	<5.0
N. TEN MILE HIGHEST	06/23/97	22.0	70	7.55	35.0	28	<10	<0.20	<1.0	103	7.1	<5.0	<5.0	<5.0
N. TEN MILE RELOCATION POND	06/23/97	22.6	56	7.22	19.8	79	<10	<0.20	2.1	227	13.3	<5.0	<5.0	8.2
N. TEN MILE UPPER	06/23/97	21.9	63	7.42	30.4	132	<10	<0.20	1.5	583	48.9	<5.0	<5.0	16.2
N. TEN MILE UPPER LITTLE POND	06/23/97	21.8	105	7.5	58.2	80	<10	<0.20	<1.0	1356	122.2	<5.0	<5.0	42.8
N. TENMILE CREEK NT4,5,&6	06/22/98					34	<10	<0.20	1.6	120	9.2	<5.0	<5.0	<5.0
N. TENMILE CREEK NT5	06/22/98					18	<10	<0.20	<1.0	151	30.2	<5.0	<5.0	<5.0
N. TENMILE CR-LOWER BREEDING	07/20/00		72	7.15	36.0	38	<15	<0.15	<1.0	166	<10	<2.0	<2.0	<10
N. TENMILE CR-UPPER BREEDING	07/20/00		78	7.12	42.2	17	<15	<0.15	<1.0	150	<10	<2.0	<2.0	<10
N. TENMILE UPPER	06/07/00		59	7.55	35.4	34	<15	<0.15	<1.0	67	<10	<2.0	<2.0	<10
NFS3	07/16/99		6.95	109	41.8	37	<15	<0.15	2.5	880	37	<2.0	<2.0	<10
NORTH WILLOW CREEK	07/09/98	16.0	25.1	6.5	13.0	152	<10	<0.20	1.9	299	15.3	<5.0	<5.0	<5.0
NT2	07/02/99		74	7.12	40.4	109	<15	<0.15	3.3	2907	213	2.4	2.8	<10
NT4	05/28/99		53	6.91	26.2	143	<15	<0.15	5.6	341	19	2	<2.0	<10
NT4	06/11/99		59	6.97	29.2	43	<15	<0.15	2.2	55	<10	<2.0	<2.0	<10
NT4	07/02/99		59	7.08	36.6	132	<15	<0.15	1.5	179	13	<2.0	<2.0	<10
PC1	06/09/99		63	6.78	15.8	68	<15	0.66	7.4	696	95	<2.0	<2.0	424
PERU CREEK	07/15/97	22.3	80	7.12	18.2	58	<10	0.41	10.3	410	61	5.3	<5.0	242.5
PERU CREEK	06/25/98					30	<10	0.41	6.8	500	42	<5.0	<5.0	343.5
PERU CREEK	05/23/00		57	6.98	11.8	26	<15	0.24	4.7	519	64	<2.0	<2.0	388
PINGREE PARK TWIN LAKES U POND	07/24/98	13.0	23.6	7.81	15.4	1704	<10	<0.20	3.6	1212	19.3	<5.0	<5.0	10.9
POLE CREEK	06/02/00		71	7.35	34.6	418	<15	<0.15	2.6	985	231	<2.0	<2.0	<10
POLE CREEK	08/12/00		187	7.70	105.8	238	<15	<0.15	2.1	1009	123	<2.0	<2.0	<10
POLE CREEK #15	06/15/97					225	<10	<0.20	1.5	286	24.5	<5.0	<5.0	<5.0
POLE CREEK #4	06/09/97	21.2	61	7.10	28.6	306	<10	<0.20	1.1	301	15	<5.0	<5.0	<5.0
POLE CREEK HOLE 15	06/02/00		54	7.31	28.0	121	<15	<0.15	<1.0	212	19	<2.0	<2.0	<10
POND AT EGGELSTON	08/03/98	12.5	74.2	8.27	42.4	117	<10	<0.20	1.0	100	10.8	<5.0	<5.0	<5.0
POND S OF MESA/DELTA LINE E65	08/04/98	12.5	17.1	7.87	8.8	67	<10	<0.20	2.0	122	11.4	<5.0	<5.0	6.6
RAINBOW LAKE	05/22/99					<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
RAINBOW LAKE	05/14/00		79	7.44	42.8	89	<15	<0.15	1.6	2047	76	<2.0	<2.0	<10

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
ROCK CREEK PARK	08/05/99					18807	28	0.79	19.8	28041	540	14.6	4.6	57
S. COTTONWOOD	07/19/00		95	7.12	44.8	96	<15	<0.15	<1.0	414	58	<2.0	<2.0	<10
S. COTTONWOOD BREEDING	05/16/00		92	7.48	49.2	226	<15	<0.15	1.9	467	<10	<2.0	<2.0	<10
S. COTTONWOOD NEW POND	07/21/00		103	7.10	50.0	109	<15	<0.15	2.1	1919	272	<2.0	<2.0	<10
S. COTTONWOOD NON -BREEDING	06/23/00		108	7.13	53.4	20	<15	<0.15	1.2	243	12	<2.0	<2.0	<10
S. COTTONWOOD NON-BREEDING	05/16/00		93	7.27	48.2	33	<15	<0.15	1.1	294	<10	<2.0	<2.0	<10
S. COTTONWOOD WEST	05/16/00		110	7.37	48.0	55	<15	<0.15	1.6	1193	53	<2.0	<2.0	<10
S. COTTONWOOD WEST NEW POND	06/22/00		99	7.25	40.4	41	<15	<0.15	2.0	778	83	<2.0	<2.0	<10
S. COTTONWOOD WEST OLD POND	06/22/00		148	7.27	56.8	66	<15	<0.15	2.1	1323	39	<2.0	<2.0	<10
SAYRES GULCH CHAFFEE CO	09/06/97	10.5	92	7.1	57.4	68	<10	<0.20	<1.0	45	1.7	<5.0	<5.0	<5.0
SAYRES GULCH CHAFFEE CO	09/13/98		112.1		59.2	56	<10	<0.20	1.8	203	19.2	<5.0	<5.0	<5.0
SAYRES GULCH CHAFFEE CO	06/17/99					30	<15	<0.15	4.1	507	26	<2.0	<2.0	<10
SECOND POND ABOVE TRICK215GMI2	08/03/98	13.2	38.5	7.66	17.6	40	<10	<0.20	1.3	208	21.1	<5.0	<5.0	<5.0
SNAKE RIVER SR2	06/25/98					13	<10	<0.20	1.5	75	18	<5.0	<5.0	10.8
SNAKE RIVER SR3	06/25/98					14	<10	<0.20	2.4	18	15.9	<5.0	<5.0	110.9
SODA CREEK	06/09/97	21.5	20	7.17	7.4	174	<10	<0.20	2.1	110	9.5	<5.0	<5.0	7.4
SODA CREEK	05/24/99		018	4.5	8.4	132	<15	<0.15	<1.0	70	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD	06/23/00		92	7.04	42.8	57	<15	<0.15	1.2	230	10	<2.0	<2.0	18
SOUTH COTTONWOOD CHAFFEE CO	09/04/99		152	7.78	99.4	32	<15	<0.15	1.9	218	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD CHAFFEE, CO	06/01/98		109.7		57.6	66	<10	<0.20	1.4	221	5.7	<5.0	<5.0	<5.0
SOUTH COTTONWOOD CHAFFEE, CO	09/07/98		106.1		55.6	84	<10	<0.20	2.6	463	7.6	<5.0	<5.0	<5.0
SOUTH COTTONWOOD CHAFFEE, CO	06/03/99		63	6.99	28.2	48	<15	<0.15	5.1	129	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD CHAFFEE, CO	06/07/99					41	<15	<0.15	2.5	1094	43	<2.0	<2.0	<10
SOUTH COTTONWOOD WEST CHAFFEE	08/30/98					24	<10	<0.20	<1.0	1800	109.5	<5.0	<5.0	<5.0
SOUTH COTTONWOOD WEST CHAFFEE	09/04/99		136	7.59	75.8	26	<15	<0.15	1.6	887	52	<2.0	<2.0	<10
SOUTH FORK BIRD CREEK	07/02/99					91	<15	<0.15	2.4	799	120	<2.0	<2.0	13
SR6	06/08/99					414	<15	2.55	1.5	515	428	8.7	<2.0	2021
SR7	07/16/99		102	7.05	7.2	197	<15	0.72	3.5	875	265	3.3	<2.0	211
STAIRWAY	07/14/00		23	6.30	9.6	46	<15	<0.15	<1.0	225	13	<2.0	<2.0	<10
STAIRWAY POND 1	08/03/00		21	5.79	9.8	113	<15	0.54	7.9	270	15	<2.0	<2.0	24
STAIRWAY POND 3	06/29/00		27	5.05	3.2	38	<15	<0.15	<1.0	139	<10	<2.0	<2.0	<10
STAIRWAY ROUTT CO.	06/20/00		14	7.43	6.0	20	<15	<0.15	<1.0	58	<10	<2.0	<2.0	<10
STAIRWAY ROUTT N. FOREST	08/04/99		020	4.5	10.6	48	<15	<0.15	1.5	120	<10	<2.0	<2.0	<10
STAIRWAY ROUTT N. FOREST	08/23/99		018	5.0	9.4	49	<15	<0.15	1.9	184	<10	<2.0	<2.0	<10
STAIRWAY SITE ROUTT CO.	06/12/00		16	6.81	7.6	24	<15	<0.15	<1.0	50	<10	<2.0	<2.0	<10
STRAIT CREEK	06/26/98					38	<10	<0.20	3.7	81	10.3	<5.0	<5.0	<5.0

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
TA BRAGG	08/05/99					97	<15	<0.15	3.0	649	44	<2.0	<2.0	21
TEXAS CREEK GUNNISON CO	08/21/99		042	7.65	23.8	88	<15	<0.15	1.1	364	13	<2.0	<2.0	<10
TRIANGLE PASS	06/29/98	11.2	23.4	8.39	5.0	54	<10	<0.20	<1.0	217	4.7	<5.0	<5.0	<5.0
TRIANGLE PASS	07/27/98	12.0	29.0	7.56	3.6	137	<10	<0.20	1.1	373	19.3	<5.0	<5.0	<5.0
TRIANGLE PASS	08/24/98		40.5		19.8	83	<10	<0.20	1.6	287	23.2	<5.0	<5.0	<5.0
TRIANGLE PASS	09/30/98		55.1		28.6	67	<10	<0.20	1.6	464	17.3	<5.0	<5.0	6.9
TRIANGLE PASS	06/23/99		72	7.74	46.4	78	<15	0.23	4.1	341	25	<2.0	<2.0	<10
TRIANGLE PASS	08/12/99		031	7.75	15.4	108	<15	<0.15	1.6	463	30	<2.0	<2.0	<10
TRIANGLE PASS	09/02/99		021	7.61	34.8	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
TRIANGLE PASS	06/09/00		50	7.40	25.4	183	<15	<0.15	<1.0	615	33	<2.0	<2.0	<10
TRIANGLE PASS	07/20/00		42	6.49	16.6	24	<15	<0.15	<1.0	657	35	<2.0	<2.0	<10
UPPER BUCK MTN POND #1	08/07/00		37	6.73	15.2	105	<15	<0.15	3.2	4220	25	<2.0	<2.0	<10
UPPER BUCK MTN POND #2	08/07/00		34	6.75	16.0	52	<15	<0.15	6.3	553	<10	<2.0	<2.0	25
UPPER N. FORK SNAKE	05/23/00		96	7.09	36.2	36	<15	<0.15	<1.0	81	<10	<2.0	<2.0	<10
UPPER N. TENMILE	06/09/00		59	6.86	32.0	60	<15	<0.15	<1.0	99	<10	<2.0	<2.0	<10
URAD-HENDERSON 1-POND	08/19/99		200	7.18	70.8	99	<15	0.15	3.3	101	879	<2.0	<2.0	65
URAD-HENDERSON 2 POND	05/14/97		1128	7.68	268.0	3111	<10	1.89	5.4	1055	<1.0	7.7	6.3	1285
URAD-HENDERSON 2 POND	07/22/98	14.2	181.3	7.20	51.2	306	<10	0.78	3.1	44	1484.2	<5.0	<5.0	225.8
URAD-HENDERSON ANN'S POND	06/10/97					2553	<10	2.21	13.2	1706	1264.6	57.8	<5.0	412.5
URAD-HENDERSON ANN'S POND	05/27/98	10.9	112.6	5.8	5.6	2856	<10	7.28	20.2	292	4344.5	14.6	<5.0	1226
URAD-HENDERSON ANN'S POND	07/08/98	12.3	39.7	7.04	14.0	537	<10	0.26	8.0	545	278.8	20.5	<5.0	48.5
URAD-HENDERSON ANN'S POND	07/13/99		44	7.40	13.4	337	<15	0.16	4.9	182	103	3.7	<2.0	37
URAD-HENDERSON DONUT	05/27/98	10.8	57.1	7.28	4.8	372	<10	0.72	3.2	139	1549.6	6.1	<5.0	185.3
URAD-HENDERSON DONUT	07/07/98	13.3	57.1	7.19	15.8	212	<10	<0.20	2.9	84	148.1	<5.0	<5.0	41
URAD-HENDERSON DONUT	07/13/99		51	7.48	15.8	239	<15	<0.15	3.0	45	112	<2.0	<2.0	17
URAD-HENDERSON DONUT	08/19/99		55	7.42	17.8	1155	<15	0.21	5.8	923	346	7.4	<2.0	61
URAD-HENDERSON DONUT	05/16/00		49	6.52	4.4	390	<15	0.21	2.8	410	795	15.3	<2.0	115
URAD-HENDERSON DONUT	07/13/00		63	7.62	20.0	133	<15	<0.15	<1.0	96	136	3.6	<2.0	17
URAD-HENDERSON ECLAIR	10/02/97	11.5	1154	3.2		34710	18	13.38	31.9	<10	8850.7	55	5.9	<5.0
URAD-HENDERSON ERIN'S POND	05/15/97		307	7.04	14.0	1492	<10	0.93	9.8	937	2612.6	12	<5.0	330.5
URAD-HENDERSON HESBO	05/15/97		80	7.72	22.8	421	<10	<0.20	1.5	221	244.4	<5.0	<5.0	8.2
URAD-HENDERSON HESBO	05/28/98	11.5	102.6	7.45	44.4	106	<10	<0.20	1.8	75	258.1	<5.0	<5.0	9.7
URAD-HENDERSON HESBO	07/21/98	14.3	100.0	6.94	99.4	284	<10	<0.20	3.6	250	2397.9	<5.0	<5.0	38.4
URAD-HENDERSON HESBO	07/09/99		179	7.18	68.8	50	<15	<0.15	1.1	46	672	<2.0	<2.0	<10
URAD-HENDERSON HESBO	08/19/99		184	7.04	71.2	31	<15	<0.15	1.8	29	401	<2.0	<2.0	<10
URAD-HENDERSON HESBO	05/16/00		103	7.7	39.6	223	<15	<0.15	<1.0	193	405	<2.0	<2.0	12

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
URAD-HENDERSON HESBO	07/13/00		280	7.33	62.6	242	<15	<0.15	<1.0	208	740	2.2	<2.0	21
URAD-HENDERSON JS POND	08/19/99		169	7.09	72.4	122	<15	<0.15	7.2	866	3044	<2.0	2.0	15
URAD-HENDERSON JS POND (STMNT)	07/22/98	14.2	181.3	7.20	51.2	262	<10	0.33	17.5	601	3437.9	5.7	<5.0	32.1
URAD-HENDERSON POWER ALLEY	05/15/97		64	7.79	2.04	1112	<10	<0.20	2.4	606	49	<5.0	<5.0	28.6
URAD-HENDERSON POWER ALLEY	05/28/98	11.1	102.6	7.40	20.6	142	<10	<0.20	2.4	129	55.7	<5.0	<5.0	30.3
URAD-HENDERSON POWER ALLEY	07/08/98	13.1	58.8	6.98	25.6	23	<10	<0.20	2.0	236	150.3	<5.0	<5.0	32.1
URAD-HENDERSON TREATMENT	05/28/98	11.7	100.0	7.23	35.8	176	<10	0.35	5.6	77	279	<5.0	<5.0	24.9
URAD-HENDERSON TREATMENT	07/08/98	13.2	100.0	6.75	97.2	29	<10	1.27	8.6	160	2662.7	<5.0	<5.0	52.3
URAD-HENDERSON TREATMENT	07/09/99		1087	7.26	60.0	74	<15	0.88	6.1	45	357	<2.0	<2.0	50
URAD-HENDERSON TREATMENT	08/19/99		836	7.16	93.4	132	<15	0.50	7.3	194	294	<2.0	<2.0	54
URAD-HENDERSON UPPER URAD	06/25/97					553	<10	1.14	6.1	239	<1.0	<5.0	<5.0	468.2
URAD-HENDERSON UPPER URAD	07/08/98	15.4	100.0	6.66	11.8	390	<10	0.62	5.9	143	<1.0	5.9	10.7	426.9
URAD-HENDERSON UPPER URAD	07/13/99		546	6.75	12.0	559	<15	1.99	16.8	133	33800	3.1	4.3	788
URAD-HENDERSON UPPER URAD	06/07/00		549	6.72	10.0	1453	<15	1.49	2.7	75		<2.0	5.8	545
VINTAGE BELOW HEND CLEAR CREEK	05/15/97		135	7.41	20.2	267	<10	0.43	3.5	209	218.9	<5.0	<5.0	144.8
WEST BRUSH CREEK	06/24/99		32	6.58	9.8	104	<15	<0.15	1.7	433	17	<2.0	<2.0	<10
WEST BRUSH CREEK	08/31/99		185	7.87	133.8	17	<15	<0.15	1.2	558	45	<2.0	<2.0	<10

APPENDIX B

Draft Candidate Conservation Agreement Between Climax Molybdenum Company, Colorado Division of Wildlife, and U.S. Fish and Wildlife Service.

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CANDIDATE CONSERVATION AGREEMENT WITH ASSURANCES

FOR THE BOREAL TOAD

BETWEEN

CLIMAX MOLYBDENUM COMPANY,

COLORADO DIVISION OF WILDLIFE

AND

U.S. FISH AND WILDLIFE SERVICE

February 2001

CANDIDATE CONSERVATION AGREEMENT WITH ASSURANCES

FOR THE BOREAL TOAD

BETWEEN
CLIMAX MOLYBDENUM COMPANY

COLORADO DIVISION OF WILDLIFE

AND

U.S. FISH AND WILDLIFE SERVICE

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CANDIDATE CONSERVATION AGREEMENT WITH ASSURANCES

FOR THE BOREAL TOAD

BETWEEN
CLIMAX MOLYBDENUM COMPANY

COLORADO DIVISION OF WILDLIFE

AND

U.S. FISH AND WILDLIFE SERVICE

I. Introduction

On June 17, 1999, the U.S. Fish and Wildlife Service (Service) and National Marine Fisheries Service jointly issued a Final Policy for Candidate Conservation Agreements with Assurances (CCAA's or Agreements) under the Endangered Species Act of 1973, as amended (64 FR 32726). The Agreements are intended to provide landowners incentives to promote implementation of voluntary conservation actions for proposed and candidate species, or those species that are likely to become candidates or proposed species. Ultimately, these Agreements will preclude or remove the need to federally list a species should all necessary property owners enter into Agreements.

Once common in the southern Rocky Mountains, the boreal toad has experienced dramatic declines in population over the past 15 to 20 years. Reasons for declines have not been definitively identified, and may be various, including effects of acidification of water, effects of heavy metals and other toxins in waters, new or more virulent strains of pathogens, habitat disturbance, or a combination of factors, leading to stress-induced immunosuppression, and, hence, increased susceptibility to naturally occurring pathogens. Recent developments point strongly toward pathogens, specifically a species of chytrid fungus (*Batrachochytrium dendrobatitis)* – as being a major causative agent in declines of certain species of amphibians, including the southern Rocky Mountain boreal toads.

The boreal toad is presently listed as an endangered species by both Colorado and New Mexico, and is a protected species in Wyoming. The U.S. Fish and Wildlife Service has classified the southern Rocky Mountain population of the boreal toad as a candidate species which is "warranted but precluded" for federal listing – meaning that there is adequate justification and information to warrant federal listing as threatened or endangered, but listing has been postponed, as there are presently other species in greater need of listing, and the U.S. Fish and Wildlife Service has limited resources to prepare and process listing packages.

Pursuant to the listing of the boreal toad as endangered in Colorado, a recovery plan for the boreal toad was developed by the Colorado Division of Wildlife (CDOW) in 1994 (revised 1997), and an interagency recovery team was formed that same year. In 1998, the existing Recovery Plan was updated and combined with an existing draft Conservation Strategy to create a comprehensive *Boreal Toad Conservation Plan and Agreement* (Conservation Plan) for the southern Rocky Mountains. As part of the conservation planning process, Conservation Agreements have been signed by eight involved state and federal agencies and by the

Colorado Natural Heritage Program, outlining and confirming their respective roles in implementing the Conservation Plan.

The recovery criteria and conservation actions described in the Conservation Plan helped form the population and habitat conservation goals and conservation measures in this Agreement. The conservation goals are the minimum population level and habitat characteristics to be maintained on Climax's Henderson Mine and Urad properties covered in this Agreement.

Since 1995, Climax and the CDOW have been signatories to an MOU. This MOU allows the CDOW and other cooperating agencies on the Boreal Toad Recovery Team access to the boreal toad breeding sites located at Henderson Mine and Urad. By signature of this Agreement, Climax Molybdenum Company ("Climax") enters into an Agreement for the Henderson Mine that Climax operates in Clear Creek County, Colorado, and for the reclaimed Urad Mine that Climax owns in Clear Creek County, Colorado. These two properties will be referred to collectively as the Henderson Mine and Urad. The Henderson Mill property in Grand County, Colorado, will be discussed later in this document.

II. Habitat Requirements

The southern Rocky Mountain population of boreal toads occupy forested wetland and upland habitats between 7,500 feet and 12,000 feet. Forest types consist of lodgepole pine or spruce-fir forests. A few toads have been found in ponderosa pine and willow/sage communities at their lower elevational limits.

Habitat for the boreal toad can be divided into three types: breeding, summer (juvenile and adult), and over-winter habitat. Based on research in Colorado, all three habitat types are variable indicating flexibility in habitat requirements by the boreal toad. Breeding habitat may consist of anything from a puddle in the road to a warm bay in a lake or reservoir. Eggs are often laid on the northern shore of water bodies; likely as a result of higher water temperature due to southern exposure to the sun. The key to successful breeding habitat is the availability of warm shallow water and water level stability through October. Riparian habitats are used sporadically by adults during the summer and riparian vegetation is important to toadlet survival following metamorphosis. Metamorphs must have vegetative protection to successfully disperse and seek out overwinter locations their first year. In locations without riparian vegetation the metamorphs often form post-metamorphic aggregations and eventually freeze or desiccate.

If the toads successfully metamorphose and overwinter the first year they generally disperse to upland montane habitats where they seek out spring seeps and underground burrows where they remain until they reach sexual maturity and return to breed. Similarly, adult boreal toads seek out upland habitats where they spend most of the summer and hibernate. Hibernacula generally consist of ground squirrel burrows or holes under stream banks where they can maintain temperatures above freezing through the winter. Often the hibernacula are within 800 yards of breeding sites, especially for males. The male boreal toads of breeding age return to the same site each year whereas female boreal toads may only breed once or twice in their lifetime.

III. Existing Conditions

The Henderson Mine and Urad are located west of Empire, Colorado, at an elevation range of 10,000 to 10,500 feet. The Henderson Mine and Urad, which are adjacent to each other, encompass a total of 2,897 acres.

Figure 1 (with inset) illustrates the approximate location and habitat of the Henderson Mine and Urad. The Henderson Mine is operated by Climax in accordance with a reclamation permit from the Colorado Department of Natural Resources, Mined Land Reclamation Board. The reclamation permit is current and in compliance with Colorado Division of Minerals and Geology, Office of Mined Lands, Minerals Program Rules and Regulations. Urad was operated by Climax between 1967 and 1974 prior to the Colorado Mined Land Reclamation Act and was closed and reclaimed voluntarily in the 1970s. Surface disturbance at the Henderson Mine is approximately 121 acres. At the mine site, it is unlikely that there will be any additional disturbance. The only current activity at Urad is the operation of a water treatment plant to treat seepage from the two Urad tailings facility and water generated in the dewatering of the Henderson Mine. The Henderson Mine has been in operation since 1976 and has an approximate mine life of an additional 20-25 years.

For the purposes of this Agreement the sites are divided into the following three categories of land use:

- Disturbed Land
- Undisturbed Land
- Reclaimed Land

Disturbed land is the land temporarily disturbed by the Henderson Mine during the mine life. Undisturbed land is that land which has not been and will not be disturbed by Henderson Operations. Reclaimed land is land disturbed by Henderson Operation activities and later reclaimed. All disturbed land will be reclaimed under the terms of the reclamation permit (CMLRB Permit #M-77-342). No additional reclamation activities are planned or required by permit at Urad. However, a wetlands mitigation plan has been submitted and approved by the U.S. Army Corp of Engineers to enhance an earlier wetlands mitigation project at Urad (Corps File No. 199280323). The original project was completed in the early 1990's and does encompass a large portion of the boreal toad habitat at Urad. The original mitigation plan called for the creation of 2.15 acres of wetlands. This plan was to offset wetland disturbances from the construction of a water collection system designed to eliminate water quality impacts to Woods Creek from the Urad tailings. However, due to several problems that won't be discussed here, only 0.48 acres of wetlands were successfully created during the original project.

The revised mitigation plan, submitted to the Corps on October 30, 2000 and accepted later that year, seeks not only to fulfill the original wetland requirements, but also to work cooperatively with the SERVICE and DOW to accomplish this task while improving boreal toad habitat. Many improvements can be incorporated into the wetlands mitigation plan to enhance breeding and winter/hibernation habitat for the boreal toads. See the revised mitigation plan for further details in Appendix A.

The Henderson Operation's boreal toad breeding locality consists of numerous ponds and wetlands in an area that has been heavily disturbed due to molybdenum mining by Climax. The specific breeding sites at this locality have been designated as follows: Power Alley, Hesbo, Treatment Pond, Donut, Anne's Pond, and Upper Urad. All breeding locations except for Power Alley exist on land previously disturbed in some way by mining activity. None are fed by treated or untreated process water. See Appendix B (pg. 79-84) in the April 2000 *Colorado Division of Wildlife Boreal Toad Research Progress Report, 1999* by Mark S. Jones for water quality data at these breeding sites. Figure 1 illustrates the approximate location of the existing breeding sites and other habitat at Henderson Operations.

Hesbo and 2-Pond were the main breeding locations in 1995 and 1996. Hesbo was the primary breeding site from 1997 to 1999. In 1995 and 1996 both sites were influenced by pretreated mine effluent running through them at an elevated temperature of 19-21 °C. Climax finished a new water treatment facility on the Urad side of the facility in 1997. As a result, 2-Pond is no longer an active breeding site and Hesbo has reduced water temperatures in the spring and no long term source of water. As a result of the water supply changes to Hesbo, water was pumped to the site weekly during the summers of 1998 and 1999. In an attempt to remedy this situation, Henderson Operations provided a backhoe to install a dam and water control structure to increase the depth of the channel in October 1998. Anne's Pond was also modified in 1998. The Hesbo modifications worked well in 1999 but Anne's Pond continued to dry up quickly. Even though Hesbo has the largest population of breeding adult toads, this site had no recruitment from 1995 to 1997. In 1998 and 1999, Lauren Livo removed Dyticid beetle larvae as part of her research, which resulted in substantial survival to metamorphosis in both years.

Power Alley is a beaver pond complex along the West Fork of Clear Creek and is the most natural breeding site in the area. It is not directly influenced by mine effluent and therefore the water temperature is colder than the previously mentioned sites and breeding occurs one to two weeks later. This site, however, has dried up during the last three years and desiccated the egg masses present.

Treatment pond is a man-made wetland complex that is dissected by the Urad Mill Road located north of the water treatment facility. Breeding activity is restricted to the pond(s) on the west side of the road. It does not have a large number of adults during breeding season but produced 10,000-15,000 toadlets each year from 1996 to 1999. Recruitment at this site is low as there is minimal overwinter refuge for toadlets.

Donut is a newer pond above the water treatment facility that serves as a catch basin for some of the upstream runoff. This site typically has 5-6 egg masses but because it is higher in elevation that the other sites, breeding occurs later making weather conditions post metamorphosis critical to toadlet survival and dispersal. In addition, there are few suitable hibernaculum close to this site. All toadlets froze in 1995 and 1996. We believe some toadlets survived in 1997. Survival of toadlets was good in 1998 and 1999, presumably a result of increased vegetation and small mammal burrows on the islands.

Anne's Pond is a small wetland area south of Donut that is fed by ground water and runoff. Because the average depth is less than 10 cm, the water temperature stays warm and tadpoles grow quickly. In 1996 this pond had several thousand tadpoles but dried up in July. At the request of the DOW, Henderson Mine personnel put in a water supply pipe to keep the water level constant, which resulted in successful recruitment in 1997 and 1998. In October 1998, a backhoe was utilized to increase the main channel depth and a side channel was added. Both of these features drain to a deep-water thermal refuge. As a result of water levels decreasing too quickly in 1999, all egg masses desiccated.

Upper Urad is a large, man-made wetland area at the west end of the valley at an elevation of 10,500 feet. Due to the elevation, this is the last site for breeding activity each year. It produced toadlets in 1995 and 1996 but they froze in 1995 and were eaten by sandpipers in 1996. No successful reproduction occurred at this site from 1997 to 1999.

Research in this geographical area to date has focused on habitat and hibernacula use, toad movements, and population structure and dynamics. Boreal toad breeding at Henderson Operations usually begins around the second week of May each year at the lower elevation sites and progresses into June at the higher breeding sites. All of the habitat preference work completed thus far by Mark Jones indicates a summer preference for rock/grass areas (primarily in reclaimed areas). Research also indicates that the man-made breeding sites have always been more successful than Power Alley, the one natural site. The limiting factors of all breeding sites are listed in the April 2000 *Colorado Division of Wildlife Boreal Toad Research Progress Report, 1999* by Mark S. Jones under the site descriptions and background section on pages 1-2. In summary, Hesbo and Anne's Pond lack stable water, Power Alley lacks stable water due to beavers, Treatment lacks protection for metamorphs and hibernaculum for metamorphs, while Donut is very close to optimum - could use a little better protection for metamorphs. Again, reference the above-mentioned report by Mark S. Jones, Table 5 on page 21 for the latest population estimates The population estimates for 2000 indicate there are only about 40 breeding age males left in the population.

Reproduction and survival through 1999 [prior to chytrid fungus] appeared to be adequate to sustain the population and, in fact, this population was one of two on the Front Range that qualified as a viable population under the Conservation Plan. Despite the reproductive success and viability of this population, all breeding locations at Henderson Operations have some aspect that is sub-optimum. For example, the lack of a stable water supply at Anne's Pond and Hesbo throughout the summer frequently causes egg masses to desiccate as the pools dry up. Treatment Pond has had successful reproduction and metamorphosis since at least 1995 but lacks overwinter refugia and, therefore, does not recruit. Problems with the remaining sites are listed in the previously paragraph. Very good summer and over-winter habitat appears to exist over the rest of the undisturbed and reclaimed land areas at the Henderson Operations. Riparian vegetation only occurs around Power Alley, Hesbo, Donut, Lower Urad Reservoir, and the Upper Urad Wetlands. The newly revised wetlands mitigation plan with the Corps should increase riparian vegetation around Treatment and Donut Ponds, as well as add a new breeding site to the area known as the Main Depression. See Appendix A for further plan details.

IV. Conservation Goals Agreed to be Maintained

The following conservation goals are the minimum population level and habitat characteristics agreed to be maintained by Climax. It is hoped that conservation measures will increase the population and acreage of habitat types above these goals. There are three habitat conservation goals agreed to be maintained. It is intended that these habitat conservation goals will be achieved within three years and maintained in perpetuity or until the boreal toad no longer requires State or Federal protection. The first is to maintain six breeding ponds of at least 10 feet in diameter. The ponds will be maintained in a condition no worse than they currently exist in with a good faith effort to improve their conditions and to maintain a stable water supply to them. All suitable breeding sites at Henderson are currently being utilized. The same conditions shall apply to any breeding and/or winter hibernation sites that are developed through the revised wetlands mitigation plan with the Corps. The second habitat goal is to maintain 2.15 acres of riparian habitat at Henderson Operations. Similar goals for the Henderson Mill will be established later after an evaluation has been completed and an agreement to introduce boreal toads has been reached. The third habitat goal is to maintain all existing hibernacula within 800 yards of breeding ponds at Henderson Operations. Henderson will encourage the production of hibernacula by adding root balls and soil adjacent to breeding ponds during the Corps mitigation work, but cannot be held accountable for work that can only be accomplished by small mammals. No conservation goal will be established for upland habitat as it is abundant and not a limiting factor for toad survival.

Due to an uncontrollable decrease of toads from chytrid fungus or other diseases it may be difficult to maintain the Henderson/Urad breeding locality (and Henderson Mill if established then stricken with disease) even with suitable habitat conditions. As long as the population faces a threat from chytrid fungus, it cannot be considered viable under the definitions posed in the Conservation Plan and Agreement (Loeffler 9ed.) 2000). However, as a goal, Climax agrees to maintain a population of 20 breeding adults. Population monitoring will continue to play an important role in tracking and researching the chytrid fungus and determination of recovery objectives

V. Conservation Measures Agreed Upon

Conservation measures in this Agreement will support the habitat conservation goals and are compatible with suitable habitat described in the Conservation Plan. If measures attributable to land management actions, do not maintain or increase the boreal toad population then additional conservation measures may be added to the Agreement or existing conservation measures altered with agreement of Climax. New or altered conservation measures will be developed by using the most up-to-date information and will be developed in cooperation with parties to this Agreement and members of the Boreal Toad Recovery Team. The following conservation measures should achieve the population and habitat conservation goals for the Henderson Operations covered under this agreement. Conservation measures, expected implementation dates, expected costs, and parties carrying out the measures [etc.] are identified in the Implementation Schedule in Appendix D.

Climax will incorporate terms of the 1998 MOU with CDOW [See Appendix E] into this Agreement, to allow access by CDOW and others to the non-industrial areas of Henderson Operations. Continuation of access will allow collection of environmental samples for the continued evaluation of disease, habitat, hibernacula, toad movement, and population structure and dynamics. However, access to the industrial area has been limited to date to assure that all Mining Safety and Health Administration (MSHA) training requirements have been met for all CDOW employees entering the property. Although few, there have been toad sightings on industrial areas.

Translocation of boreal toads to the Henderson Mill may proceed if and when approved by the Boreal Toad Recovery Team and Climax. If translocation occurs, this Agreement shall be amended to include the Henderson Mill and to set conservation measures and population goals specifically for the Mill. Such introduction will depend on a determination that it is consistent with the Boreal Toad Conservation Plan and would not result in harm to Climax and potential future land uses. Since tailings deposition occurs at the mill site, there will be on going land disturbance at that property. Minimal future impacts are anticipated at the mine property during the life of Henderson.

Climax will, under the direction of the CDOW and recommendations of the Recovery Team and Conservation Plan, and within the restrictions of water rights, make improvements to the breeding sites identified as Anne's Pond and Hesbo. Improvements will be directed towards sustaining a sufficient water supply to the sites from May to October, as water is only critical during breeding, to attempt to prevent desiccation of the egg masses and tadpoles. Pumping water, installing permanent supply pipes and/or other options will be considered to maintain the water supply. Improvements at other existing breeding ponds as recommended by CDOW and the Recovery Team will be considered by Climax. Evaluation of water management needs for the Henderson Mill area will be determined when and if translocation is agreed upon.

Treatment Pond recruitment problems will attempt to be addressed by providing artificial hibernacula through placement of logs, root wads, rocks, boring of holes or other methods. Three to five hibernacula will be created per acre. Little research has been done on creation of artificial hibernacula so this will be an experiment requiring monitoring. The Recovery Team should review the monitoring methodology. Artificial hibernacula may be created around Donut and Anne's Pond where the upland or riparian habitat has been disturbed if this process is successful around Treatment Pond.

Based on the approved Revised Mitigation Plan with the Corps, riparian habitat will be increased to 0.22 acres around Constructed Pond (Donut Study Site), 0.49 acres around the Main Depression (Donut Study Site), 0.15 acres around the Catchment Basin (Donut Study Site), and 1.05 acres around the Tailings Wetlands (Treatment Pond area) for a total of 2.15 additional acres of riparian habitat.

Existing breeding sites will only be disturbed to mitigate sub-optimum conditions as mentioned above.

VI. Expected Conservation Benefits

1. The conservation measures will maintain stated habitat conservation goals that, in turn, will maintain a viable population into the future, or opportunity for a viable population into the future should disease temporarily suppress the population.

2 Suitable habitats identified herein which currently exist at the Henderson Mine and Urad will be maintained.

3 Continued access by Recovery Team personnel will provide a location to study the chytrid fungus and it's effect on boreal toads.

4. Based on the recommendations of the Recovery Team, the CDOW, and USFWS, Climax may allow for future translocation to the Henderson Mill to preserve existing genetic stock and to potentially create another breeding locality and viable population. However, only if such translocation will not interfere with Climax's ability to operate the Henderson Mill.

VII. Assurances Against Future Regulation

Should the boreal toad become federally listed, additional conservation measures other than those identified herein will not be required and additional restrictions will not be imposed by the Service on their own accord or through consultation on future environmental permits. Authorization for incidental take back to levels consistent with the habitat and population conservation goals are disclosed in the section 10 (a) (1) (A) Enhancement of Survival Permit attached as Appendix F.

In addition, the USFWS and CDOW recognize that Climax secures water for its Henderson Operations through specific rights and decrees. That water may be used and diverted for its operations in accordance with those rights and decrees and further that such rights and decrees may limit Climax's ability to carry out certain enhancement projects or recommended actions. Likewise, existing and future environmental or reclamation permits regulating the Henderson Operations may preclude Climax from undertaking certain enhancement projects or recommended actions.

VIII. Habitat and Population Monitoring and Reporting

Habitat monitoring will be conducted by CDOW annually the first five years to determine if progress towards, and maintenance of, habitat goals are being achieved. Once habitat goals are achieved, monitoring will take place every three years until it is determined that the boreal toad no longer requires State or Federal protection. Monitoring of breeding pond water maintenance will only require ocular observation by CDOW, Climax, or other researchers every month from May to October or whenever water management changes are made. Upland habitat monitoring will not be required beyond visual observations as upland habitat is not a limiting factor. Riparian habitat monitoring will require length and width measurements of major habitat types and a species inventory. Hibernacula monitoring will require specific mapping and marking of artificial and natural hibernacula. Determination of artificial hibernacula use will occur through radio tracking, ocular observation, manual search with hands or nets, optic cable video-camera, or a combination of the above. Population monitoring will take place annually by the CDOW at Henderson Operations and the Henderson Mill site when and if translocation is conducted. Standardized breeding site monitoring will be conducted as specified in the Boreal Toad Conservation Plan. Monitoring of compliance with this Agreement will take place by the Service through annual reports, due December 31st of each year, and site inspections coordinated with Climax and CDOW when necessary.

IX. Notification of Take

Before any action that may incidentally take boreal toads occurs on the property, Climax shall notify the Service or the CDOW, so the agencies will be afforded a reasonable opportunity to rescue individual specimens of boreal toads before any authorized incidental taking occurs.

X. Transfer of Benefits

The benefits of this Agreement shall transfer automatically to any subsequent owner of the property. However, any subsequent owners must obtain a new section 10(a)(1)(A) permit since these are non-transferable in accordance with 50 CFR part 13.25. Climax agrees to notify the Service upon any transfer of land under this Agreement.

XI. Funding of Conservation Measures

Where appropriate, Climax may provide assistance at the site using existing personnel, equipment and facilities and, where appropriate will cooperate in efforts to secure funding for the recommended activities. The CDOW will provide funding and personnel for many of the management activities, research on chytrid fungus, habitat, and population monitoring. It is expected that supplies, equipment, and staff time for habitat conservation measures will cost $5,000 per year for Climax and approximately $90,000 per year for the CDOW. Staff time is expected to be 100 hrs/year for Climax employees and 700 hrs/year for CDOW employees. CDOW funding will likely come from Go Colorado (GOCO). The Service may be able to provide funding through the Partners for Fish and Wildlife Program, Endangered Species Act section 6 funds provided to the States (CDOW), or through the Landowner Incentive Program. Staff time for the Service is expected to be 24 hrs/year for review of conservation actions and compliance with the Agreement.

XII. Duration of Agreement

This Agreement will remain in effect until the boreal toad no longer has a special designation under State and Federal species lists.

XIII. Termination of the Agreement

Should Climax wish to discontinue this Agreement they may do so at any time by providing written notice to the Service.

Appendix A

Revised 404 Mitigation Plan

(To be supplied by Climax)

Appendix C

Finding of the Benefits of the Conservation Measures

The Service finds that the benefits of conservation measures implemented by Climax at its Henderson Operations, when combined with those benefits that would be achieved it is assumed that conservation measures were also to be implemented on other necessary properties, would preclude or remove any need to list the boreal toad. The conservation measures should maintain habitat goals possible for continued support of a viable population. Barring further declines by the chytrid fungus, the conservation measures should maintain or increase the number of adult toads observed there in recent years. The population conservation goal of 150 adult toads is much greater than the minimum number considered a viable population in the Conservation Plan but should be attainable barring sustained declines by the chytrid fungus or other diseases. It is hoped that, at a minimum, the population will be maintained over a five year period and will maintain an average total of at least twenty (20) breeding adults producing an average of four (4) viable egg masses per year in concert with the second definition below of a viable population. All known habitat and environmental threats, under control of Climax, would be removed from the Henderson Operations' toads through this Agreement.

Recovery Objectives and Status

The objectives of the management and conservation actions outlined in the *Boreal Toad Conservation Plan and Agreement* and in this Candidate Conservation Agreement are to (1) prevent the extirpation of boreal toads from the area of their historic occurrence in the southern Rocky Mountains. This area includes eleven mountain ranges or geographic areas, covering southern Wyoming, much of Colorado, and a portion of northern New Mexico and (2) to avoid the need for federal listing of the boreal toad under the ESA, and (3) to recover the species to a population and security level that will allow it to be de-listed from its present endangered status in Colorado and New Mexico.

The present recovery objectives and criteria are based on objectives for boreal toad recovery formulated and previously approved by the interagency Boreal Toad Recovery Team in Colorado's *Boreal Toad Conservation Plan and Agreement.* The Colorado Division of Wildlife has already adopted these criteria and is pursuing conservation actions described in this plan for recovery of the boreal toad in Colorado. Should federal listing of this species occur, these criteria should be incorporated into any subsequent federal recovery plan for this species.

The following are criteria for downlisting and delisting of the boreal toad in the State of Colorado

To downlist from "endangered" to "threatened", there must be at least two (2) viable breeding populations of boreal toads in each of at least six (6) of the eleven (11) areas, or mountain ranges, of its historic distribution, AND the number of viable breeding populations throughout the historic range must total at least fifteen (15).

To delist the boreal toad in Colorado, there must be at least two (2) viable breeding populations of boreal toads in each of at least nine (9) of the eleven (11) areas, or mountain ranges, of its historic distribution, AND the number of viable breeding populations throughout the historic range must total at least twenty-five (25).

In order for a population of boreal toads to be considered "viable", it must meet the following criteria:

There must be documented breeding activity and recruitment to the population in at least two (2) out of the past five (5) years. However, if breeding activity has not been documented in the past three (3) years, there must be reliable observations of toads, including at least one sub-adult age class, in the area during at least two (2) of those three (3) years.

OR

There has been an average total of at least twenty (20) breeding adults at the breeding locality, producing an average of at least four (4) viable egg masses per year, and the number of breeding adults observed at the locality has remained stable or increased over a period of at least five (5) years.

AND

The population faces no known, significant and imminent threat to its habitat and environmental conditions.

For the purpose of interpreting the above criteria, the following definitions will apply:

Breeding population: Toads associated with one or more breeding localities which are located within a common second or third order drainage, and separated by no more than five (5) miles (approximately 8 km).

Breeding Locality: A geographic area containing one or more breeding sites with are separated by a distance of no more than ½ mile (approximately 0.8 km).

Breeding Site: A specific location in any body of water where toads congregate to breed and deposit eggs.

Recruitment: The presence of one-year-old toads in any given year will be considered to be successful recruitment from the previous year's breeding activity.

Appendix D

Implementation Schedule

(Make one that includes actions to be taken, by whom, when, cost estimates, and any other information necessary.)

Appendix E

1998 Memorandum of Understanding between Climax and CDOW

(To be supplied by CDOW)

APPENDIX F

Enhancement of Survival Permit for Incidental Take of Boreal Toads

(To be supplied by USFWS)