Colorado Division of Wildlife

Boreal Toad Research Progress Report

1995-1997

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Preface

The Boreal Toad Recovery Team was first convened in 1994 by John Goettl in an attempt to give direction to a multitude of independent efforts aimed at boreal toad research and recovery. Goettl pulled together a group which was made up of every "expert" he could find on boreal toad biology as well as representatives of interested state and federal agencies. The culmination of recovery team efforts are summarized in the Boreal Toad Recovery Plan (Goettl [ed] and the Boreal Toad Recovery Team 1997). The Boreal Toad Recovery Team is currently coordinated by Chuck Loeffler of the Colorado Division of Wildlife Aquatic Section.

John Goettl conducted field research on various aspects of boreal toad ecology until his retirement in January 1997. In addition, he initiated multiple contracts (MOU's) to leverage his time and the available resources to learn as much as possible as quickly as possible in the face of the severe boreal toad population declines which had been reported.

This report represents an attempt to consolidate boreal toad research sponsored by the Colorado Division of Wildlife from 1995 to 1997 into a single document to make this information available to members of the Boreal Toad Recovery Team and other interested parties. The various sections of this report cover preliminary results of :

- Research conducted under a Colorado Division of Wildlife (CDOW) MOU with Colorado State University on the experimental reintroduction of boreal toads to Lost Lake, Boulder County. This work is being conducted by Kirsta Scherff-Norris, a graduate student at CSU.

- Research conducted by the CDOW on habitat use, movements, and general life history aspects of boreal toads at the Climax Molybdenum Company mine near Empire, Colorado. John Goettl (before 1/97) and Mark Jones are the principal investigators.

- Research conducted by the CDOW on the toxicity of various metals to boreal toads. Steve Brinkman is the principal investigator.

- Research conducted by the CDOW on boreal toad tadpole ecology. The principal investigator is Lauren J. Livo.

- Research conducted under a CDOW MOU with the University of Colorado at Boulder on the molecular genetic determination of management units within the Southern Rocky Mountain population of boreal toads. The principal investigator is Anna M. Goebel.

Funding for boreal toad research and recovery efforts in Colorado have been provided by Great Outdoors Colorado.

REINTRODUCTION OF VARIOUS YEAR CLASSES OF BOREAL TOADS TO LOST LAKE, BOULDER COUNTY, COLORADO

Note: One of the research needs originally identified by the Boreal Toad Recovery Team was to conduct research related to reintroduction actions which includes determining rearing and propagation techniques and conducting experimental reintroduction. Reintroduction and captive propagation and rearing may be a vitally important technique in the future management of the boreal toad. Therefore, it was essential that the methods for reintroduction, propagation, and rearing be investigated and well-defined. Research on the captive propagation and breeding of boreal toads was conducted during 1995 and 1996 and has been previously reported (Scherff-Norris 1997).

INTRODUCTION

Amphibians appear to be declining worldwide (Blaustein et al. 1994, Pounds and Crump 1994, Beebee 1992, Griffiths and Beebee 1992, Wake 1991, Blaustein and Wake 1990), and more species in the Bufonidae family are in peril than in any other anuran family (Johnson 1992). In Colorado, the boreal toad, *Bufo boreas boreas*, is state-listed as "endangered" (Buhlmann 1994) and federally-listed as "warranted but precluded" (USFWS 1995). The endangered status in Colorado is defined as "any species of native wildlife whose prospects for survival or recruitment within the state are in immediate jeopardy" (CDOW 1985). The dramatic declines in boreal toad populations over the last 25 years that led the Colorado Division of Wildlife (CDOW) to list this species also prompted it to establish the Boreal Toad Recovery Team. One of the research needs identified by the Boreal Toad Recovery Team was experimental reintroduction (Goettl, ed. and the Boreal Toad Recovery Team 1997). Reintroductions of declining species are quickly becoming an important tool of conservation biology and wildlife management (Armstrong et al. 1994, ANZECC 1994, Bridgewater and Walton 1994, Johnson 1992, Chivers 1991, Reading et al. 1991, Stuart 1991, Mlot 1989). For example, in its indigenous fauna and flora transfer guidelines, the New Zealand Department of Conservation says that despite previous unsuccessful attempts, transfer of animals is a valuable technique that will continue to be used in the future (NZDC 1994). Factors affecting the success of reintroductions are poorly known (Armstrong et al. 1995, Griffith et al. 1989, Scott and Carpenter 1987), and because reintroduction may be a vitally important technique in future management of the boreal toad, it is essential that reintroduction methods be investigated and well-evaluated.

This study addresses this issue with an experimental reintroduction of various ages of boreal toads raised by different husbandry methods. This reintroduction was experimental as defined by Lindburg (1992) because it was geared toward developing expertise to be used if/when the wild population of boreal toads reaches a crisis stage. Toads were reintroduced in the summers of 1996 and 1997 and an area surrounding the reintroduction site was intensively monitored 6/97-8/97. The primary goal of this study was to determine whether various year classes of reintroduced boreal toads had different survival probabilities. This information would aid in determining the most efficient method for toad reintroductions to establish three self-sustaining populations in all mountain ranges of the toad's historic distribution, as outlined in the Boreal Toad Recovery Plan (Goettl, ed. and the Boreal Toad Recovery Team 1997). Additionally, I wanted to determine whether the different groups of toads showed relative preferences for different substrates. This could lead to a better understanding of habitat requirements of boreal toads. Finally, because toads were marked by toe clipping, I wanted to evaluate the effect of this common marking technique.

Background

Lost Lake was surveyed for boreal toads in years prior to reintroduction. Museum records report boreal toads at Lost Lake in 1960 and 1961 (Corn et al. 1989). Breeding was observed in 1987 (2 visits) and 1988 (3 visits), with 3 egg strands reported in 1988 (Corn et al. 1989). The last year that breeding was

reported was 1991 or 1992 (A. Goebel in Livo 1995) and the lake was thought to be devoid of a breeding population of toads. No toads or tadpoles were found in 1993 (A. Goebel and L. Livo in Livo 1995). No toads were found in four 1994 surveys (Livo 1995) or in a 1995 survey (M. Gasaway, pers. comm.) In 1996, one adult female was found on 5/30 and again on 7/2 (L. Livo, pers. comm.). A 7/25/96 survey found no toads (L. Livo, pers. comm.).

Site Description

The reintroduction site was Lost Lake, Boulder Co., Colorado, at 2,987m elevation. It is 1 km north of Bryan Mountain, Boulder County, Township 1 South, Range 74 West, Northeast Quarter, Section 24, USGS Quad: Nederland. Lost Lake is a high mountain lake in the Roosevelt National Forest. There is an active beaver at the lake, and the lake is heavily used for recreational fishing and hiking. No amphibians other than boreal toads were observed at Lost Lake during this study.

Reintroduction Guidelines

There are many guidelines evolving that outline necessary steps prior to, during, and following, reintroductions. Beebee (1996) listed several criteria as necessary for an amphibian reintroduction to proceed. This reintroduction met Beebee's criteria that habitat requirements be understood, the reintroduction site be suitable, and donor population be as near, geographically, as possible, to the reintroduction site, given current scientific knowledge. The restriction that the reintroduction should be monitored for more than five years has been met to date, and is anticipated to be fully met in the future. Beebee's requirement that tadpoles should be reintroduced was met, and they were from a single population with numerous parents. Reintroduction for at least two consecutive years was achieved in the newly metamorphosed year class (1996 and 1997 young of year toadlets), in accordance with Beebee's guidelines. The stipulation that the cause of species decline and absence from the reintroduction site be understood were not met, as the cause of decline of the boreal toad is still unknown. Although there are hypotheses (i.e., Carey 1993), the true cause of the decline remains unsolved. Through reintroductions of various year classes of toads, I can investigate whether the cause of decline was transitory and no longer in place, or is still present. If mortality is observed, I can run pathological tests in an attempt to determine the causes of mortality, and possibly causes of population decline at this site. Also, as pointed out by Armstrong et al. (1995), the requirement to eliminate the original cause of decline for a species before reintroduction is flawed in that it assumes that without the elimination the animals will perish. This does not take into account that the original cause of extinction may be undeterminable, and even if determined, it may not be the current limiting factor. In selecting the most suitable habitat for reintroduction, Southgate (1994) found that only experimental reintroductions can actually verify suitability.

Brambell (1977) outlined conditions in addition to those of Beebee which must be met before considering reintroduction of any captive endangered species. The condition that the reintroduction site can support more toads than at present can be assumed to have been met in this reintroduction, as the reintroduction site no longer supports a breeding population of boreal toads. The prerequisite that there was no needless introduction of "foreign" blood in the animals for reintroduction and that the captive stock maintained original genetic variability were met because reintroduced individuals were either first generation captive offspring or wild bred offspring reared in captivity. Brambell refers primarily to large mammals in his discussion, which explains his belief that individuals must be acclimated prior to release. There was little to no acclimation for the groups of toadlets reintroduced in this study. Finally, there was adequate funding for this project, as recommended by Brambell.

The International Union for Conservation of Nature and Natural Resources' (IUCN) definition of reintroduction is "the intentional movement of an organism into a part of its native range from which it has disappeared or become extirpated in historic times as a result of human activities or natural catastrophe" (IUCN 1987). The IUCN's guidelines for reintroduction include knowledge and elimination of the cause of

extinction of the animal and satisfaction of species' habitat requirements at the reintroduction site. Also, following reintroduction, monitoring is identified as a key component to the reintroduction program, as well as long-term research, if possible. The application of these guidelines to this reintroduction is discussed above. The IUCN calls for a feasibility study and preparation stage, in which the aforementioned requirements are met, as well as an investigation of local people's attitudes towards the reintroduction and establishment of sufficient funds for the project. There was no investigation of human attitudes to this reintroduction.

A review of 145 reintroductions of captive-born animals (mammals, birds, reptiles and amphibians, fish, and invertebrates) revealed that herpetological reintroductions were slightly more likely to be successful (17% versus 11% for mammals and birds) (Beck et al. 1994). The authors concluded that successful reintroductions extended over many years and released large numbers of animals. Although this reintroduction was only two years in duration, there were relatively large numbers of animals released (ca. 24,000).

In a survey of bird and mammal releases between 1973 and 1986, Griffith et al. (1989) stated that translocation may be an important technique for rare species due to increasing rates of extinction and declining biodiversity. Additionally, they pointed out a need for analyzing methods, results, and strategies of species translocations. According to their survey, translocations of endangered, threatened, and sensitive species had low success, even when translocations were into excellent habitat. They concluded that translocation must be considered before it is a last resort for a species with low density and declining populations. While these statements were made in reference to birds and mammals, they apply as well to amphibians. This reintroduction is such an attempt to investigate reintroduction strategies before reintroduction becomes the last option for conservation of the boreal toad. Burke (1991) stated that translocations (as well as relocations and repatriations) should be an option in any recovery program, regardless of the fact that it may not be a panacea. Campbell (1978) reported that wild-caught or first generation captive-bred animals are more likely to be successful after introduction than animals from several generations of captive breeding. All animals involved in this reintroduction were wild-caught or first generation captive-bred.

METHODS

The following age classes of boreal toads were reintroduced to Lost Lake: tadpoles, 1-4 week old toadlets, 9-10 month old toadlets, 22 month old toadlets, and adult toads. Health checks were performed on individuals prior to reintroduction, as recommended by Haebler (1992), Dodd and Seigel (1991), and Stanley Price (1991). Tadpoles were visually examined for stunting, abnormal swimming, deformities, or fungal infections. All metamorphosed individuals were checked for redness in the groin area, yellowish or dark skin, or excessive sloughing of skin, any of which could be indicators of bacterial or fungal infections. No deformed individuals were knowingly reintroduced.

Researchers disinfected their hands with Nolvalsan (Fort Dodge Laboratories, Inc.) and rinsed with water prior to handling toads. All toads were toe clipped with sterilized (dipped in Nolvalsan) iris or craft scissors. The entire toe on the front foot was removed and the toe to the edge of the webbing on the back foot was removed. The exposed area was treated with Bactine ® (Bayer Corp.). Martin and Hong (1991) found that anurans with open wounds treated with Bactine ® healed much quicker than those not treated. Bactine ® contains 2.5% lidocaine hydrochloride, and lidocaine (1-2%) is recommended as a local anesthetic for amphibians (Crawshaw 1992, Johnson 1991). Because of the uncertain effect of general anaesthesia on ectotherms, it may be advisable to avoid general anaesthesia if the procedure is minor (ASIH et al. 1987). I

used a batch mark for each group of toads, so that only one toe was cut per individual[1], due to Clarke's (1972) conclusion that recapture probability in Fowler's toad, *Bufo woodhousei fowleri*, decreased with the number of toes removed. The thumb, first toe on the front foot, and the longest toe on the back foot were not cut, as they are important in feeding, mating, and swimming. Upon reviewing Clarke's data, Gelder and Strijbosch (1996) concluded that Clarke would not have reached the above conclusion had he clipped only 2, 3, or 4 toes. The toe clip numbering scheme used was that of Martof (1953). Toe clips were saved for possible future DNA analysis and aging as recommended by Gonser and Collura (1996).

All reintroduced toads were monitored by Visual Encounter Surveys (Crump and Scott 1994) approximately three times per week, from 6/3/97 to 8/18/97. However, the data presented here are for the three sampling periods 6/30-7/14, 7/14-8/4, and 8/4-8/18, because the initial sampling period 6/3-6/27 was considered a pilot period and did not have adequate area coverage. The areas censused were: the perimeter of the lake (including vegetation on the water's edge), the area below the beaver dam along which the toadlets were reintroduced, and beaver ponds in the drainage below the lake. All areas (with the exception of the beaver ponds below the lake) were identified on a map with a UTM coordinate grid (1 or 5-m square cells). These areas were searched by walking transects 1-m wide (looking ½ m on either side of the transect). Vegetation was pushed aside in searching, but there was no destruction (i.e., moving logs or cutting vegetation) of the habitat. Surveys were conducted between mid-morning and mid-afternoon, generally on sunny days. When a toadlet was captured, the following was recorded: location (on a grid or map), substrate (grass, log, moss, mud, rock, or log), whether the toadlet was in full sun (yes or no), the toadlet's toe clip number, toe condition (good, fair, or poor[2]), and whether the toe was regenerating (yes, no, ?). If a toe was regenerating, the length of the regeneration was noted and the toe was re-clipped according to the above protocol. There were two night surveys to identify potential breeding activity by remnant resident toads. Any resident toads found were toe clipped (toes 2400 and 1), weighed, and length recorded. All aspects of monitoring and collecting followed protocol outlined in the Boreal Toad Recovery Plan (Goettl, ed. and the Boreal Toad Recovery Team 1997). Protocol for rearing all captive individuals was taken from the Hatchery Manual for the Rearing and Propagation of Captive Boreal Toads, *Bufo boreas* (Scherff-Norris 1997). The maximum time in captivity for any group of toadlets was one year. This should have helped minimize genetic adaptation to captivity, as suggested by Frankham (1994).

Tadpoles (1997 year class): Tadpoles were collected from a pond ("Hesbo") at the Climax Molybdenum Mine near Georgetown, Colorado. (The 1-4 week old toadlets [1997 year class] mentioned below are from the same egg masses as these tadpoles). The tadpoles were moved to the CDOW Watson Hatchery, Bellvue, Colo. on 5/28/97, where they were raised in an outdoor raceway until 6/23/97 when they were reintroduced to Lost Lake. Tadpoles were backpacked into Lost Lake in heavy plastic fish transport bags and released into one of six enclosures. The enclosures measured 183cm (length) x 56cm (width) x 46cm to 91cm (height). The height tapered down towards the shore to provide a deep to shallow water profile in which the tadpoles could thermoregulate. The deepest water in the enclosure was ca. 72cm. The enclosures were constructed of nylon mesh siliconed to a PVC pipe frame and lined around the top with aluminum flashing to prevent toadlets from escaping. On the shore there was an area of approximately 465cm^2 of dry area onto which the tadpoles could emerge to metamorphose. Some tadpoles (originally 200/enclosure) were cannibalized en route to Lost Lake, so the approximate number of tadpoles introduced into each of six enclosures was 192,

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[1]With the exception of a one year old group with toes 300 and 20 clipped for purposes of studying a tetracycline mark on the front and rear toes.

[2] "Good"= wound completely healed over, little to no swelling or redness; "Fair"=wound healed over but with moderate to extreme swelling and/or redness; "Poor"=wound has exposed raw skin or bone

191, 191, 187, 185, and 188, respectively. Tadpoles were Gosner stage 27-32 (Gosner 1960) and had a mean weight of 0.32 g (0.085 SD). There was no statistically significant difference in the mean weights of tadpoles in each enclosure ($F=1.04$, $p=0.3971$). All six enclosures had natural vegetation added as a food source, and three of the enclosures also had a mixture of fish and amphibian food added. Natural food was added to enclosures on 6/30/97, 7/3/97, and 7/28/97. The enclosures that received supplemental food were replenished three times per week. Shortly after putting the tadpoles in the enclosures (7/2/97), I realized we were feeding substantially more supplemental food than the tadpoles were eating (possibly 2-3 times more), which was apparently causing water quality problems that led to tadpole death. On 7/3/97, all food was removed (supplemental and natural) from the supplementally fed enclosures and amount of feed added was reduced. The amount to be added was determined by the amount consumed since the previous feeding. If more than 95% of the food was gone, feed amount was increased slightly. If less than 85% was gone, feed amount was decreased slightly. Otherwise, food amount was left unchanged. Each day before feeding, excess food from the previous feeding was removed. The feeding assignments per enclosure were made by mechanical randomization. When tadpoles metamorphosed and developed to at least Gosner's stage 44 and had a tail stub between 0.0 and 2.5mm in length, they were weighed, measured, toe clipped, and released. The toe clip was age and group specific (enclosure 1=toe 20, enclosure 2=toe 10 , enclosure 3=toe 300, enclosure 4=toe 1, enclosure 5=toe 50, enclosure 6=toe 2).

1-4 week old toadlets (1996 year class): These toadlets were collected as eggs from parts of six egg masses at Hesbo and reared at the CDOW Fish Research Hatchery, Bellvue, Colo. Toadlets (n=12,000) were reintroduced between 8/6/96 and 8/28/96, once they had metamorphosed and showed reaction to movement. This group of toadlets was neither toe clipped nor weighed.

1-4 week old toadlets (1997 year class): One group of toadlets was collected as eggs and/or tadpoles at Hesbo and the other was collected from eggs produced by captive boreal toads at the CDOW Fish Research Hatchery. Both groups were reared at the CDOW Watson Hatchery in adjacent raceways (mean temp=17.7°C (2.2°C SD)). There was no statistically significant difference in the temperatures of the two raceways ($t=-0.4902$, $p=0.6240$). The wild eggs from Hesbo came from parts of 6 egg masses and the captive eggs came from three egg masses. Toadlets (n=4,235 wild bred, n=7,071 captive bred) were reintroduced to Lost Lake between 7/16/97 and 8/22/97, once they had metamorphosed and showed reaction to movement. Wild bred toadlets had a mean weight of 0.43g (0.065 SD) and captive bred toadlets had a mean weight of 0.36g (0.077 SD). This was a statistically significant difference ($F=11.90$; $p=0.0073$). Because date had a statistically significant effect on toe clipping ($F=13.60$; $p=0.0001$), I treated the dates as a random sample of dates from which I could have sampled. Prior to reintroduction, toadlets were batch marked with a year class and origin (wild or captive) specific toe clip (wild =toe 5, hatchery=toe 2400).

9-10 month old toadlets (1996 year class): Three groups of 9-10 month old toadlets were reintroduced to Lost Lake on 6/9/97. One group (n=77) had been intensively hibernated overwinter in a Percival Environmental Chamber and the second group (n=520) were extensively hibernated overwinter in outdoor raceways with insulated and/or heated boxes at the CDOW Fish Research Hatchery. Neither of these two groups was fed during the winter. The third group (n=48) of toadlets was experimentally marked by the USGS-BRD with tetracycline and were not hibernated, but fed through the winter. The average weights of the different groups of toadlets were: intensively hibernated group=2.36g (0.66 SD) , extensively hibernated group=1.14g (0.73 SD), and non-hibernated group= 2.37g (0.56 SD). The statistical significance between these groups, along with the 22 month old toadlets is given in Table 1. Each group was uniquely toe clipped prior to reintroduction (intensively hibernated=toe 3200, extensively hibernated=toe 3, non-hibernated=toes 300 & 20).

22 month old toadlets (1995 year class): Thirty-nine two-year-old toadlets were reintroduced to Lost Lake on 6/9/97. These toadlets were brought into captivity in 7/96 from Hesbo and overwintered in the Percival Environmental Chamber. The average weight of the toadlets was 11.02g (3.14 SD). Prior to reintroduction, they were batch marked with an year class specific toe clip (toe 30).

Adult toads: Five male adults were collected from Hesbo and released at Lost Lake on 6/27/97, 7/18/97, and 7/25/97. The adults were fitted with radio transmitters (Holohil Systems Ltd.; weight=1.8g) to track movement patterns in the summer months and determine overwintering temperature of hibernacula with temperature-sensing radios (Holohil Systems, Ltd.; weight=2.5g). The weights (g) of the five toads at reintroduction, including radios, were: 31.3, 33.6, 37.5 35.6, and 27.6.

Table 1. Least squared mean weights and statistical significance of 9-10 month old and 22 month old toadlets.

YEAR CLASS	WEIGHT (LSMEAN)	Pr > |T| H_0 : LSMEAN (I) = LSMEAN (j)			
		1995 22 mo. old	1996 Intensively hibernated 9-10 mo. old	1996 Extensively hibernated 9-10 mo. old	1996 Non-hibernated 9-10 mo. old
1995 22 mo. old	11.02	----	0.0001	0.0001	0.0001
1996 Intensively hibernated 9-10 mo. old	2.36	0.0001	----	0.0172	0.9700
1996 Extensively hibernated 9-10 mo. old	1.14	0.0001	0.0172	----	0.0201
1996 Non-hibernated 9-10 mo. old	2.37	0.0001	0.9700	0.0201	----

RESULTS

Average daily survival probability for each group of toadlets was computed and is given in Table 2, along with simultaneous 95% confidence intervals computed exactly using the binomial distribution. Wild and captive toadlets (1997 year class) were not included in the survival analysis because the areas to which they were reintroduced, and the immediately surrounding areas, were not adequately sampled prior to the end of the sampling season. They will be used in next season's survival analysis.

Table 2. Average daily survival probability and simultaneous 95% confidence intervals for each year class of toadlets for 1997 sampling periods.

SAMPLING PERIOD	YEAR CLASS				
	1995 22 mo. old	1996 96 release 1-4 wk. old	1996 Intensively hibernated 9-10 mo. old	1996 Extensively hibernated 9-10 mo. old	1996 Non- hibernated 9-10 mo. old
2: 6/30-7/14	0.9728 (0.9717,0.9738)	0.9860 (0.9852,0.9867)	0.9940 (0.9936,0.9942)	0.9717 (0.9716,0.9718)	0.9993 (0.9987,0.9995)
3: 7/14-8/4	0.9408 (0.9366,0.9456)	0.9714 (0.9711,0.9718)	0.9614 (0.9605,0.9623)	0.9417 (0.9413,0.9421)	0.9419 (0.9403,0.9438)
4: 8/4-8/18	0.9057 (0.8835,0.9292)	0.8477 (0.8440,0.8537)	0.8248 (0.8071,0.8489)	0.8156 (0.8068,0.8287)	0.0000 (0.0000,0.8396)

Survival probabilities were compared using the Akaike Information Criterion (AIC) (Burnham and Anderson 1992 and Lebreton et al. 1992). AIC is a model selection method that attempts to find the optimal model for the data without over fitting (too many parameters) or under fitting (too few parameters). The formula for the AIC incorporates a measure of the difference between the data and the model, as well as a penalty term as the number of parameters in the model increases. The most parsimonious model will be the one with the lowest AIC value. In this study, it was found that the global model (including all data) had the lowest AIC value of all models tested. Other models tested and their resulting AIC values are listed in Table 3.

Tadpoles reared to metamorphosis were not included in the survival analysis because there was not adequate searching following their release. Of the non-fed enclosures, only one produced toadlets (n=13). All three of the supplementally fed enclosures produced toadlets (n=100 total; enclosure 3=23, enclosure 5=33, enclosure 6=42.) All tadpoles that did not metamorphose were presumed dead, because there was no sign that tadpoles escaped, numerous carcasses were found throughout the summer, and there were no live individuals at the end of the summer. There was a statistically significant difference in the least squared mean weight of the metamorphosed toadlets from the different enclosures ($F=45.27$, $p=0.0001$). The least squared mean weight for each enclosure was as follows: enclosure 3=0.304g; enclosure 4=0.128g; enclosure 5=0.315g; enclosure 6=0.371g). There was no interaction between enclosure number and date of clipping with regard to tadpole weight ($F=2.26$, $p=0.0863$). There was also no date effect ($F=0.15$, $p=0.9615$).

There was a difference noted in mortalities between the fed and unfed enclosures during the period of overfeeding described in the Methods section. There were 58 mortalities in enclosure 3, 26 in enclosure 5, and 34 in enclosure 6. There were no mortalities observed in the non-fed enclosures on the same date.

Substrate preference of each group relative to the other groups was recorded by noting whether individuals were found on grass, log, moss, mud, rock, or log substrate. A Chi-Square test of independence between the substrate an individual was found on and the year class of that individual was run for each

substrate and group. The residuals (observed minus expected) for each cell are given in Table 4.

Table 3. Survival probability models run with associated AIC values.

MODEL	AIC VALUE	COMPARISON
GLOBAL	3384.844	global (all values unique)
SUR13	3389.927	setting survival probabilities for 1996 9-10 mo. old intensively hibernated and 1996 9-10 mo. old non- hibernated equal across all sampling periods
SUR13M4	3390.524	setting survival probabilities for 1996 9-10 mo. old intensively hibernated and 1996 9-10 mo. old non- hibernated equal in sampling periods 2 and 3
SUR7	3392.418	setting survival probabilities for 1996 1-4 wk. old 96 release and 1996 9-10 mo. old intensively hibernated equal across all sampling periods
SUR7M4	3394.185	setting survival probabilities for 1996 1-4 wk. old 96 release and 1996 9-10 mo. old intensively hibernated equal in sampling periods 2 and 3
SUR16	3419.332	setting survival probabilities for 1996 1-4 wk. old 96 release and 1996 9-10 mo. old non- hibernated equal across all sampling periods
SUR16M4	3419.782	setting survival probabilities for 1996 1-4 wk. old 96 release and 1996 9-10 mo. old non- hibernated equal in sampling periods 2 and 3
SUR3	3429.59	setting survival probabilities for 1996 9-10 mo. old intensively hibernated and 1996 9-10 mo. old extensively hibernated equal across all sampling periods
SUR3M4	3431.561	setting survival probabilities for 1996 9-10 mo. old intensively hibernated and 1996 9-10 mo. old extensively hibernated equal in sampling periods 2 and 3
SUR14	3441.916	setting survival probabilities for 1996 9-10 mo. old extensively hibernated and 1996 9-10 mo. old non- hibernated equal across all sampling periods
SUR14M4	3443.17	setting survival probabilities for 1996 9-10 mo. old extensively hibernated and 1996 9-10 mo. old non- hibernated equal in sampling periods 2 and 3
SUR8	3511.434	setting survival probabilities for 1996 1-4 wk. old 96 release and 1996 9-10 mo. old extensively hibernated equal across all sampling periods
SUR8M4	3512.643	setting survival probabilities for 1996 1-4 wk. old 96 release and 1996 9-10 mo. old extensively hibernated equal for sampling periods 2 and 3
EXAMPLE	3550.295	setting survival probabilities for all groups equal within sampling periods; looking for sampling period effect
SEASON2	3554.694	setting survival probabilities for all groups equal for sampling periods 2 and 3; looking for sampling period effect
RAIN	3557.838	setting survival probabilities equal within groups over sampling periods 2 and 3; looking for group effect
GROUP	3986.45	setting survival probabilities equal within groups over all sampling periods; looking for group effect
TOAD1	4344.28	setting survival probabilities for all groups equal across all sampling periods

Table 4. Relative substrate preference by year class. (⊕ = residual more than 2 SD from expected (in positive direction); + = residual more than 1 SD from expected (in positive direction); ⊖ = residual more than 2 SD from expected (in negative direction); - = residual more than 1 SD from expected (in negative direction)).

YEAR CLASS	GRASS	LOG	MOSS	MUD	WATER
1995 22 mo. old	-	+	⊕	⊖	+
1996 96 release 1-4 wk. old	⊖	-	⊕		⊕
1996 Intensively hibernated 9-10 mo. old	⊕	-		-	-
1996 Extensively hibernated 9-10 mo. old			⊖		⊕
1996 Non-hibernated 9-10 mo. old	⊕	+		-	
1997 Captive 1-4 wk. old	+	-		-	+
1997 Wild 1-4 wk. old	-	+	-	⊕	⊖

Toe clip condition for each group was recorded by noting the condition of the toe at time of capture. A Chi-Square test of independence between the toe clip condition of an individual and the year class of that individual was run for each toe clip condition and group. The residuals (observed minus expected) for each cell are given in Table 5.

Table 5. Toe clip condition for each year class (⊕ = residual more than 2 SD from expected (in positive direction); + = residual more than 1 SD from expected (in positive direction); ⊖ = residual more than 2 SD from expected (in negative direction); - = residual more than 1 SD from expected (in negative direction)).

YEAR CLASS	GOOD	FAIR	POOR
1995 22 mo. old			
1996 Intensively hibernated 9-10 mo. old	+	⊖	⊖
1996 Extensively hibernated 9-10 mo. old	⊕	⊖	⊖
1996 Non- hibernated 9-10 mo. old	+	⊖	-
1997 Captive 1-4 wk. old			
1997 Wild 1-4 wk. old	⊖	⊕	⊕

Whether a toe was regenerating or not was recorded by noting that the toe was "yes," "no," or "?" (questionably) regenerating. A Chi-Square test of independence between toe regeneration of an individual and the year class of that individual was run for each toe regeneration category and group. The residuals (observed minus expected) for each cell are given in Table 6.

Table 6. Toe regeneration by year class. (⊕ = residual more than 2 SD from expected (in positive direction); + = residual more than 1 SD from expected (in positive direction); ⊖ = residual more than 2 SD from expected (in negative direction); - = residual more than 1 SD from expected (in negative direction)).

YEAR CLASS	YES	?	NO
1995 22 mo. old			
1996 Intensively hibernated 9-10 mo. old	⊕	+	-
1996 Extensively hibernated 9-10 mo. old	-		
1996 Non- hibernated 9-10 mo. old	⊕	+	-
1997 Captive 1-4 wk. old	+	⊕	
1997 Wild 1-4 wk. old	⊖	-	+

Length of toe regeneration for each group was recorded by noting the length of the regeneration when the individual was captured. Results are in Table 7.

Table 7. Toe regeneration by year class (mm).

YEAR CLASS	N	MEAN (mm)	STD DEV (mm)	MINIMUM (mm)	MAXIMUM (mm)
1996 Intensively hibernated 9-10 mo. old	8	1.54	0.24	1.13	1.81
1996 Extensively hibernated 9-10 mo. old	7	1.44	0.63	0.81	2.41
1996 Non-hibernated 9-10 mo. old	11	1.49	0.38	1.00	2.00
1997 Captive 1-4 wk. old	3	1.29	0.51	0.79	1.81

There were toadlets found on the trail below Lost Lake that were not included in the analysis and give some indication that there may be toadlets emigrating from the area. These data are summarized in

Table 8.

Table 8. Toadlets found on trail below Lost Lake.

DATE	TOE CLIP	LOCATION
7/25/97	1996 96 release 1-4 wk. old	25-30 m above road bridge next to stream
7/28/97	1995 22 mo. old	100 m below fork to lake at stream
8/04/97	1996 Intensively hibernated 9-10 mo. old	100 m below fork to lake near stream
8/13/97	1997 Captive 1-4 week old	100 m below fork to lake on trail where stream crosses
8/18/97	1996 96 release 1-4 wk. old	75 m below fork to lake on trail
8/22/97	1996 Extensively hibernated 9-10 mo. old	In stream below fork to lake
9/19/97	1996 96 release 1-4 wk. old	50 m below stream that crosses trail below fork to lake

Of the 5 adult toads reintroduced to Lost Lake, only one was tracked through the entire summer. It was last found on 10/17/97 and its temperature sensitive radio indicated that it was in a location with a temperature of 1.3°C next to a stream bed under vegetation next to a dead stump. Two other adults slipped out of their radio harnesses. Another toad presumably went out of radio range or its radio quit working, as no signal was received a week after the toad's release. Finally, one toad was severely cut by its radio harness and died. Average and longest distances moved have yet to be determined.

There were two night surveys to monitor any breeding activity by remnant resident toads (6/3/97 and 6/11/97). No toads were found in the night surveys. However, there were 4 female, resident boreal toads found at Lost Lake in the summer of 1997 (6/18, 6/20, 7/14, 8/1). Their mean weight was 88.15g (7.15 SD) and they were toe clipped at the time of initial capture.

There was breeding at Lost Lake in 1997, presumably between a resident female and reintroduced male toad. Tadpoles appeared in large numbers (300-400) on 7/23, but then quickly disappeared (30-40 seen on 8/4). These individuals failed to metamorphose, or to even grow full hind legs, before winter.

DISCUSSION

In attempting to find the best model to describe the average daily survival probabilities of the different groups of toadlets in this study, setting average daily survival probabilities equal within each group for all sampling periods ("Group" model) did not produce a model with a lower AIC value than the global model. This means that there was not a clear indication that one group had a higher survival across time than any other group. Setting average daily survival probabilities equal for all groups within a given sampling period ("Example" model) also failed to produce an AIC value lower than that of the global model. However, it does have a lower AIC value than the "Group" model, indicating that a sampling period effect is more likely than a group effect in this data.

Many of the other models were run in an attempt to identify how average daily survival probabilities

related within the 1996 year class of toadlets which included: toadlets released as new metamorphs (1-4 weeks old) in 1996, toadlets released in 1997 following a winter of extensive hibernation, toadlets released in 1997 following a winter of intensive hibernation, and a group of toadlets released in 1997 following a winter with no hibernation. It is not surprising that the groups with the most controlled winter (intensively hibernated and non-hibernated) had the most similar average daily survival probabilities (AIC=3389.927). However, the model with the intensively hibernated group and the group released in 1996 had an AIC value close to the previous model (AIC=3392.418). This is surprising because the toadlets released in 1996 would have presumably had the most harsh hibernation conditions, so I would have expected them not to have average daily survival probabilities similar to any of the other groups. The next model, comparing 1996 toadlets not hibernated with those that spent the winter at Lost Lake had a larger increase in AIC value (to 3419.332), which indicates the model does not have a very good trade-off between variance and bias. The same can be said for the final three models that compared: extensively hibernated toadlets with intensively hibernated toadlets (AIC=3429.590); extensively hibernated toadlets with non-hibernated toadlets (AIC= 3441.916); and extensively hibernated toadlets with 1996 released toadlets (AIC=3511.434). It is interesting that in this final model, the average daily survival probabilities of the two groups that spent the winter outside (albeit at very different elevations) were the most dissimilar.

Although none of the models tested produced an AIC value lower than that of the global model, the worst AIC value comes from setting average daily survival probabilities equal across all groups and sampling periods. This gives some indication that there are differences in the average daily survival probabilities presented in Table 2, but that this is clouded by unknown factors. It should be noted that in addition to the models mentioned above, I tried running each of the models setting average daily survival probabilities equal only in sampling periods 2 and 3, as there appeared to be much lower average daily survival in sampling period 4 (Table 2). However, as almost all of the AIC values in Table 3 indicate, this did not result in a better trade-off between bias and variance than the original models.

Although I cannot distinguish a difference in the survival probabilities of the various groups of toadlets, it is encouraging that similar reintroductions in the literature have been successful. Following a 1992 reintroduction of metamorphs (number not reported) of the Majorcan Midwife toad, *Alytes muletensis*, Bush (1994) reported that at three of eight reintroduction sites, breeding populations had been established and that males were heard calling at another four sites. In Latvia, European tree froglets were introduced and grew to be successful breeders (Zvirgzds et al. 1995). Unfortunately, Houston toads, *Bufo houstonensis*, which were the subject of massive captive rearing and reintroduction in the 1980s have fared far worse in reintroduction attempts. After the release of 62 adults, 6,985 metamorphs, and 401,384 eggs, at 10 ponds over numerous years, only a few calling males and occasional eggs were found by the late 1980s. Possible reasons for this are predation of tadpoles by snakes (*Nerodia erythrogaster* and *Thamnophis proximus*) and ant (*Solenopsis invicta*) predation on toadlets (Beebee 1996).

In this study, I noted a statistically significant difference in mean weight between fed and unfed tadpole groups. This may be an indication of food limitation on tadpoles at Lost Lake. However, it is impossible to say with certainty, as the tadpoles were restricted to the areas within the enclosures for their food searching. There may have been areas of higher food abundance elsewhere in the lake that the tadpoles would have used had they had access to them. In a similar study on caged natterjack toads, *Bufo calamita*, the authors reported a significant difference in mean length between fed (rabbit pellets) and unfed groups of tadpoles (t=1.80, df=10, p=c.0.05) Beebee et al. 1982).

Based upon the extremely poor survival of tadpoles to metamorphosis reintroduced to Lost Lake (13/570 [non-supplementally fed] , 100/564 [supplementally fed]), I believe that tadpoles are not the best age class for reintroduction. The individuals that did metamorphose were smaller and less robust than their siblings that were raised just past metamorphosis in a hatchery and then released to Lost Lake. Incidentally, these tadpoles were at an advanced stage (Gosner stage 30 [Gosner 1960]) when released, not newly hatched, which would most likely have decreased their recruitment even more. The first metamorphosis of tadpoles at

Lost Lake occurred nearly a month later than in captivity. Additionally, wild tadpoles disappeared prior to metamorphosis for an unknown reason. In a reintroduction of the fire-bellied toad, *Bombina bombina*, in Sweden, tadpoles as well as toadlets (raised in a laboratory) were used to establish a "fairly stable" population over a six year period (Andren and Nilson 1995a). The tadpoles were raised in net cages until metamorphosis, and then released at the release sites (not left unprotected). However, it is difficult to compare that reintroduction to the current study, because the authors did not identify the proportions of tadpoles versus toadlets that contributed to the establishment of the population, nor the proportions of tadpoles that survived to metamorphosis at the reintroduction site.

Numerous authors (Andren and Nilson 1995b, Bloxam and Tonge 1995, Cooke and Oldham 1995, and Reinert 1991) reported that reintroduction of early life stages was most successful. This may appear to contradict my finding that tadpoles are unsatisfactory for reintroduction. However, in the study by Cooke and Oldham (1995) on the common toad, *Bufo bufo*, this distinction was made in comparing eggs to adults, not eggs to metamorphs, which was the largest group released in the current study. Due to their poor survival and difficulty in tracking in this study, adult boreal toads were not judged to be satisfactory for reintroduction, either. In a reintroduction of *Ambystoma tigrinum*, Reinert (1991) defined early life stages as "eggs, larvae, and neonates," as did Andren and Nilson (1995b) for the fire-bellied toad. Because these two studies did not clearly distinguish between tadpoles/larvae and metamorphs, it is difficult to determine whether tadpoles had higher survival than in my study.

There are, however, some examples in the literature of successful reintroductions using tadpoles/larvae. Following the release of 400 European tree frog tadpoles, *Hyla arborea*, in the Netherlands, a viable breeding population was established (no quantification given) (Beebee 1996). Great crested newts, *Triturus cristatus*, have been captively reared and reintroduced as larvae in Britain, and it was found that 100-200 large larvae can establish a population of mature adults within 2-3 years (Beebee 1996). Following reintroduction of wood frogs, *Rana sylvatica*, over a 10-year period to a series of human-made ponds in Illinois, a breeding population was established and growing. There were 27,300 eggs, 32 tadpoles, and 19 adults reintroduced (Thurow 1994). Reproduction was observed following introduction of eggs and/or larvae of *Ambystoma maculatum*, *Ambystoma annulatum*, and *Rana sylvatica* (Sexton and Phillips 1986), and *Bufo calamita* (Charlton 1984).

The results presented in Table 4 reveal no clear pattern of substrate preferences of groups of toadlets relative to other groups. However, the two groups of 1997 toadlets appeared to have nearly opposite relative substrate preferences. Reasons for these differences are unknown.

The toe clip conditions from this study, presented in Table 5, are very positive. All of the 1996 toadlets showed more "good" conditions than expected and fewer "fair" and "poor" conditions than expected. The 1995 toadlets and 1997 captive bred toadlets had toe clip conditions that deviated less than one from expected, which is also good. However, the 1997 wild bred toadlets' toe clip condition showed fewer "good" conditions than expected, and more "fair" and "poor" conditions than expected. The reason for this difference is unknown. Although there were instances of toe loss in this study that did not correlate with the clipping scheme, such toadlets were distinguishable by of the size of the individual and/or the type of cut on the missing toe (i.e. not a clean cut at the base of the toe). Of the toadlets recaptured, there did not appear to be a negative impact of toe clipping (with the possible exception of the 1997 wild bred toadlets).

These findings are consistent with Clarke's (1972) study of the effect of toe clipping on Fowler's toad, *Bufo woodhousei fowleri*, in which he reported that healing was rapid and wounds on recaptured animals were not swollen three to seven days after marking. Clarke also found that tendons reinserted in well-healed toes and that there were not major long term effects on muscle control. In a study of natterjack toads, *Bufo calamita*, toe clipping was speculated to cause stress and momentary pain, and the authors advised against toe clipping when possible (Denton and Beebee 1993). However, the authors reported that the wounds from toe clipping were completely healed within 2 days. In a toe clip marking experiment of 61 common toads, *Bufo bufo*, no toads showed any sign of inflammation during the 10 month experiment and all

wounds healed within a week (Gelder and Strijbosch 1996). The authors checked to be certain that no bone protruded out of the wound following toe clipping. We attempted to do the same, although it was difficult with some of the smallest toadlets (mean weight = 0.43g and 0.36g, 1997 wild 1-4 week olds and 1997 captive 1-4 week olds, respectively). Toe clipping is likely the fastest, least expensive, most reliable, and least stressful marking technique at present (Halliday 1995). With regard to marking anurans, Paine et al. (1989) stated that it is extremely difficult to do for permanent identification and that the most widely used technique is toe clipping. Olson's (1992) experience with toe clipping of western toads, *Bufo boreas*, and Cascades frogs, *Rana cascadae*, was that it appeared to have no effect and that individuals quickly resumed breeding. There was no toe-clipping induced mortality, and over the five years of the study, hundreds of study animals returned to the study sites. In a study investigating the effects of toe-clipping on Australian frogs, *Crinia signifera*, Lemckert (1996) found that increasing the number of toes clipped (from 2 to 4) apparently did not significantly affect the chance of recapture 30 days later. Toe clipping did not affect survival of the frogs, and infection was rare (6/500 frogs). Lemckert did note a behavioral change in clipped individuals that involved the individuals leaving the breeding pond immediately following clipping and returning a few days later to breed. Because there have been conflicting reports on the effects of toe clipping, it is important that studies involving toe clipping clearly describe the methods employed so that studies can be compared (Reaser 1995). It appears that toe clipping likely had little effect on the toadlets in this study.

There was toe regeneration in toadlets reintroduced to Lost Lake (Tables 6 and 7). There was more regeneration than expected for 1996 intensively hibernated toadlets, 1996 non-hibernated toadlets, and 1997 captive bred toadlets. There was no difference from expected in regeneration of toes for the 1995 toadlets, and less than expected in both 1996 extensively hibernated toadlets and 1997 wild bred toadlets. Reasons for these differences are unknown.

In a study of Fowler's toad, *Bufo woodhousei fowleri*, Clarke (1977) found no significant toe regeneration. He sampled a total of 1,346 immature and adult toads. Smith (1987) noted that chorus frogs, *Pseudacris triseriata*, toe clipped as emerging froglets (n=771) showed no signs toe regeneration after 1-2 years. In a study of 6,396 juvenile and adult Manitoba toads, *Bufo hemiophrys*, Kelleher and Tester (1969) reported that toe regeneration was rare and could be detected by short length and light color. An obvious, small fleshy growth was the only sign of regeneration that Clarke (1972) observed following toe clipping, even in animals marked over a year. Campbell (1970) reported that there was not "confusing regrowth" of clipped digits for boreal toads. I do not know why there is more toe regeneration in this study than the above studies. Although there has been regeneration, it has been recognized and the toe has been reclipped to prevent confusion.

The discovery of four resident adult females and no males at Lost Lake is not surprising, if boreal toads have population dynamics similar to those of the natterjack toad, *Bufo calamita*. Beebee (1993) reported that in the final stages of natterjack toad extinction, the only surviving toads may be females. He attributed this to females having longer life spans (due to their "safer" lifestyle) than males.

CONCLUSIONS

At this point in the study, I would conclude that neither tadpoles nor adult toads are the best age class for establishing a new population of boreal toads. I cannot detect a difference in survival between the various age classes of toadlets reintroduced, nor in relative substrate choice of the reintroduced toadlets. Toe clipping does not appear to be detrimental to the boreal toads in this study. In 1998, weights of toadlets will be recorded to compare growth between groups over last season. Due to the fact that it takes boreal toads four to six years to mature, continued monitoring will be necessary to more completely evaluate the success of this reintroduction.

Because this reintroduction had no replicate site, the conclusions are site specific. Additional research at more sites is necessary to attempt to account for habitat variability. Although differences in precipitation, cloud cover, wind, and temperature could have affected our ability to detect all individuals,

attempts were made to census for toads in similar climatic conditions, as per the guidelines of the Visual Encounter Survey (Crump and Scott 1994). New sites should be chosen and experimental reintroduction of the same age classes, as well as eggs, should be completed.

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SITE DESCRIPTION AND BACKGROUND

Research on population size, stability, movement, and habitat use is currently being conducted at the Henderson/Urad Mine where the largest known metapopulation of boreal toads in Colorado exists. The Henderson Mine toad breeding sites consist 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: 2-pond, Power Alley, Hesbo, Treatment Pond, Donut, Ann's Pond, and Upper Urad (Figure 1).

Hesbo and 2-Pond were the main breeding locations in 1995 and 1996. Hesbo was the primary breeding site in 1997. In 1995 and 1996 both sites were influenced by pre-treated mine effluent running through them at an elevated temperature of 19-21°C. Climax has just finished a new water treatment facility on the Urad side of the facility; untreated mine effluent now flows into 2-Pond and then through Hesbo in a plastic pipe. 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. Hesbo has the largest population of adult toads during breeding but has not recruited in the last three years.

Power Alley is a beaver pond complex along the west branch 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 two previously mentioned sites and breeding occurs one to two weeks later.

Treatment is the eastern most of several ponds used to treat water from the tailings dams in this valley. It does not have a large number of adults during breeding but produced 10,000-15,000 toadlets in both 1996 and 1997.

Donut is a newer pond above the water treatment facility. 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.

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 pipe to keep the water level constant which resulted in successful recruitment in 1997.

Upper Urad is a large 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 in 1997 at this site.

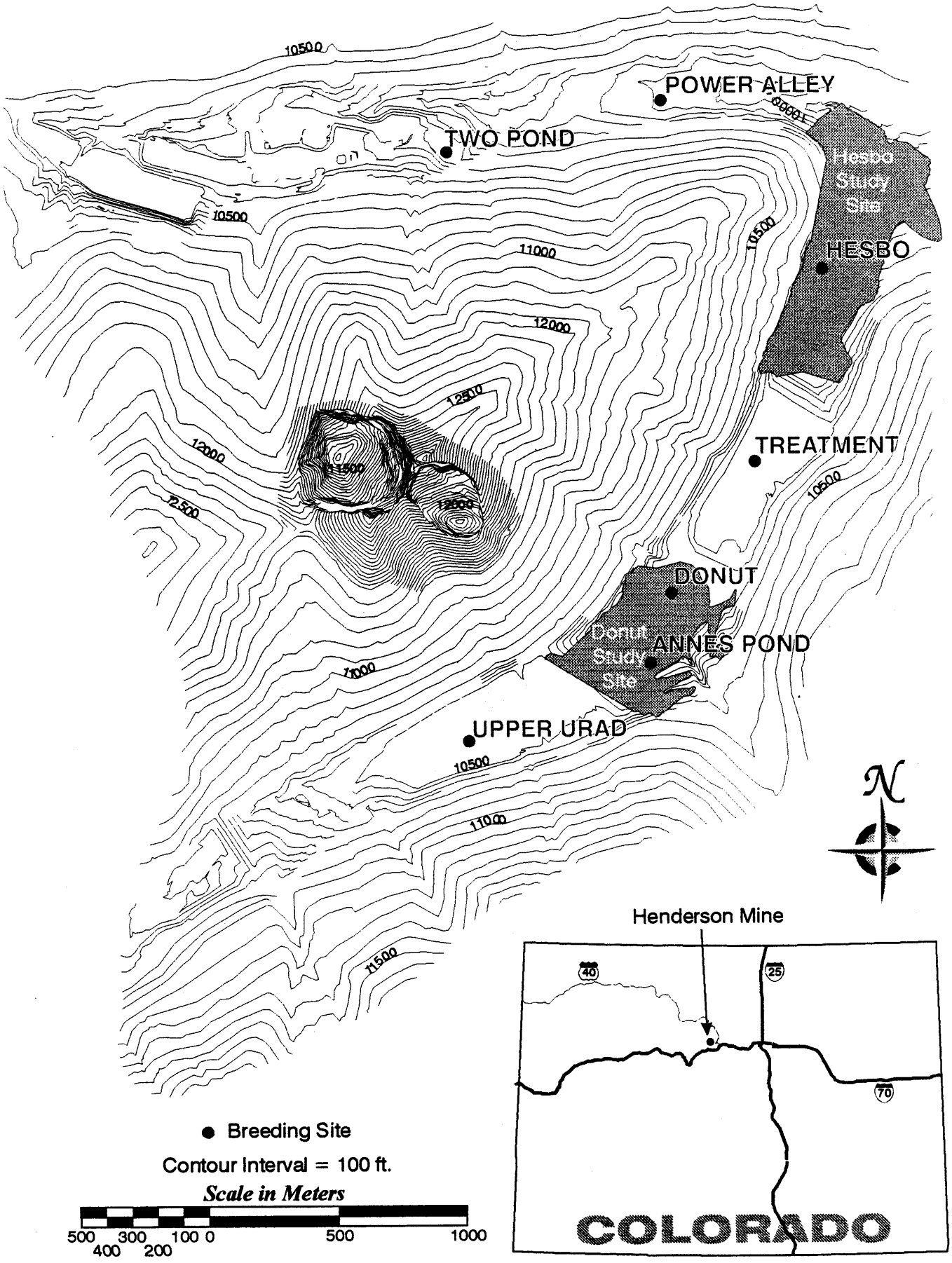

Figure 1: Henderson Mine Study Area

MATERIALS AND METHODS

The Henderson/Urad metapopulation breeding sites were monitored by J. Goettl in 1995 and 1996. Data collected in 1995 was mostly exploratory in nature, as little was known about the status of these breeding sites and field techniques were being refined. This project was transferred to M. Jones in 1997.

Starting in 1996, all breeding sites were 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 Boreal Toad Recovery Plan (Goettl [ed.] 1997).

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 Pittag numbers were recorded along with the other pertinent data on individuals and site. 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.

Ten toads (six males and four females) were radio tagged in May and June, 1997 at Hesbo 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 0.96mm medical polyethylene tubing. Because of problems with this tubing cutting the toads it was later covered with 2mm vinyl tubing which solved this problem. During the course of the 1997 field season nine additional toads were radio tagged as replacements, four of these in the Donut/Anne's Pond area. All but two of these replacements was needed as a result of the harness falling off the toad, only toads with four or more weekly contacts were used in analyses (Table 1). There is a small margin of error between the harness being too loose and falling off and too tight and injuring the toad, therefore we tended to be conservative with the fit of the harness. One of the transmitters failed after one day and another toad migrated down Clear Creek in a short period of time and was lost.

Each radioed toad was located one time per week from May until they went into hibernation. Prior to hibernation, all radios were replaced with Holohil BD-2GT temperature sensitive transmitters so hibernation temperature could be monitored monthly during the winter. 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.

Table 1. Contact statistics for radio tagged boreal toads in the Henderson/Urad study area.

Toad ID	Start date	Sex	Contacts	Monitored (days)	Comments
915	6/10/97	F	12	86	Radio harness slipped off after 86 days.
919	6/10/97	M	16	106	Hibernating in same hole as 922.
148	6/10/97	M	8	45	Radio harness slipped off after 45 days.
336	6/10/97	M	16	112	Hibernating in hole above Hesbo.
703	6/10/97	M	15	99	Winter radio harness slipped off after change over.
771	5/28/97	F	19	132	Hibernating in hole under rock pile above Hesbo.
773	5/28/97	F	16	111	Hibernating in hole under road above Hesbo.
774	5/28/97	F	9	56	Escaped radio detection, was moving down Clear Creek.
918	6/10/97	M	4	14	Radio fell off.
920	6/10/97	M	5	20	Radio fell off.
924	7/07/97	M	11	85	Hibernating in same hole as 925.
925	6/30/97	M	12	91	Hibernating in same hole as 924.
927	8/07/97	M	8	48	Hibernating below Hesbo by spring.
922	9/29/97	F	1	--	Hib. at tagging location, only used to monitor hib. temp.

Habitat and slope coverages were developed in ARC/INFO starting with a photo interpreted CAD file obtained from the Henderson Mine and then ground truthing and making corrections by walking the perimeter of each habitat area with a GPS unit. Toad location data was overlaid on the habitat and slope coverages to try to determine if preferences existed. The habitat categories for the Hesbo study area were defined as aspen/conifer, road, spring, stream, water (lentic), wet area (terrestrial), and rock. The categories for the Donut study area were conifer, road, spring, stream, water (lentic), wet area (terrestrial), and rock. Only toads which had eight or more habitat locations were included in the analysis. As there were only three toads in the Donut area with eight locations, this data was not included in the statistical analysis, it is presented in tabular format. To test whether toads used a habitat category in greater or lesser proportion than its availability in the study area, a univariate t-test was used in SAS (1994) which tests whether the difference between the mean of the proportion of habitat availability and the mean of the proportion of habitat use equaled zero ($\propto$=0.05).

Capture-recapture methods were used to estimate population numbers of males at each breeding site from 1995 to 1997. 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.

Movement was calculated by plotting sequential locations for each toad on a 3 m^2 cell digital elevation model in ARC/INFO. In this way, the extreme elevational unevenness of the terrain could be incorporated into the calculations. Total distance moved/time for each toad and average daily movement in meters was calculated. Differences between male and female movements were tested using the Mann-Whitney U test.

RESULTS

Breeding Site Monitoring:1995 and 1996

Summaries of breeding site surveys conducted by Goettl and others may be found in Appendix 2. Summaries of breeding site water quality data from 1995 and 1996 may be found in Appendix 3.

Breeding Site Monitoring:1997

Hesbo- Hesbo was monitored at night weekly from May 12 to June 17 , 1997. The peak of breeding activity occurred on May 20 with 87 adults observed (82 male, 5 female). Eleven egg masses were laid, but we never observed more than 1500 tadpoles. All but 50 tadpoles disappeared during the last week of June. It is hypothesized that predation by Dytiscid larvae may be factor. The remaining tadpoles disappeared by August 15. No recruitment occurred at this site in 1997.

2-Pond- 2-Pond was night monitored weekly from May 14 to June 17, 1997. No breeding was observed. Four adults and 14 juveniles from the 1995 year-class were observed. Poor water quality as a result of direct inflow of untreated mine effluent was the primary factor.

Power Alley- Power Alley was night monitored weekly from May 14 to June 17, 1997. The most adults observed at this site was 60 (1 female, 59 males) on June 3. Seven egg masses were laid at this site: five desiccated, one was moved to Fort Collins, Watson Lake Hatchery, and one laid in an upper pond produced approximately 2,000 tadpoles which developed normally.

One additional egg mass was transferred to the upper pond from Ann's Pond. Approximately 2,000 toadlets metamorphosed and dispersed from this site by mid-September in 1997.

Upper Urad- Upper Urad was night monitored weekly from June 4 to July 9, 1997. Eleven adults (two females) was the highest number of toads observed on any occasion. One egg mass was laid but never developed, cause undetermined. No successful reproduction in 1997.

Donut- Donut was night monitored weekly from June 4 to June 24, 1997. Five egg masses were deposited at this site: one was taken to Fort Collins, Watson Lake Hatchery, portions of two were moved across the road to another pond (these later died). The remainder hatched and developed normally (approx. 4,000). Lauren Livo conducted tadpole ecology experiments at this site. Although many toadlets died from desiccation and exposure at this site, we believe up to several hundred may have located suitable hibernaculum.

Treatment- Treatment was night monitored from May 20 to June 25, 1997. The most adults observed in one night was three. Four egg masses were observed, but based on the number tadpoles observed on July 24 (>15,000), we suspect more egg masses were present. Monitoring was continued at this site throughout the summer and an estimated 10,000 toadlets survived to metamorphosis. It is unlikely many will survive the winter as there are no suitable hibernaculum areas around this site.

Anne's Pond- Anne's Pond was monitored from June 2 to July 2, 1997. Four to five egg masses were observed and based on the history of this pond drying up in previous years, one egg mass was moved to a stable site in upper Power Alley and two were moved to the pond by donut (these later died- water too cold). Anne's Pond contained approximately 1,000 tadpoles which reached metamorphosis. We have worked with Henderson staff to keep water in this pond and most toadlets dispersed successfully. We consider Anne's Pond a new breeding location.

Habitat Use and Movement

Locational data was collected on a total of 14 radio tagged boreal toads (Table 1). One of these was a female radioed on September 29 (#922), this individual never moved from the tagging site and hibernated at this site in the roots of a large engleman spruce *Picea engelmannii*. Movement and habitat use data was collected on all other radioed toads. It should be noted that major heterogeniety between individual toads was observed in both habitat use and movement data.

Of the 129 toad locations recorded in the Hesbo study area in 1997, 11.7% were on 0-20% slope (18.3% of total study area), 25.8% were on 21-40% slope (33.2% of total), 46.1% were on 41-60% slope (38.9% of total), and 16.4% were found on 61-80% slopes (9.7% of total). No toad locations were recorded on slopes >80%; slopes of this magnitude comprised only 0.04% of the study area. Of the 29 toad locations in the Donut study area, 31.0% were on 0-20% slope (31.0% of total study area), 41.4% were on 21-40% slope (27.4% of total), 17.2% were on 41-60% slope (27.0% of total), and 10.3% were on 61-80% slope (5.1% of total). There were no slopes greater than 80% in the Donut study area. Boreal toads do not appear to use slopes in proportion to their availability at either the Hesbo ($P<0.0159$) or the Donut study areas ($P<0.0003$) although we feel that locations were chosen for reasons other than slope. The primary objective of this analysis was to show that slope is not a deterrent to toad movement and that boreal toads commonly frequent upland habitats not associated with the relatively flat wetland areas.

The habitat areas for the Hesbo study area were defined as aspen/conifer, conifer, road, spring seep, water, wet area, and rock. Of the 129 toad locations recorded in the Hesbo area in 1997, 10.9% were in aspen/conifer (4.4% of total habitat), 9.3% were in spring seep areas (4.5% of available habitat), 34.9% were in wet areas (0.8% of habitat), and 45.0% were in rock areas (26.5% total habitat). No toads locations were recorded in conifer or road areas, which represented 47.8 and 4.7 percent of the total available habitat respectively. The incidence of toad locations in each habitat type are shown in Table 2. The Aspen/Conifer category was not used significantly out of proportion to its availability (N=8, T=1.001, P=0.3499). Wet areas were used significantly more than their proportion of available habitat (N=8, T=3.0586, P=0.0184). Springs were not used significantly out of proportion to their availability (N=8, T=1.0524, P=0.3278). Rocky areas were used significantly more than their proportion in the study area (N=8, T=2.7795, P=0.0273). No toad locations were recorded in pure conifer or roads in the Hesbo area. It should be stressed that although certain habitat elements were not used significantly more or less than available when the data is lumped, there were major differences in individual toad habitat preferences and categories which did not show group significance such as aspen/conifer and springs were used extensively by certain individuals (Table 2.). Maps of individual toad habitat use may be found in Appendix 1.

The habitat areas for the Donut study area were defined as conifer, road, spring seep, stream, water (other than stream), wet area, and rock. Of the 29 toad locations (three individuals) recorded in the Donut area in 1997, 20.7% were in conifer (34.8% of total area), no toads were located in road areas (6.6% of total area), 17.2% were found by springs (1.2% of total area), 10.3% were recorded by streams (0.9% total area), 6.9% were located in lentic water (3.2% total area), no toads were found in wet terrestrial areas (0.5% of available habitat), and 44.8% were found in rocky areas (52.8% total habitat). Table 2. illustrates the individual preferences exhibited by the Donut toads. Toad 922 was found at the base of an engleman spruce by a spring in the middle of a conifer stand and stayed at this location into hibernation. Toad 915 was located in a wide variety of habitat types and toad 919 was also located in a variety of habitat types but was located most often in conifer and rocky areas.

Table 2. Number of boreal toad locations recorded in each habitat type at the Hesbo and Donut study area in 1997.

Habitat Category	Toad Radio Number																	
	148	332	336	703	771	772	773	774	814	820	914	915[b]	918	919[b]	920	922[b]	924	925	927
Aspen/Conifer[a]			2	6	3		1	1	1			1		5					
Road												2		1					
Spring					4		3					2		2		1			4
Stream												2		1					
Water												2							
Wet Area	5		5	6	1			3		1			4		5		9	6	
Rock	3	1	9	3	11	1	12	4			2	3		7			2	6	4

[a] Category was Aspen/Conifer for Hesbo site and Conifer for Donut site.
[b] These are toads in the Donut study area, all others are Hesbo.

Movement was calculated for each toad weekly on a 3 m^2 digital elevation model as previously described. One hundred thirty eight individual weekly movement measurements were calculated for 13 toads. The average distance moved per day was 11.83 m (min=0.08m, max=135.69m, SD=20.42). Male toads moved an average of 10.60 m per day (SD=17.62, N=86) and females moved an average of 13.93 m per day (SD=24.52, N=50). The greatest distance moved by any one individual was number 774, a female which moved 643 m in only 28 days. Due to individual heterogeneity, it could not be shown that movement by female boreal toads was significantly different than males (Z=-0.852, P=0.394). Movement by female toads was variable than males (Figure 2.) and maximum distances moved were far greater in some cases.

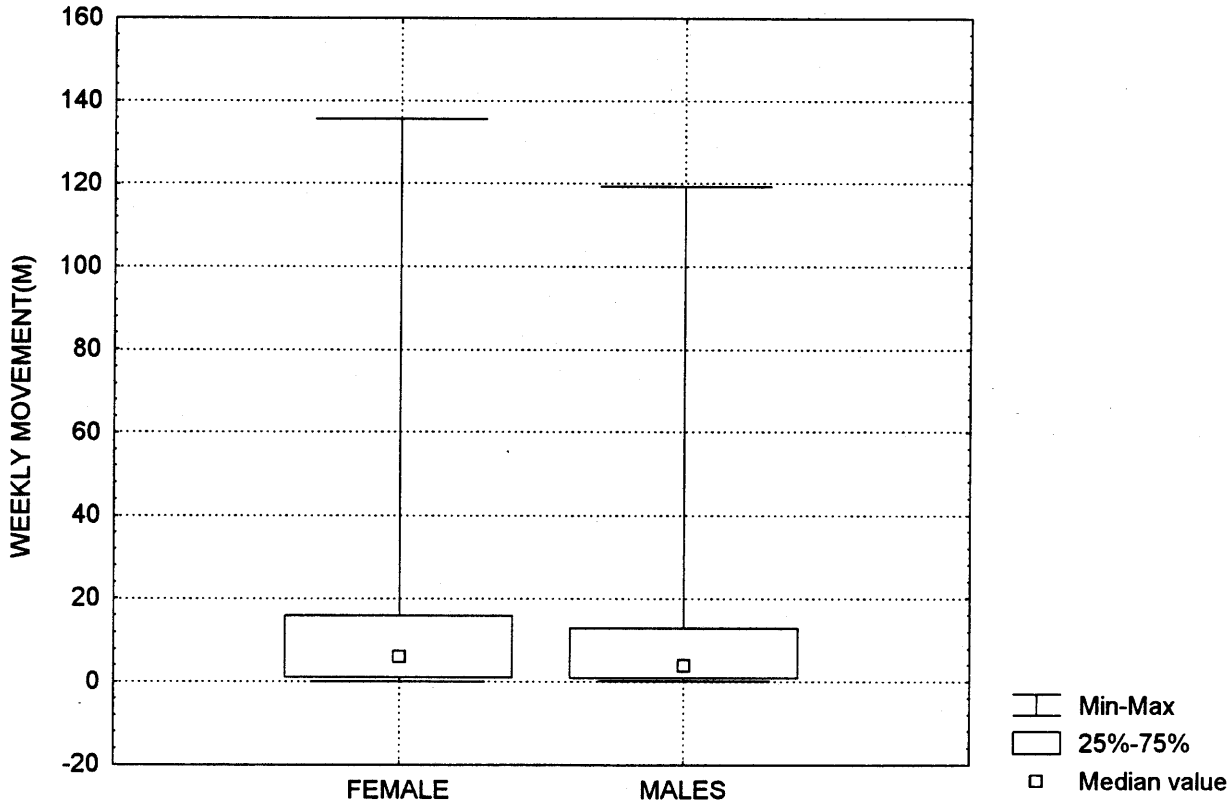

Figure 2. Comparison of male (N=86) and female (N=86) boreal toad movement increments at the Henderson/Urad study site in 1997.

Hibernation Sites and Temperatures

The boreal toads in the Henderson/Urad metapopulation depend extensively on golden- mantled ground squirrel *Spermophilus lateralis* burrows as hibernaculum. In 1995, Goettl radio tracked four toads to their hibernaculum and subsequently monitored their external temperature. One hibernated at the base of an engleman spruce north of Anne's Pond, one female hibernated in a burrow associated with a spring below

Hesbo, one female hibernated 40 meters above the Urad road in a burrow complex (several other adults were observed in this burrow). One hibernated under a bank which had a spring seep flowing from under it in the Power Alley area. November to April hibernation temperatures of the four toads ranged from 3.0°c to 9.0°c with a mean of 4.9°c (n=24, SD=1.94).

In 1996, Goettl tracked three toads to hibernaculum, one female hibernated in a complex of ground squirrel burrows below Hesbo, one female hibernated in a clump of willows associated with a spring seep east of Hesbo on the south side of Clear Creek, and one hibernated in a clump of willows at the west end of the Upper Urad Wetland. All transmitters died before they could be recovered in May. November to April hibernation temperatures of the four toads ranged from 3.0°c to 9.0°c with a mean of 5.0°c (n=24, SD=1.97).

In 1997, eight toads are being monitored in hibernaculum. Two of these are hibernating together in the same ground squirrel burrow below Hesbo, two are hibernating together at the base of an engleman spruce in a ground squirrel burrow north of Anne's Pond (same site as 1995 and 1996), one is under the Urad road in deep hole (this toad may not be able to get out), two are in ground squirrel burrows in the aspen/conifer area up the hill from Hesbo, and one is hibernating below Hesbo in a burrow associated with a spring seep (same location as 1995). November to April hibernation temperatures of the eight toads ranged from 3.5°c to 11.0°c with a mean of 6.0°c (n=31, SD=1.26).

Breeding Site Population Estimates

Boreal toads at the Urad/Henderson breeding sites were PIT tagged during 1995, 1996, and 1997 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.
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 3. Population estimates for male boreal toads at the breeding sites in the Urad/Henderson area from 1995 to 1997.

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
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
Upper Urad	1996	M_{tb}	41	0.26	40 to 41
Upper Urad	1997	M_o	34	7.59	27 to 59
Donut	1997	M_{th}	19	4.32	16 to 37

In all cases, the estimate derived from the Capture model (Table 3.) 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 individuals and 233 in 1997. There was not enough tags implanted at all sites to calculate estimates for every site in 1995. This type of work is critical in defining what is natural fluctuation in breeding numbers over time and identifying declines.

As stated earlier, the number of female boreal toads in the Henderson/Urad area is difficult estimate because they were never recaptured again in the same year, and only rarely in subsequent years. From 1995 to 1997, the only site with female recaptures was Hesbo. There was one female tagged in 1995 that returned in 1996 and one tagged in 1996 that returned in 1997. No females that were tagged at one site ever showed up at a different site. There is speculation that females do not breed every year. There is also evidence that male:female capture rates are skewed toward male dominance (Campbell 1976). If you were to assume that every female that frequented a breeding site left an egg mass, which is probably a reasonable assumption, ten or eleven females visited the Hesbo site in 1997. Nine of these were found during breeding site monitoring which indicates we are seeing most of the females visiting the site. From 1995 to 1997 a total of 37 individual females (all years and all sites combined) were handled in comparison to 221 males in 1995, 223 males in 1996, and 209 males in 1997 (all sites combined). The yearly male:female sex ratios were 20:1 in 1995, 32:1 in 1996, and 10:1 in 1997 which supports the hypotheses that females do not breed every year. More research needs to be conducted on the biology and population dynamics of female boreal toads as this information may be a key link in population declines.

Site Fidelity

As previously discussed there is insufficient recapture data available at this time to estimate breeding site fidelity of adult female boreal toads. There was, however a fair amount of data collected from 1995 to 1997 on Henderson/Urad adult male boreal toads to suggest a high level of breeding site fidelity. In 1996 at Hesbo, 81 of 131 male toads handled had been tagged at this site in 1995. In 1997 a total of 124 males were handled, 53 were from individuals tagged in 1995 and 78 were individuals handled in 1996 (1995 and 1996 tags); 49 of the 78 individuals were handled in 1995, 1996, and 1997. In 1996 at Power Alley, 23 of 54 toads handled were tagged at this site in 1995. In 1997, out of a total of 79 individuals handled, 27 were returns from 1995 and 17 were tagged in 1996; 20 of these individuals were handled in 1995, 1996, and

1997. Five of the males handled in 1997 were tagged in 1995 but did not appear in 1996.

DISCUSSION

Trends in population size and breeding success at all known boreal toad breeding sites is being monitored on an ongoing basis. This information will permit rapid identification of changes in abundance which could influence recovery. It is obvious that not all sites recruit every year and this fluctuation is natural. In most cases, individual breeding sites recruit in one out of three years at best.

Climatic conditions each year have a major impact on recruitment. Spring storms frequently kill egg masses and early fall freezing conditions either directly kill toadlets or negatively impact dispersal to suitable hibernaculum. The egg masses at both Upper Urad and Mizpah (down Clear Creek from Henderson) were killed in 1997 by late spring storms, similar conditions existed in 1996. Breeding sites at higher elevations are more susceptible to negative climatic conditions. In addition, cooler than average summer temperatures slow tadpole development which makes fall conditions critical to timely metamorphosis and dispersal. Water level fluctuation resulting in desiccation of egg masses is also very common. In 1997, all five egg masses in the main pool at Power Alley desiccated due to the water level dropping prior to hatch. We have been able to mitigate this situation in a number of cases by artificially manipulating water level or by moving egg masses to stable sites which resulted in substantial recruitment that otherwise would not have occurred.

Variation in yearly recruitment causes natural fluctuations in populations through the absence of sequential year classes. These short term fluctuations are tempered by the fact that boreal toads are relatively long lived (8-15 yrs. based on preliminary analysis of skeletochronology of samples from the Henderson/Urad population). Long term research is needed to define possible long term fluctuations and to distinguish between natural and anthropogenically caused declines (Pechmann et al. 1991).

Limited work has been done on trying to define habitat requirements of the boreal toad after they leave the breeding site. This information is needed both in recovery efforts to identify suitable translocation sites and as an aid in land use decisions. Bartelt and Peterson (1994) conducted similar radio telemetry studies on the Targhee National Forest in which they quantified use of various habitat components. They found that boreal toads occupied terrestrial habitats 90 percent of the time and their daily movements were significantly influenced by the distribution of suitable cover (usually shrubs). Our data also show that upland habitats are used extensively by boreal toads after the breeding season and therefore it is not adequate to only protect wetland areas (breeding sites) from development or disturbance, the surrounding upland habitats are also critical to population survival.

Bartelt and Peterson (1994) found ground squirrel burrows were used extensively by toads and that the toads used a wide variety of microhabitat types. Our work at Henderson also emphasized the importance of ground squirrel burrows both as shelters which were used daily, presumably for thermal and hydroregulation, and as hybernaculum. Our data shows that the toads do indeed use a wide variety of habitat types and that there was high variability between individuals in habitat selection. The activity and subsequent use of habitats by ectotherms is closely tied to their body temperatures (Huey 1991) which may explain the disproportionately high use of rocky areas. Toads were commonly found basking in rocky areas, but they were always within a couple of meters of a burrow or vegetative shelter.

The same toads were observed repeatedly in different areas in the same burrows or general areas they were previously recorded at, ie. the toads would move to a different area 10 to 50 meters away and then return to the same exact place a week or two later. In this respect, male boreal toads appear to have very distinct home ranges and seem to know where all the suitable burrows are within this area. Other authors have also noted distinct home range areas in anuran populations (Brattstrom 1962; Campbell 1976; Parker and Gittins 1979; Bartelt and Peterson 1994). Females on the other hand moved further away from the breeding site in a short period of time, and based on our PIT tag returns we cannot say whether they return to the same breeding site. This behavior by female boreal toads was also noted by Bartelt and Peterson (1994).

Boreal toad movement seems to be highly variable between individuals, or as Campbell (1976) put it "...some few animals tend to be wanderers" . Female toads which we radio tagged at a breeding site left the location immediately after egg deposition and generally moved further away from the breeding site quicker than did males. Again habitat use heterogeneity among females was observed with some finding suitable summer locations within 400 to 600 m from the breeding wetland while other individuals moved up to several km (Goettl, unpubl. data, Lost Lake data) or even out of the study area. Bartelt and Peterson (1994) recorded a female traveling 2.5 km and Campbell (1976) described a female that moved 3 km and crossed the Continental Divide. Campbell (1970) observed many boreal toads move up to 900 m during the summer. The males we tracked typically stayed within several hundred yards from the breeding site, moving back and forth in limited areas as described above.

Hibernation by boreal toads in ground squirrel burrows is common and they seem to be able to find suitable temperature and humidity conditions in them to survive the harsh winters at high elevation. Evaluation of the hibernation data from 1995 to 1997 at Henderson/Urad shows that boreal toads hibernate communally in many cases and the same hibernaculum are used year after year by different individuals. Communal hibernaculum were also observed by Campbell (1970). Most upland hibernaculum are ground squirrel burrows and many are in close proximity to spring seeps. We have seen toads in the same hole as the ground squirrels, so they are not necessarily abandoned. Observations have been made of up to five and six toads in the same burrow. As an added note, 15- year old toadlets were located under a rock in spring 1997 just after the snow melted. The rock protruded no more than an inch into the ground and therefore the toadlets experienced near freezing if not freezing temperatures and survived the winter. More research needs to done on temperature tolerances of toadlets since in many cases they metamorphose so late in the year that they cannot disperse to areas with reasonable hibernation structure.

The sex ratios observed during breeding site monitoring at the Henderson/Urad sites (1995- 5% female, 1996- 3% female, 1997- 10% female) are troubling in that we do not know if these are true sex ratios or if only a few females actually breed in any given year. Similarly skewed sex ratios have been reported in other boreal toad studies. Sex ratio information in Table 4. comes from two sources: Divide Lake, Trout Lake (both in Hinsdale County) are from Campbell (1976), while the remaining data is from Corn et al. (1997). The "5-year average" for Campbell's Front Range observations were mostly the late 1960s. Sex ratio percentages were calculated from Corn et al. (1997) from the numbers they reported (omitting the juveniles). The "Away from breeding sites" column shows close to a 50% ratio which favors the hypotheses that the females do not breed every year. Campbell (1976) also stated that female boreal toads may be less likely to be captured since they move much further away from the breeding site after the breeding season. We plan on monitoring more intensively for females in 1998 to get a better idea of true sex ratio in the Henderson Mine area.

Table 4. Comparison of boreal toad breeding site sex ratios from several populations in Colorado.

Sex ratio	Year	Males	Females	Juv.
Divide Lake	1971	82%	18%	
Divide Lake	1972	70%	30%	
Trout Lake	1971	75%	25%	
Trout Lake	1972	78%	22%	
Front Range	5-year average	73%	27%	
Lost Lake, RMNP	1991-1996	401 (82%)	87 (18%)	
Kettle Tarn, RMNP	1991-1996	316 (84%)	62 (16%)	5
Lake Husted (RMNP)	1991-1996	20 (74%)	7 (26%)	
Halfway (RMNP)	1991-1996	32 (84%)	6 (16%)	
Away from breedings sites (RMNP)	1991-1996	17 (47%)	19 (53%)	8
North Fork (all the above RMNP)	1991-1996	776 (81%)	181 (19%)	

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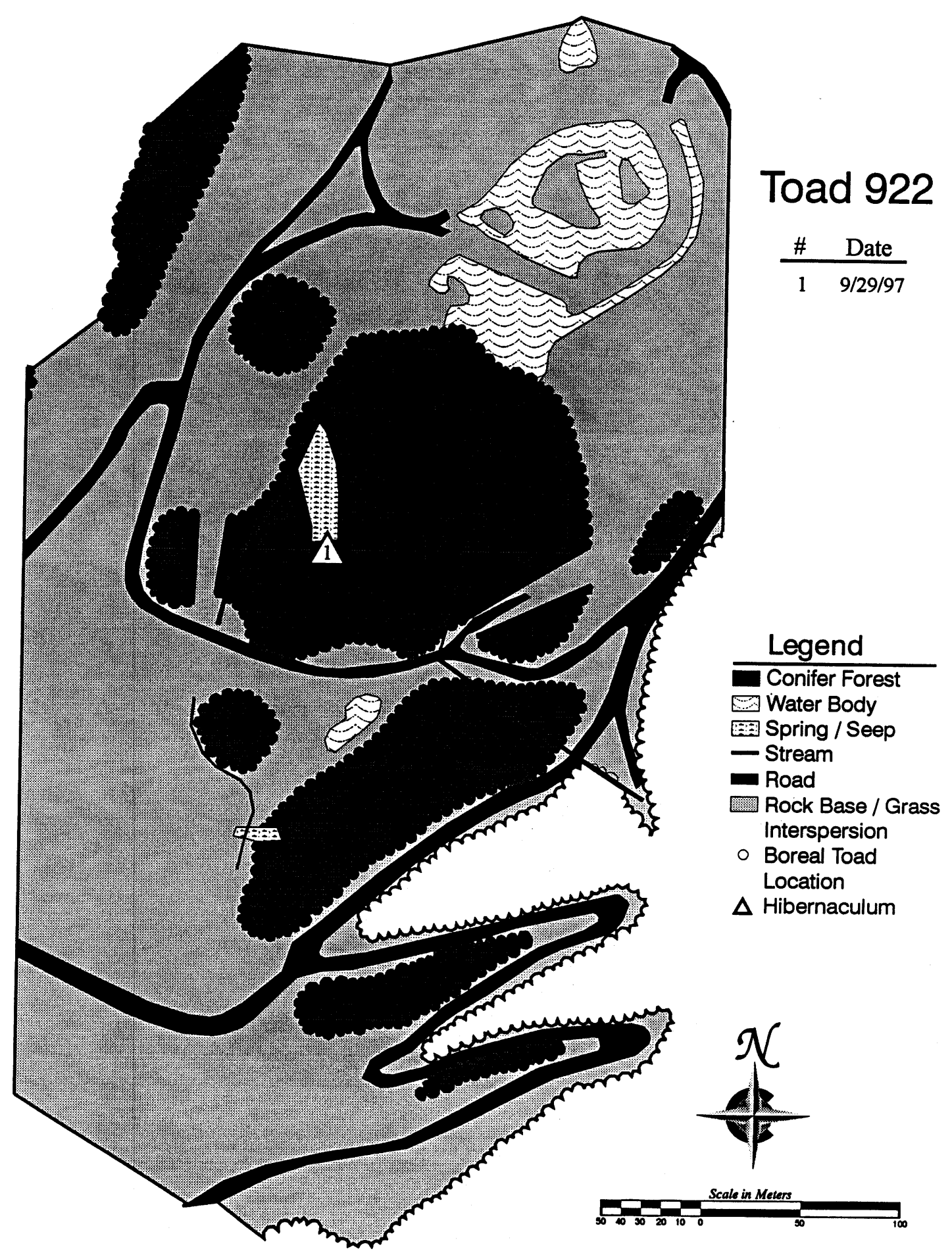
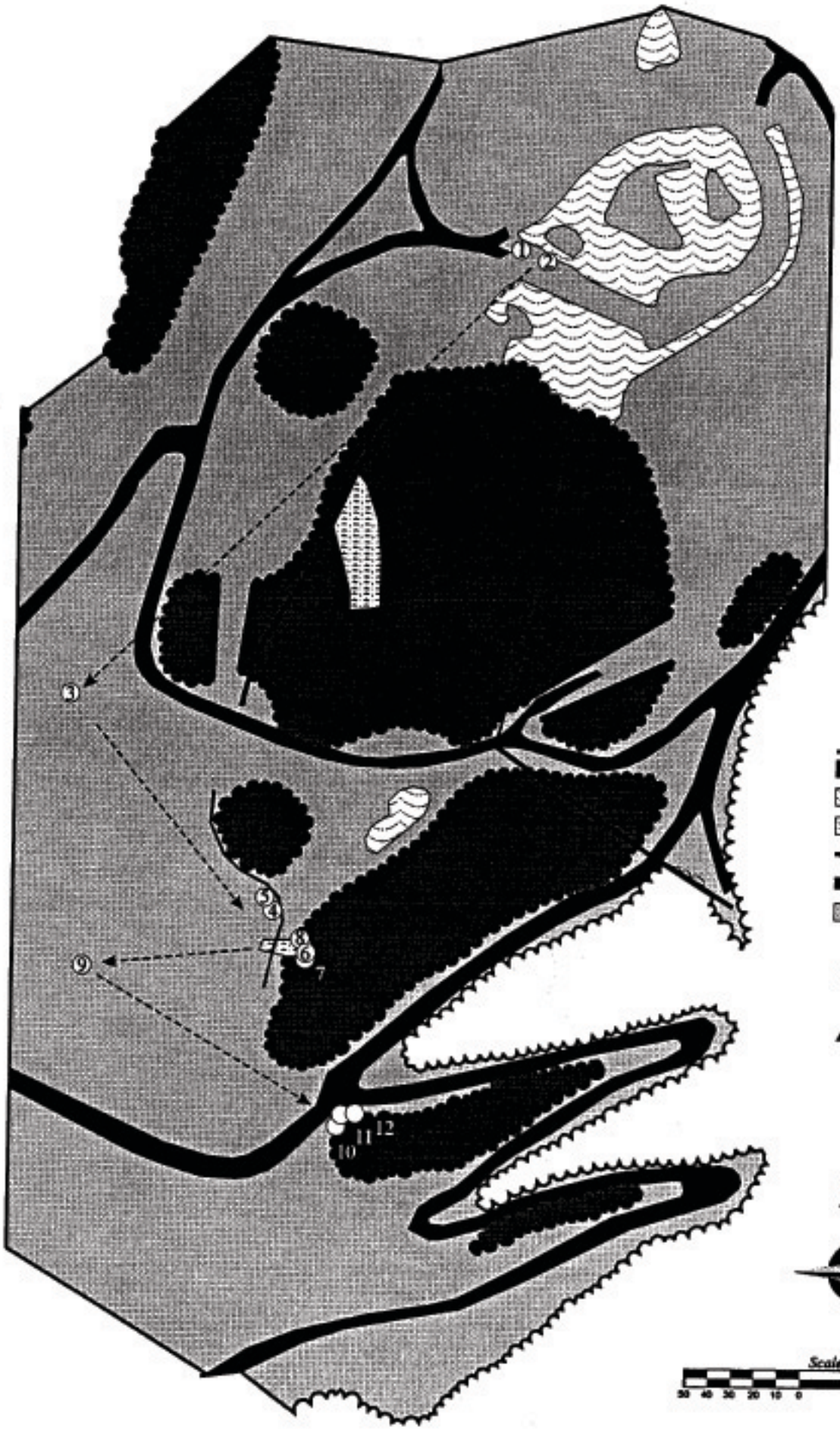
APPENDIX 1. Boreal toad radio telemetry contact locations in the Henderson/Urad study area, 1997.


Toad 922
Date
1 9/29/97
1
Legend
Conifer Forest
Water Body
Spring / Seep
Stream
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
N
Scale in Meters
50 40 30 20 10 0 50 100



Toad 915

#	Date
1	6/10/97
2	6/12/97
3	6/17/97
4	6/23/97
5	6/30/97
6	7/7/97
7	7/16/97
8	7/23/97
9	8/7/97
10	8/14/97
11	8/19/97
12	9/3/97

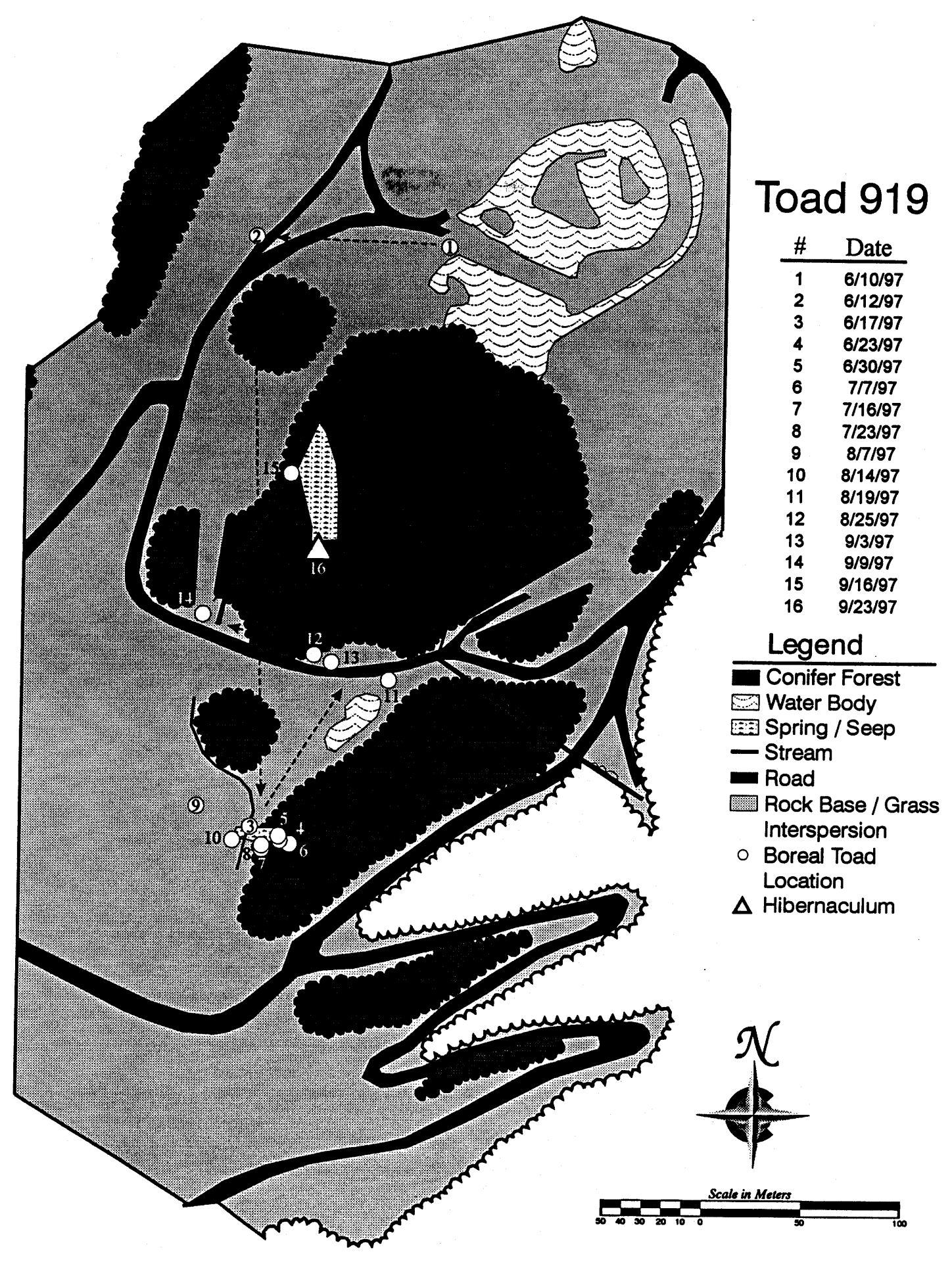
Legend

- Conifer Forest
- Water Body
- Spring / Seep
- Stream
- Road
- Rock Base / Grass Interspersion
- ○ Boreal Toad Location
- △ Hibernaculum

N

Scale in Meters

50 40 30 20 10 0 50 100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Date
1 6/10/97
2 6/12/97
3 6/17/97
4 6/23/97
5 6/30/97
6 7/7/97
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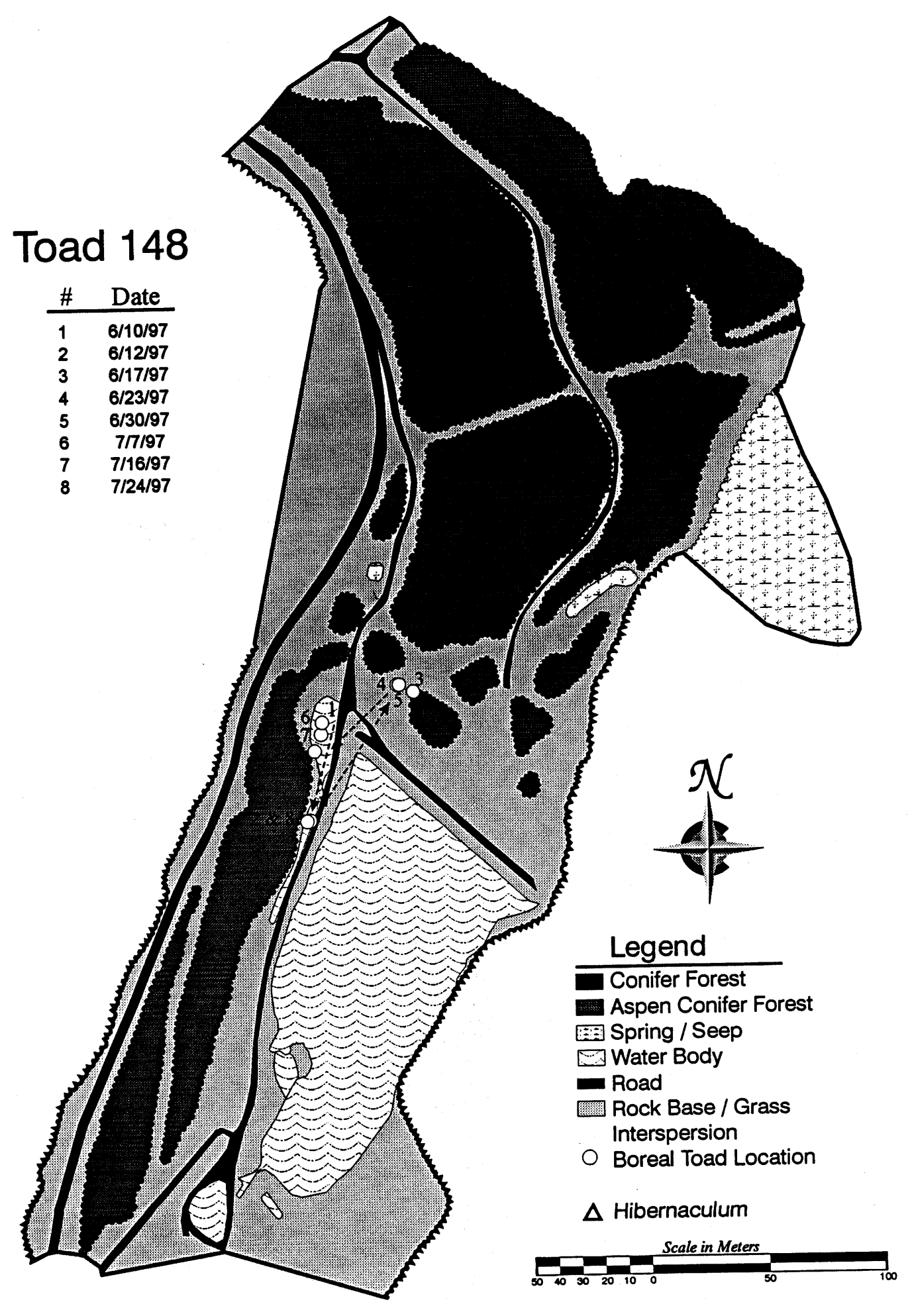
8 7/23/97
9 8/7/97
10 8/14/97
11 8/19/97
12 8/25/97
13 9/3/97
14 9/9/97
15 9/16/97
16 9/23/97
Legend
Conifer Forest
Water Body
Spring / Seep
Stream
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
N
Scale in Meters
50 40 30 20 10 0 50 100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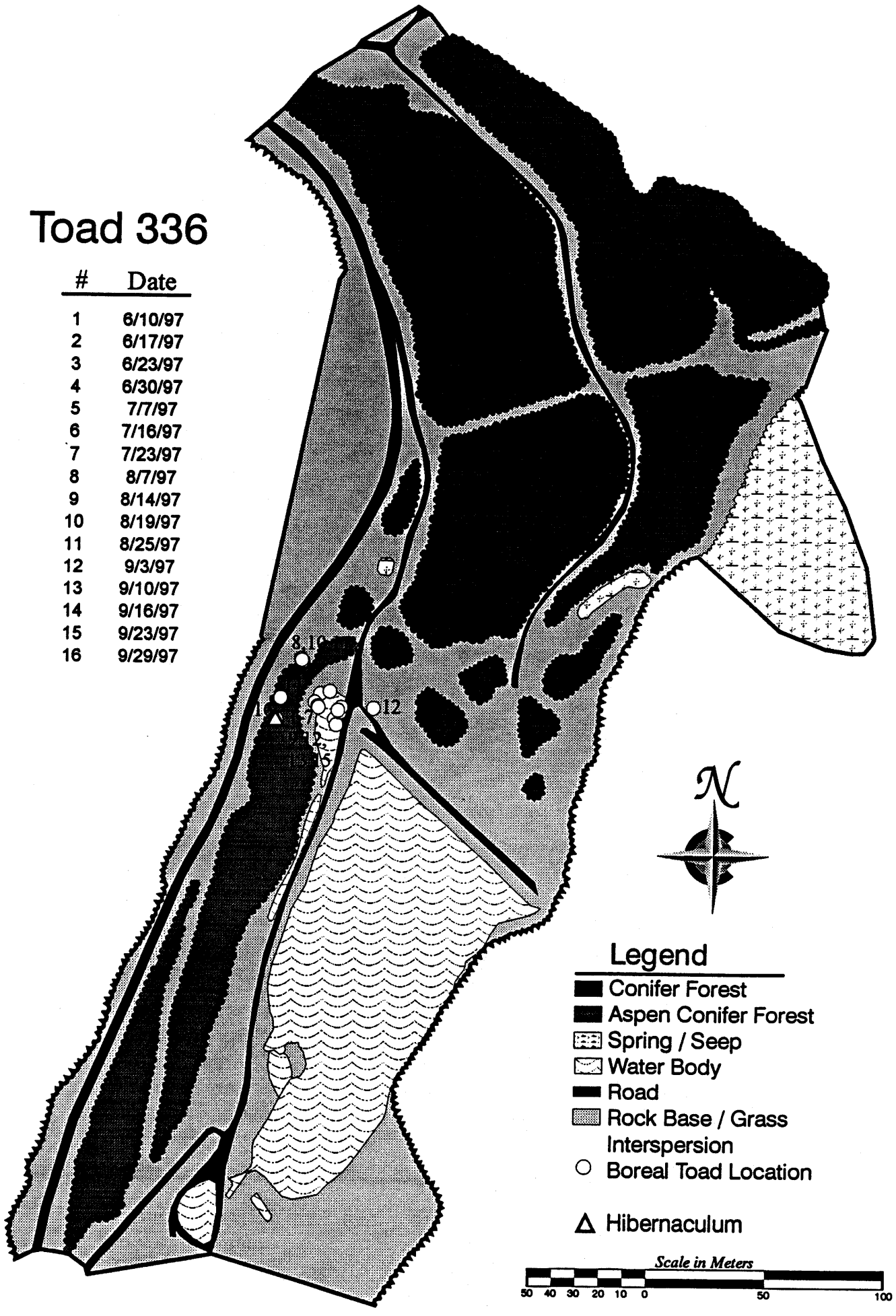
Date
1 6/10/97
2 6/12/97
3 6/17/97
4 6/23/97
5 6/30/97
6 7/7/97
7 7/16/97
8 7/24/97
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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3 6/23/97
4 6/30/97
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6 7/16/97
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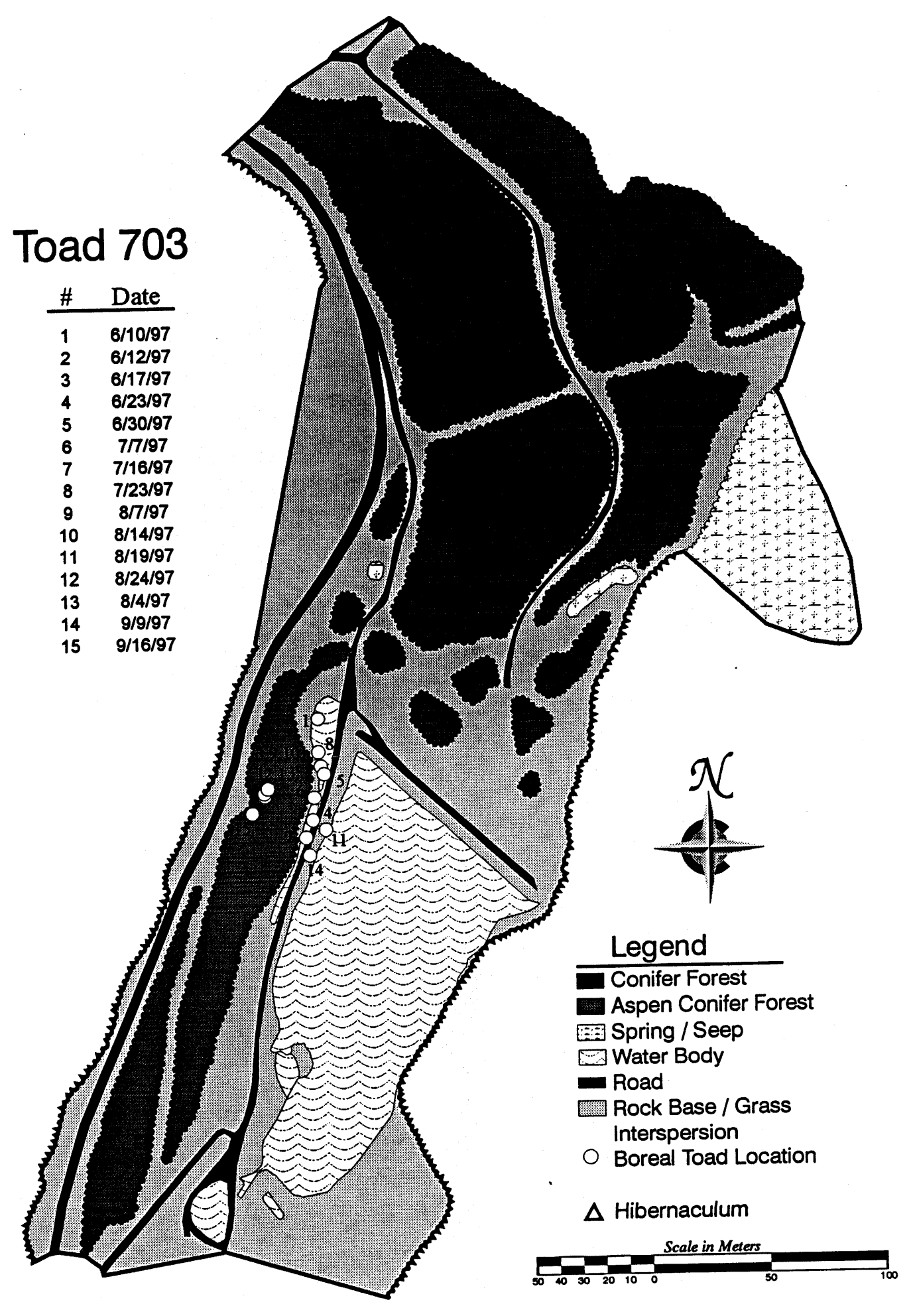
8 8/7/97
9 8/14/97
10 8/19/97
11 8/25/97
12 9/3/97
13 9/10/97
14 9/16/97
15 9/23/97
16 9/29/97
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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4 6/23/97
5 6/30/97
6 7/7/97
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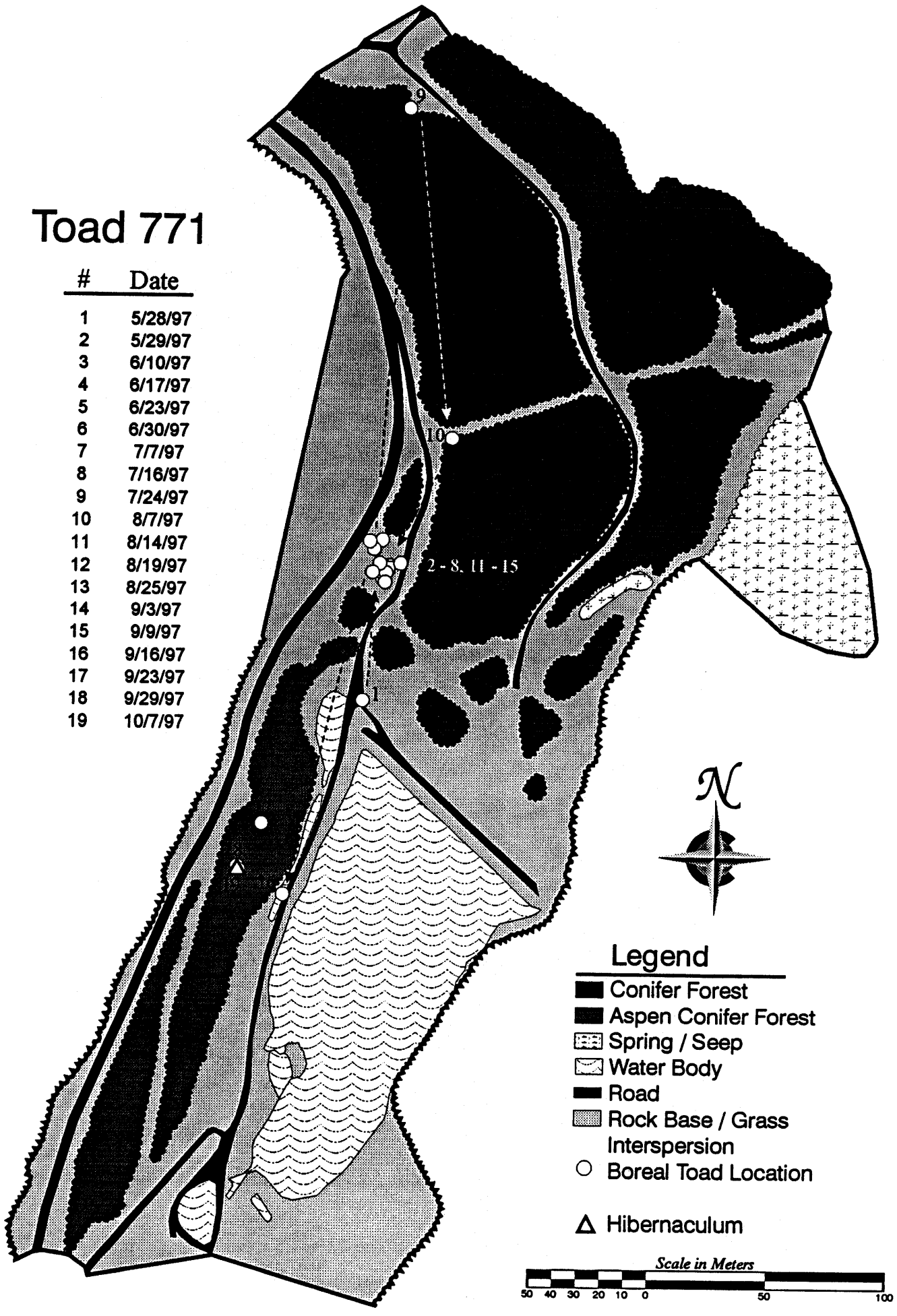
8 7/23/97
9 8/7/97
10 8/14/97
11 8/19/97
12 8/24/97
13 8/4/97
14 9/9/97
15 9/16/97
1
8
5
4
11
14
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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2 5/29/97
3 6/10/97
4 6/17/97
5 6/23/97
6 6/30/97
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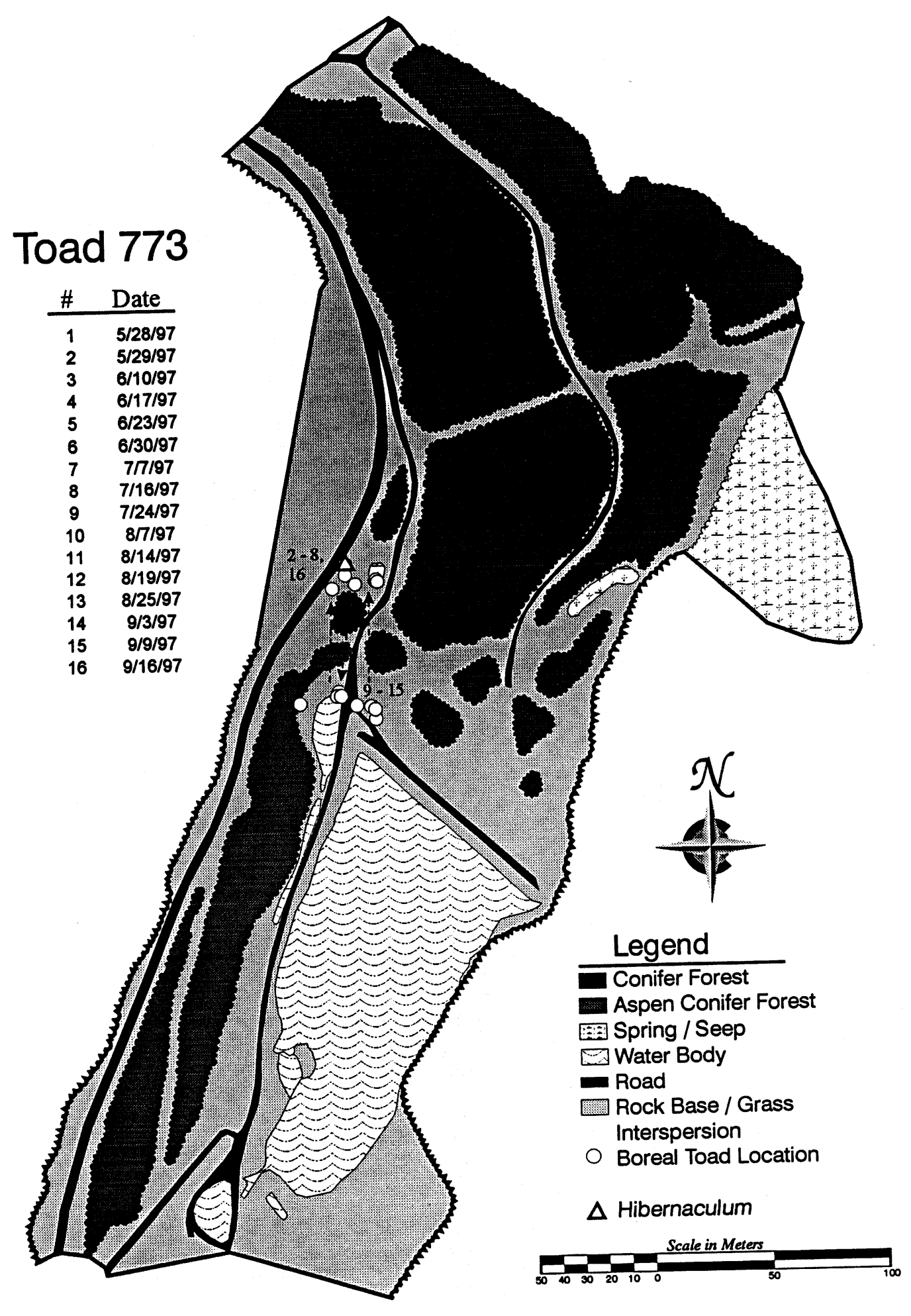
8 7/16/97
9 7/24/97
10 8/7/97
11 8/14/97
12 8/19/97
13 8/25/97
14 9/3/97
15 9/9/97
16 9/16/97
17 9/23/97
18 9/29/97
19 10/7/97
9
10
2 - 8, 11 - 15
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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2 5/29/97
3 6/10/97
4 6/17/97
5 6/23/97
6 6/30/97
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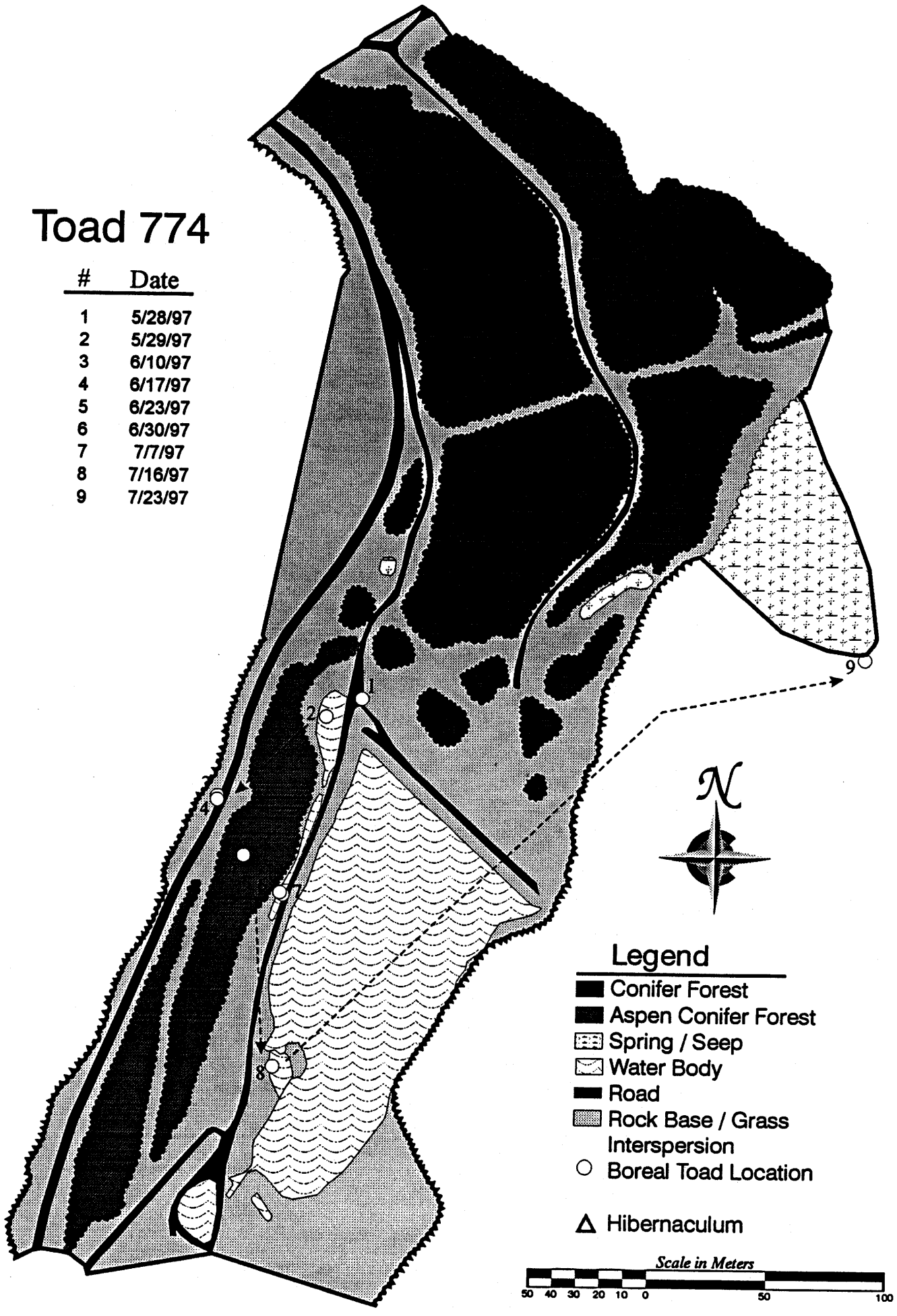
8 7/16/97
9 7/24/97
10 8/7/97
11 8/14/97
12 8/19/97
13 8/25/97
14 9/3/97
15 9/9/97
16 9/16/97
2 - 8, L6
9 - L5
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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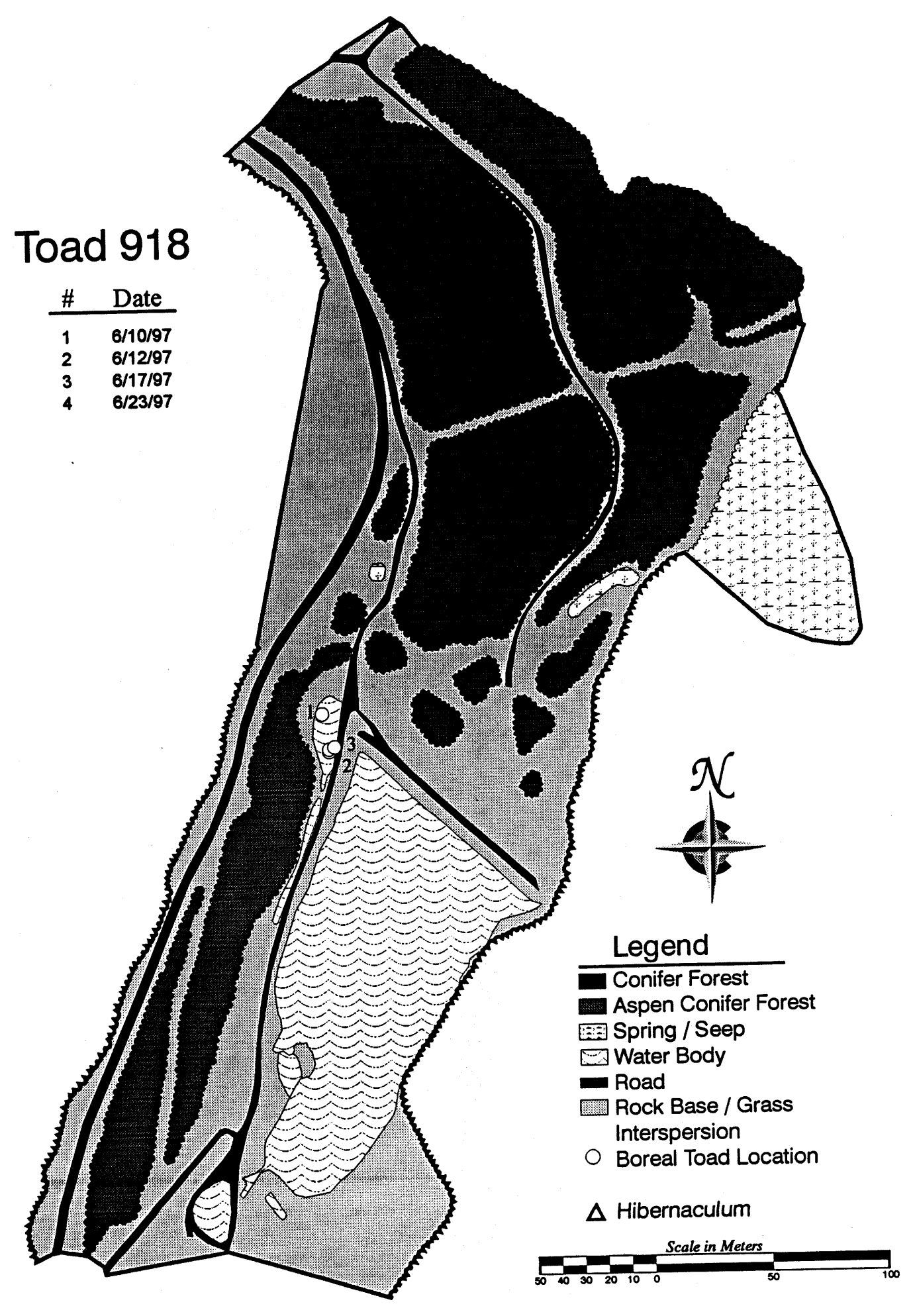
3 6/10/97
4 6/17/97
5 6/23/97
6 6/30/97
7 7/7/97
8 7/16/97
9 7/23/97
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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Date
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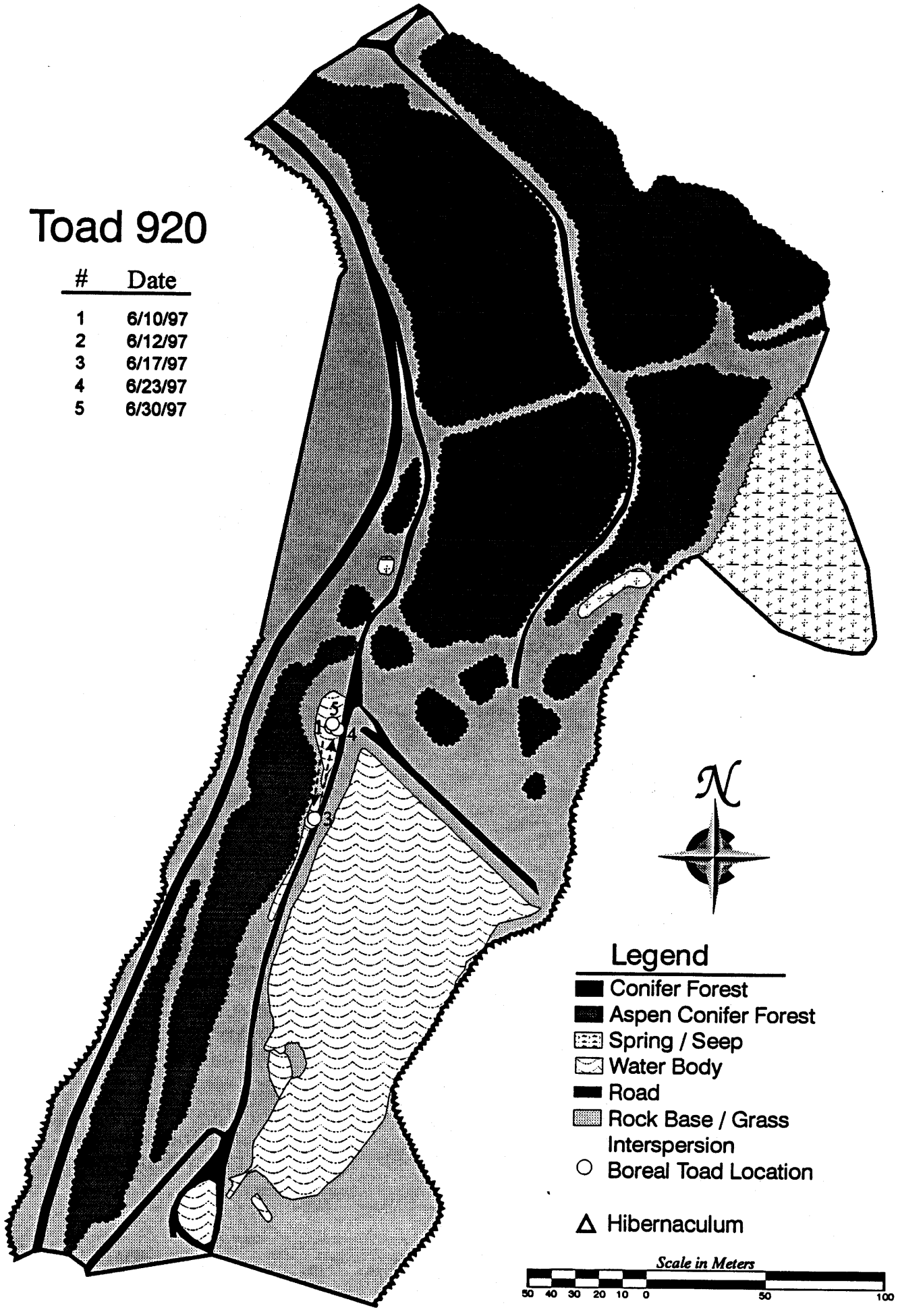
2 6/12/97
3 6/17/97
4 6/23/97
1
3
2
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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2 6/12/97
3 6/17/97
4 6/23/97
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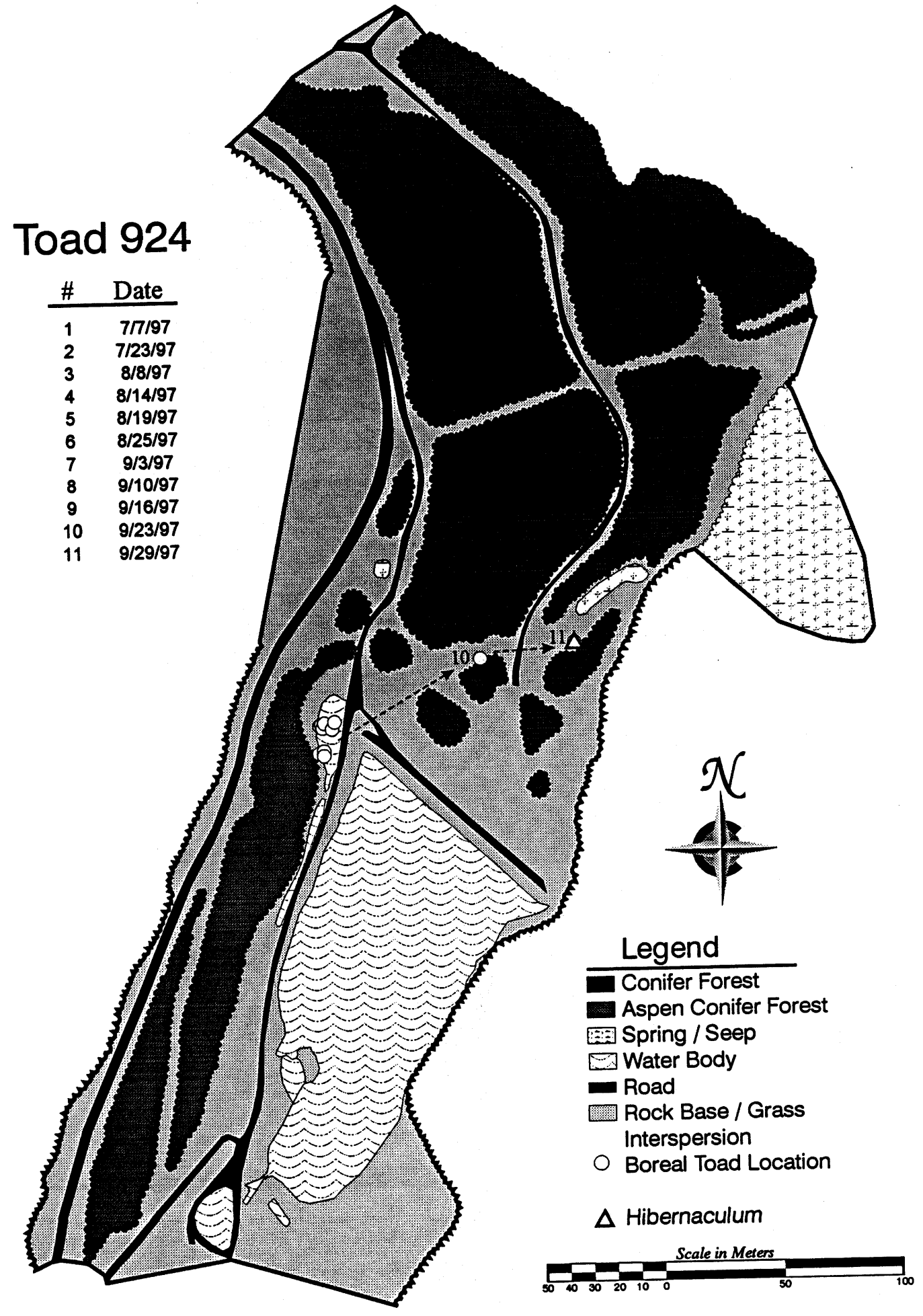
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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8 9/10/97
9 9/16/97
10 9/23/97
11 9/29/97
10
11
N
Legend
Conifer Forest
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Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
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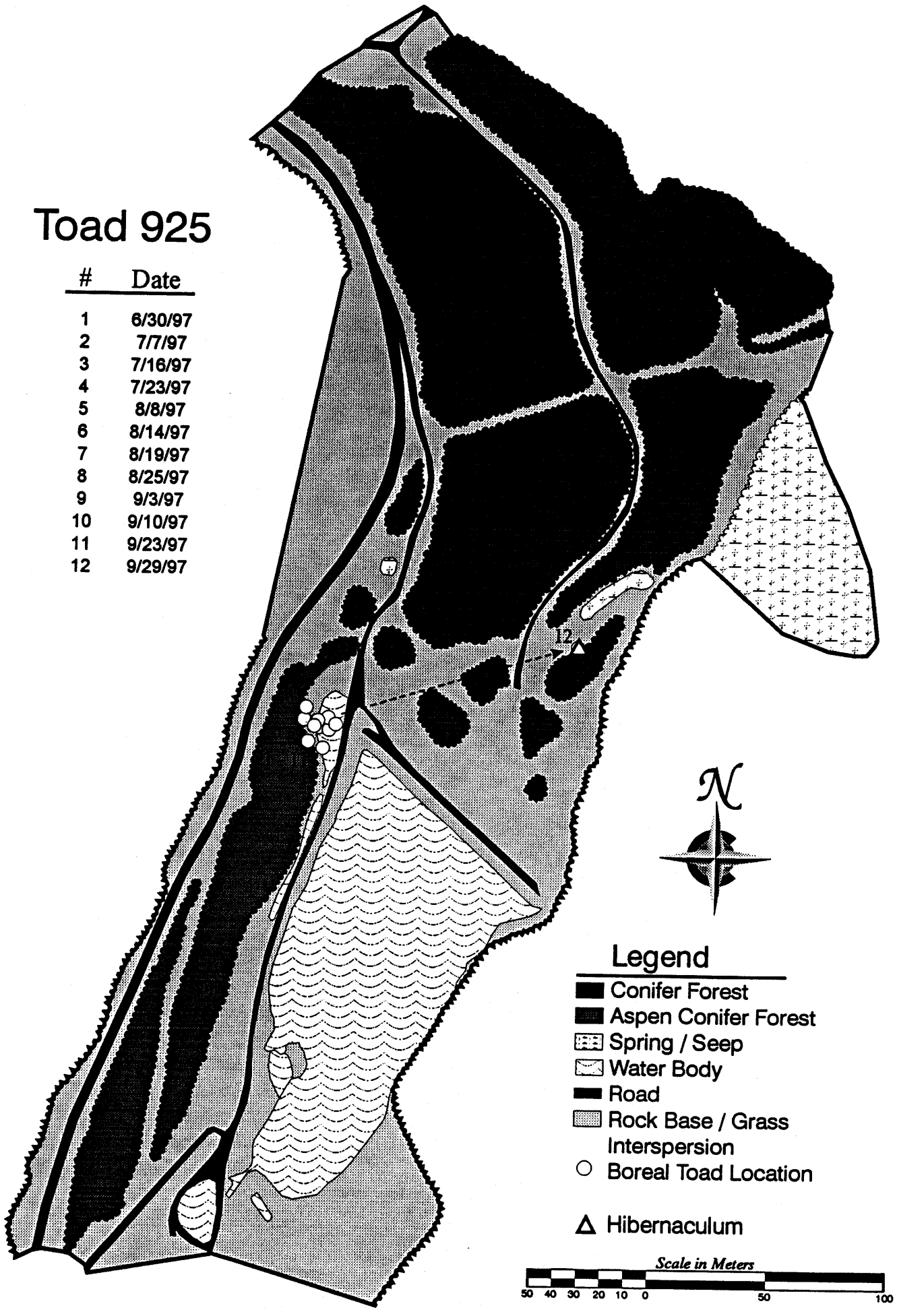
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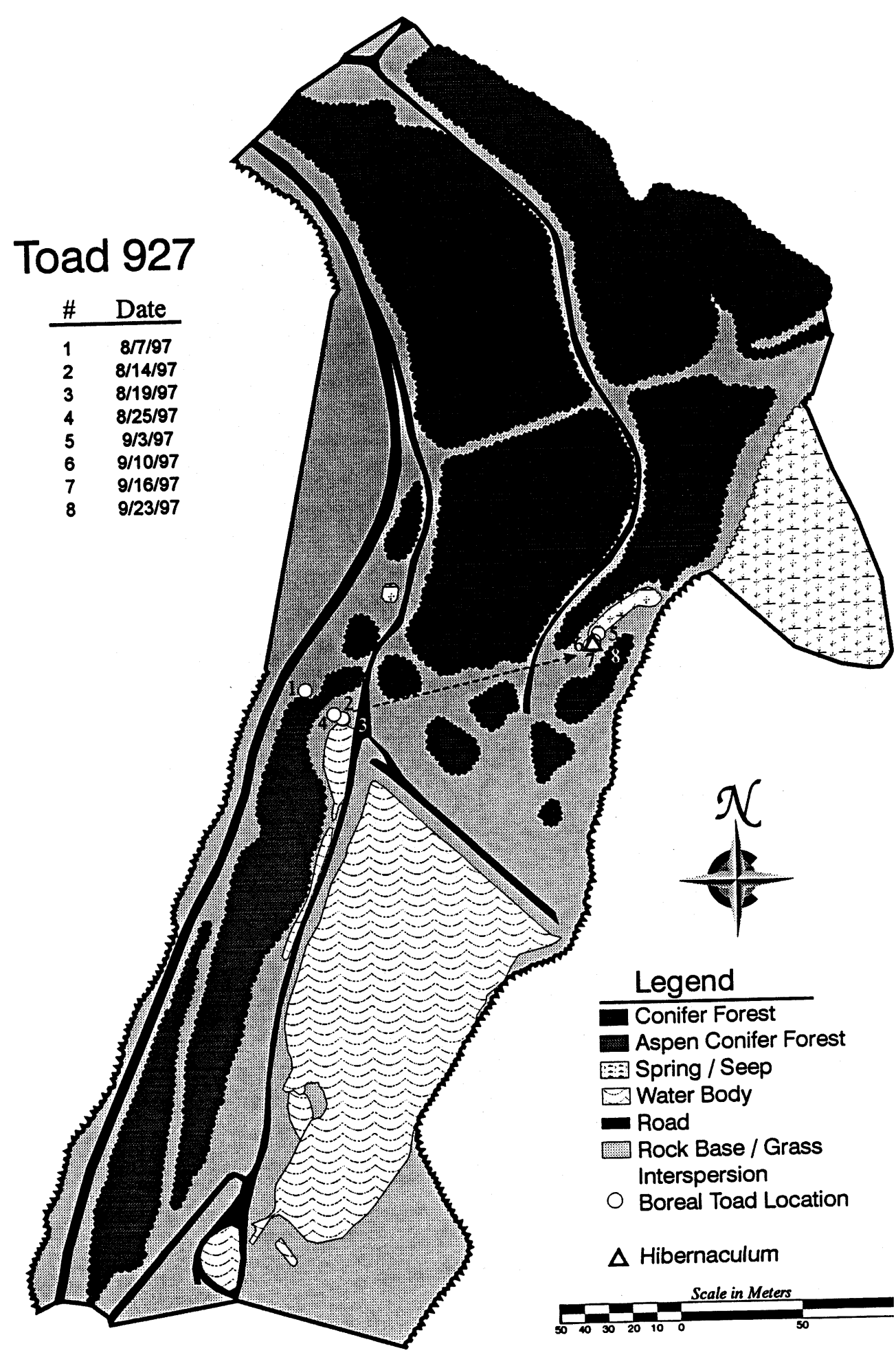
10 9/10/97
11 9/23/97
12 9/29/97
12
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50 100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8 9/23/97
1
2
3
4
5
6
7
8
N
Legend
Conifer Forest
Aspen Conifer Forest
Spring / Seep
Water Body
Road
Rock Base / Grass Interspersion
Boreal Toad Location
Hibernaculum
Scale in Meters
50 40 30 20 10 0 50

APPENDIX 2. Summaries of breeding activity at various sites in 1995, 1996.

1995
Clear Creek
Herman Gulch

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	H_2O Temp	Comments
06/01/95	1550	6	6	21	6		66	
06/02/95	2045	3	3	39	3	1	8	
06/02/95	2145	8	8	50	8		8	
06/05/95	2110	10	10	40	10	3	10	
06/06/95	1205	11	11	42	11	11	66	drying out
06/08/95	2110	1	1	41	1	12	42	
06/13/95	2143			49		1	9	clutch in road
06/14/95	2215	1	1	38	1	1 new	58	
06/16/95	1215					6 new	13	
06/19/95	2155	1	1	25	1		14	
06/22/95	2225	1	1	18	1		12	
06/28/95	2220			7			12	
07/05/95	1055	5					13	est. 200,000 tadpoles
07/10/95	1515			2			23	
07/24/95	1445							tadpoles drying
07/28/95	1330							drying bad
07/29/95	1135						72	
07/30/95	0830			1			52	
08/08/95	1050						57	tadpoles moved
08/17/95	1000						54	1st metamorph observed
08/24/95	1115						55	
09/06/95	1020						57	
09/15/95	1230						-	good toadlet production

1995
Triangle Pass

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	H_2O Temp	Comments
08/05/95	Day-Night	1		0	0	1	5-28	1 sub-adult laid 6/30
08/15/95	Day-Night	NO DATA					26	
08/30/95	Day-Night	1	0	0	0	0	20	1-2 KTP
09/23/95	1210	NO DATA					Frozen	TP Frozen

No recruitment in 1995, evidence of recent recruitment with sub adult.

1995
Clear Creek
Mt. Bethel

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	H_2O Temp	Comments
06/06/95	1055			2			47	
06/08/95	2150			1			34	
06/13/95	2105			5		1		Laid 6/12?
06/14/95	2130			4			41	
06/19/95	1130	3				1	-	obs shedding skin
06/19/95	2120			4		1	8	
0622/95	2140	3		1			8	clutch hatched
06/23/95	1000			2			7	
06/28/95	2130			2			8	TP development Good 500 TP
07/10/95	1450	1					25	50-100 TP remaining
07/24/95	1145			1			66	2 juveniles sighted; 10 TP remain
07/28/95	1000						20	3 juveniles; 9 TP remain
07/31/95	1000			1				3 juveniles; 5 TP
08/08/95	0945	1					60	2 juveniles; 1 TP
08/09/95	0945			2			17	3 juveniles; 0 TP
08/11/95	1100			3			72	2 juveniles; 0 TP
08/17/95	0940	1					59	1 TP re-appears
08/18/95	1800			1				Birds got the last TP
08/24/95	1015							1 new metamorph; 2 juveniles
09/06/95	1000	1						
09/15/95	1130			1				

Peak of breeding 06/13/95; 5 adults
No recruitment - observers assumed avian predation

1995
Clear Creek
Georgetown/Silverdale

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	H_2O Temp	Comments
06/14/95	2045							Area flooded
06/22/95	2055							Big pond blown out
07/17/95	1520						68	3 Juveniles
07/24/95	1620	1		1				5 Juveniles
09/06/95	1120							No toads

No breeding in 1995
Evidence of past breeding in sub adults observed in July

1995
Summit County
North Tenmile

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
07/19/95	1415	1	1			6	2000	22	1 sub adult
07/28/95	1630	1				5	3-4000	66	some drying
08/02/95	1200		1				3-4000	60	
08/13/95	1200	1	1				2-3000	60	active beaver pond
08/20/95	1115			2			3-4000	51	water rising
08/30/95	1020						3-4000	54	
09/05/95	1110			2			3-4000	46	
09/10/95	1300						2000	60	garter snakes obs. regularly
09/23/95	1300						2000	46	
09/30/95	1230						2-300	40	metamorphosis unlikely
10/07/95	1200						2-300	40	mostly frozen
10/14/95							1-200	41	pond ice covered; tadpoles under ice

Many adults in area
No recruitment in 1995

1995
Summit County
Montezuma

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
06/15/95	1415	1						53	still some ice on ponds
06/21/95	1440	4	1	1	1			26	sub adults present
06/22/95	2100	5				1		19	
06/24/95	1800	3				1		8	sub adults present
06/27/95	1030	7				1		13	
06/29/95	1030					1		10	lowering water level stranding eggs
07/04/95	1430					1			slow development
07/06/95	1315	7				1		21	
07/11/95	1030	6				1	20-30	14	hatching in progress
07/13/95	1350	4				1	50	18	50% dead eggs
07/18/95	0945	1				1	20	12	pond dropping
07/24/95	1145	3				1	20-30		
07/31/95	1215	1				1	1	56	1 toadlet obs
08/01/95	1415	1				1	0	62	
08/09/95	1545						0	60	
08/15/95	1700	1					0	64	
08/21/95	1500	1					0	58	
08/30/95	1320						0		clutch unsuccessful
09/05/95	1450								no toads

No recruitment in 1995 - evidence of past recruitment
Maximum number of toads (7) observed 06/27/95 and 07/06/95

1995
Summit County
Cucumber Gultch

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
07/12/95	1400	1				1		31C	clutch 3/4 weeks old
07/14/95	1100					1		54	
07/18/95	1620	1				1	100	17	Good tadpole growth
07/23/95	1415					1	20	79	WSE declining
07/27/95	1630	1				1	6	70F	
07/31/95	1545	1				1	4	76	
08/04/95	1000	1				1	3	66	
08/08/95	1015	1				1	2	50	
08/13/95	1430					1	20-40	64	sub adults present
08/15/95	1415	1				1	20	74	TP with both sets of legs
08/21/95	1625						20	66	
08/23/95	1450						20	68	
08/29/95	1730						20	64	6 new toadlets
09/05/95	1340							68	
09/10/95	1615								metamorphosis complete?

Possibility of 20 toadlets produced only recruitment in Summit County in 1995

1995
Pitkin County
Conundrum Creek

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
06/29/95	1330	3					1000	54	TP hatched late May or early June
07/08/95	1230	2	1		1	1	2000	59	
07/23/95	1130	1		1			3000	61	Sub adults present

Recruitment in 1995 unknown; multiple ages present

1995
Pole Creek Golf Course

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
06/01/95	2100	5	2		2			55	
06/03/95	1300	4				2		60	
06/04/95	1430			2		2		64	
06/08/95	1130			3		3		58	
06/10/95	1200			3		3		62	
06/12/95	1215			1				78	Active hatching; water receding
06/13/95	2030			1			500	62	
06/17/95	1300	1					500	72	
06/19/95	1130						1000	71	1 clutch & 150 tadpoles moved
06/22/95	1630	1						80	some tadpoles left high and dry
06/23/95	1600							80	
06/26/95	0930						100	61	
07/02/95	1500	1						52	
07/05/95	1700	1						64	
07/06/95	1530	1					1000	74	
07/08/95	1030	1						64	tadpoles throughout the pond
07/12/95	1500			1				82	pond filling with "moss"
07/18/95	0730						0	56	no tadpoles observed
07/21/95	1245						350	74	
07/22/95	1330			1			300	73	TP in clumps of 50-100
07/25/95	1130						13	67	water up 1 ft.
08/04/95	1230						35	74	tadpoles spread out
08/10/95	1200						100	71	
08/13/95	1045	1					150	63	start of metamorphosis
08/17/95	1330						25	67	some dead metamorphs
08/19/95	1130			1			25	66	
08/22/95	1000						15	65	lots of new toadlets obs.
08/25/95	1300						3	73	metamorphosis continues
08/29/95	1400						2	70	
09/09/95	1200						0	64	metamorphosis complete

Good recruitment breeding peak in early May

1995
Cottonwood Creek
Collegiate Peaks Campground CH1

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
05/18/95	1500	1							middle pond 2" snow
05/23/95	1700	1	1		1			15	lower
05/25/95	1700	1	1		1	1		56F	lower
06/02/95	1500	2				1		62	middle
06/05/95	1300	1	1		1	1		15	lower
06/05/95	1230	3	1		1	1		16	upper sub adults present
06/12/95	1300						1000	65F	middle
06/13/95	2130			16			1000	13	middle
06/13/95	2130			10				6	upper
06/20/95	1100					1			upper duck predation
06/21/95	1600			2			1	17	middle
06/27/95	1330	2				1	0	18	middle sub adults present
07/05/95	1350					1		19	middle
07/07/95	1840					1	300	18	middle
07/12/95	1345						300		middle
07/17/95	1510					1	0	15	middle all TP gone
07/24/95	1450			1		2	1000	16	lower
07/31/95	1220					2	1000	19	lower
08/21/95	1500					2	1000	23	lower start of metamorphosis
09/09/95	1030					2	50	14	lower many toadlets
09/15/95	2300	2	1					12	upper
09/16/95	1045					2		17	lower metamorphosis complete
09/23/95								9	lower no toads seen

first toad 5/18; first breeding 5/23; first eggs 5/29; fish hatching 6/9; last hatching 6/29; metamorphosis 8/26; estimated production 1000 toadlets; maximum # adults = 26 6/13

1995
Cottonwood Creek
Denny Creek

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
06/20/95	1515	8	5	15	2	3		7	sub adults present
06/23/95	1230	4				3	2500	9	high egg mortality
06/27/95	1440	1							
06/30/95	1100	5				3		11	6 sub adults
07/06/95	2100	8	10			3	2500	11	
07/12/95	1415					3	2500	15	toads on trail obs. in G.S. burrow
07/17/95	1900			3		3	2500	15	3 sub adults
07/24/95	1900			3			2500	12	
07/31/95	1500			2		3	1500	13	
08/08/95	1030					3	800	22	
08/14/95	0900					3	1000	12	sub adults present
08/21/95	1630					3	1000	16	start of metamorphosis
09/09/95	1140					3	300	18	good production
09/16/95	1030					3	150	15	4 metamorphs observed
09/23/95	1330					3	150	6	cold killing tadpoles

access restricted by snow
1st toad amplexus and egg 06/20
first and last hatching 07/02 - 07/09
onset of metamorphosis 08/15
production 1000 toadlets

1995
Cottonwood Creek Drainage
Hartenstein Lake

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
07/07/95	2030	2		33				11	
07/13/95	1000	3	2		1			12	
07/20/95	1515			10	1	1		17	
07/24/95	1710					1		12	
07/31/95	1300					1		18	
08/08/95	1130						400	18	
08/14/95	1045					1	300	17	
08/21/95	1830	1				3	600	16	
09/09/95	1340	3				6	600	23	
09/16/95	1230					5	500	16	

1st load 7/7; 1st amplexus 07/13; 1st eggs 07/20; 1st hatch 8/1; 1st metamorphosis 9/15; production likely poor

1995
Cottonwood Creek Drainage
South Cottonwood Creek

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
06/06/95	1500	2						7	sub adults present
06/20/95	2200			27				7	
06/27/95	1200					3			
07/05/95	1442					3	6000	18	
07/12/95	1150					3	6000	30	
07/17/95	1715			2		3	600	14	
07/24/95	1310					3	2000	23	
07/31/95	1130	1				3	600	15	sub adults present
08/07/95	1300					3	1000	12	WSE decreasing
08/15/95	1215		1			3	1000	24	
08/21/95	1330					3	500	19	
09/09/95	1550	1	1			3	150	21	65 new toadlets present
09/16/95	0930					3	100	14	30-40 toadlets
09/23/95	1100						0	8	metamorphosis complete

1st toads 06/06; amplexus < 06/22; eggs 06/27; eggs 06/22 - 07/04; metamorphosis est. 8/15; production good > 500 toadlets produced

1995
Cottonwood Creek Drainage
Browns Creek

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles (embryos)	H_2O Temp	Comments
06/13/95	1850						2000	17.8	Juveniles present, relocated garter snake
06/14/95	0930	1					2000	10.7	Tadpoles moved
06/20/95	0930						2000		
06/23/95	2200	2	3				1500	13.1	
07/05/95	1030						1500	15.0	
07/08/95	1300						1500	23	Predation by garter snake
07/17/95	1725						500	13.5	
07/24/95	1500						500	18.0	
07/31/95	1130						150	19.4	Start of metamorphosis
08/07/95	0930						30	20.0	Snakes taking metamorphs
08/15/95	1500						10	21.0	
08/21/95	0930						0	16.8	No production

1st toad 6/13/95, hatching 06/02/95 metamorphosis 07/31/95, very poor production due to garter snake predation

1995
Cottonwood Creek Drainage
Kroenke Lake

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles (embryos)	H_2O Temp	Comments
08/08/95	1400		1				1000	17.8	Desiccation a problem
08/10/95	1200			5			300	20.4	
08/15/95	1200						1200	17.0	
08/22/95	0930	1					1200	17.8	
09/10/95	1130						300	20.2	
09/17/95	1130						300	17.3	

Production unlikely due to the impending cold temps. Site first observed after breeding and hatching.

1995
Cottonwood Creek Drainage
Fourmile Creek

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
07/18/95	2230	3	1					11.7	
08/25/95	0900	1							No embryos observed

No production

1995
Clear Creek Drainage
Hesbo

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
05/30/95	2100	27	2					15	
06/01/95	2110	56	2		2			15	
06/06/95	2200	62	2		1			18	
06/15/95	2155	60						18	2 egg masses
06/19/95	2335		1	52					
06/22/95	2220	43	1				many	20	
06/27/95	2330	36					many	21	
07/06/95	2215	71		1			10,000	21	
07/11/95	2209	18						24	
07/18/95	2200	21						21	
07/24/95	2115	21						20	blood taken from 10 males
08/04/95	1215	1	1					21	

First toad 05/30/95; first amplexus 06/01/95; first eggs 06/02/95; hatching prior to 06/13/95; last egg mass ≈ 7/13/95

Over the course of the breeding season there may have been as many as 8-10 egg masses - counts were not possible because of water movement mixing eggs and tadpoles; production was poor due to predation, desiccation and entrainment to lower Urad Reservoir

1995
Clear Creek Drainage
2 Pond

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
06/06/95	2100	5	1	2	1			22	
06/13/95	2200	21	1	1			1	21.5	
06/15/95	2105	13					1 TP	19	
06/19/95	2125	18					2 TP	19	
06/22/95	2115	18					lots of tadpoles	20	
06/27/95	2200	8					lots of tadpoles	20	
07/06/95	2120	18	1				500 TP	22	
07/11/95	2145	8					2,000 TP	25	
07/18/95	2115	3						21	
07/24/95	2150			1				20	
08/04/95	1030							21	lots of 15mm toadlets

First toad 06/06/95; first amplexus 06/06/95; first eggs 06/7/95; first hatching 06/14/95; first metamorphosis 08/01/95; production very good > 1,000K toadlets

1995
Clear Creek Drainage
Power Alley

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
06/13/95	2300	27	6				8 EM	14	
06/19/95	2220	28	1				8 EM	19	
06/22/95	2115			14			3 EM	21	egg masses dried out
06/27/95	2240	18	2					18	
07/06/95	2245	33	3					22	start of limbs
07/11/95	2230	23	4				500 TP	24	
07/18/95	2310	14						20	
07/24/95	2250	6	1					20	males bled
08/04/95								24	

First toads 06/13/95; first amplexus and eggs before 06/13/95; first hatchings before 06/19/95; first metamorphosis ≈ 08/04/95

1995
Clear Creek Drainage
Vintage

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
06/20/95	1030	2	2		2		1		
06/22/95	2330			5			1		
06/27/95								17	start of hatching
07/06/95	1100						2 groups	22	
07/24/95	1100						500 TP	18	
08/15/95	1200						2,000 TP	19	Pond drying

First toads 06/20/95; first eggs before 06/20/95; hatching 06/27/95; very few metamorphs because of pond drying

1995
Clear Creek Drainage
Upper Urad

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
07/11/95	1810	5	1	1					
07/24/95	1100						5,000 TP	21	
08/01/95	1000						5,000	21	
08/08/95	1000						200	17	
08/15/95	1100						400	22	
08/23/95	1000							17	TP very large
08/29/95	1000							23	a few toadlets
09/13/95	1100							17	a few toadlets
09/20/95	1000						300		
09/27/95	1200								20-30 good toadlets
10/04/95	1200								1 ft of snow; many frozen toadlets

First toads 07/11/95; first tadpoles before 07/24/95; first metamorphosis 09/13/95; poor success due to late of metamorphosis

Northwest Region Summary

1996
12 areas (18 breeding sites)

First Creek

Tadpoles discovered 08/16/96 in pool within stream; metamorphosis completed by 09/23/96

Diamond Park

150 tadpoles found 07/25/96; tadpoles were gone by 08/12/96, some metamorphs were present

Soda Creek

Four metamorphs observed 08/08/96 and 08/09/96; one sub-adult seen on 08/11/96; water levels fluctuate violently

Pole Creek

Generally blasted by Memorial Day storm; Hole 4 produced 2 egg masses on 07/05/96; 3-4000 metamorphs produced by 9/01/96

Hole 15 had 5 adults and 25 tadpoles, less than 12 metamorphs were produced

Jim Creek

No breeding observed, however four ages of toads are present

Peru Creek / Montezuma

Peru Creek - eight sub-adults observed on 06/20/96; 200 tadpoles found on 07/24/96; 08/16/96 metamorphosis in full swing, as high as 1000 metamorphs produced

Montezuma - One dead toad (Basidiobilus) collected on 07/12/96 as many as 9 adults observed in the area over the summer, but no reproduction in 1996

Cucumber

Many adults in the area but no breeding in 1996

North 10 Mile (6 sites)

Perhaps only 2 toadlets produced at sites 1 and 2, Site 3 was impacted by cold and showed no recruitment, site 4 produced 1 toadlet mortality related to cold and desiccation. No production at pond 5; 13 male adults observed 07/08/96; site 6 the highest pond produced 200 toadlets from as many as 3 egg masses and 4000 tadpoles

East Lake Creek (2 sites)

Sites found 08/13/96, some recruitment, metamorphosis over a six week period, toadlets very small, much more exploration in this area is needed

Holy Cross City

Too cold for metamorphosis, Grey Jays observed preying on large tadpoles

Conundrum

Good production in 1996, adult mortality due to Basidiobilus observed

1996
Clear Creek Drainage
Hesbo

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
05/14/96	2100	48	7		7			12	
05/19/96	2140	55	3		3			14	
05/23/96	2230	66	1		1		6+	14	all eggs & TP moved to research hatchery
05/30/96	2200	54						15	
06/04/96	2200	67					4 EM	17	
06/11/96	2148	48						17	
06/18/96	2155	59		1			2,000 TP	18	
06/25/96	2140	22					8,000 TP	19	tadpoles in adjoining ditches
07/02/96	2200	11					75 TP	20	
07/16/96	1750						300 TP in 3 spots	20	
07/23/96	1130	1	2				200 TP	20	1 new egg mass
09/04/96									No recruitment

First eggs 05/15/96; hatching 05/20/96; no recruitment due to entrainment, predation and desiccation

1996
Clear Creek Drainage
Power Alley

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
05/14/96	1920								No Toads
05/19/96	2320	44	1	1	1			12	
05/23/96	2145	45					2	10	Egg masses dry
05/30/96	2340	30						3	Freezing
06/04/96	2200	43						15	
06/11/96	2217	33						13	
06/18/96	2050	24		4				17	no live egg masses
06/25/96	2050	24						14	
07/02/96	2120	16		3				18	Pond almost dry
07/16/96									No recruitment

1996
Clear Creek Drainage
2 Pond

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
05/14/96	2045							12	No toads observed
05/19/96	2100	3		1				16	
05/23/96	2048	2						14	
05/30/96	2125	4							
06/04/96	2100	3						17	3 subadults
06/11/96	2125	4					1 EM	15	
06/18/96	2115	4					4,000 TP	19	
06/25/96	1615						500 TP	19	May be 2 clutches
07/02/96	1400						1,000 TP	21	4 Groups
07/16/96	1720						0		

1996
Clear Creek Drainage
Upper Urad

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Tadpoles	H_2O Temp	Comments
05/24/96	0930							7	No toads observed
06/12/96	0845	1						10	
06/18/96	2250	21	1					11	Hesbo pit tag return
06/25/96	2235	18	1					8	
07/02/96	2200	21							
07/09/96	2215	8							
07/16/96	1130	4	3						
07/18/96	0930	6					4,000 TP		From 3 egg masses
07/23/96	1310	7					8,000 TP	19	
08/16/96							2,000 TP		

Good reproduction, poor recruitment due to sandpiper predation.

1996
Clear Creek Drainage
Vintage

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
05/19/96	0002								No toads
05/24/96	0830							11	No toads
06/12/96	1125						1 EM	18	Hatching eggs
06/26/96	1125						1,000 TP	19	Tadpoles being lost to entrainment to Clear Creek
07/18/96	1100						80TP	16	About to go dry

No production

1996
Treatment

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
05/31/96	0930	1						2	
06/04/96	1500							15	No toads
07/10/96	0920	1		1			1,000 TP	16	
07/16/96	1000	2	1						
07/17/96	1125						700 TP		
08/09/96	1300								Metamorphosis start about 8/3
09/04/96	1040								

First eggs before 07/01/96, metamorphosis starting 08/03/96; good recruitment at least 400 toadlets

1996
Donut

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
06/25/96	2155	1	1		1			16	Eggs hatching 06/21/96
07/03/96	1130	3	1		1		10,000 TP	19	
07/09/96	2130	2					10,000 TP		
07/16/96	1045								
07/23/96	1235						10,000 TP	20	May have been a new egg mass
09/04/96	1100						500 TP		500 toadlets

First eggs 06/14/96; hatching 06/21/96; poor recruitment due to lack of cover for metamorphosis - hot, cold, dry
First eggs 06/18/96, first hatching 06/21/96

1996
Ann's Pond

Date	Time	# Males	# Females	# Unknown	Pr. amplex	Clutches	# Egg Masses or # Tadpoles	H_2O Temp	Comments
07/08/96	0940						2,000 TP	15	Very large tadpoles
07/23/96	1145						2,000 TP		Pond drying
07/26/96	1150						1,000 TP		Move 1000 to Upper Urad

No recruitment dried up

APPENDIX 3. Water chemistry data form 1995 and 1996.

SITE	DATE	TEMP	COND	PH	BG_ALK	PHTH_ALK	HARD	CD_TOT	CU_TOT	FE_TOT	MN_TOT	PB_TOT	ZN_TOT	F	CL	NO3	PO4	SO4
2 POND H. MINE	07/16/96	15.5	1194	9.2	71.6	10.0	184.4	<0.10	<1.0	<50	1645	<1.0	16	21.1	11.3	10.2	<0.5	1268.0
2 POND H. MINE	05/29/96	12.2	1178	9.07	241.6	10.8	100.6	<0.10	<1.0	<50	726	59	26	10.0	16.1	8.1	<0.5	425.5
ABOVE COTTONWOOD LAKE	06/12/96	10.5	115	7.53	61.2	0	70.8	<0.10	<1.0	131	<10	<1.0	<10	<0.5	<0.5	<0.5	<0.5	6.7
ANN'S POND H. MINE	06/27/96	10.2	1533	8.15	137.2	0	58.4	<0.10	<1.0	<50	11400	<1.0	17	12.4	14.7	11.4	<0.5	758.1
BROWN'S CREEK	06/12/96	10.8	77.3	7.25	39.6	0	48.2	<0.10	<1.0	367	<10	<1.0	<10	<0.5	<0.5	1.5	<0.5	6.2
BROWNS CREEK	07/07/96	11.8	39.6	7.52	15.2	0	22.6	<0.10	13	158	<10	<1.0	18	<0.5	<0.5	1.0	<0.5	5.4
COLLEGIATE PEAKS	07/16/96	14.0	86.4	7.01	7.8	0	39.2	0.33	5.5	428	303	5.3	54	1.6	1.1	<0.5	<0.5	28.0
COLLEGIATE PEAKS	06/12/96	12.0	113.2	7.12	60.0	0	62.2	<0.10	1.2	923	281	<1.0	<10	<0.5	<0.5	0.9	<0.5	2.1
COTTONWOOD CREEK	06/24/96	11.4	39.2	7.59	21.2	0	32.6	<0.10	1.2	235	13	<1.0	<10	<0.5	<0.5	<0.5	<0.5	1.8
CREEDE HATCHERY	05/20/96	11.6	66.7	7.61	38.09	0	29.0	<0.10	2.1	212	48	<1.0	20	<0.5	1.6	<0.5	<0.5	7.1
DENNY CREEK	06/06/96	12.4	125.0	7.28	51.0	0	60.6	0.12	3.6	236	78	2.8	27	<0.5	2.4	<0.5	<0.5	18.4
DENNY CREEK	06/25/96	14.0	408	7.66	85.2	0	247.4	0.27	15	407	3070	8.6	122	2.6	1.1	<0.5	<0.5	145.3
DENNY CREEK	06/25/96	13.0	48.0	8.05	16.4	0	22.6	0.16	2.6	98	273	<1.0	38	<0.5	<0.5	0.5	<0.5	8.1
DONUT H. MINE	06/06/96	11.0	82.0	7.14	37.6	0	34.6	<0.10	2.0	730	53	<1.0	<10	<0.5	2.6	<0.5	<0.5	5.0
DONUT H. MINE	05/20/96	11.6	1161	9.08	244	7.8	93.2	<0.10	<1.0	<50	657	<1.0	22	9.9	15.7	8.1	<0.5	423.5
FERN CREEK	05/17/96	11.2	41.8	7.38	20.8	0	28.2	<0.10	<1.0	131	<10	<1.0	<10	0.7	<0.5	<0.5	7.9	<0.5
FERN CREEK	05/17/96	12.9	164.3	6.98	20.4	0	58.2	<0.10	<1.0	<50	726	<1.0	26	12.4	14.7	11.4	<0.5	758.1
FOUR MILE CREEK	06/24/96	10.9	503.0	7.13	67.6	0	157.2	<0.10	1.6	1186	223	<1.0	<10	<0.5	156.3	<0.5	<0.5	9.4
FOUR MILE CREEK	06/25/96	14.6	1456	8.89	154.6	2.7	60.2	<0.10	<1.0	<50	5820	<1.0	14	11.2	14.1	9.4	<0.5	762.0
GOTHIC NATURAL AREA	07/09/96	10.8	50.7	7.55	16.4	0	26.0	0.27	9.8	184	278	5.6	72	0.7	<0.5	<0.5	7.9	<0.5
HARTENSTEIN LAKE	06/20/96	11.0	101.6	7.88	61.2	0	65.4	<0.10	<1.0	125	<10	<1.0	<10	0.7	<0.5	<0.5	<0.5	2.1
HARTENSTEIN LAKE	07/15/96	9.2	41.6	7.00	19.8	0	43.2	<0.10	<1.0	56	<10	<1.0	<10	1.8	<0.5	0.7	<0.5	1.6
HERMAN GULCH	06/03/96	9.5	29.4	7.23	16.0	00	23.6	<0.10	<1.0	101	<10	<1.0	<10	<0.5	<0.5	<0.5	<0.5	2.2
HERMAN GULCH	07/15/96	8.3	70.3	7.18	40.4	0	48.8	<0.10	<1.0	70	<10	<1.0	<10	<0.5	<0.5	1.4	<0.5	5.6
HESBO H. MINE	06/26/96	8.2	67.9	7.39	41.0	0	24.9	<0.10	<1.0	61	<10	<1.0	11	2.0	<0.5	0.7	<0.5	1.8
HESBO H. MINE	08/24/96	8.4	43.2	7.27	25.8	0	30.8	<0.10	<1.0	211	<10	<1.0	<10	<0.5	<0.5	<0.5	<0.5	2.4
KETTLE TARN	07/12/96	22.0	241.0	8.39	114.2	0	136.6	<0.10	<1.0	687	26	<1.0	<10	<0.5	0.6	<0.5	<0.5	17.9
KROENKE LAKE	08/24/96	15.8	62.9	7.21	25.4	0	30.0	<0.10	<1.0	1509	202	<1.0	<10	0.5	1.3	<0.5	<0.5	7.4
KROENKE LAKE	08/07/96	22.0	51.8	7.56	23.6	0	23.6	<0.10	<1.0	515	22	<1.0	<10	<0.5	0.7	<0.5	<0.5	2.2
LOST LAKE	07/12/96	21.9	265.0	8.06	98.0	0	146.6	<0.10	<1.0	311	27	<1.0	<10	<0.5	0.5	<0.5	<0.5	48.2
LOVE LAKE	07/12/96	21.9	157.2	7.91	62.8	0	87.6	<0.10	<1.0	255	22	<1.0	16	<0.5	0.8	0.7	<0.5	12.2
MOUNT BETHEL	08/20/96	11.1	35.5	6.43	12.2	0	26.4	<0.10	<1.0	1123	<10	1.0	<10	<0.5	0.7	<0.5	<0.5	2.2
MOUNT BETHEL	07/12/96	22.0	76.8	7.83	37.4	0	36.0	<0.10	<1.0	144	<10	<1.0	<10	<0.5	0.7	<0.5	<0.5	3.9
MT. BELLVIEW	08/05/96	11.4	114.7	6.54	11.0	0	44.0	<0.10	1.1	516	93	<1.0	<10	<0.5	1.0	<0.5	<0.5	54.0
MT. GOTHIC	07/15/96	11.0	108.2	7.53	60.2	0	75.2	<0.10	<1.0	281	<10	<1.0	<10	<0.5	0.7	0.7	<0.5	6.9
POWER ALLEY H. MINE	08/24/96	10.0	40.3	6.33	14.8	0	36.8	<0.10	<1.0	3461	228	<1.0	<10	<0.5	0.6	0.5	<0.5	0.9
RUSTLER'S GULCH	07/16/96	10.4	29.9	6.47	9.8	0	20.0	<0.10	1.0	1803	57	<1.0	<10	<0.5	0.8	0.5	<0.5	1.2
SITE	07/15/96	13.4	56.7		23.2	0	28.4	<0.10	<1.0	1499	136	<1.0	<10	<0.5	1.8	<0.5	<0.5	6.6
SOUTH COTTONWOOD	08/01/96	10.0	15.9	6.67	14.6	0	10.0	<0.10	<1.0	204	<10	<1.0	<10	<0.5	0.6	<0.5	<0.5	1.2
SOUTH COTTONWOOD	08/24/96	8.4	64.7	7.03	34.0	0	41.2	<0.10	<1.0	66	49	<1.0	<10	<0.5	0.8	2.4	<0.5	6.1
TRAIL 401 (GOTHIC AREA)	07/15/96	8.0	60.5	7.80	37.8	0	45.4	<0.10	<1.0	167	<10	<1.0	<10	<0.5	<0.5	<0.5	<0.5	2.4
TROUT CREEK	07/10/96	22.1	191.5	8.33	90.0	0	139.4	<0.10	<1.0	232	<10	<1.0	<10	<0.5	<0.5	<0.5	<0.5	1.6
UPPER URAD H. MINE	08/22/96	11.3	110.6	7.05	64.6	0	71.0	0.11	<1.0	353	<10	<1.0	<10	<0.5	0.7	0.7	<0.5	5.6

SITE	DATE	TEMP	COND	PH	BG_ALK	PHTH_AL	HARD	CU_TOT	CD_TOT	FE_TOT	MN_TOT	PB_TOT	ZN_TOT	F	CL	NO3	PO4	SO4
HESBO	05/11/95	15	1490	9	387	0	143	59	<5	319	112	<50	112	21.0	10.9	16.8	<0.5	1021.0
HESBO	05/23/95	15.0	1490	9.0	388	0	23	2.0	0.12	352	142	<1.0	142					
HESBO	05/30/95	15.4	1400	9.62	324	0	91	<10	<0.10	<50	46	<50	46					
HESBO	06/06/95	17.9	830	8.92	210	0	116	1.0	<0.10	<50	71	<1.0	71					
HESBO	06/13/95	18.2	*	9.08	266	0	134	<10	<0.10	<50	36	<50	36					
HESBO	06/19/95	18.6	1160	9.09	218	0	141	<1.0	<0.10	<50	29	<1.0	29					
HESBO	06/27/95	20.6	1200	9.57	148	0	135	1.6	<0.10	<50	20	<1.0	20					
HESBO	07/06/95	21.1	1690	8.55	141	0	63	<1.0	<0.10	<50	11	<1.0	11	13.2	9.6	10.0	<0.5	932.8
HESBO	07/11/95	23.6	1780	8.90	177	15	*	<1.0	<0.10	85	28	<1.0	28	14.7	9.1	11.1	<0.5	798.7
HESBO	07/18/95	20.7	1940	9.33	110	17	16	<10	<0.10	<50	7660	<50	<10	21.8	11.3	10.3	<0.5	999.0
HESBO	07/24/95	20.4	1910	9.09	105	10	15	<1.0	<0.10	<50	9160	<1.0	10	22.1	12.2	14.2	<0.5	970.4
HESBO	08/01/95	21.0	1710	8.85	123	0	29	<1.0	<0.10	<50	9950	<1.0	26	20.0	12.1	10.3	<0.5	1008.0
HESBO	08/08/95	22.0	1650	8.86	151	14	32	<1.0	<0.10	<50	6630	<1.0	<10	16.7	9.6	11.8	<0.5	850.1
HESBO	08/15/95	21.2	1590	8.92	175	17	36	<1.0	<0.10	<50	4260	<1.0	<10	15.6	11.4	9.6	<0.5	764.3
HESBO	08/23/95	20.8	1580	9.31	181	35	118	<1.0	<0.10	<50	1496	<1.0	12	14.5	12.3	11.0	<0.5	724.2
HESBO	08/29/95	21	1510	8.85	170	17	22	<1.0	<0.10	<50	8580	<1.0	10	15.6	15.7	9.1	<0.5	773.7
HESBO	09/13/95	18.2	1460	8.94	184	15	22	<1.0	<0.10	<50	4240	<1.0	13	14.8	9.6	9.6	<0.5	730.0
2 POND	05/30/95	16.3	1390	9.61	316	0	95	<10	<5	<50	78	<50	78					
2 POND	06/06/95	22.3	1140	8.89	282	0	99	1.1	<0.10	<50	61	<1.0	61					
2 POND	06/13/95	21.1	*	9.05	226	0	136	<10	<5	<50	33	<50	33					
2 POND	06/19/95	18.9	1100	9.22	210	0	144	1.3	<0.10	96	52	<1.0	52					
2 POND	06/27/95	20.3	1530	9.52	178	0	144	<1.0	<0.10	<50	58	<1.0	58					
2 POND	07/06/95	21.6	1740	8.62	136	6	79	<1.0	<5	<50	26	<1.0	26	14.1	9.8	10.3	<0.5	885.1
2 POND	07/11/95	25.3	1790	9.07	166	16	92	1.0	<0.10	<50	26	<1.0	26	18.1	9.0	11.3	1.0	853.3
2 POND	07/18/95	20.5	1920	9.38	111	19	*	<10	<5	<50	10090	<50	<10	22.0	11.1	10.3	1.0	1033.0
2 POND	07/24/95	20.3	1910	9.11	108	14	31	<1.0	<0.10	<50	10170	<1.0	17	22.0	12.0	14.0	<0.5	1030.0
2 POND	08/01/95	20.9	170	8.97	122	0	25	<1.0	<0.10	<50	9550	<1.0	<10	16.8	12.1	10.0	<0.5	990.0
2 POND	08/08/95	22.1	1650	8.90	156	6	56	<1.0	<0.10	52	7860	<1.0	17	16.7	9.5	11.5	<0.5	855.2
2 POND	08/15/95	21.2	1620	8.92	177	24	17	<1.0	<0.10	<50	5150	<1.0	11	15.2	11.3	9.3	<0.5	752.5
2 POND	08/23/95	20.8	1590	9.35	180	39	131	<1.0	<0.10	<50	1680	<1.0	<10	14.0	12.5	9.5	<0.5	725.7
2 POND	08/29/95	20.7	1520	8.83	171	18	27	<1.0	<0.10	<50	8960	<1.0	16	15.3	16.4	9.0	<0.5	771.3
2 POND	09/13/95	17.6	1470	8.97	181	17	18	<1.0	<0.10	<50	5100	<1.0	21	15.0	9.7	9.2	<0.5	732.8
UPPER URAD	07/24/95	21.2	73	8.46	113.	0	506	3.0		262	262	<1.0	62	2.0	0.9	<0.5	<0.5	376.1
UPPER URAD	08/01/95	21.4	79	8.33	116.	0	560.	3.8		408	576	<1.0	100	2.9	0.9	0.5	0.7	403.0
UPPER URAD	08/08/95	16.8	82	7.84	122.	0	565	2.8		580	645	<1.0	69	3.4	0.9	<0.5	0.5	439.5
UPPER URAD	08/15/95	22.3	85	8.23	124.	0	577	2.9		710	322	<1.0	69	3.6	1.6	<0.5	<0.5	457.2
UPPER URAD	08/23/95	17.0	84	8.04	119.4	0	566.4	1.2		492	392	<1.0	42	3.1	1.3	<0.5	0.6	440.4
VINTAGE	06/27/95	17.3	160	7.35	22.8	0	52	1.8	<0.10	190	39	<1.0	39					
VINTAGE	07/06/95	21.7	180	7.28	24.8	0	58	3.1	0.17	371	67	1.0	67	<0.5	28.5	<0.5	<0.5	4.8
VINTAGE	07/24/95	17.8	120	7.72	21.8	0	47	3.9	0.76	416	1380	1.2	82	<0.5	25.5	<0.5	0.7	3.7
VINTAGE	08/15/95	18.6	280	7.50	80.4	0	177	1.7	<0.10	3410	9790	1.7	31	0.5	47.1	<0.5	<0.5	2.9
POWER ALLEY	06/06/95	14.0	70	7.94	20.6	0	31.2	<1.0	<0.10	155	17	<1.0	17					
POWER ALLEY	06/14/95		60	7.11	23.2	0	30.8	2.0	<0.10	344	59	1.0	59					
POWER ALLEY	06/19/95	18.7	70	7.89	22	0	33.6	1.8	<0.10	321	24	<1.0	24					
POWER ALLEY	06/27/95	17.6	60	7.96	27.2	0	39.6	1.4	<0.10	165	31	<1.0	31					
POWER ALLEY	07/06/95	22.1	70	7.97	28.4	0	36.2	1.8	0.19	249	22	<1.0	22	<0.5	1.1	0.9	<0.5	6.8
POWER ALLEY	07/11/95	25.6	100	8.42	52.8	0	117	1.6	<0.10	343	25	<1.0	25					
POWER ALLEY	07/18/95	19.5	70	7.96	31.4	0	35.8	<10	<5	711	32	<50	32	<0.5	0.8	1.9	1.0	6.2
POWER ALLEY	07/24/95	19.7	90	7.93	39	0	43	1.8	<0.10	1184	798	2.0	30	<0.5	0.9	<0.5	<0.5	7.4
POWER ALLEY	08/01/95	24.4	120	8.04	116.	0	61.4	2.3	<0.10	1953	565	3.5	23	0.6	2.1	0.5	<0.5	9.7
POWER ALLEY	08/08/95	23.4	170	7.43	75.8	0	36.8	1.7	<0.10	3247	6090	5.7	34	0.5	2.5	<0.5	<0.5	13.8
BROWNS CR. LOWER POND	06/14/95		50	7.57	22.0	0	23.8	<1.0	<0.10	76	<20	<1.0	<10	1.7	0.5	<0.5	1.0	2.9
BROWNS CREEK	07/03/95		40	7.74	20	0	26	2.7	<0.10	65	<20	<1.0	<10	2.1	<0.5	<0.5	0.8	1.9
BROWNS CREEK	07/24/95		40	7.30	19.6	0	23.6	<1.0	<0.10	56	<20	<1.0	<10	2.0	<0.5	<0.5	<0.5	1.9
BROWNS CREEK	08/07/95		40	7.67	21	0	23.6	4.3	<0.10	68	<20	<1.0	<10	2.1	<0.5	<0.5	<0.5	1.9
BROWNS CREEK	08/15/95		40	7.22	20.4	0	20.0	1.1	<0.10	94	<20	<1.0	<10	2.0	<0.5	<0.5	<0.5	1.7
BROWNS CREEK	08/22/95	11.1	40	7.56	15.8	0	22.4	<10	<5	123	<20	<50	10	1.9	<0.5	<0.5	<0.5	1.6
COLLEGIATE PEAKS	07/17/95		60	7.47	30.2	0	36	2.5	<0.10	293	<20	<1.0	<10	<0.5	0.8	1.4	<0.5	8.2
COLLEGIATE PEAKS	07/31/95		130	7.26	33.6	0	74	<1.0	<0.10	256	<20	<1.0	<10	<0.5	<0.5	<0.5	<0.5	37.8

COLLEGIATE PEAKS	08/14/95		150	7.16	39.6	0	84.6	2.1	<0.10	472	26	<1.0	<10	<0.5	0.7	<0.5	<0.5	47.6
COLLEGIATE PEAKS LOWER	08/21/95	10.9	110	8.09	33.8	0	60.2	<10	<5	398	<20	<50	<10	<0.5	<0.5	<0.5	<0.5	25.2
COLLEGIATE PEAKS LOWER	08/28/95	11.5	100	7.71	33.8	0	59.2	<10	<5	846	<20	<50	16	<0.5	<0.5	<0.5	<0.5	21.6
COLLEGIATE PEAKS MIDDLE POND	06/13/95		70	7.88	32.3	0	36.0	5.4	<0.10	100	<20	<1.0	<10	<0.5	0.9	0.6	<0.5	7.1
COLLEGIATE PEAKS UPPER POND	06/13/95		70	7.81	30.4	0	33.8	1.2	<0.10	57	<20	<1.0	<10	<0.5	<0.5	1.6	1.1	5.9
DENNY CREEK	06/20/95		20	7.75	13.2	0	15.0	<1.0	<0.10	186	<20	<1.0	<10	<0.5	<0.5	<0.5	1.0	2.1
DENNY CREEK	07/03/95		10	8.18	7.2	0	9.6	<1.0	<0.10	86	<20	<1.0	<10	<0.5	0.6	<0.5	<0.5	1.4
DENNY CREEK	07/17/95		20	7.17	15	0	16.6	1.7	<0.10	135	<20	<1.0	<10	<0.5	0.7	<0.5	<0.5	1.9
DENNY CREEK	07/24/95		30	7.38	16.8	0	20.8	<1.0	<0.10	94	<20	3.9	<10	<0.5	0.5	<0.5	<0.5	1.9
DENNY CREEK	08/14/95		30	7.32	19	0	19.6	1.2	<0.10	138	<20	<1.0	<10	<0.5	0.5	<0.5	<0.5	2.0
DENNY CREEK	08/21/95	9.7	60	7.97	34.6	0	29.6	<10	<5	225	<20	<50	<10	<0.5	<0.5	<0.5	<0.5	2.2
DENNY CREEK	08/28/95	9.6	50	7.65	18.6	0	21.0	<10	<5	690	37	<50	<10	<0.5	<0.5	<0.5	<0.5	1.9
HARTENSTEIN LAKE	07/03/95		30	7.67	16.4	0	16.6	2.7	0.15	116	<20	<1.0	<10	<0.5	0.8	<0.5	<0.5	2.0
HARTENSTEIN LAKE	07/20/95		10	6.95	7.2	0	19.4	2.9	0.15	90	<20	<1.0	<10	<0.5	<0.5	<0.5	<0.5	1.4
HARTENSTEIN LAKE	07/24/95		10	6.80	7	0	10.0	<1.0	<0.10	103	<20	<1.0	<10	<0.5	<0.5	<0.5	<0.5	1.5
HARTENSTEIN LAKE	08/14/95		10	6.92	7.2	0	9.8	1.8	<0.10	82	<20	<1.0	<10	<0.5	0.6	<0.5	<0.5	1.4
HARTENSTEIN LAKE	08/21/95	11.1	10	7.49	6.8	0	11.0	<10	<5	110	<20	<50	<10	<0.5	<0.5	<0.5	<0.5	1.2
HARTENSTEIN LAKE (REPLICATE)	08/28/95	11.1	10	7.49	6.8	0	11.0	<10	<5	169	<20	<50	12					
KROENKE LAKE	08/10/95		20	6.66	8.2	0	16.6	3.1		1817	30	1.9	<10	<0.5	<0.5	<0.5	<0.5	1.7
KROENKE LAKE (LOWER BREEDING	08/15/95		20	6.49	10.4	0	18.6	1.2		1815	27	1.3	<10	<0.5	0.6	<0.5	<0.5	1.9
KROENKE LAKE (UPPER BREEDING P	08/15/95		30	7.08	18.4	0	19.2	<1.0		845	27	<1.0	<10	<0.5	<0.5	<0.5	<0.5	1.9
KRONKE LAKE WETLANDS (LOWER)	08/21/95	11.1	50	7.97	28.0	0	18.4	<10		981	21	<50	10	<0.5	<0.5	<0.5	<0.5	1.6
KRONKE LAKE WETLANDS (UPPER)	08/28/95	12.2	60	7.40	34.4	0	38.2	<10		1112	70	<50	<10	<0.5	<0.5	<0.5	<0.5	2.0
SOUTH COTTONWOOD CREEK	06/20/95		70	7.97	39.4	0	46.2	<1.0		88	<20	<1.0	<10	<0.5	<0.5	<0.5	1.0	9.7
SOUTH COTTENWOOD CR.	07/03/95		60	7.31	32	0	37.6	2.9		75	<20	2.3	<10	<0.5	<0.5	<0.5	<0.5	6.6
SOUTH COTTONWOOOD	07/17/95		110	7.49	63.2	0	66.2	2.9		398	<20	<1.0	<10	<0.5	1.5	<0.5	<0.5	5.3
SOUTH COTTONWOOD	07/24/95		70	7.22	35.6	0	34.6	9.8		116	<20	<1.0	15	<0.5	2.2	<0.5	<0.5	6.1
SOUTH COTTONWOOD	08/07/95		70	7.65	42	0	47.4	1.3		132	<20	<1.0	11	<0.5	<0.5	<0.5	<0.5	6.9
SOUTH COTTONWOOD CREEK	08/15/95		100	7.39	53.6	0	57.2	<1.0		242	<20	<1.0	23	<0.5	<0.5	<0.5	<0.5	6.6
SOUTH COTTONWOOD	08/21/95	10.4	90	8.52	46.2	0	55.8	<10		82	<20	<50	<10	<0.5	<0.5	<0.5	<0.5	7.1
SOUTH COTTONWOOD	08/28/95	12.4	110	7.79	57.4	0	62.8	<10		245	<20	<50	<10	<0.5	0.6	<0.5	<0.5	6.4
CONUNDRUM	06/30/95		520	9.33	74.6	0	362	<1.0	<5	275	26	<1.0	<10	<0.5	0.9	<0.5	<0.5	210.0
CONUNDRUM	08/02/95		600	8.11	52.6	6.2	393.4	<10	<5	1873	65	<50	<10	<0.5	0.8	<0.5	<0.5	338.1
CUCUMBER GULCH	07/14/95	21.5	40	7.06	12.6	0	16	<1.0	<0.10	166	<20	<1.0	<10	<0.5	1.0	<0.5	<0.5	6.4
CUCUMBER GULCH	07/28/95		10	7.00	14.4	0	16.8	2.4	0.17	4427	59	3.7	34	<0.5	1.0	<0.5	<0.5	6.7
CUCUMBER GULCH	08/04/95	14.2	60	6.68	4.0	0	16.0	<10	<5	150	<20	<50	<10	<0.5	1.2	12.9	<0.5	6.5
CUCUMBER GULCH	08/15/95	13.7	40	7.22	10.6	0	18.0	<10	<5	169	<20	<50	<10	<0.5	2.1	<0.5	<0.5	6.1
CUCUMBER GULCH	08/29/95	14.0	50	7.30	12.2	0	18.0	<10	<5	183	<20	<50	<10	<0.5	2.2	<0.5	<0.5	5.9
CUCUMBER GULCH	09/10/95	.	50	6.52	13.4	0	25.2	<10	<5	824	35	<50	<10	<0.5	2.9	<0.5	<0.5	6.2
MONTEZUMA	06/27/95		140	6.60	7.6	0	52	2.9	0.45	437	351	2.3	352	<0.5	1.0	1.9	<0.5	47.4
MONTEZUMA	07/06/95		100	6.60	7.2	0	50.8	1.5	0.38	209	425	<1.0	266	<0.5	1.3	<0.5	<0.5	43.3
MONTEZUMA	07/18/95		60	6.38	7.2	0	36.8	3.1	0.38	379	219	1.3	236	<0.5	<0.5	0.8	<0.5	29.3
MONTEZUMA	07/31/95		80	7.29	6.6	0	36.8	<10	<5	578	220	<50	184	<0.5	0.7	<0.5	<0.5	31.0
MONTEZUMA	08/15/95		80	7.26	5.6	0	34.8	<10	<5	308	147	<50	149	<0.5	0.6	<0.5	<0.5	29.4
NORTH TEN MILE	07/19/95		60	7.02	38.6	0	41.6	1.5	<5	428	<20	<1.0	<10	<0.5	<0.5	0.6	<0.5	2.0
NORTH TEN MILE	07/28/95		60	7.06	41.2	0	39.2	2.1	<5	397	<20	<1.0	<10	<0.5	<0.5	<0.5	<0.5	2.0
NORTH TEN MILE	08/13/95		80	7.32	38.8	0	43.6	<10	<5	541	27	<50	<10	<0.5	0.5	1.6	<0.5	1.5
NORTH TEN MILE	08/20/95		100	7.51	50.0	0	57.8	<10	<5	718	43	<50	<10	<0.5	2.3	1.6	<0.5	1.5
NORTH TEN MILE	08/30/95	14.2	100	7.53	49.2	0	56.8	<10	<5	822	30	<50	<10	<0.5	<0.5	0.6	<0.5	1.4
NORTH TEN MILE	09/10/95		100	7.03	38.8	0	54.6	<10	<5	452	<20	<50	10	<0.5	<0.5	2.0	<0.5	1.4
NORTH TEN MILE	09/23/95		70	6.72	37.0	0	41.2	<10	<5	677	22	<50	<10	<0.5	<0.5	1.1	<0.5	1.6
NORTH TEN MILE	10/14/95		90	6.21	40.4	0	44.6	<10	<5	900	35	<50	<10	<0.5	<0.5	<0.5	<0.5	1.8
POLE CREEK	06/03/95	22.3	90	7.64	41.6	0	46.6	1.0	<0.10	254	<20	<1.0	<10					
POLE CREEK	06/12/95	22.3	70	7.46	29.2	0	30.6	<1.0	<0.10	187	<20	<1.0	<10					
POLE CREEK	06/26/95	22.4	63	7.46	25.6	0	25.6	<1.0	<0.10	143	25	<1.0	<10					
POLE CREEK	07/10/95		80	8.33	46.6	0	44.2	1.3	0.41	333	<20	<1.0	<10	<0.5	0.7	<0.5	<0.5	3.3
POLE CREEK	07/21/95		110	7.52	54	0	50	1.4	<0.10	412	32	<1.0	<10	<0.5	0.5	<0.5	<0.5	3.1
POLE CREEK	08/04/95		130	7.94	66.4	0	65.4	<10	<5	797	59	<50	10	<0.5	1.1	0.6	<0.5	3.2
POLE CREEK	08/13/95		130	6.78	76.2	0	75.6	<10	<5	86	<20	<50	<10	<0.5	1.4	<0.5	<0.5	3.2
POLE CREEK	08/25/95		150	7.04	79.6	0	75.4	<10	<5	1405	169	<50	<10	<0.5	1.5	0.5	<0.5	3.2

HERMAN GULCH MAIN POOL	06/02/95	12.2	280	6.85	23.2	0	90.6	<10	<5	1521	241	<50	15	<0.5	80.6	<0.5	<0.5	7.3
MOUNT BETHEL MAIN POOL	06/06/95	14.0	50	7.23	23.6	0	35.4	<10	<5	163	<20	<50	<10	<0.5	0.6	0.6	<0.5	2.5
MAIN POOL HERMAN GULCH	06/12/95	20.0	31	6.75	16.2	0	4.85							<0.5	103.0	<0.5	<0.5	8.2
MAIN POOL N. SHORE HERMAN GLC	06/12/95	9.7	64	6.57	3.52	0	5.11							<0.5	21.1	<0.5	<0.5	4.1

TOXICITY OF CADMIUM TO BOREAL TOAD TADPOLES

INTRODUCTION

Boreal toad (*Bufo boreas*) numbers have declined in Colorado over the last quarter century. They have been state-listed as endangered since November 1993 and federally listed as "warranted but precluded" since March 1995 (Goettl [eds.] and the Boreal Toad Recovery Team 1997). The Colorado Division of Wildlife Aquatic Toxicology Laboratory is assisting with investigations into possible causes of this decline by evaluating water quality characteristics that may limit survival and distribution of boreal toad tadpoles. These efforts include analysis of water samples collected from current and historic breeding ponds, developing techniques to measure effects of toxicants (heavy metals, pesticides, deicing compounds) to tadpoles, and conducting experiments to determine toxicity of selected metals to boreal toad tadpoles. Here we report the effects of short and long term exposure of boreal toad tadpoles to cadmium. Cadmium was investigated because of its high toxicity to aquatic organisms (Eisler 1985).

MATERIALS AND METHODS

Static Renewal

A 10 day static 24-hr renewal test was conducted to estimate acute toxicity of cadmium and to set concentrations for use in the long term chronic exposure experiment. Egg masses containing between 70 and 120 Boreal toad eggs were placed in 300 ml polyethylene beakers containing 200 milliliters of 1000, 500, 100, 50, or 0 μg Cd/l added as cadmium sulfate to dechlorinated Fort Collins city tap water. The beakers were placed in an incubator, covered with a watch glass and gently aerated with compressed air. Temperature of the incubator was maintained at 25°C during a 12 hour day and 15°C during a 12 hour night. Eggs/larvae/tadpoles were transferred to 300 ml polyethylene beakers containing 200 mls of freshly prepared solutions daily using a brine shrimp net. Mortalities were monitored daily and removed using a pipet. Tadpoles were fed daily with Mazuri amphibian feed beginning eight days after start of cadmium exposure.

Water quality characteristics were measured weekly. Hardness and alkalinity were determined according to Standard Methods (APHA 1985). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Anions were determined using a Dionex 4005i ion chromatograph. Water samples for cadmium analysis were collected from exposure solutions at the start and end of each 24 hr renewal. This was to measure any loss of cadmium from the solution as a result of sorption, precipitation or uptake by organisms. After collection, water samples for cadmium analysis were preserved by acidification to pH<2 using Ultrex nitric acid. Cadmium concentrations in water samples were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction.

After ten days, surviving tadpoles within each exposure concentration were terminally anesthetized with tricaine methane sulfonate (MS-222), pooled, dried with a paper towel and weighed in preweighed polypropylene centrifuge tubes. The tubes containing the tadpoles were placed in a drying oven at 80°C and dried to constant weight. The tadpoles were then digested with trace metal grade nitric acid and heated for eight hours in a water bath at 60°C. Trace metal grade hydrogen peroxide was added and the tubes heated an additional eight hours. The digests were diluted to volume with deionized water and analyzed for cadmium content as described above.

Flow-Through

A serial diluter (Benoit et al. 1982) delivered seven concentrations of cadmium (as cadmium sulfate) and a dilution water control to completely randomized 2.2 liter glass exposure chambers at a rate of twenty mls/minute. The experiment was replicated four times with nominal cadmium exposure concentrations of 216, 72, 24, 12, 6, 3, 1.5 and 0 μg Cd/l. Source water consisted of dechlorinated Fort Collins city tap water. The exposure chambers were placed in a water bath maintained near 20°C using a Remcor water recirculating heater/cooler. Two fluorescent blacklights and two florescent full spectrum (Repta Sun) bulbs suspended approximately 12 inches above the exposure chambers provided artificial lighting with 16 hour light and 8 hour dark photoperiod.

Twenty boreal toad tadpoles about 10 days post hatch were randomly placed in each exposure chamber. Tadpoles were fed a mixture of Mazuri, Silver Cup trout food and frozen romaine lettuce *ad libitum*. Exposure chambers were checked daily for mortality. Dead tadpoles were removed, blotted dry with a paper towel and weighed.

Water quality characteristics were measured weekly in alternating replicates as described above. Water samples for cadmium analysis were collected weekly in each exposure level from alternating replicates. Acid leachable as well as dissolved cadmium (passing through a 0.45 micron filter) were analyzed. Water samples for cadmium analysis were preserved by acidification to pH<2 using Ultrex nitric acid. Cadmium concentrations in water samples were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame or Thermo Jarrell Ash SH4000 with CTF 188 graphite furnace. Water samples analyzed by furnace used 0.1% ammonium phosphate as a matrix modifier. Both instruments used Smith-Hieftje background correction.

Five tadpoles were randomly removed from each exposure chamber after 13, 28 and 56 days of exposure and terminally anesthetized with MS-222. Total length, snout-vent length, weight, and Gosner amphibian development stage (Gosner 1960) were recorded. Total length and snout-vent length were not measured on tadpoles collected after 56 days because of the effect metamorphosis had on these measures. The tadpoles were digested and analyzed for cadmium content using methodologies described for the acute exposures.

Statistics

Median lethal concentrations (LC50s) were based on initial exposure concentrations and calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977). Initial and 24 hour cadmium concentrations were compared using one-tailed t-test. Analysis of variance (ANOVA) was performed on mortality and wet weight data using SAS computer software (SAS 1989). Mortality data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Assumptions of normality and homogeneity of variance were evaluated using Shapiro Wilk's test and Bartlett's test respectively ($p<0.10$). Treatment means were compared using one-tailed Dunnett's multiple comparison test ($p<0.05$) to evaluate differences from control.

RESULTS

Static Renewal

Water quality characteristics are presented in Table 1. Mean cadmium concentrations of initial and 24 hour old exposure solutions are presented in Table 2. With the exception of the control, cadmium concentrations of 24 hour old solutions are significantly lower than the initial concentrations. Mortality due to cadmium exposure did not occur until about three days (Figure 1). Median lethal concentration (LC50) for 72 hours exposure was 815 μg Cd/L with a 95% confidence interval of 741-895 μg Cd/L. The 96 hr LC50 was 582 μg Cd/L with a 95% confidence interval of 539-628 μg Cd/L. Mean wet weights, dry weights and cadmium content of surviving tadpoles are reported in Table 3. The experiment was not replicated therefore

statistical inferences are limited but some conclusions can be drawn from the data. The weight data suggests that cadmium exposure reduces growth. A visual inspection of mortality data suggests the lowest observed effect concentration (LOEC) was 500 μg Cd/L with a no observed effect concentration (NOEC) of 100 μg Cd/L (Figure 1 and Table 3). The geometric mean of the LOEC and NOEC is 223 μg Cd/L.

Flow-Through

Water quality characteristics were similar to those in the static renewal test (Table 4). All parameters were stable throughout the test with the exception of dissolved oxygen. Dissolved oxygen in the source water was relatively low (3.25-5.34 mg/L) during the first two weeks of exposure but this did not seem to adversely affect the tadpoles. Aeration of the source water after the first two weeks increased dissolved oxygen levels to near saturation for the remainder of the test. Total and dissolved cadmium concentrations and associated mortality are shown in Table 5. A large majority of total cadmium present exists in the dissolved fraction which is typical of the soft water used in this experiment. Survival of boreal toad tadpoles was not affected by cadmium exposure concentrations ($p>0.05$). However, cadmium exposure had profound effects on development and growth. Though unaffected at 13 days of exposure, gosnerian development was significantly reduced after 28 days exposure to 241 and 71 μg Cd/L and after 56 days was reduced by 7.2 μg Cd/L (Figure 2). Total length was significantly reduced at cadmium concentrations of 7.2 μg/L or more after 13 days exposure and reduced at 4.9 μg/L or more after 28 days exposure (Figure 3). Snout-vent length was significantly reduced above exposure concentrations of 16.2 and 4.9 μg/L after 13 and 28 days exposure respectively (Figure 4). Wet weight was reduced in tadpoles exposed to a cadmium concentration of 4.9 μg/L or greater after thirteen and 28 days. After 56 days of exposure, wet weight was reduced at cadmium exposures greater than of 37.5 μg/L (Figure 5). Figure 6 shows that cadmium is rapidly accumulated by boreal toad tadpoles. Whole body cadmium content increased in all exposure levels compared to the control (Figure 6). The influence of duration of exposure on whole-body cadmium content did not follow a clear trend. Cadmium content increased between thirteen days and 28 days of exposure but decreased considerably between 28 and 56 days of exposure. Casual observation revealed that tadpoles exposed to the higher concentrations of cadmium were less active spending less time feeding and swimming than controls. A relatively high percentage of tadpoles had scoliosis; about 10-15%. The magnitude of scoliosis ranged from mild to severe but did not seem to interfere with feeding or survival. Incidence of scoliosis was present in all exposure levels including controls and did not seem dose-related.

DISCUSSION

Boreal toad tadpoles were much more resistant to the acute lethal effects of cadmium than other aquatic vertebrates. The 96 hour LC50 for boreal toad tadpoles was 582 μg/L compared to 2.7 and 3.0 μg/L for rainbow trout, *Oncorhynchus mykiss*, in water with similar characteristics (Davies et al. 1993). The LC50 reported for boreal toad tadpoles was based on the initial exposure concentrations. Because of decreases in cadmium concentrations over time, actual exposure concentrations (and so the LC50) would be somewhat less. The geometric mean of the LOEC and NOEC from the 10 day acute exposure was 223 μg Cd/L. Exposure to cadmium concentrations as high as 241μg Cd/L for 56 days did not affect survival. Longer exposures to cadmium did not seem to increase mortality. This suggests that short exposure (10 days) adequately predicted the lethal effects associated with chronic exposure to cadmium.

The ten-day acute exposure indicated that cadmium may affect growth of tadpoles. This was verified by the chronic test. Cadmium exposure dramatically decreased growth within two weeks of exposure. Development of tadpoles was affected by the fourth week of exposure. Gosner development stage, weight, total length, and snout-vent length were all affected at much lower concentrations than survival. Weight was the most sensitive endpoint of cadmium effects on growth and also the easiest to measure. Reduction of tadpole weight due to cadmium was detectable at less than 1% of the 96 hr LC50 and was manifested within 13 days of exposure. It is unknown whether reductions of growth and development resulted from decreased

feeding activity or changes in metabolism brought on by cadmium exposure.

In the static renewal experiment, tadpoles accumulated substantial levels of cadmium even after only ten days of exposure. During chronic exposure, whole body content of cadmium was dose-dependent and overall increased between 13 and 28 days. However cadmium content decreased between 28 and 56 days for most treatments. The reason for this decrease is unknown. The most likely explanation is that significant excretion of cadmium occurs during metamorphosis. This is supported by the fact that tadpoles in the highest exposure, 241 g Cd/L, did not lose cadmium between 28 and 56 days and also were the only treatment that did not metamorphose. Whole body content of cadmium can be a useful measure of exposure to cadmium as it includes exposures in both water and diet. Whole body cadmium content can also reflect influences of episodic events and exposures often missed by collection of water samples. However, whole body metal content data should be interpreted with care. Many factors can modify bioavailability (and hence uptake) of metals including pH, hardness, presence of other metals, temperature, and dissolved organic matter. For amphibians, stage of development may be important. As toads metamorphose, the relative water content decreases so metal content should be expressed on a dry weight basis. Also, ingested sediment in the gut can cause overestimation of whole body metal content.

The concentrations of cadmium required to kill boreal toad tadpoles are quite high and much greater than would be found in breeding areas in Colorado. However, the sublethal effects observed here occurred at concentration well below lethal levels. These sublethal effects could be detrimental to survival of tadpoles in their actual environment. For example, reduced growth may result in increased risk of predation and decreased overwinter survival of toadlets. A delay in development could lead to recruitment failure if tadpoles are unable to metamorphose to toadlets prior to onset of winter; a likely possibility given the short summers at the higher elevations where boreal toads tadpoles develop.

Overall, the approaches and techniques used in these experiments proved suitable as a means to study the effects of metal exposure to tadpoles. For the most part, the tadpoles seemed to grow well and thrive in the exposure chambers. The cause of the scoliosis is unknown. Possible causes include genetics and diet. Scoliosis occurs in trout fed diets deficient in ascorbic acid (Poston 1967, Halver 1969) and tryptophan (Shanks et al. 1962, Halver 1970). Cadmium concentrations in the static 24-hr renewal experiment declined substantially over the course of 24 hours due to cadmium uptake, precipitation and/or sorption. Loss of toxicant over time represents a major limitation of static renewals which is overcome using flow-through diluters. When conducting static renewal toxicity tests, water samples should be collected from solutions at the beginning and end of renewals so that toxicant losses can be estimated. The acute test was fairly successful at predicting chronic mortality. However, short term exposure did not detect sublethal effects such as reductions of growth or development and would probably fail to detect other possible effects such as deformities. The chronic test proved successful and sensitive to such effects. This experimental approach is limited in its ability to expose boreal toads past metamorphosis.

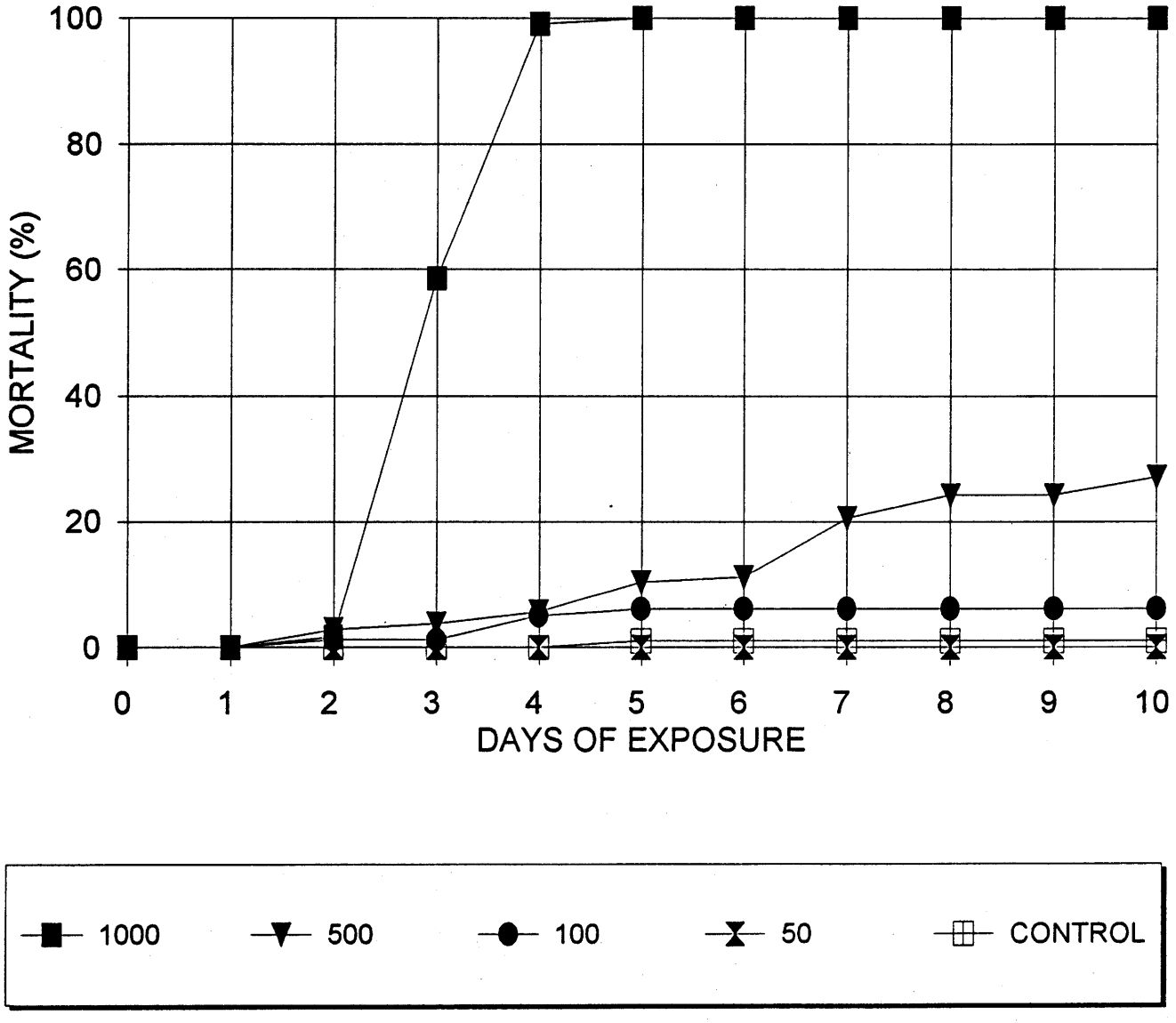

Figure 1. Mortality (%) of Boreal toad tadpoles exposed to waterborne cadmium during the 10 day static renewal.

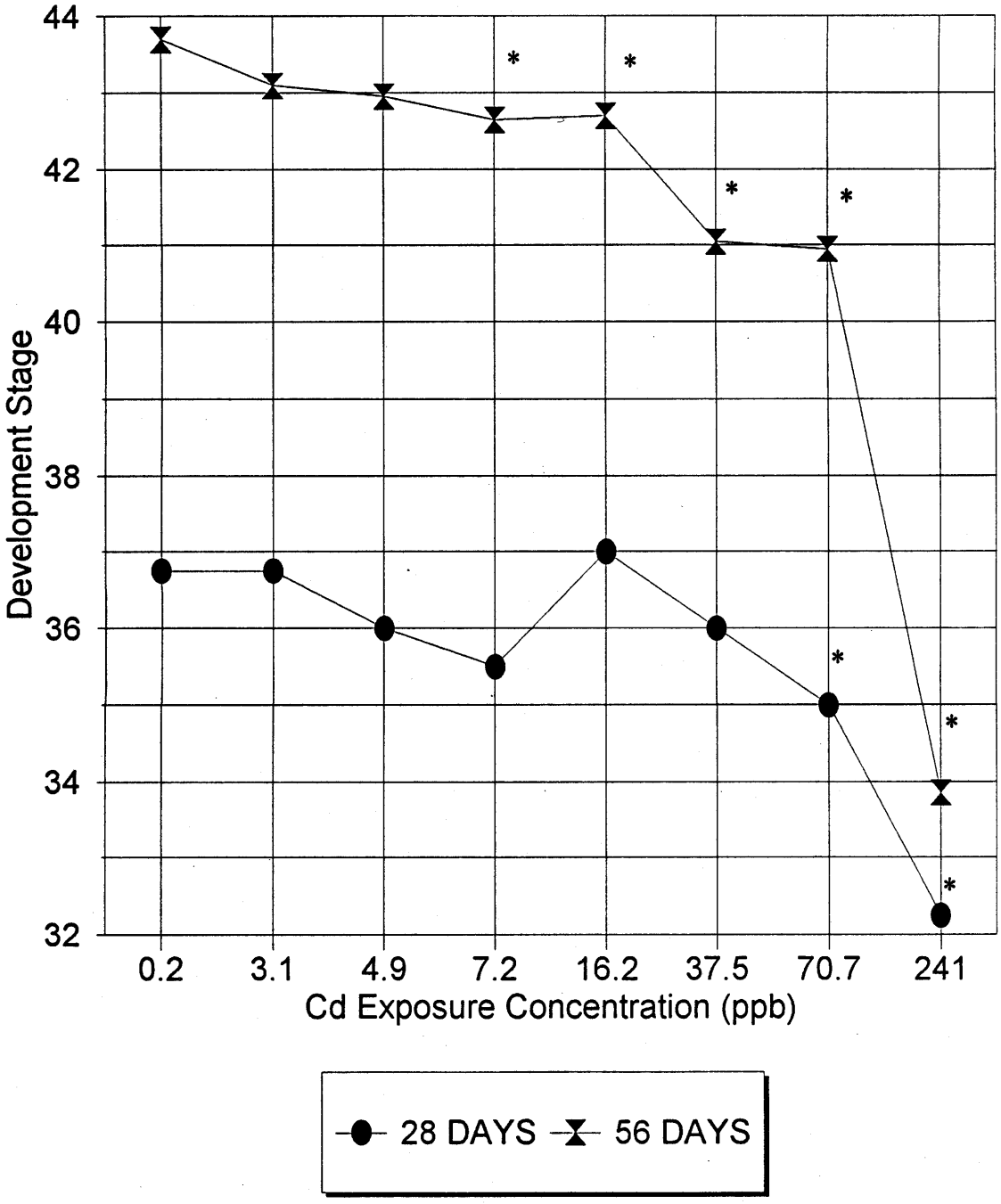

Figure 2. Gosner development of boreal toad tadpoles exposed to cadmium. Asterisk indicates treatment significantly less than control ($p<0.05$).

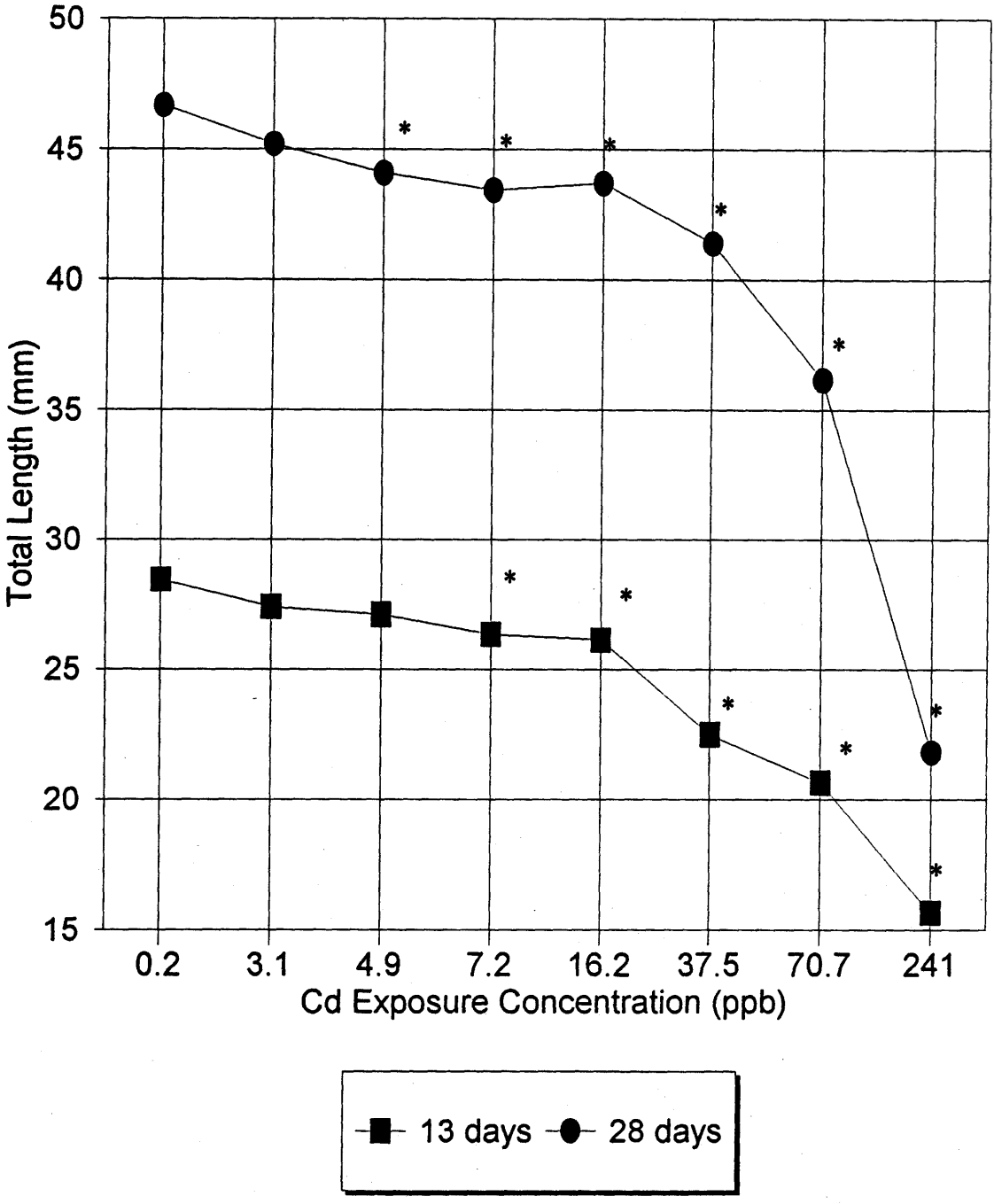

Figure 3. Total length (mm) of boreal toad tadpoles exposed to cadmium for 13 and 28 days. Asterisk indicates treatment significantly less than control ($p<0.05$).

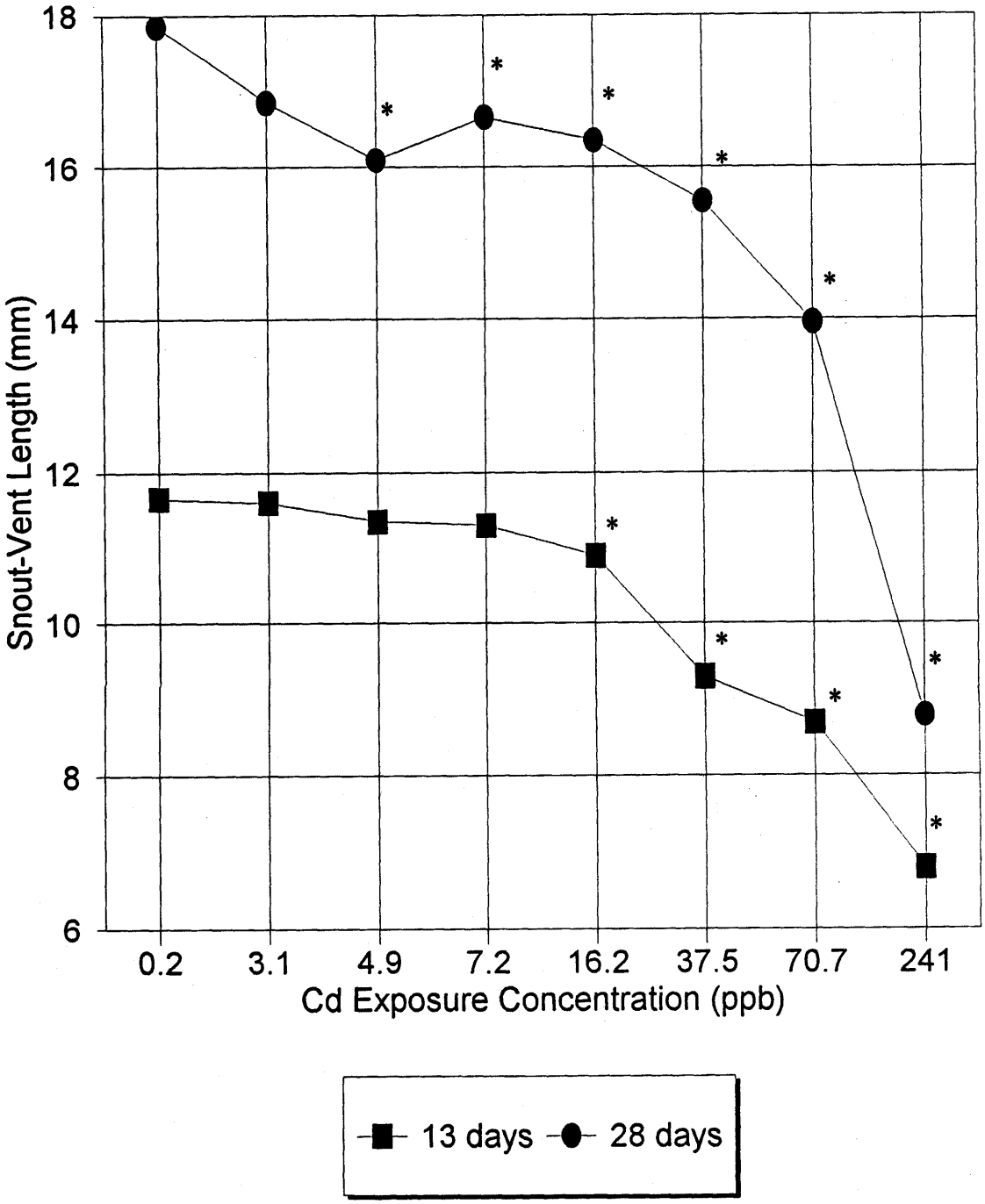

Figure 4. Snout-vent length (mm) of boreal toad tadpoles exposed to cadmium for 13 and 28 days. Asterisk indicates treatment significantly less than control ($p<0.05$).

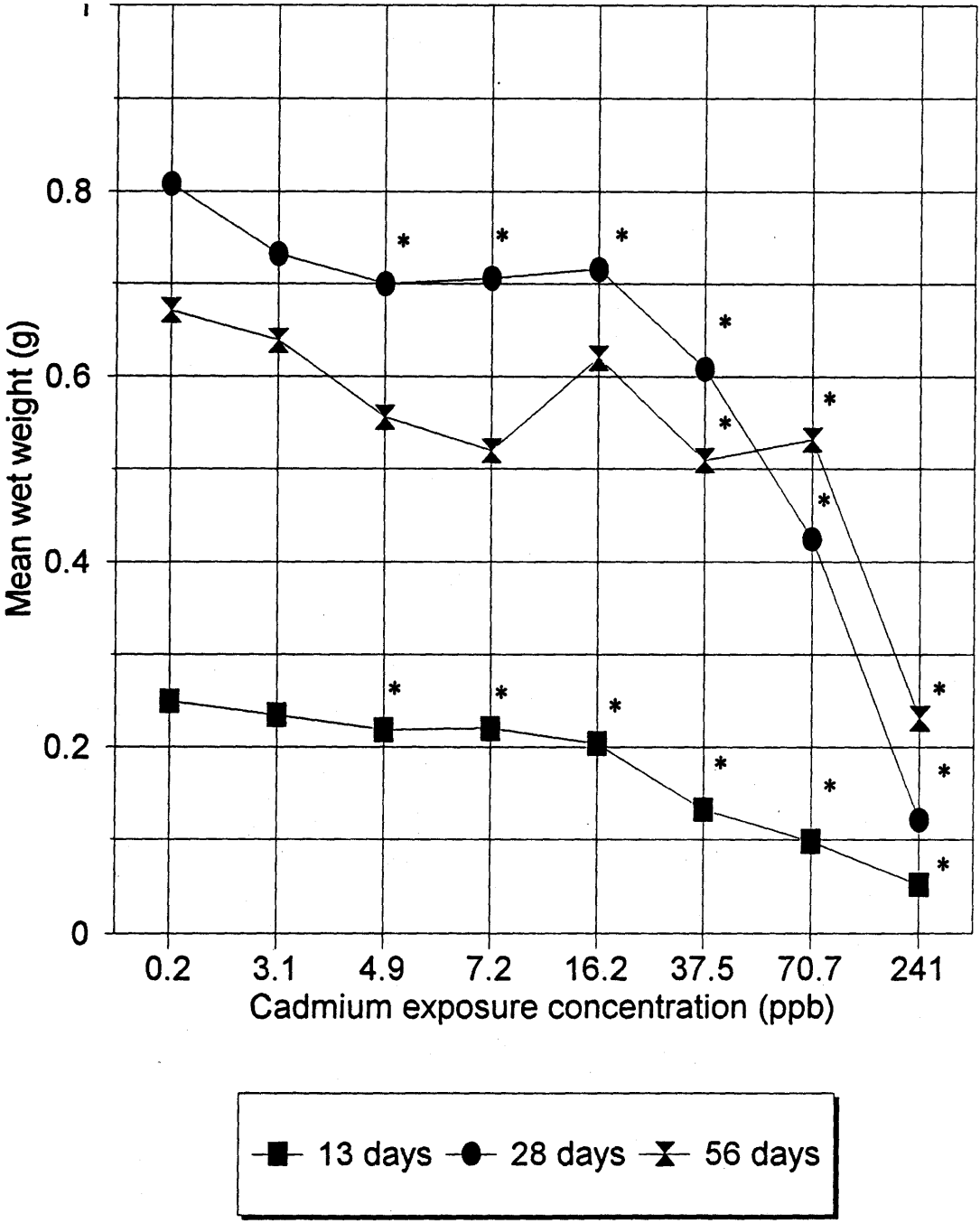

Figure 5. Mean wet weight (g) of boreal toad tadpoles exposed to cadmium for 13, 28, and 56 days. Asterisk indicates treatment significantly less than control ($p<0.05$).

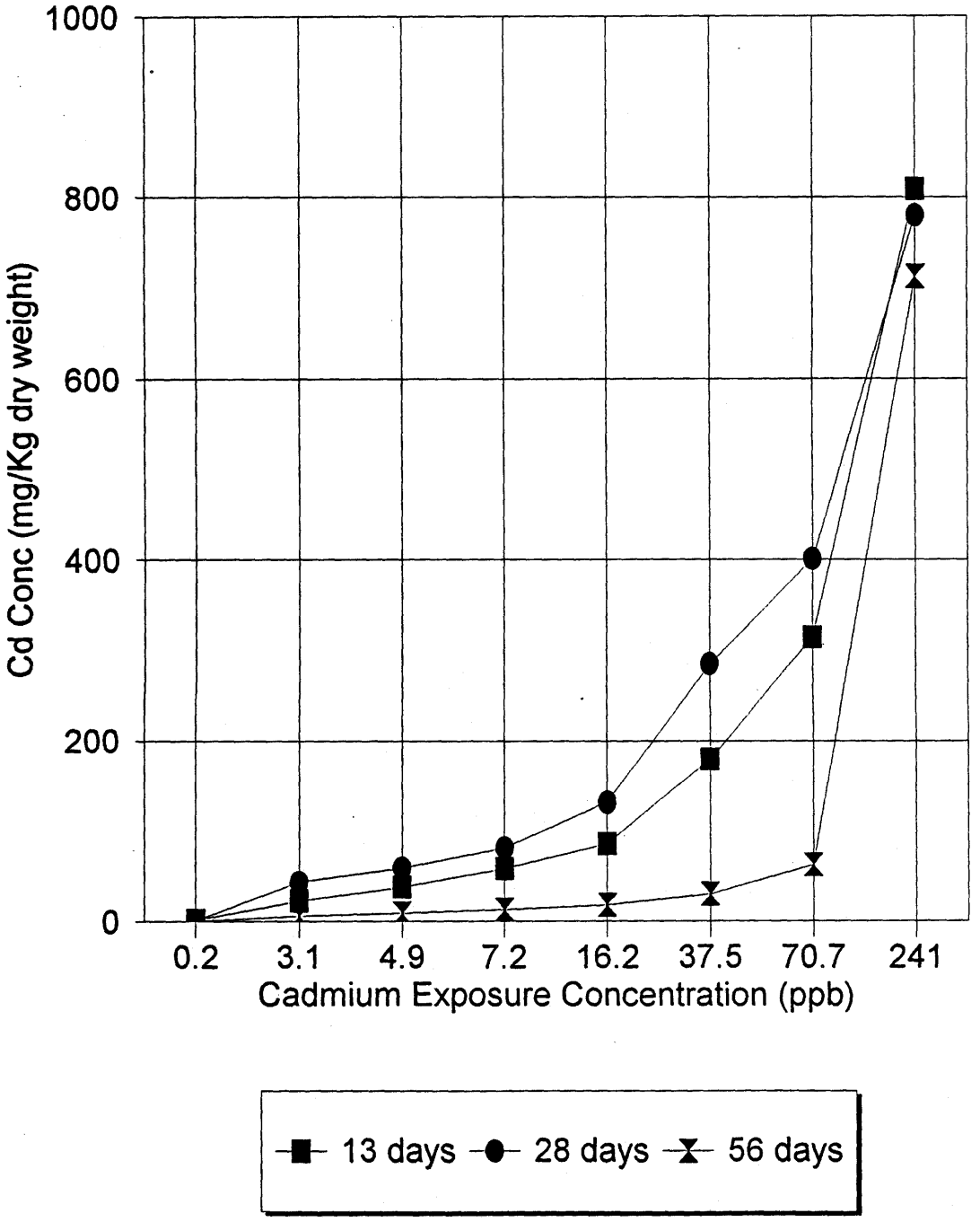

Figure 6. Whole body cadmium content (mg/Kg dry weight) of boreal toad tadpoles exposed to cadmium for 13, 28, and 56 days. All treatments greater than control ($p<0.05$).

Table 1. Water quality characteristics of exposure water used during acute static renewal toxicity test.

Hardness (mg $CaCO_3$/l)	49.9
Alkalinity (mg $CaCO_3$/l)	36.6
pH (S.U.)	7.16
Conductivity (μS/cm)	111.6
Fluoride (mg/l)	1.3
Chloride (mg/l)	3.0
Nitrate (mg/l)	<0.5
Phosphate (mg/l)	<0.5
Sulfate (mg/l)	30.6

Table 2. Mean, standard deviation, and ranges of cadmium concentrations (μg/l) in freshly prepared and 24 hour-old exposure solutions from acute static renewal toxicity test.

Nominal (μg/l)	0		50		100		500		1000	
	Initial	24 hours	Initial	24 hours	Initial	24 hours	Initial	24 hours	Initial	24 hours
Mean (μg/l)	<5	<5	45.9	31.9*	91.6	51*	457	295*	908	655*
Std Dev	0	0	1.7	4.2	4.8	14.3	12.1	61.3	10.5	145
Range	<5	<5	44-48	25-38	83-100	28-67	433-477	161-350	895-920	407-785

*Significantly less than initial ($p<0.01$)

Table 3. Mortality (%), mean wet and dry weight (mg) and cadmium content (μg/g dry weight) of tadpoles following ten day acute static renewal toxicity test.

Nominal cadmium concentration (μg/l)	0	50	100	500	1000
Mortality (%)	1.1	0.0	6.2	27.1	100
Mean wet weight (mg)	10.9	10.0	10.9	5.8	--
Mean dry weight (mg)	0.651	0.579	0.592	0.397	--
Cadmium content (μg/g dry weight)	0.12	6.34	13.83	34.89	--

Table 4. Water quality characteristics of exposure water used in chronic flow-through cadmium toxicity test conducted on boreal toad tadpoles.

	MEAN	STANDARD DEVIATION	RANGE
HARDNESS (mg $CaCO_3$/L)	50.4	2.2	44.0-55.0
ALKALINITY (mg $CaCO_3$/L)	37.1	4.7	32.8-65.8
pH (S.U.)	7.25	0.13	6.97-7.46
DISSOLVED OXYGEN (mg O_2/L)	5.65	1.36	3.21-7.70
TEMPERATURE (°C)	19.1	0.6	18.1-20.0
CONDUCTIVITY (μS/cm)	102.8	3.6	95.0-114.0
FLUORIDE (mg/L)	0.88	0.21	0.37-1.14
CHLORIDE (mg/L)	2.08	0.35	1.54-2.76
NITRATE (mg/L)	<0.5	0.36	0.00-1.2
PHOSPHATE (mg/L)	<0.5	0.17	0.00-0.62
SULFATE (mg/L)	11.82	0.25	11.5-12.3

Table 5. Total and dissolved cadmium exposure concentrations and associated survival of boreal toad tadpoles subjected to chronic flow-through toxicity test. Standard deviations in parentheses.

Nominal Cd Conc (μg/L)	Measured Total (μg/L)	Measured Dissolved (μg/L)	Percent Survival
216	241.0 (38.3)	202.3 (16.3)	85.0 (0.6)
72	70.7 (11.1)	63.5 (10.6)	87.5 (0.5)
36	37.5 (7.0)	32.8 (6.7)	90.0 (0.8)
18	16.2 (2.6)	14.2 (2.5)	87.5 (0.5)
9.0	7.2 (2.3)	6.6 (1.9)	92.5 (0.5)
4.5	4.9 (1.9)	3.7 (1.2)	87.5 (1.3)
2.25	3.1 (1.2)	2.7 (1.1)	87.5 (1.3)
0	0.2 (0.1)	<0.1 (0.0)	92.5 (0.5)

TOXICITY OF COPPER TO BOREAL TOAD TADPOLES

INTRODUCTION

Boreal toad (*Bufo boreas*) numbers have declined in Colorado over the last quarter century. They have been state-listed as endangered since November 1993 and federally listed as "warranted but precluded" since March 1995 (Goettl [eds.] and the Boreal Toad Recovery Team 1997). The Colorado Division of Wildlife Aquatic Toxicology Laboratory is assisting with investigations into possible causes of this decline by evaluating water quality characteristics that may limit survival and distribution of boreal toad tadpoles. These efforts include analysis of water samples collected from current and historic breeding ponds, developing techniques to measure effects of toxicants (heavy metals, pesticides, deicing compounds) to tadpoles, and conducting experiments to determine toxicity of selected metals to boreal toad tadpoles. Here we report the effects of short and long term exposure of boreal toad tadpoles to copper. Copper has been reported to be highly toxic to boreal toad tadpoles (John Goettl Jr., Colorado Division of Wildlife, personal communication).

MATERIALS AND METHODS

Acute exposure

A 10 day static 24 hr renewal test was conducted to estimate acute toxicity of copper and to set concentrations for use in a long term chronic exposure experiment. Boreal toad tadpoles approximately 10 days old were placed in 300 ml polyethylene beakers containing 200 milliliters of 100, 50, 20, 10, or 0 μg Cu/l added as copper sulfate pentahydrate to dechlorinated Fort Collins city tap water. The beakers were placed in an incubator, covered with a watch glass and gently aerated with compressed air. Temperature of the incubator was maintained at 25 °C during a 12 hour day and 15 °C during a 12 hour night. Tadpoles were transferred to 300 ml polyethylene beakers containing 200 mls of freshly prepared solutions daily using a brine shrimp net. Mortalities were monitored daily and removed using a pipet. Tadpoles were fed daily with Mazuri amphibian feed and frozen lettuce. Duration of exposure was ten days.

Chronic exposure

A serial diluter (Benoit et al. 1982) delivered seven concentrations of copper (as copper sulfate pentahydrate) and control to completely randomized 2.2 liter glass exposure chambers at a rate of twenty mls/minute. The experiment was replicated four times with nominal copper exposure concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0 μg Cu/L. Source water consisted of dechlorinated Fort Collins tap water. The exposure chambers were placed in a water bath maintained near 20 °C using a Remcor water recirculating heater/cooler. Two fluorescent blacklights and two florescent full spectrum (Repta Sun) bulbs suspended approximately 12 inches above the exposure chambers provided artificial lighting with 16 hour light and 8 hour dark photoperiod.

Twenty-five boreal toad tadpoles about 10 days post hatch were randomly placed in each exposure chamber. Tadpoles were fed a mixture of Mazuri, Silver Cup trout food and frozen romaine lettuce *ad libitum*. Exposure chambers were checked daily for mortality. Dead tadpoles were removed, blotted dry with a paper towel and weighed.

Water quality parameters were measured weekly in alternating replicates. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Anions were determined using a Dionex 4005i ion chromatograph.

Water samples for copper analysis were collected weekly in each exposure level from alternating replicates. Acid leachable as well as dissolved copper (passing through a 0.45 micron filter) were analyzed. Water samples for copper analysis were preserved by acidification to pH<2 using Ultrex nitric acid. Copper concentrations in water samples were measured using an Instrumentation Laboratory Video 22 atomic

absorption spectrometer with air-acetylene flame or Thermo Jarrell Ash SH4000 with CTF 188 graphite furnace. Both instruments used Smith-Hieftje background correction.

Five tadpoles were randomly removed from each exposure chamber after 14 and 42 days of exposure and terminally anesthetized with MS-222. Total length, snout-vent length, weight, and Gosner amphibian development stage (Gosner 1960) were recorded. The tadpoles were rinsed with deionized water, blotted dry with a paper towel, pooled and weighed in preweighed polypropylene centrifuge tubes. Tubes containing the tadpoles were placed in a drying oven at 80°C and dried to constant weight. Trace metal grade nitric acid was added to the tubes which were then heated for eight hours in a water bath at 60°C. Trace metal grade hydrogen peroxide was added and the tubes heated an additional eight hours. The digests were then diluted to volume with deionized water and analyzed for copper content using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction.

Statistics

The 96-hour median lethal concentration (LC50) was determined using Spearman-Karber (Hamilton et al. 1977, Hamilton et al. 1978). Analysis of variance (ANOVA) was performed on mortality and wet weight using SAS computer software (SAS 1989). Mortality data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Assumptions of normality and homogeneity of variance were evaluated using Shapiro Wilk's test and Bartlett's test respectively ($p<0.10$). Treatment means were compared using one-tailed Dunnett's multiple comparison test ($p<0.05$) to evaluate differences from control.

RESULTS

Acute exposure

Water quality characteristics are presented in Table 1. Measured copper concentrations and associated mortality are shown in Table 2. Copper concentrations were stable throughout the 24 hour period between renewals. All mortalities occurred within 24 hours of exposure. The 24, 48, 72, 96 and 240 hour median lethal concentrations (LC50) are 57.11 μg Cu/L with a 95% confidence interval of 47-69 μg Cu/L. This experiment like the cadmium toxicity test was not replicated therefore statistical inferences are again limited. However clearly the lowest observed effect concentration (LOEC) based on mortality is 57.7 μg Cu/L with a no effect concentration (NOEC) of 24.2 μg Cd/L (Table 2). The geometric mean of the LOEC and NOEC is 37.4 μg Cu/L.

Chronic exposure

Water quality characteristics of exposure water are shown in Table 3. Water quality characteristics were stable throughout the test. Total and dissolved copper concentrations and associated mortality during the first 96 hours of the chronic exposure are shown in Table 4. A majority of total copper present in exposure water is dissolved which is characteristic of the soft water used in this experiment. The 96-hour median lethal concentration (LC50) from the flow-through test is 69.4 μg Cu/L based on total copper concentrations. Copper concentrations and associated mortality following 43 days of exposure are shown in Table 5. Copper concentrations were consistent throughout the test. The lowest observed effect level (LOEC) based on mortality occurred at a copper concentration of 33.8 μg Cu/L with the no observed effect level (NOEC) at 15.2 μg Cu/L. The maximum allowable toxicant concentration (MATC) is 22.7 μg Cu/L based on the geometric mean of the LOEC and NOEC. Weight of tadpoles was unaffected by copper exposure after 14 days but was reduced at a concentration of 15.2 μg Cu/L after 42 days (Table 5 and Figure 1). Development of tadpoles was unaffected by copper exposure. Whole body copper content was higher than controls at all copper exposures. Whole body copper content did not change between 14 and 42 days when based on wet weight (Figure 2) but decreased over time when based on dry weight (Figure 3).

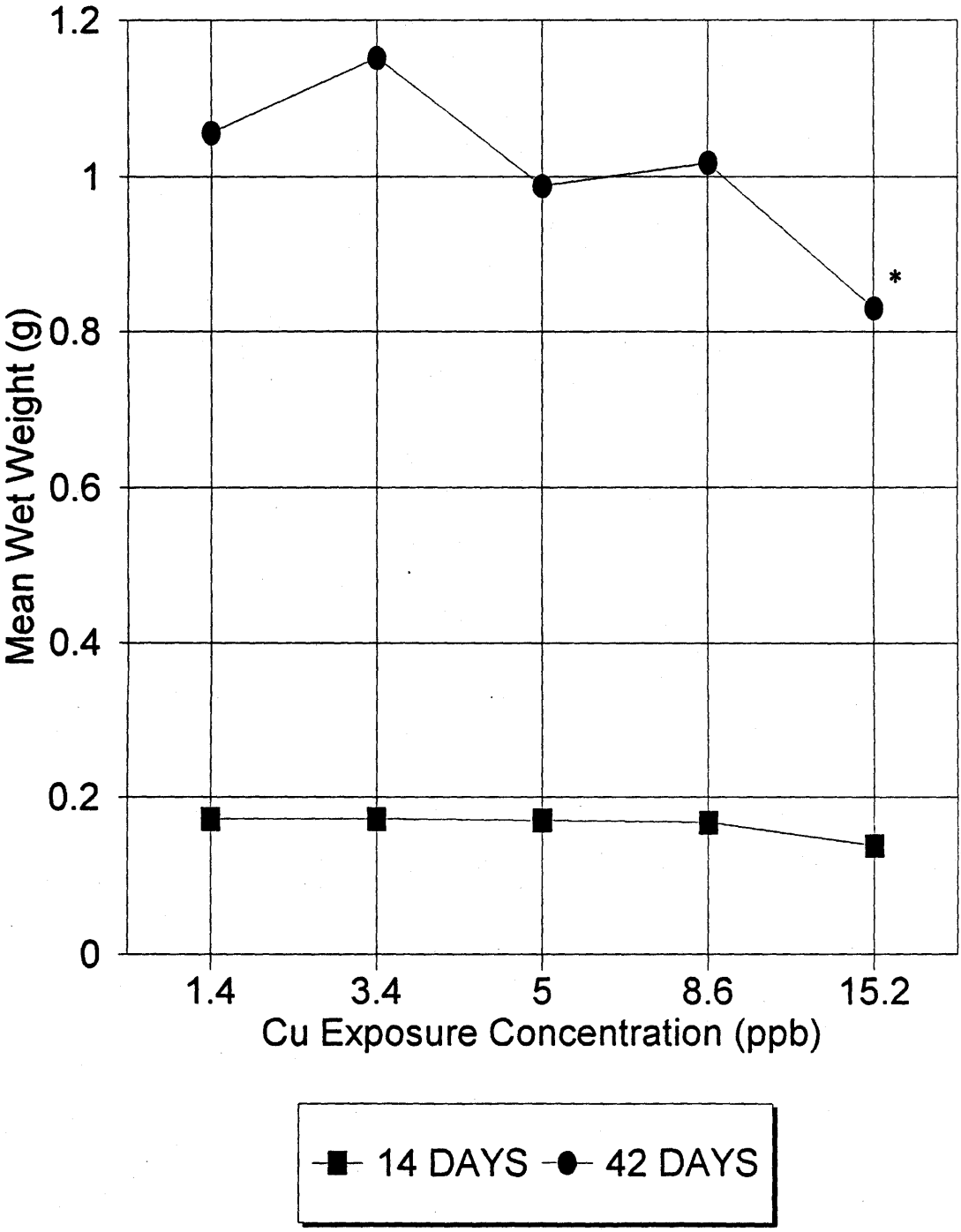

Figure 1. Mean wet weight (g) of boreal toad tadpoles after 14 and 42 days of exposure to copper. Asterisk indicates significantly less than control.

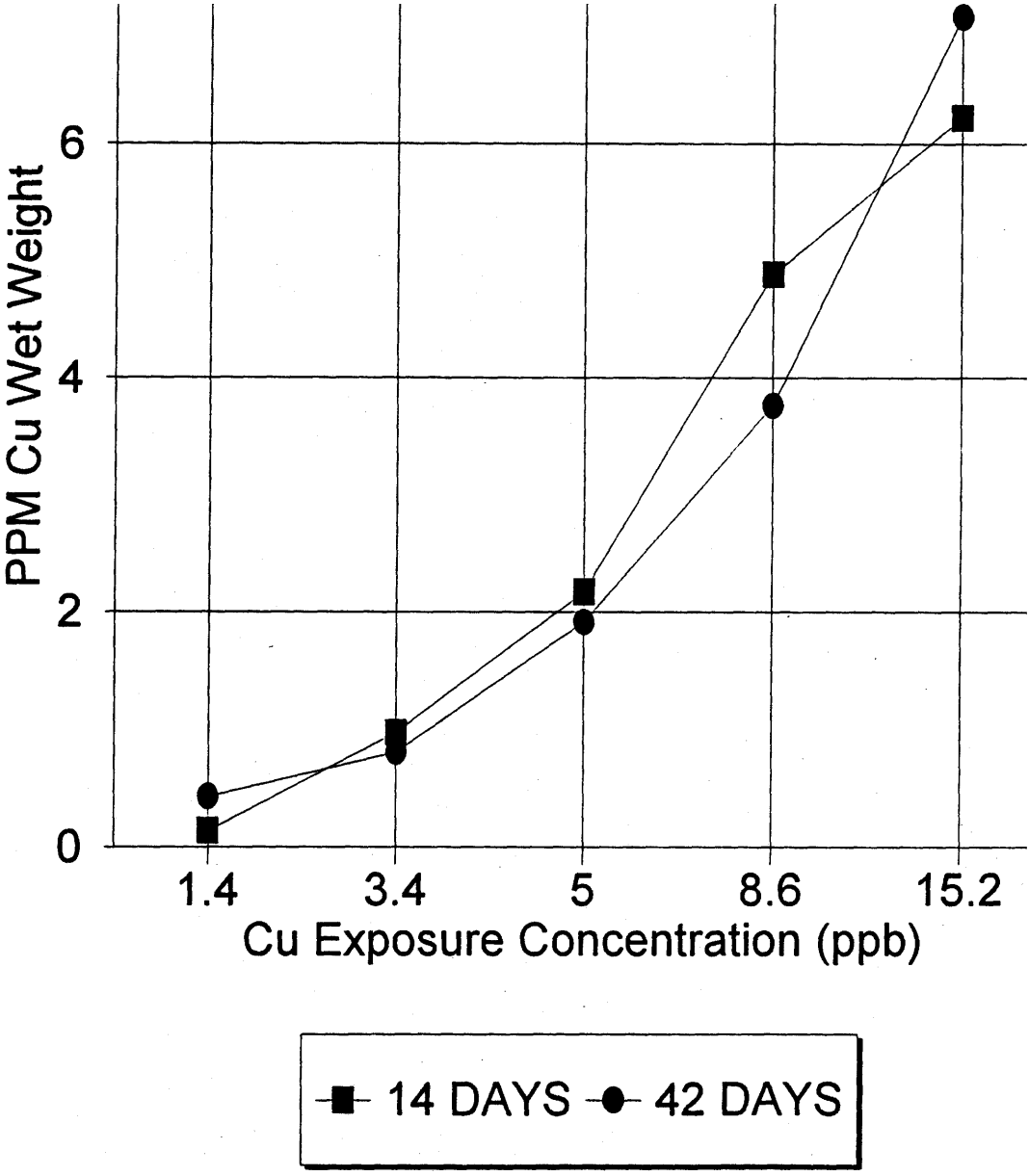

Figure 2. Whole body copper content (mg/Kg wet weight) of boreal toad tadpoles exposed to copper for 14 and 42 days. All treatments greater than control.

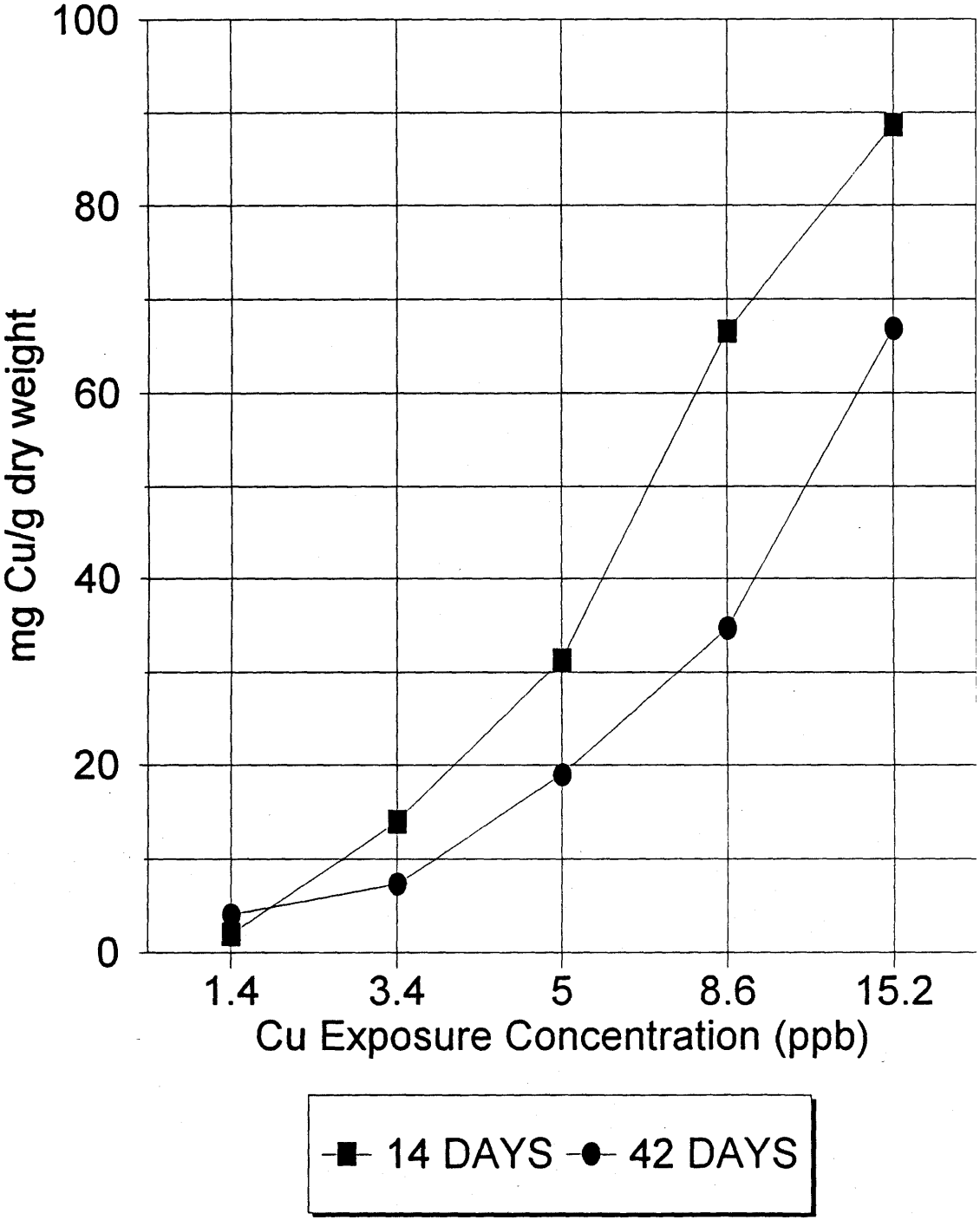

Figure 3. Whole body copper content (mg/Kg dry weight) of boreal toad tadpoles exposed to copper for 14 and 42 days. All treatments greater than control.

DISCUSSION

The results of the static renewal and flow-through tests are quite consistent. The 96 hr LC50s from the static renewal and flow-through exposures were 57.1 and 69.4 μg Cu/L, respectively. The geometric means of the LOEC and NOEC are 37.4 and 22.7 μg Cu/L for the static renewal and flow-through tests, respectively. This is good agreement considering the difference in duration of exposure (10 verses 42 days) and also the sensitivity of this measure to selection of exposure concentrations. The similar results of the ten-day static-renewal and 42 day flow-through test indicate that short-term toxicity tests may provide accurate estimates of chronic tadpole mortality for some metals. Short-term exposures would not detect sublethal effects such as reductions of growth and development. Exposure to copper for 42 days reduced the growth of tadpoles exposed to copper concentrations of 15.2 and 33.8 μg Cu/L. While not accompanied by reduced development, reduced growth may increase risk of predation while in the tadpole stage or reduce overwinter survival following metamorphosis. The copper concentrations that affected growth and survival are similar. This contrasts with cadmium where growth and development were affected at cadmium concentrations well below lethal levels. The acute toxicity of copper to boreal toad tadpoles is remarkably similar to rainbow trout (*Oncorhynchus mykiss*). Davies and Brinkman (1997) reported 96 hr LC50s of 46.6-55.7 μg Cu/L to rainbow trout exposed to copper in similar water quality. Since boreal toad tadpoles are so sensitive, use of copper pipes or brass fittings should be minimized when rearing boreal toads in captivity.

Whole-body copper content followed a dose-dependent relationship with copper concentrations. Whole-body copper content did not change over time when based on wet weight indicating that copper content was regulated by the tadpoles. However, copper content decreased between 14 and 42 days if based on dry weight. The difference of results between these two approaches arises from a decrease in water content as tadpoles develop and start to metamorphose. Whole body copper content is a sensitive measure of copper exposure and one which includes dietary and waterborne sources as well as influences of episodic exposures. However, as with cadmium, metal content data collected from the field should be interpreted with care. Many factors can modify bioavailability (and hence uptake) of metals including pH, hardness, presence of other metals, temperature, and dissolved organic matter. Also, ingested sediment in the gut can cause overestimation of whole body metal content. Different stages of development can also affect interpretation due to changes in water content of amphibians as they metamorphose.

Table 1. Water quality parameters of exposure water.

Hardness (mg $CaCO_3$/l)	51.2
Alkalinity (mg $CaCO_3$/l)	38.6
pH (S.U.)	7.21
Conductivity (μS/cm)	102.6
Fluoride (mg/l)	1.1
Chloride (mg/l)	3.3
Nitrate (mg/l)	<0.5
Phosphate (mg/l)	<0.5
Sulfate (mg/l)	31.4

Table 2 . Mean and standard deviation of copper exposure concentrations, associated mortality, mean wet and dry weights and copper content of boreal toads subjected to a static 24 hr renewal toxicity test for ten days. Standard deviations in parentheses.

Nominal (μg/l)	0	10	20	50	100
Mean Cu Concentration (μg/l)	<5.0 (0)	13.6 (3.0)	24.2 (3.6)	57.7 (9.6)	132 (0)
% Mortality	0	0	0	50	100
Mean Wet Weight (mg)	136.7	132.3	136.4	101	--
Mean Dry Weight (mg)	8.3	8	7.9	7.2	--
Cu Content (μg/g dry wt)	9.8	19.5	19.4	46.9	---

Table 3. Water quality characteristics of exposure water used in copper toxicity test conducted on boreal toad tadpoles.

	MEAN	STANDARD DEVIATION	RANGE
HARDNESS (mg $CaCO_3$/L)	49.5	1.5	47-52
ALKALINITY (mg $CaCO_3$/L)	37.1	5.1	33.8-64.0
pH (S.U.)	7.36	0.14	7.08-7.62
DISSOLVED OXYGEN (mg O_2/L)	6.70	0.90	5.0-8.1
TEMPERATURE (°C)	18.4	0.4	17.6-19.1
CONDUCTIVITY (μS/cm)	102.2	2.0	97-106
FLUORIDE (mg/L)	0.9	0.2	0.5-1.2
CHLORIDE (mg/L)	2.0	0.2	1.5-2.3
NITRATE (mg/L)	<0.5	0.3	<0.5-0.6
PHOSPHATE (mg/L)	<0.5	0.0	<0.5
SULFATE (mg/L)	11.9	0.2	11.6-12.2

Table 4. Total and dissolved copper exposure concentrations and associated acute (96 hour) mortality of boreal toad tadpoles.

Nominal Conc (μg/L)	Measured Total (μg/L)	Measured Dissolved (μg/L)	Percent Mortality
100	100	85	94.0 (5.0)
50	60	55	26.0 (8.0)
25	34	32	1.0 (2.0)
12.5	19.4	12.4	0.0 (0.0)
6.25	5.9	5.4	0.0 (0.0)
3.12	3.4	2.0	1.0 (2.0)
1.56	2.2	1.1	1.0 (2.0)
0	<0.5	<0.5	0.0 (0.0)

LC50 (95% C.I.) = 69.4 (65.6-73.5) μg Cu/L

Table 5. Total and dissolved copper exposure concentrations (standard deviations) and associated survival of boreal toad tadpoles chronically exposed for 43 days. Standard deviations in parentheses.

Nominal Conc (μg/L)	Measured Total (μg/L)	Measured Dissolved (μg/L)	Percent Mortality	Mean Weight (g)
100	92.0 (11.3)	82.5 (3.5)	100 (0)*	--
50	63.5 (4.9)	58.5 (4.9)	100 (0)*	--
25	33.8 (1.0)	32.8 (2.2)	97 (4)*	0.560 (0.244)‡
12.5	15.2 (2.1)	13.0 (0.8)	24 (17)	0.830 (0.172)‡
6.25	8.6 (1.7)	7.1 (1.1)	18 (20)	1.017 (0.096)
3.12	5.0 (1.3)	3.9 (1.3)	10 (10)	0.988 (0.097)
1.56	3.4 (1.6)	2.8 (1.4)	16 (11)	1.159 (0.195)
0	1.4 (1.7)	<0.5 (0.9)	11 (6)	1.056 (0.063)

* Significantly higher than control ($p<0.05$)
‡ Significantly lower than control ($p<0.05$)

Maximum allowable toxicant concentration (MATC) = 22.7 μg Cu/L

TOXICITY OF MANGANESE TO BOREAL TOAD TADPOLES

INTRODUCTION

Boreal toad (*Bufo boreas*) numbers have declined in Colorado over the last quarter century. They have been state-listed as endangered since November 1993 and federally listed as "warranted but precluded" since March 1995 (Goettl [eds.] and the Boreal Toad Recovery Team 1997). The Colorado Division of Wildlife Aquatic Toxicology Laboratory is assisting with investigations into possible causes of this decline by evaluating water quality characteristics that may limit survival and distribution of boreal toad tadpoles. These efforts include analysis of water samples collected from current and historic breeding ponds, developing techniques to measure effects of toxicants (heavy metals, pesticides, deicing compounds) to tadpoles, and conducting experiments to determine toxicity of selected metals to boreal toad tadpoles. Here we report the effects of short term exposure of boreal toad tadpoles to manganese.

MATERIALS AND METHODS

Static Renewal

A 10 day static 24 hr renewal test was conducted to estimate acute toxicity of manganese and to set concentrations for use in a long term chronic exposure experiment. Ten boreal toad (*Bufo boreas*) tadpoles (Gosner stage 18, Gosner 1960) were randomly placed in 300 ml polyethylene beakers containing 100 milliliters of 100, 50, 10, 5, 1 or 0 mg Mn/L added as manganese sulfate monohydrate to dechlorinated Fort Collins city tap water. The beakers were placed in an incubator, covered with a watch glass and gently aerated with compressed air. Temperature of the incubater was maintained at 25 °C during a 12 hour day and 15 °C during a 12 hour night. Tadpoles were transfered to 300 ml polyethylene beakers containing 100 mls of freshly prepared solutions daily using a brine shrimp net. Mortalities were monitored daily and removed using a pipet. Tadpoles were fed daily with Mazuri amphibian feed and frozen lettuce. Duration of exposure was ten days.

Flow-through

A serial diluter (Benoit et al. 1982) delivered seven concentrations of manganese (as manganese sulfate monohydrate) and control to completely randomized 2.2 liter glass exposure chambers at a rate of twenty mls/minute. The experiment was replicated four times with nominal manganese exposure concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0 μg Mn/L. Source water consisted of dechlorinated Fort Collins tap water. The exposure chambers were placed in a water bath maintained near 20 °C using a Remcor water recirculating heater/cooler. Two fluorescent blacklights and two florescent full spectrum (Repta Sun) bulbs suspended approximately 12 inches above the exposure chambers provided artificial lighting with 16 hour light and 8 hour dark photoperiod. Twenty boreal toad tadpoles about 10 days post hatch were randomly placed in each exposure chamber. Tadpoles were fed a mixture of Mazuri, Silver Cup trout food and frozen romaine lettuce *ad libitum*. Exposure chambers were checked daily for mortality.

Water quality parameters were measured in each exposure. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Hardness and conductivity were measured in control exposures only due to interference of manganese.

Water samples for manganese analysis were collected weekly in each exposure level from alternating replicates. Samples were stored in 2 oz. high density polyethylene bottles and preserved by acidification to pH<2 using Ultrex nitric acid. Manganese concentrations were measured using an Instrumentation

Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame with Smith-Hieftje background correction.

Statistics

The 96-hour median lethal concentration (LC50) was determined using Spearman-Karber (Hamilton et al. 1977, Hamilton et al. 1978). Analysis of variance (ANOVA) was performed on mortality using SAS computer software (SAS 1989). Mortality data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Assumptions of normality and homogeneity of variance were evaluated using Shapiro Wilk's test and Bartlett's test respectively ($p<0.10$). Treatment means were compared using one-tailed Dunnett's multiple comparison test ($p<0.05$) to evaluate differences from control.

RESULTS

Static Renewal

Water quality characteristics are presented in Table 1. Measured manganese concentrations and associated mortality are shown in Table 2. Manganese concentrations were stable throughout the 24 hour period between renewals. Mortality did not occur in manganese exposures of 10 mg Mn/L or less. In the 50 mg/L exposure, 40% mortality occurred by 96 hours leading to complete mortality by 168 hours. Mortality in the 100 mg/L exposure started as soon as 24 hours and resulted in complete mortality by 96 hours. The 96 and 168 hour LC50 were 42.3 and 20.8 mg Mn/L respectively.

Flow-through

Water quality characteristics of exposure water are shown in Table 1. Manganese concentrations and associated mortality for the first 96 hours and after 10 days of exposure are shown in Table 3. Mortality patterns from the first 96 hours precluded calculation of a reliable LC50. After ten days, percent mortality of tadpoles exposured to manganese concentrations greater than 4.9 mg/L were significantly greater than control ($p<0.05$).

DISCUSSION

The results of this experiment indicate that boreal toad tadpoles are relatively tolerant of manganese. The 96 hour LC50 for rainbow trout in similar water quality was 4.8 mg Mn/L (Davies and Brinkman 1994); about ten times lower than tadpoles at 42.3mg Mn/L. However, the duration of exposures of this experiment were relatively short (10 days). The toxicity of manganese increased substantially over time. For example, the LC50 at 168 hours was less than half of the 96 hour LC50. Longer exposures would be required to assess chronic and sublethal effects.

The cause of the high mortality in all treatments (including controls) is unknown. The techniques used in this experiment were virtually identical to those used successfully in previous experiments investigating toxicity of cadmium and copper. This should eliminate diet, temperature and light regimes, diluter system, and handling as potential causes for the mortality. In any case, the results of this experiment should be interpreted with extreme care since the unknown cause(s) of the mortality may have interacted with manganese exposure resulting in overestimation of toxicity. This experiment should be repeated to confirm the toxicity of manganese during short term exposures and to investigate effects from long term exposures.

Table 1. Water quality parameters of exposure water during static renwal and flow-through toxicity test.

	Static Renewal	Flow-through
Hardness (mg $CaCO_3$/l)	52.6	51.8
Alkalinity (mg $CaCO_3$/l)	38.8	40.0
pH (S.U.)	7.30	7.18
Conductivity (μS/cm)	115.3	110.5

Table 2. Manganese concentrations (mg/L) and associated mortality (%) of boreal toad tadpoles during the static 24 hr renewal toxicity test.

Nominal Concentration (mg/L)	0	1	5	10	50	100
Measured Concentration (mg/L)	<0.02	0.95	4.55	9.23	46.9	98.6
24 Hr Mortality (%)	0	0	0	0	0	10
48 Hr Mortality (%)	0	0	0	0	0	40
96 Hr Mortality (%)	0	0	0	0	40	100
168 Hr Mortality (%)	0	0	0	0	100	100

96 hour LC50 42.3 mg Mn/L
168 hour LC50=20.8 mg Mn/L

Table 3. Manganese concentrations (mg/l) and associated mortality (%) of boreal toad tadpoles during the flow-through toxicity test. Standard deviations in parentheses.

Nominal Concentration (mg/L)	96 Hour Acute		10 Day	
	Measured Concentration (mg/L)	Mortality (%)	Measured Concentration (mg/L)	Mortality (%)
0	<0.020	2.5 (2.9)	<0.020 (0.0)	23.8 (8.5)
1.2	2.3	11.2 (12.5)	1.9 (0.24)	20.0 (8.2)
2.5	3.3	3.8 (4.8)	3.1 (0.37)	37.5 (11.9)
5.0	5.2	10.0 (5.7)	5.0 (0.5)	68.8 (21.7)*
10	9.6	10.0 (10.8)	9.6 (0.8)	60.0 (10.8)*
20	18.7	36.2 (14.4)	18.9 (0.3)	76.2 (14.9)*
40	39.8	52.5 (27.2)	39.7 (0.1)	98.8 (2.5)*
80	83.8	35.0 (16.3)	83.8 (0.0)	100.0 (0.0)*

Unable to calculate 96 hr. LC50.
*Significantly greater than control ($p<0.05$)

TOXICITY OF ZINC TO BOREAL TOAD TADPOLES

INTRODUCTION

Boreal toad (*Bufo boreas*) numbers have declined in Colorado over the last quarter century. They have been state-listed as endangered since November 1993 and federally listed as "warranted but precluded" since March 1995 (Goettl [eds.] and the Boreal Toad Recovery Team 1997). The Colorado Division of Wildlife Aquatic Toxicology Laboratory is assisting with investigations into possible causes of this decline by evaluating water quality characteristics that may limit survival and distribution of boreal toad tadpoles. These efforts include analysis of water samples collected from current and historic breeding ponds, developing techniques to measure effects of toxicants (heavy metals, pesticides, deicing compounds) to tadpoles, and conducting experiments to determine toxicity of selected metals to boreal toad tadpoles. Here we report the effects of short term exposure of boreal toad tadpoles to zinc.

MATERIALS AND METHODS

Static Renewal

A 10 day static 24 hr renewal test was conducted to estimate acute toxicity of zinc and to set concentrations for use in a long term chronic exposure experiment. Ten boreal toad (*Bufo boreas*) tadpoles (Gosner stage 18, Gosner 1963) were randomly placed in 300 ml polyethylene beakers containing 100 milliliters of 10, 5, 1 or 0 mg Zn/L added as zinc sulfate heptahydrate to dechlorinated Fort Collins city tap water. The beakers were placed in an incubator, covered with a watch glass and gently aerated with compressed air. Temperature of the incubator was maintained at 25°C during a 12 hour day and 15°C during a 12 hour night. Tadpoles were transferred to 300 ml polyethylene beakers containing 100 mls of freshly prepared solutions daily using a brine shrimp net. Mortalities were monitored daily and removed using a pipet. Tadpoles were fed daily with Mazuri amphibian feed and frozen lettuce. Duration of exposure was ten days.

Flow-through

A serial diluter (Benoit et al. 1982) delivered seven concentrations of zinc (as zinc sulfate heptahydrate) and control to completely randomized 2.2 liter glass exposure chambers at a rate of twenty mls/minute. The experiment was replicated four times with nominal zinc exposure concentrations of 4000, 2000, 1000, 500, 250, 125, 62.5 and 0 μg Zn/L. Source water consisted of dechlorinated Fort Collins tap water. Two fluorescent blacklights and two florescent full spectrum (Repta Sun) bulbs suspended approximately 12 inches above the exposure chambers provided artificial lighting with 16 hour light and 8 hour dark photoperiod. Twenty boreal toad tadpoles about 10 days post hatch were randomly placed in each exposure chamber. Tadpoles were fed a mixture of Mazuri, Silver Cup trout food and frozen romaine lettuce *ad libitum*. Exposure chambers were checked daily for mortality.

Water quality parameters were measured in each exposure. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Hardness and conductivity were measured in control exposures only due to interference of zinc.

Water samples for zinc analysis were collected weekly in each exposure level from alternating replicates. Samples were stored in 2 oz. high density polyethylene bottles and preserved by acidification to pH<2 using Ultrex nitric acid. Zinc concentrations were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame with Smith-Hieftje background correction.

Statistics

Median lethal concentrations (LC50) were determined using Spearman-Karber (Hamilton et al. 1977, Hamilton et al. 1978). Analysis of variance (ANOVA) was performed on mortality using SAS computer software (SAS 1989). Mortality data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Assumptions of normality and homogeneity of variance were evaluated using Shapiro Wilk's test and Bartlett's test respectively ($p<0.10$). Treatment means were compared using one-tailed Dunnett's multiple comparison test ($p<0.05$) to evaluate differences from control.

RESULTS

Static Renewal

Water quality characteristics are presented in Table 1. Measured zinc concentrations and associated mortality are shown in Table 2. Zinc concentrations decreased significantly during the 24 hour period between renewals. The highest exposure concentration, 10,000 μg /L, resulted in complete mortality within 24 hours. In the 5,000 μg /L exposure, there was ninety percent mortality through 96 hours of exposure when complete mortality was achieved. There was no other mortality except for a single tadpoles exposed to 1,000 μg /L for 96 hours. The 24 and 48 hour LC50 concentration based on initial concentrations was 2310 μg /L. The 96 and 168 hour LC50 was somewhat lower at 1460 μg /L.

Flow-through

Water quality characteristics of exposure water are shown in Table 1. Zinc concentrations and associated mortality for the first 96 hours and after 10 days of exposure are shown in Table 3. The 96 hour LC50 derived from the flow-through exposure was 1326 μg /L. After 10 days of exposure, the no observed effect concentration (NOEC) was 617 and the lowest observed effect concentration (LOEC) was 1108 μg /L. The geometric mean of the NOEC and LOEC is 826 μg /L.

DISCUSSION

Boreal toad tadpoles were more tolerant of zinc than rainbow trout. The median lethal concentration for rainbow trout in similar water ranges between 370 and 756 μg/L (Holcombe and Andrew 1978). The duration of exposures of this experiment were relatively short (10 days) and longer exposures would be required to assess chronic and sublethal effects.

The cause of the high mortality in all treatments (including controls) is unknown. The techniques used in this experiment were virtually identical to those used successfully in previous experiments investigating toxicity of cadmium and copper. This should eliminate diet, temperature and light regimes, diluter system, and handling as potential causes for the mortality. In any case, the results of this experiment should be interpreted with extreme care since the unknown cause(s) of the mortality may have interacted with zinc exposure resulting in overestimation of toxicity. This experiment should be repeated to confirm the toxicity of zinc during short term exposures and to investigate effects from long term exposures.

Table 1. Water quality parameters of exposure water during static renewal and flow-through toxicity test.

	Static Renewal	Flow-through
Hardness (mg $CaCO_3$/l)	53.9	54.6
Alkalinity (mg $CaCO_3$/l)	36.8	37.3
pH (S.U.)	7.31	7.29
Conductivity (μS/cm)	101.8	98.1

Table 2. Zinc concentrations (mg/L) and associated mortality (%) of boreal toad tadpoles during the static 24 hour renewal toxicity test.

Nominal Concentration	0		1		5		10	
	Initial	24 Hrs	Initial	24 Hrs	Initial	24 Hrs	Initial	24 Hrs
Measured Concentration	<0.01	<0.01	0.89	0.56*	4.72	3.68*	9.41	5.38*
24 Hr Mortality	0		0		90		100	
48 Hr Mortality	0		0		90		100	
96 Hr Mortality	0		10		100		100	
168 Hr Mortality	0		10		100		100	

*Significantly less than initial ($p<0.01$)
24 hour LC50=2.31 mg Zn/L
48 hour LC50=2.31 mg Zn/L
96 hour LC50=1.46 mg Zn/L
168 hour LC50=1.46 mg Zn/L

Table 3. Zinc concentrations (μg/l) and associated mortality (%) of boreal toad tadpoles exposed to zinc for 96 hours and ten days in the flow-through test. Standard deviations in parentheses.

Nominal Concentration (μg/L)	96 Hour Acute		10 Day	
	Measured Concentration (μg/L)	Mortality (%)	Measured Concentration (μg/L)	Mortality (%)
0	<10	6.2 (7.5)	<10	20.5 (11.5)
62	98	0.0 (0.0)	104 (14)	4.0 (4.9)
125	162	3.8 (2.5)	196 (35)	13.0 (10.3)
250	308	3.8 (4.8)	314 (13)	7.5 (5.0)
500	585	5.0 (7.1)	617 (41)	30.0 (9.2)
1000	1038	27.5 (12.6)	1108 (31)	76.2 (17.3)*
2000	1880	97.5 (5.0)	1880	100.0 (0.0)*
4000	3800	100.0 (0.0)	3800	100.0 (0.0)*

96 hour LC50=1330 μg Zn/L
*Significantly greater than control ($p<0.05$)

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INVESTIGATIONS OF BOREAL TOAD TADPOLE ECOLOGY

Within the past 20 years, boreal toads (*Bufo boreas*) have undergone inexplicable population declines in distribution and abundance in Colorado (Carey 1993, Corn et al. 1989). Because of these declines, the Colorado Division of Wildlife listed this species as endangered in 1993 (Goettl 1997). Predation on eggs, tadpoles, or metamorphosed toads has not been suggested as a direct cause of population declines in this species in Colorado. However, many formerly large boreal toad populations have been eliminated or reduced to small remanents. With the reduced abundance of boreal toads, natural predation events may be a threat to some remaining toad populations (Corn 1993).

In addition, reintroduction of boreal toads to previously occupied habitat is among the possible recovery activities for the species (Goettl 1997). Analysis of factors affecting the survival of early life stages has conservation implications, since increased recruitment could enhance the recovery of toad populations. In addition, investigation of habitat characteristics associated with successful survival to metamorphosis, including predator communities, can aid in selection of sites for reintroduction efforts.

This report contains the preliminary results from a series of experiments and field observations focused on the early life history stages of boreal toads. Sections include:

- Boreal toad tadpole predators
- Tadpole color variation
- Tadpole density effects
- Post-metamorphic aggregations
- Fluctuating asymmetry

BOREAL TOAD TADPOLE PREDATORS

INTRODUCTION

Because of noxious compounds in the skin, tadpoles in the genus *Bufo* are generally regarded as unpalatable to many predators (Voris and Bacon 1966, Kruse and Stone 1984, Hews and Blaustein 1985). However, several avian predators have been documented as consuming *Bufo boreas* tadpoles and newly metamorphosed toadlets, including gray jays, ravens, and spotted sandpipers (Beiswenger 1981, Goettl, pers. com., pers. obs.). The purpose of this study was to identify additional tadpole predators in both laboratory and field settings.

MATERIALS AND METHODS

I collected potential predators from a variety of montane habitats, including historical and current boreal toad sites. *T. elegans* were collected from boreal toad breeding sites in Chaffee County, Colorado. All other predator collection sites were above 8,000 feet elevation in Clear Creek County or western Boulder County, Colorado. Potential predators included tiger salamander (*Ambystoma tigrinum*) larvae, western terrestrial garter snakes, and a variety of invertebrates, including predaceous diving beetle (*Dytiscus* sp.) larvae. All invertebrate predators were used in only one trial and, except where noted, used within 24 hours of capture. Three *A. tigrinum* larvae were each used in two trials, the first within 24 hours of capture and the second three days later.

Prey animals included *B. boreas* tadpoles and toadlets from Clear Creek County and chorus frog (*Pseudacris triseriata*) tadpoles from two sites in western Boulder County. Except where noted, tadpoles were used within 24 hours of capture.

For all predators except *T. elegans*, trials took place in plastic 15 x 30-cm containers filled with 1.5 liters of pond water. Trial containers for tadpoles contained food flakes and softened lettuce. Tadpoles were allowed to acclimate for 30 to 60 minutes before I introduced the predator. I tallied surviving tadpoles on an

hourly basis for the first four hours. Trials ended at 24 hours unless noted.

Trials with *T. elegans* took place in a 30 x 60-cm tank. The floor of the tank was covered with a layer of damp pea gravel. Ten newly metamorphosed toadlets were allowed to acclimate for 15 minutes before predator introduction. In the *T. elegans* trial with tadpoles, 10 tadpoles were in water that filled the tank to a depth of 7 cm, and the pea gravel was pushed against one end of the tank so that it provided an area above water for the snake. Trials with *T. elegans* and toadlets lasted 10 minutes. The trial with *T. elegans* and tadpoles lasted 4 hours.

Before introducing the tadpoles to the trial containers, I measured their snout-vent and total lengths in mm and scored developmental stage using Gosner (1960). Depending on the experiment, either 5 or 10 tadpoles were used in each trial. For trials comparing *B. boreas* and *P. triseriata* tadpoles, I matched snout-vent lengths of the tadpoles as closely as possible to present potential predators with tadpoles of equivalent value as prey. However, the tadpoles of these two species have different relationships between snout-vent lengths, total lengths, and developmental stage. Trials with *A. tigrinum* took place later in the season than those with *Dytiscus* sp. larvae, resulting in larger mean lengths and stage of development for all prey animals. Table 1 compares the means for Gosner stages, snout-vent lengths, and total lengths of these two species in trials with *Dytiscus* sp. and *A. tigrinum* larvae. All tadpoles were Gosner stage 40 or less (pre-metamorphic). Tadpoles not used in trials were kept as controls in the same conditions. Toadlets used in the *T. elegans* trials were Gosner stages 44 through 46.

Since tadpoles could not be returned to the pond of origin because of the possibility of introducing pathogens, I conducted one trial with a *Dytiscus* larva using 27 boreal toad tadpoles that were either survivors from previous trials or used as controls.

Four trials were conducted with a variety of medium-sized adult dytiscid beetles and each had a duration of 48 hours. Two trials each used five boreal toad tadpoles (snout-vent size range of 8-12 mm). Two additional trials each used 10 boreal toad tadpole, five small (5-7 mm snout-vent length) and five large (10-15 mm snout-vent length).

Additional trials included: leech (*Nephelopsis obscura*, three trials, each with five large and five small boreal toad tadpoles that had been in captivity > 7 days); backswimmer *Notonecta undulata* (one 48-hour trial with five large and five small boreal toad tadpoles); and unidentified caddis fly larva (one trial with five boreal toad tadpoles).

I also made opportunistic observations of predation on boreal toad tadpoles at several known boreal toad breeding sites.

Table 1. Comparisons of mean (± standard errors) of tadpole SV lengths, total lengths, and developmental stages. *Dytiscus* sp. trials used a total of 15 tadpoles of each species; *Ambystoma tigrinum* trials used 30 tadpoles of each species.

	Dytiscus sp. trials			*Ambystoma tigrinum* trials		
	Gosner	SV (mm)	Total length (mm)	Gosner	SV (mm)	Total length (mm)
B. boreas tadpoles	29.1±0.2	9.7±0.2	23.1±0.4	32.9±0.4	12.0±0.2	30.5±0.6
P. triseriata tadpoles	32.7±0.4	10.0±0.3	26.4±0.6	35.8±0.4	11.6±0.3	33.2±0.9

RESULTS

Three predators readily consumed large numbers of *B. boreas* tadpoles or toadlets in laboratory trials: predaceous diving beetle (*Dytiscus* sp.) larvae, *Ambystoma tigrinum* larvae, and *Thamnophis elegans*. Various medium-sized adult dytiscid beetles (*Agabus tristis*, *Rhantus binotatus*, and *Graphoderus occidentalis*) were minor tadpole predators. The leech *Nephelopsis obscura*, backswimmer *Notonecta undulata*, and caddis fly larva did not consume any tadpoles in the laboratory trials. Specific trial results are discussed in more detail below.

In four trials with *Dytiscus* sp. larvae, each comparing five large (mean snout-vent length 13.3±0.4 mm, mean Gosner stage 33.2±0.4) with five small (mean snout-vent length 8.7±0.2 mm, mean Gosner stage 27.9±0.2) *B. boreas* tadpoles, there was no difference in survival at 24 hours (χ^2= 0.47, df=1, $p > 0.05$).

In three trials comparing relative vulnerabilities of *B. boreas* and *P. triseriata* tadpoles to *Dytiscus* larvae, the *B. boreas* tadpoles were significantly more vulnerable than were *P. triseriata* tadpoles (χ^2=26.25, df=1, $p < 0.01$). Of the 15 *B. boreas* tadpoles, none survived 24 hours, while all but one of the 15 *P. triseriata* tadpoles survived. After these trials were complete, I offered *P. triseriata* on forceps to two of the *Dytiscus* larvae; the larvae readily consumed the tadpoles.

In a single trial with a *Dytiscus* larva in a container with 27 boreal toad tadpoles, the *Dytiscus* larvae killed 26 of the 27 tadpoles within 11 hours; by the end of the 24-hour trial, all tadpoles had been killed.

In two trials with medium-sized adult dytiscid beetles and tadpoles of 8-12 mm snout-vent length, the beetles consumed no tadpoles by 24 hours, but each had consumed a single tadpole by 48 hours. When two trials with adult beetles were repeated with five large (10-15 mm snout-vent length) versus five small (5-7 mm snout-vent length) tadpoles, each beetle had consumed one small tadpole by 24 hours and two small tadpoles by 48 hours. All the large tadpoles survived.

In six trials with *A. tigrinum* larvae, *B. boreas* and *P. triseriata* tadpoles were about equally vulnerable to predation (χ^2=1.15, df=1, $p > 0.05$). Each tiger salamander larva consumed 4 to 8 tadpoles (mean 6.3 ± 1.6 tadpoles) in each of the six trials.

In four 10-minute trials with *T. elegans* (2 adults, 2 neonates), a mean of 4.2±1.9 of the 10 toadlets in each trial survived. Even the two neonate snakes consumed newly metamorphosed toadlets, although they appeared to have more difficulty than the adult snakes in handling this prey.

Boreal toad breeding sites frequently contained *Dytiscus* larvae, and I often observed these invertebrates capturing tadpoles. At least one site in Clear Creek County with numerous *Dytiscus* larvae showed steep declines in tadpole numbers. Although observed at some historical *B. boreas* breeding sites, no *T. elegans* were observed at current sites in the Front Range. However, at least two sites in Chaffee County contained this snake, and predation on *B. boreas* was documented at one of those sites. Finally, although *A. tigrinum* continues to occupy several historical *B. boreas* breeding sites, this salamander was not observed at any current breeding sites.

DISCUSSION

The most striking observation in this series of experiments was the great vulnerability to *Dytiscus* larvae of *B. boreas* tadpoles compared to *P. triseriata* tadpoles. Several factors are known to be associated with vulnerability to predation, including differences in palatability, size, developmental stage, activity levels, and the ability of tadpoles to perceive and avoid alarm compounds released by injured conspecifics.

The observed differential vulnerability probably is not due to differences in palatability, since the *Dytiscus* larvae appeared to accept *P. triseriata* readily when individuals were offered to them on forceps. Also, *A. tigrinum*, which could be expected to "taste" any differences since the tadpole's skin comes into contact with oral tissues of the salamander, exhibited no difference in predation rates between the two species.

The two tadpole species differed somewhat in size and developmental stage. Although both tadpole species had similar snout-vent lengths, *P. triseriata* were somewhat more advanced in development and had

longer total lengths, both of which may have positively affected their ability to escape from predatory attacks. However, this same size and development advantage was present in the trials with *A. tigrinum*, but there was no difference in vulnerability to this predator. Also there was no significant difference in vulnerability to predation of small versus large *B. boreas* tadpoles by *Dytiscus* larvae.

Peterson and Blaustein (1992) observed similar higher vulnerability to *Dytiscus* larvae of *B. boreas* versus *Pseudacris regilla* tadpoles. They attributed this differential predation to possible higher activity levels of *B. boreas* tadpoles. Activity levels and chemosensory perception differences were not evaluated in the trials reported here. However, a subjective observation during the trials was that *P. triseriata* tadpoles were much more reactive to the proximity of *Dytiscus* larvae than were *B. boreas* tadpoles. In contrast, neither tadpole species appeared to avoid contact with *A. tigrinum* larvae, and *B. boreas* tadpoles did not appear to avoid contact with *Dytiscus* larvae (including when a larva was consuming a conspecific).

Dytiscid beetle larvae may be responsible for some of the sharp declines in tadpole populations observed at several boreal toad breeding sites. Approximately 450 Dytiscid beetle species are present in the United States and Canada (Spangler 1987). However, based on observations at boreal toad breeding sites, the most important boreal toad tadpole predators of the dytiscid beetles are in the genus *Dytiscus*. At least five species in this genus occur in Colorado: *D. hybridus*, *D. marginicollis*, *D. cordieri*, *D. alaskanus*, and *D. dauricus*, with *D. dauricus* having the most widespread records of occurrence in the state (Roughley 1990). A generalized life cycle for *Dytiscus* includes mating in the fall or spring, overwintering in water by adults, oviposition (sometimes in plant tissues) in the spring when ponds become ice-free, hatching of eggs into the first of three aquatic larval instars, appearance of the third and largest instar by July and persisting until late July or early August, followed by terrestrial pupation and emergence of adults (Larson 1975, Holomuzki 1985, Stehr 1987, Roughley 1990). Adult *Dytiscus* are strong fliers, and capable of considerable dispersal (Roughley 1990)

In 1997, I observed large (third instar) *Dytiscus* larvae between July 3 and August 4 at various montane sites in Boulder and Clear Creek counties; however two moderate-sized larvae identified as *Dytiscus* (one observed with a small tadpole in its mandibles) were present at a site in Clear Creek County as late as September 11.

The phenology of *Dytiscus* larvae and that of boreal toad tadpoles overlaps broadly in both space and time. *Dytiscus* larvae and boreal toad tadpoles frequently occur in the same pond. Although adult dytiscid beetles are primarily nocturnal, both *Dytiscus* larvae and boreal toad larvae are diurnal (Holomuzki 1985, pers. obs.). In the Cascade Mountains, Peterson and Blaustein (1992) observed *Dytiscus* larvae 1 to 4 weeks after *B. boreas* larvae hatched. The first appearance of *Dytiscus* larvae at boreal toad breeding sites in Colorado is not known, but if they hatch at about the same time as boreal toad tadpoles, it is possible that the tadpoles are susceptible to predation from these insects throughout most of their development. By July, diving beetle larvae have grown large and are capable of preying on the largest of the tadpoles.

The experiments and field observation reported here demonstrate that pre-metamorphic boreal toad tadpoles are palatable to *Dytiscus* larvae. Further, at many sites tadpoles likely are exposed to *Dytiscus* larvae throughout much of their development.

Dytiscus larvae at Front Range sites probably begin pupation by early August. Many tadpole species become unpalatable during metamorphosis due to the increase in skin glands and noxious compounds (Brodie et al. 1978). Any such increase in unpalatability occurs after *Dytiscus* begins pupation, since tadpoles in this area usually begin metamorphosis around mid-August (pers. obs.).

Boreal toad breeding ponds often contain a variety of adult dytiscid beetles, especially medium and small individuals of genera other than *Dytiscus*. These were occasionally observed consuming boreal toad tadpoles, but they may have been acting as scavengers rather than as predators. From the limited number of trials, it appears that small tadpoles may be more vulnerable to these predators than larger tadpoles. Elsewhere, backswimmers have been documented as *B. boreas* tadpole predators (Kiesecker et al. 1996). These invertebrates are common at several boreal toad breeding sites, however the single trial I conducted did

not result in any predation.

Limited trials indicate that both *T. elegans* and *A. tigrinum* can act as major predators of *B. boreas* tadpoles and toadlets. Both of these vertebrates are broadly sympatric with *B. boreas*, however neither of these species was recorded at current boreal toad breeding sites in Clear Creek County. Both occur at some historical boreal toad breeding sites in the Front Range, and *T. elegans* occupies at least two current toad breeding sites in Chaffee County, where predation by this snake has been documented. Although larval and adult *A. tigrinum* are sometimes encountered at the same site as adult boreal toads, the most recent record of *A. tigrinum* larvae at a site with *B. boreas* tadpoles was from West White Slide Lakes, Jackson County, Colorado in June, 1988 (USGS/Biological Resources Division database).

Boreal toads in Colorado often select temporary pools and other ephemeral sites in which to breed. In recent years, toads have deposited eggs in tire ruts in roads, shallow pools below a more permanent pool, a shallow pool at the foot of an avalanche track, newly constructed artificial wetlands in an area heavily altered by mining, shallow pools surrounding a lake, backwaters in streams, and abandoned beaver ponds (pers. obs.). Permanent and semi-permanent lakes and active beaver ponds are also employed. When both permanent and temporary pools are available in the same small area, the toads may prefer to breed in the temporary pools. For example, at the Herman Gulch site, greater numbers of toads breed in shallow pools that dry up by late June rather than in the more permanent pool. At Lost Lake, Rocky Mountain National Park, toads breed in pools surrounding the lake rather than in the lake itself (Corn et al. 1997). This use of ephemeral pools probably is associated with the rapid tadpole development possible in shallow, sunny bodies of water. However, these sites also may be relatively free of tadpole predators compared to more permanent aquatic habitats, including the three major tadpole predators identified by this study, *Dytiscus* larvae, *A. tigrinum* larvae, and *T. elegans*.

Efforts to remove and relocate *T. elegans* and *Dytiscus* larvae were initiated at some boreal toad breeding sites. These efforts should continue and be expanded to include other sites where these predators may decrease boreal toad tadpole populations. In addition, the presence of *Dytiscus* larvae, *A. tigrinum* larvae, and *T. elegans* should be among the factors considered in evaluating potential boreal toad reintroduction sites.

TADPOLE COLOR VARIATION

INTRODUCTION

Typical *Bufo boreas* tadpoles in the southern Rocky Mountains exhibit jet black coloration throughout most of their development to metamorphosis (Livo 1995). However, tadpoles from at least one site (Brown's Creek, in Chaffee County, Colorado) have an atypical pattern of color development. Observations conducted since 1995, when the Brown's Creek site was first surveyed, indicate that tadpoles at this site consistently become olive brown well before metamorphosis (pers. obs.).

This study provides details about the development of tadpole color at the Brown's Creek site and compares it to the more typical tadpole color development at the Donut site in Clear Creek County. We also discuss possible functional relationships associated with the color development observed at Brown's Creek.

MATERIALS AND METHODS

We sampled *Bufo boreas* tadpoles at two boreal toad breeding sites, Brown's Creek and Donut. The Brown's Creek site is a series of abandoned beaver ponds located at approximately 9,760 feet elevation in the San Isabel National Forest, Chaffee County, two of which contained tadpoles in 1997. Although the egg masses were not observed prior to hatching, based on the size and location of recently hatched tadpoles, we believe the tadpoles were derived from at least three clutches. The Donut site is an artificial wetland located at approximately 10,280 feet elevation in the Wood's Creek drainage, Clear Creek County. Tadpoles at this site

were derived from at least five egg masses.

We captured tadpoles with a dip net. For each tadpole, we measured the total length in mm and scored the developmental stage using Gosner (1960). We compared the dorsal color of each tadpole to soil color charts (Munsell Color 1994) and recorded scores for chart number, value, and chroma. Absolute black would have a value score of 0, although on chart 1 (achromatic colors), the darkest available comparison for value was 2.5. Hence, tadpoles as dark or darker than 2.5 were assigned a value of 2.5. We scored tadpole colors in the field during mid-day under sunny to partly cloudy conditions.

We released the tadpoles after measuring and scoring all individuals in the sample. Consequently, no tadpole was measured twice on the same day, although it is possible that some tadpoles were measured both early and late in their development on different sampling dates.

RESULTS

For data analysis, we considered scores for Gosner stage and Munsell Soil Color value and chroma scores as ordinal (ranked) data. Chart number (hue) scores were scored as nominal data. Correlation coefficients (r_S) reported below are Spearman's rank order correlation coefficient (Sokal and Rohlf 1969, Zar 1974).

Because total tadpole length and Gosner stage were highly correlated at both sites (Brown's Creek: $r_S = 0.9824$, $n = 40$, $p < 0.01$; Donut: $r_S = 0.8532$, $n = 57$, $p < 0.01$), our analyses were conducted using Gosner stage to indicate development.

There was a highly significant difference in the chart scores of Brown's Creek versus Donut tadpoles ($\chi^2 = 24.86$, $df = 1$, $N = 97$, $p < 0.01$). Brown's Creek tadpoles in earlier stages scored on Chart 1, while later stages scored more frequently than expected on Chart 2.5Y. All Donut tadpoles scored throughout development on Chart 1.

In a comparison of color value scores of 2.5 versus scores >2.5, there was a highly significant difference in the value scores of Brown's Creek versus Donut tadpoles ($\chi^2 = 24.86$, $df = 1$, $N = 97$, $p < 0.01$). There was a significant correlation between Gosner stage and Munsell value score for tadpoles from Brown's Creek ($r_S = 0.8390$, $n = 40$, $p < 0.01$), with tadpoles scored at later Gosner stages more likely to have higher value scores. Correlation between Gosner stage and Munsell value score was not calculated for Donut tadpoles, since there was no variability in value score.

In a comparison of chroma scores, there was a highly significant difference between Brown's Creek versus Donut tadpoles ($\chi^2 = 29.720$, $df = 2$, $N = 97$, $p < 0.01$). There was a significant correlation between Gosner stage and Munsell chroma score for tadpoles from Brown's Creek ($r_S = 0.8866$, $n = 40$, $p < 0.01$), with tadpoles of later Gosner stages more likely to have higher chroma scores. There was no significant difference between Gosner stage and Munsell chroma scores ($r_S = 0.0336$, $n = 57$, $p > 0.05$), for Donut tadpoles.

DISCUSSION

Like tadpoles from normally colored populations, the tadpoles at Brown's Creek are very dark when small. However, Brown's Creek tadpoles differ significantly on all three Munsell color variables from more typical tadpoles, exemplified by the Donut tadpoles. Instead of remaining very dark throughout development, Brown's Creek tadpoles gradually become olive brown as they grow. Further, this pattern of color development has been consistent for the three years that the Brown's Creek site has been observed. Munsell Soil Color scores recorded for Brown's Creek tadpoles in 1996 are very similar to those reported here (unpubl. data).

Several possible explanations, which are not necessarily mutually exclusive, exist for the unusual pattern of color development observed in tadpoles at the Brown's Creek site:

1) The color morph is a result of genetic differences in the Brown's Creek toads,
2) The color morph is environmentally induced through tadpole exposure to compounds in the water,

substrate, or food,

3) The color morph is induced through environmental albedo, resulting in substrate matching,

4) The color morph is induced by exposure to compounds associated with predators.

While we do not have enough information at this time to exclude any of these possible causes for the color of the Brown's Creek tadpoles, each merits some consideration.

Genetic differences

The reliable appearance of brown color in tadpoles, observed over three different field seasons and resulting from multiple egg masses, suggests the possibility of a genetic difference in the Brown's Creek boreal toad population compared to populations elsewhere in the southern Rocky Mountains. Genetic work, focusing on mitochondrial DNA (mtDNA), currently is being conducted by Anna Goebel. At present, only limited information is available concerning the Brown's Creek site, but the mtDNA haplotype observed was not unique (Goebel 1997). Mutations occurring in nuclear DNA, however, would not be detected by examination of mtDNA haplotypes. Only a few adult boreal toads have been observed at the Brown's Creek site, and only a few egg masses have been produced in the last three breeding seasons, suggesting that the area supports a relatively small population. Genetic mutations, even deleterious ones, can become widespread or fixed more easily in a small population than a large one.

The black pigment on the surface of boreal toad tadpoles is melanin. If a genetic mutation occurred that affected melanin production in tadpoles, it could be associated with retarded growth, since tyrosine is required for both melanin synthesis and synthesis of thyroid growth hormones (Bagnara et al. 1978, Corn 1986).

Possible negative effects on growth and development, possibly resulting from mutations, were observed in three tadpoles at the Donut site. In 1996, when Donut had approximately 15,000 normally colored tadpoles, we observed a single brown tadpole. Of about 3,000 tadpoles at Donut in 1997, we observed two brown individuals. In all three instances, the brown tadpoles were retarded in growth and development compared to the normally colored tadpoles. However, the Brown's Creek tadpoles have growth and development rates that appear to be similar to that observed for tadpoles at other breeding sites in Chaffee County (pers. obs.), suggesting little or no deleterious effect of the brown condition for these tadpoles.

Environmental compounds

It is possible that one or more compounds present in the substrate or water at the Brown's Creek site is responsible for the brown color development of the tadpoles. However, if compounds caused tadpoles to become brown through interference with melanin production, growth and development rates would probably be retarded, as discussed above. Also, no obvious source of unusual compounds is present at the Brown's Creek site, which is located in a National Forest.

Environmental albedo

Many animals have the ability to match local environments, usually through sensing environmental albedo (Fernandez and Collins 1988). For example, Fernandez and Collins (1988) evaluated color differences of tiger salamanders (*Ambystoma tigrinum*) under different field and experimental conditions. They found that salamanders held in containers painted white became lighter, while those held in black containers became darker. In addition, field observations demonstrated that animals in turbid (high albedo) conditions were lighter than those in a clear (low albedo) environment.

Of 18 boreal toad breeding sites (13 current, 5 historic) examined, water at all was clear (pers. obs.). Soil colors varied considerably between breeding sites, although they tended to be relatively dark (Figure 1). The soil value score of 4 for Brown's Creek was among the lightest of the observed substrates, and the brown tadpoles at this site were a good match to the substrate. However, five sites, including the Donut site, had the

same or lighter soil value scores without the tadpoles showing any evidence of substrate color matching. Hence, substrate matching through environmental albedo does not appear to be a general characteristic of boreal toad tadpoles in Colorado.

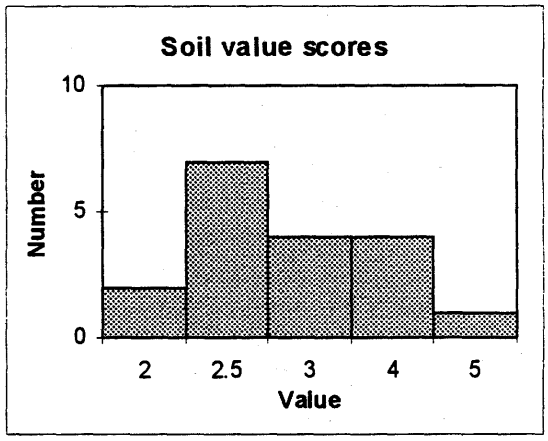

Figure 1. Distribution of Munsell soil color value scores at 18 current and historical boreal toad breeding sites.

Predator exposure

Some anuran tadpoles develop altered color patterns and morphology as a response to exposure to compounds associated with predators (McCollum and Leimberger 1997). These predator-induced changes have been demonstrated with the gray treefrog (*Hyla chrysoscelis*) in experiments that included non-lethal exposure of tadpoles to dragonfly larvae. Morphological changes were most pronounced when the dragonfly larvae were fed conspecifics (McCollum and Leimberger 1997). The predator-induced morphology may also make the tadpoles less vulnerable to predation than typical tadpoles.

It is unknown at this time what species of anuran tadpoles have the capability of detecting predators and responding with morphological changes. Further, the types of predators, both vertebrates and invertebrates, that elicit these responses is unknown.

The Brown's Creek site is somewhat unusual in that large numbers of western terrestrial gartersnake (*Thamnophis elegans*) are present. These snakes readily consume boreal toad tadpoles and toadlets, and we have documented predation by *T. elegans* at the Brown's Creek site.

In addition to their unusual color, the tadpoles at Brown's Creek exhibit a suite of unusual behaviors that may make them less susceptible to predation by gartersnakes. For example, in contrast to the large boreal toad tadpole aggregations observed at other sites, tadpoles at Brown's Creek tend to be somewhat dispersed. We also observed tadpoles burrowing into the substrate. Both these factors would increase predator search time.

At other sites, newly metamorphosed toadlets frequently remain in the vicinity of the breeding pond and sometimes form dense post-metamorphic aggregations. In contrast, newly metamorphosed toadlets rarely are observed at Brown's Creek. At this time we do no know whether this is due to the limited number of tadpoles that survive to metamorphosis or to rapid dispersal of toadlets from the breeding site.

To begin to evaluate these various hypotheses regarding brown tadpole color, in 1998, we will conduct "common garden" experiments. Samples from Brown's Creek boreal toad egg masses will be raised in the laboratory and exposed to the same water, food, and environmental conditions as samples from a control population with the typical coloration. Table 2 shows possible results and the associated

interpretation.

Table 2. Possible results of "common garden" experiment

	Possible results	Interpretation
Brown's Creek Control	Brown Brown	Brown color is environmentally induced (environmental compounds or agents responsible for the induction of brown color are present in the laboratory).
Brown's Creek Control	Brown Black	Brown color is a genetic characteristic of the Brown's Creek toad population.
Brown's Creek Control	Black Black	Brown color is environmentally induced (environmental compounds, environmental agents, or predator exposure) but the agents responsible for color induction are not present in the laboratory

Some additional possibilities exist for the common garden experiment, including an outcome with one or both populations having some brown and some black tadpoles. Altig and Channing (1993) note that at one pond in Costa Rica, some cohorts of *Rhinophrynus dorsalis* tadpoles were dark, while other cohorts were light, and it is possible that continued monitoring of the Brown's Creek site will reveal some years with normally colored tadpoles.

If the cause of the brown tadpole morph is due to exposure to compounds, the identity of the compounds should be determined. Site-specific conservation implications are quite different if the compound is deleterious and due to human agency or a consequence of exposure to natural predators and beneficial to tadpole survival.

If the cause of the brown morph is found to be genetic, it may increase the value of the Brown's Creek population in terms of conservation of the endangered boreal toad. For example, if a boreal toad reintroduction program identifies a suitable reintroduction site, it may be desirable to use eggs, tadpoles, or toadlets derived from the Brown's Creek site in preference to other potential donor populations.

TADPOLE DENSITY EFFECTS

INTRODUCTION

In contrast to the cryptic, solitary behavior of many anuran tadpoles, boreal toad (*Bufo boreas*) tadpoles often form enormous aggregations, made even more conspicuous by the intense black color of the tadpoles against the substrate (Peterson and Blaustein 1991, Koch and Peterson 1995, pers. obs.). Many of the characteristics of *B. boreas* tadpole aggregations parallel those described by Beiswenger (1975, 1997) for *Bufo americanus* tadpoles. First, the aggregations develop during daylight hours. *B. boreas* tadpole aggregations typically form in the most shallow and warmest water available, with tadpoles dispersing to deeper water at night. Tadpoles in aggregations actively feed and tend to be in nearly constant motion.

Such aggregations can be expected to interact with uptake of food, either by increasing competition for food (with a consequent lowering of rate of food ingestion) or by increasing availability of existing food (with a consequent increase in rate of food ingestion). *B. boreas* tadpole aggregations might even stimulate the growth of food, such as algae and bacteria, in any of the following ways: by increasing food growth rates through water temperature increases (through absorption of solar radiation by black tadpoles), stirring up

sediments, thus making nutrients more available for algal growth, and deposition of feces, making nutrients more available for algal growth.

Given the short growing season at the high elevation boreal toad breeding sites, tadpole aggregations that interfered with food uptake of tadpoles without some positive nutritional offset would be too costly to maintain. The purpose of this study was to determine whether different densities of boreal toad tadpoles in enclosures would result in density-related offsets of competition for nutrition. Differences in available nutrition would be expressed by differences between treatments in tadpole growth rates or differences in accumulation rates of organic material on protected surfaces.

MATERIALS AND METHODS

The experiments took place at the "Donut" boreal toad breeding from July through September 1997. Donut is a large artificial pond (maximum depth ca. 1m) located at 10,280 ft. elevation in the Woods Creek drainage, Clear Creek County, Colorado.

To construct enclosures, I attached fiberglass screening with staples and silicon glue to wood frames (width 50 cm, length 95 cm, and depth 31 cm). I covered the bottom of each enclosure with substrate from the pond, and tacked bird netting across the top of each enclosure to prevent avian predation.

I placed 20 enclosures perpendicular to shore in a section of the pond where tadpoles aggregated in previous breeding seasons. I used a randomized block design to assign five treatments (three density treatments of 50, 100, and 200 tadpoles, a "thermal tadpole model" treatment with black gravel at the shallow end, and a control treatment with no gravel or tadpoles) to each of five enclosures in four blocks. In each enclosure I also set out four glass slides to estimate deposition of organic material in the enclosure. The slides sat upright with both faces open to the water in a plastic box that I covered with mesh to prevent grazing by tadpoles or invertebrates.

At 10-day intervals, I counted all tadpoles in each enclosure by netting them and transferring them to plastic containers. Ten minutes after collecting all visible tadpoles in an enclosure, I examined it again for any tadpoles I had missed. I measured the snout-vent length, total length, and scored developmental stage (Gosner 1960) for a sample from each enclosure. Glass slides were removed and replaced at these intervals.

Water temperatures in enclosures were recorded to the nearest 0.2 °C with a Miller & Weber rapid-read cloacal thermometer. Dissolved oxygen readings were taken with a Yellow Springs Instrument Company meter.

RESULTS

Tadpoles experienced unexpectedly high mortality in some enclosures. Figure 2 shows the mean survival over time for tadpoles in enclosures of 50, 100, and 200-tadpole initial densities.

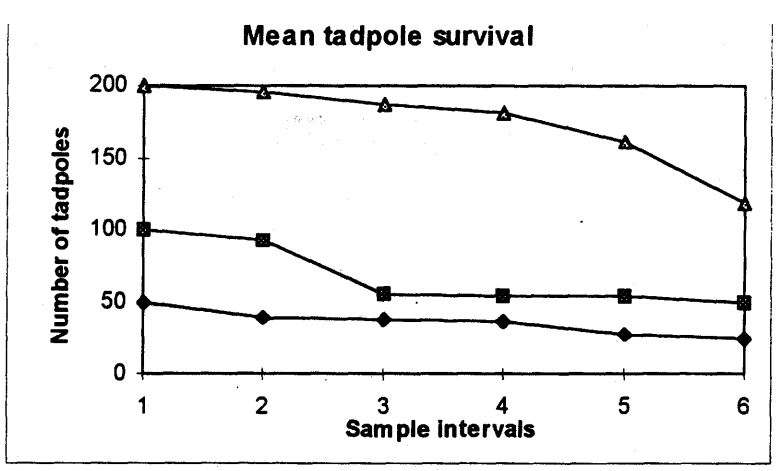

Figure 2. Mean survival of tadpoles by initial density treatment.

Analysis of variance indicated that density treatment did not have a significant effect on the percentage of tadpoles that survived, while block assignment did have a significant effect (Table 3). When I compared mean temperatures of enclosures to percentage of surviving tadpoles, there was no significant correlation (r = - 0.4743, df = 10, $p > 0.05$). I observed almost no variation in oxygen levels between enclosures.

Table 3. ANOVA: Tadpole percent survival by blocks and density

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Block	3	9481.5625	3160.5208	6.2080*	4.76
Density	2	238.875	119.4375	0.2346	5.14
Error	6	3054.625	509.1042		
Total	11	12775.0625			

When I examined tadpole snout-vent length at the end of the experiment by two-way ANOVA, significant effects were noted for block assignment, initial density treatment, and interaction between blocks and densities (Table 4). However, this analysis should be regarded as provisional, since there were unequal sample sizes due to some enclosures having fewer than 10 surviving tadpoles. Figure 3 shows mean tadpole survival by block; 100 percent survival in a block would be represented by 350 tadpoles.

Table 4. Provisional ANOVA table: Tadpole snout-vent length by blocks and density.

	Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Cells		11	302.07	27.46	22.15*	1.95
	Blocks	3	72.72	24.24	19.55*	2.76
	Densities	2	139.78	69.89	56.36*	3.15
	Blocks x Densities	6	89.58	14.93	12.04*	2.25
Error		100	124.17	1.24		
Total		111	426.25			

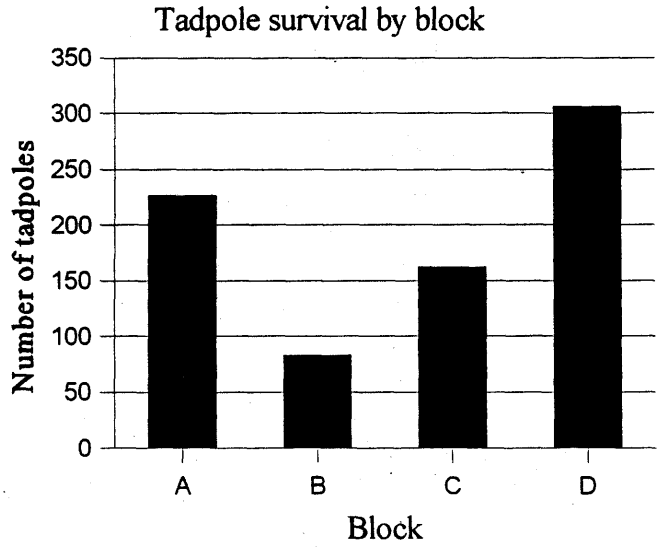

Figure 3. Mean (of three replicates) survival of tadpoles by block.

Figure 4 shows the mean snout-vent length of tadpoles of tadpoles in each enclosure (block/density combination). There was a significant negative correlation between the percent survival of tadpoles in an enclosure with mean snout-vent lengths ($r = -0.6391$, $df = 10$, $p < 0.05$).

The enclosures had very little permeability to tadpoles. On two occasions, a single tadpole was removed from a gravel or control enclosure, and both times the tadpole was very small. However, during the final tally of tadpoles, one of the 50-tadpole initial density enclosures had an unexpectedly high number of tadpoles (56). Previous scores for this container were 50 (starting density), 49, 49, 48, and 48. A photograph taken of the tadpoles from this enclosure was consistent with the count of 56, indicating that excess tadpoles had somehow contaminated the enclosure. For percentage survival of this enclosure, I used the tally from the previous interval's count.

The glass slides had very little deposition of material. There was no detectable difference in mass on a scale sensitive to 0.01 gram of slides between an initial weight and the weight after I scraped material off one side of the slide with a razor blade.

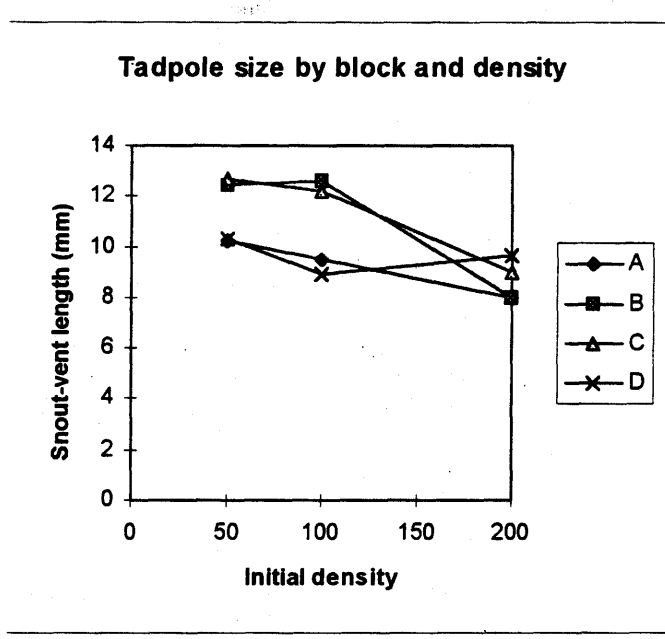

Figure 4. Tadpole size by block and density.

DISCUSSION

These experiments provide no evidence for the role of aggregation in enhancing growth or food availability for larval *B. boreal*. Instead, as density increased, tadpole growth, as reflected in mean snout-vent length, decreased. This is consistent with the observations of Wang and Hayes (1995), who found that, regardless of water volume, increasing *B. boreas* tadpole numbers were sufficient to inhibit growth and development.

Nor did these experiments support the idea that larger numbers of tadpoles promote growth of foods such as algae and bacteria. If differences in rate of accumulation of organic material exist, they are very subtle. Material deposited on the glass slides was not detectable with a balance able to record 0.01-g differences in mass.

There was a significant effect of block assignment on tadpole survival. The source of this block effect is not known. Oxygen levels did not vary much between enclosures. I found no significant association between tadpole survival in enclosures and enclosure water temperatures recorded during the day. However, nighttime water and oxygen temperatures were not recorded. Tadpoles that moved freely in the pond spent the day in shallow, warm water and retreated to deeper water at night. It is possible that nighttime conditions in the enclosures varied by block, so that tadpoles in blocks B and C were exposed to less favorable temperature or oxygen conditions than tadpoles in blocks A and D. An area of seepage into the pond was located near block B and there may have been some effect attributable to this feature.

Metamorphosis begins at Gosner stage 42. Although tadpoles in the pond began to metamorphose by early September, all the tadpoles in the enclosures as late as September 25 were far short of metamorphosis. The most advanced tadpoles in enclosures were at stage 37.

Based on these experiments, if aggregation enhances tadpole growth, it is probably through the thermal advantage of processing nutrients more rapidly than of increasing the availability of food.

INTRODUCTION

High densities of newly metamorphosed individuals have been noted for various members of the genus *Bufo (boreas, americanus, carens, cognatus, marinus, punctatus, viridis*), the ranid *Aubria subsigillata*, and spadefoot toads (*Spea intermontana, Scaphiopus holbrookii*) (Arnold and Wassersug 1978). Since the first report of post-metamorphic aggregations in *Bufo boreas* by Black and Black (1969), such aggregations have been observed throughout the species' range.

Several environmental and behavioral factors may influence the formation of post-metamorphic aggregations (PMAs): *(I)* deteriorating larval environment (Arnold and Wassersug 1978), *(ii)* inability to disperse (Arnold and Wassersug 1978), *(iii)* protection from desiccation (Arnold and Wassersug 1978; Lillywhite and Wassersug 1974), *(iv)* enhancement of insolation or thermal environment (Black and Black 1969; Arnold and Wassersug 1978; Lillywhite and Wassersug 1974), and *(v)* selfish herd/predator saturation (Arnold and Wassersug 1978; Graves et al 1993), and *(vi)* for PMAs of distasteful anurans, the aggregations also may enhance apomatic functions (Hews and Blaustein 1985). As noted by Heinen (1993), these functions are not mutually exclusive, i.e., more than one function may be in operation for PMA formation.

Heinen (1993) demonstrated the possible relation between aggregations and desiccation in a laboratory setting, in which *Bufo americanus* toadlets survived desiccating environments better when in groups than when solitary, and that the toadlets formed aggregations in desiccating, but not wet, environments.

This paper provides field evidence that for newly metamorphosed *Bufo boreas* toadlets, PMAs may be associated with a desiccating environment, thermal advantage, and inability of the toadlets to disperse.

METHODS

Localities. — I made observations of newly metamorphosed toadlets at several localities in Clear Creek county, especially Mt. Bethel and Donut in 1996 and at Donut and Anne's Pond in 1997.

The Mt. Bethel site is a small, shallow pool (maximum depth < 0.5m) located at an elevation of 3170 m (10,400 feet) in the Clear Creek drainage. Two small streams drain into the pool. A small log dam at the outlet maintains water in the pool throughout the summer. Sedges are present along the pool margins as well as along the inlets and outlet. However, there are few rocks, logs, or rodent burrows around the perimeter of the pond that could serve as temporary shelters for newly metamorphosed toadlets.

The Donut site is a large artificial pond (maximum depth ca. 1m) located at 10,280 ft. elevation in the Woods Creek drainage. There is no natural inlet or outlet; water enters and leaves the pond via pipes. The shoreline is rocky and almost completely barren of vegetation. This pond was formed as part of an artificial wetland in 1994.

Anne's Pond is a temporary pool. Movement of earth by heavy equipment formed this shallow depression in 1996. The pool fills to a maximum depth of ca. 10 cm with snowmelt. It is located at 3140 m (10,300 feet) in the Ruby Creek drainage. There is no inlet, but water exits via drainage across a dirt road where it then enters a natural channel that connects 50 m downstream with Ruby Creek. In 1996, Anne's Pond dried before tadpoles completed development. In 1997, water levels were maintained throughout the summer by using a pipe intermittently to divert water from Woods Creek into the pool until it reached the edge of the road. The north shoreline of the pool is almost devoid of vegetation, while the south shoreline is vegetated with grasses and mosses.

Mt. Bethel. — On 26 August 1996 at the Mt. Bethel site, I walked around the circumference of the pool and measured the distance to the nearest cm from shore of 20 newly metamorphosed toadlets. All toadlets were in the open. I measured snout-vent length and determined the Gosner (1960) developmental

score for these toadlets. On subsequent visits, I counted the number of toadlets and noted their distribution.

Donut. — On 7 September 1996 and several dates in September 1997, I estimated the number of toadlets in numerous PMAs. For each aggregation as well as for a selection of solitary individuals, I measured the temperature near the aggregation centers with a Miller & Weber rapid-read cloacal thermometer.

On 14 September 1996, several dead toadlets were present at Donut. I measured the distance from shore of the first 14 dead toadlets I encountered. For each of these toadlets, I also measured the distance from shore of the nearest living toadlet. To assess dispersal ability at this site, I turned all objects within 4m of the shoreline that could potentially shelter toadlets along the areas where metamorphosis was concentrated. I turned over additional objects and examined open areas for toadlets beyond this range, but did not find any. For each object sheltering toadlets, I measured the distance from shore (to the nearest 5 cm) and tallied the number of toadlets under the object.

Anne's Pond. — On several dates in September 1997, I counted toadlets around the pond and looked for evidence of their dispersal. On September 7 I measured the temperatures of PMAs using the methods described above.

Agar models. — Because desiccation appeared to be associated with dead toads at the Donut site in 1996, I used small cylinders of agar as toadlet models to test potential differences in desiccation vulnerability at Donut and Anne's Pond in 1997. Anne's Pond was selected because of its proximity to Donut, which allowed the agar models to be set out simultaneously at both sites. On three dates in September, 1997, I placed agar cylinders along transects at Donut and Anne's Pond. A small tube was used to stamp out the 10-mm diameter cylinders. Agar cylinders were weighed prior to placement to the nearest 0.01 g and ranged in mass from 0.56 to 0.82 g (mean = 0.65 ± 0.01 g). There was no difference in starting mass between cylinders at Donut and Anne's pond ($t = 0.02$, $df = 52$, $p > 0.05$). All agar used was from the same batch, and on any single day, only agar in a single plate was used for both ponds. On the same days, agar cylinders in excess of those needed for transects were stamped out and weighed in the same fashion and then placed in PMAs at Donut.

The 10-mm cylinder diameter was chosen since it roughly matched snout-vent lengths of newly metamorphosed toadlets, which usually range from about 13 to 18 mm. However, the agar blocks weighed more than newly metamorphosed toadlets, which at Donut had a mean weight of 0.39 ± 0.02 g (range 0.28 - 0.50 g, n = 13) and at Anne's Pond had a mean weight of 0.25 ± 0.01 g (range 0.19 - 0.31 g, n = 18).

Transects were placed in areas with newly metamorphosed toadlets. Three transects were placed at each pond on each of three days, each of which was partly cloudy. Each transect contained 6 agar blocks, spaced in 20 cm increments from shore (0 cm) to 100 cm from shore. The agar blocks were placed on the transects (or in PMAs) and remained in place for 3 hours, then weighed to the nearest 0.01 g. Desiccation was calculated as the ratio of end weight to starting weight.

RESULTS

Mt. Bethel. — Metamorphosis of 20 individuals was first noted on 26 August 1996. On this date, toadlets were a mean distance from the water of 49.5 ± 8.8 cm (n = 20, range = 0 - 130 cm). There was a significant negative correlation between number of toadlets and distance from shore ($r = -0.74$, $n = 20$, $p < 0.01$). With the exception of a single individual at Gosner stage 45, all toadlets were at Gosner stage 44. There was no correlation between snout-vent length (13.4 ± 0.07 mm, n = 20) and distance from shore ($r = -0.0955$, $n = 20$, $p > 0.05$).

A small (10 individual) PMA was observed at the Mt. Bethel site on 2 September 1996, when a total of 123 metamorphs were observed around the pond. The aggregation was comprised of Gosner stage 44 and 45 toadlets, with mean SV length of 13.8 ± 0.4 mm (n = 10).

The number of toadlets at this site declined over time, probably due to toadlet dispersal. No other PMAs were observed. On 11 September 1996 69 toadlets were observed. On 18 September 1996, 47 toadlets were observed, including three that had dispersed 20 to >50 m away from the pond via the outlet, and five

that dispersed 2 to 10 m up an inlet. Toadlets remained on damp soil along the pond edge and streams; none were found under objects such as downed logs. Only one toadlet was observed at this site on 14 October 1996 when nighttime temperatures dropped enough to form a partial coat of ice on the pond.

Donut. — On 25 August 1996, a single individual had reached Gosner 44. By 30 August 1996, ca. 1130 newly metamorphosed toadlets were observed, with several PMAs. On 7 September 1996, PMAs ranged in size from 5 to ca. 200 toadlets. Soil temperature was 17°C and temperatures of PMAs ranged from 17 - 24°C. There was a significant correlation between the temperature and the number of individuals in the PMAs ($r = 0.6084$, $df = 17$, $p < 0.01$). By September 14, 1996 > 5000 toadlets had metamorphosed. Several of the newly metamorphosed toadlets were dead. Comparison of distance from shore for dead and nearest living toadlets indicated that dead toadlets were significantly further from shore (Students t-test, $t = 3.243$, $n = 14$, $p < 0.01$). There was a significant negative correlation between number of toadlets and distance from shore ($r = -0.37$, $n = 42$, $p < 0.05$). Toadlets under objects were a mean distance of 99 cm from shore (range 0 - 300 cm). No toadlets were found more than 3m from the shore, although I checked under objects and examined open areas beyond this range. On 18 September 1996, PMAs continued to be present, and many individuals in them were dead and either desiccated or covered with fungus.

In 1997, I observed a single metamorphosed toadlet on August 26. By 2 September, ca. 300 toadlets had metamorphosed, including about 200 in a loose but extensive aggregation. On September 7, ca. 1300 toadlets were present, with an approximately equal number of tadpoles remaining. In 1997, correlations between PMA size and temperature varied, but two of the three were significant: September 7, $r = 0.2428$ ($df = 37$, $p > 0.05$); September 11, at 1300 h, $r = 0.513$, $df = 29$, $p < 0.01$ and at 1500h, $r = 0.403$, $df = 29$, $p < 0.05$.

At 1300 hours on 11 September 1997, I estimated that 746 toadlets were in 23 aggregations of 5 or more (mean number of toadlets per aggregation = 33 ± 7). Two hours later there were an estimated 1217 toadlets in such aggregations (mean number of toadlets per aggregation = 55 ± 10). When comparing PMAs comprised of at least 5 toadlets in small (5 to 31), medium (31-55), and large (>55) aggregations, toadlets tended to be in smaller aggregations at 1300h than at 1500h ($\chi^2 = 6.38$, $df = 2$, $n = 44$, $p < 0.05$). Post-metamorphic aggregations continued to be present through the last observation date. Large numbers of dead toadlets were frequently seen at this site.

Anne's Pond. — PMAs at Anne's Pond tended to be small and readily disrupted upon disturbance. The largest PMA observed at this site contained 15 individuals. The correlation between PMA size and temperature did not reach significance ($r = 0.649$, $n = 8$, $p > 0.05$), probably due in part to the small sample size available. I first observed metamorphosis at Anne's Pond on 18 August 1997. By 26 August 1997, ca. 400 toadlets had metamorphosed, the highest number noted at this site. Some toadlet dispersal was already evident, as on this date four toadlets were found around a puddle in the road and two at the beginning of the drainage channel. Toadlet numbers around the pond decreased, with the proportion of toadlets found into the drainage channel increasing until the last date this site was checked (Figure 5). There were significantly higher proportions of toadlets in the channel late in the season compared with early in the season ($\chi^2 = 150$, $df = 1$, $n = 772$, $p < 0.01$).

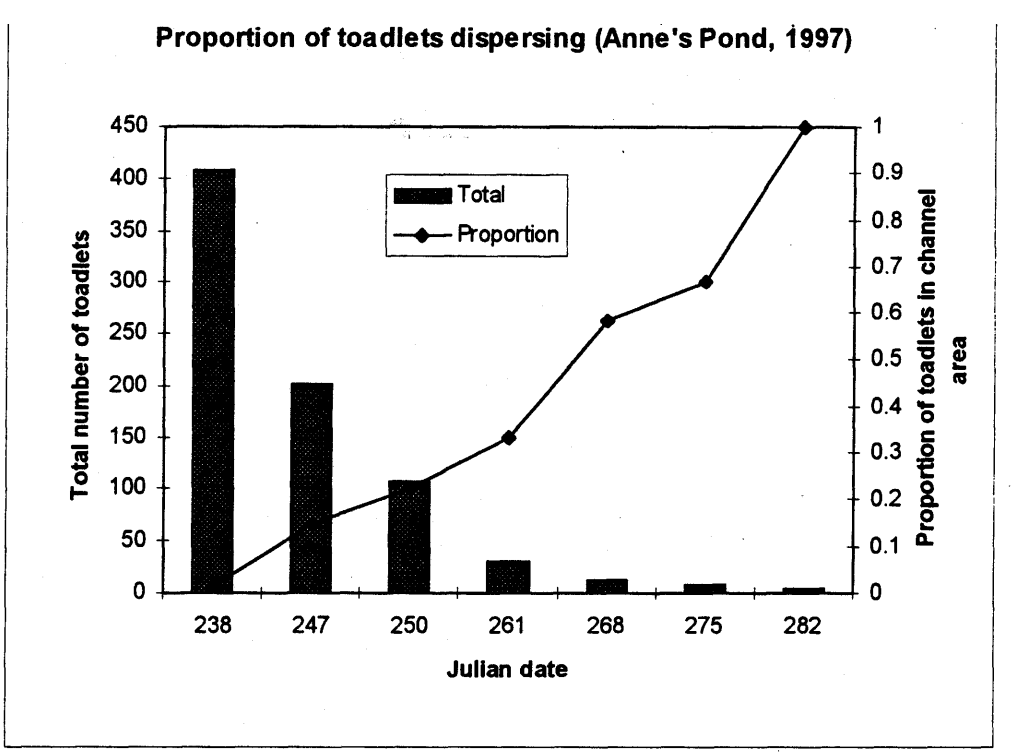

Figure 5. Increasing proportion of "dispersing" toadlets over time.

For both Donut and Anne's Pond, agar blocks tended to lose increasing proportions of their starting weight with increasing distance from shore. Two-way ANOVA indicated significant effects of distance from shore, pond, and interaction (Table 5).

Table 5. Two-way ANOVA for agar weights

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05,\ 0.01}$
Pond	1	0.2248	0.2248	15.0368**	4.00, 7.08
Distance	5	1.6794	0.3359	22.4682**	2.37, 3.34
Pond x distance	5	0.2268	0.4536	30.3411**	2.37, 3.34
Error	96	1.4352	0.01495		
Total	107	3.5662			

Comparing the mean ending weights at Donut with those at Anne's Pond, Tukey HSD (Thorne and Slane 1997) indicated that Donut was significantly more desiccating than Anne's Pond at distances of 80 and 100 cm from shore ($p < 0.05$), but not at locations closer to shore.

Comparison of agar blocks placed in PMAs at Donut with agar blocks at varying distances from shore using one-way ANOVA indicated a significant effect of group (Table 6).

Table 6. One-way ANOVA for agar weight loss at Donut

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.001}$
Between	6	2.0709	0.34512	20.1744**	4.37
Error	66	1.1292	0.0171		
Total	72	3.2001			

I made post-hoc comparisons of agar mass losses at Donut using Fisher LSD test (Thorne and Slane 1997). Agar blocks placed in PMAs differed significantly in their desiccation rates from all other treatments, and were intermediate between the 0 and 20 cm distances (Table 7). There was a highly significant difference between agar blocks placed at 0 and 20 cm. However, other groups separated by a distance of 20 cm did not differ significantly in their desiccating power. Except for the comparison of 60 cm and 100 cm, all groups 40 cm or more distant from one another were significantly different in their desiccation rates.

Table 7. Fisher LSD comparison for agar mass loss at Donut.

	Groups						
	0 cm	20 cm	40 cm	60 cm	80 cm	100 cm	PMA
0 cm	—	3.9875**	5.4274**	6.8916**	7.7138**	7.6085**	2.2960*
20 cm		—	1.4399	2.9041**	3.7263**	3.6210**	2.3493*
40 cm			—	1.4642	2.2864*	2.1811*	4.0267**
60 cm				—	0.8222	0.7169	5.7324**
80 cm					—	0.1053	6.6903**
100 cm						—	6.5676**
PMA							—

$t_{0.05} = 2.0003$ *p<0.05 df = 66
$t_{0.01} = 2.6603$ **p<0.01

Table 8 shows estimated times that toadlets of two different starting masses could remain at particular locations before losing 40% of their initial mass. Assumptions in this table include that toadlets would undergo the same mean mass loss rates as the agar for the various locations and that mass loss (g/hr) is constant over time. The two initial toadlet masses used in the table correspond to mean masses for toadlets at Donut and Anne's Pond.

Locations at shoreline (0 cm) had net mass gains, so estimates for toadlet survival at these locations were not calculated. PMAs offered approximately three times the length of survival as any location for which survival time was estimated.

Table 8. The estimated number of hours a toadlet could survive at various locations before losing 40% of the initial mass. Values were not estimated for locations at which agar blocks gained mass.

	Location	Survival (in hours); starting mass = 0.39	Survival (in hours); starting mass = 0.25
Donut	PMA	17.73	11.37
	0 cm	—	—
	20 cm	5.88	3.77
	40 cm	3.96	2.54
	60 cm	2.98	1.91
	80 cm	2.61	1.67
	100 cm	2.65	1.70
Anne's Pond	0 cm	—	—
	20 cm	5.96	3.82
	40 cm	4.85	3.11
	60 cm	5.15	3.30
	80 cm	4.94	3.17
	100 cm	4.90	3.14

DISCUSSION

Post-metamorphic aggregation formation appears to be a typical feature at *Bufo boreas* breeding sites where sufficient numbers of newly metamorphosed individuals occur. *B. boreas* in Colorado is a high-elevation form for which the thermal regime may be especially important. In captivity, toadlet aggregations form under heat lamps (pers. obs.). This paper presents evidence that there is a thermal advantage associated with larger PMAs, since in most instances, there is a positive and significant correlation between aggregation size and temperature.

Post-metamorphic aggregations frequently are small and easily disrupted. Black and Black (1969) described the apparently transient nature of PMAs in *Bufo boreas* observed in Alberta, Canada, in which most aggregations first observed in the early afternoon had dispersed by 1830 hours. The aggregation observed by Lillywhite and Wassersug (1974) dispersed after being disturbed and apparently was not reformed. Similarly, small PMAs observed at the Mt. Bethel and Anne's Pond dispersed upon disturbance.

At Mt. Bethel, Anne's Pond and other sites in the southern Rocky Mountains, toadlets appear to disperse soon after metamorphosis. At some localities with extensive vegetation in the area surrounding the breeding pool, toadlets may remain in the vicinity of the pool, even overwintering near the margins of the breeding pool (pers. obs.). Typically, few dead toadlets are observed in these situations.

In contrast, the PMAs at the Donut site were large, enduring, and not readily disrupted. Further, in both 1996 and 1997 I found large numbers of dead toadlets at this site once metamorphosis was underway. In paired observations of dead with living toadlets, dead toadlets were significantly further from shore than

living toadlets. Bufonids tend to be relatively tolerant of dehydration, with adult *Bufo boreas* reaching their critical activity point when dehydrated to 41 percent of their initial (hydrated) body mass (Hillman 1980). Schwartzkopf and Alford (1996), using agar models, estimated periods of time numbering in days for adult *Bufo marinus* to undergo a 50% mass loss. However, newly metamorphosed individuals have higher surface-area to volume ratio than adults, and so can be expected to be much more vulnerable to desiccation. At Donut and Anne's Pond, the estimated survival time before reaching a 40% mass loss varied from 1.67 to 17.73 hours, depending on location and initial toadlet mass (excluding shoreline locations, which had a net mass gain). The maximum survival time would be expected to be along the shoreline and within PMAs, which are also the locations at which most toadlets are found.

Although PMAs at Donut occurred in the same general area of the shore, the precise location and composition of individual PMAs shifted over time. Water loss for agar blocks placed in PMAs was intermediate compared to agar blocks on shore and at 20 cm from shore. However, it is likely that toadlets actually experience more favorable moisture conditions in the aggregations since the "churning" within the aggregations by the toadlets resulted in movement of 13 of 19 agar blocks to the aggregation margins. When PMA sizes were compared on the same day at different times, more toadlets were in large PMAs later in the day, which was also when the breeze had become stronger, thereby increasing vulnerability to desiccation.

The active movement by toadlets to aggregations and within aggregations may serve an additional function: minimization of excessive exposure to dangerous levels of UV radiation in an area with scant shade. Under sunny conditions, *Bufo boreas* metamorphs at this elevation approach their tolerance level to UV-B (C. Carey, pers. com.).

Also in contrast to other sites, there was little evidence of dispersal at the Donut site. Although toadlets at this site frequently sought shelter under rocks or other surface objects, no toadlets were found under objects >3m from the shoreline. In comparison, toadlets at other sites were found > 50m from the breeding pool. The lack of an inlet or outlet channel (with the associated moisture conditions) combined with desiccating soils resulted in the inability of the toadlets to move away from the pond. These conditions may be modified in the future to permit toadlet dispersal.

I suggest that large and enduring PMAs indicate that newly metamorphosed anurans are unable to disperse.

FLUCTUATING ASYMMETRY

INTRODUCTION

There are four major patterns of asymmetry in paired structures in bilateral organisms (Van Valen 1962; Richard Jones, pers. comm.): 1) Directional asymmetry, in which one side is always larger, 2) Antisymmetry, in which one side is always asymmetric, but it can be either the left side or the right side, 3) Alternating asymmetry, in which one side is always asymmetric, but within an individual the side that is larger alternates through time, and 4) Fluctuating asymmetry (FA), in which there is no tendency for one side to be larger than the other. A fifth pattern, of cyclical asymmetry, is a type of fluctuating asymmetry in which there are temporary within-individual variations in asymmetry, often related to periodic events such as menstrual cycles (Manning et al. 1996).

Distinguishing FA from other forms of asymmetry requires statistical evaluation of the data. When the frequency distribution for a trait with FA is examined, the mean of the right side minus the left side (R - L) equals 0 and the distribution is binomial (Palmer 1996). In contrast, the R - L mean for a trait is significantly greater than or less than zero in the case of directional asymmetry, and has a platykurtic or bimodal distribution in the case of antisymmetry (Palmer and Strobeck 1986, Møller and Pomiankowski, 1993).

The degree of FA for a trait is usually very small, often representing $\leq$ 1-5% of the total variation in

the trait (Merilä and Björklund 1995). Several factors influence the level of FA observed for a trait. Genetic factors, including reduced genetic variation, hybridization, and incorporation of mutations with major effects have all been associated with higher levels of FA (Leary and Allendorf 1989). Secondary sexual characteristics, such as feather ornaments in birds, may have FA values that are "an order of magnitude larger than that of ordinary morphological characters of the same individuals" (Møller 1995). Møller and Pomiankowski (1993) also suggest that ornament symmetry should increase with increasing ornament size. However, Evans et al. (1995) found a U-shaped relationship between FA values and trait size for four traits of a Jamaican hummingbird, including the male tail ornaments.

FA has been suggested as a way to measure developmental stress (Clark 1992; Leary and Allendorf 1989; Parsons 1990; Sarre and Dearn 1991). Organisms exposed to various environmental pollutants have been reported to have higher levels of FA (Leary and Allendorf 1989; Ryabov and Kryshev 1991). Some studies have shown that, even within individuals, different morphological features frequently possess different levels of FA (Palmer and Strobeck 1986). In his comparisons of feather ornaments on birds from marginal versus central populations, Møller (1995) found that levels of FA in these ornaments were higher in birds from marginal populations, where the birds were subject to both increased environmental stress and reduced genetic variability.

Boreal toads are among the amphibian species that have undergone population declines in the past two decades (Carey 1993; Corn et al. 1989). Several possible causes have been proposed for these declines, including acid precipitation, increased UV radiation, pesticide applications, and spread of pathogens (Carey 1993; Corn 1994). However, to date the population declines have not been linked to any specific factor or factors. If these toads were subjected to increasing levels of environmental or genetic stress that resulted ultimately in the reported declines in this species, it may be possible that examination of variation of FA will show biologically meaningful differences between populations.

The purpose of this preliminary study is to assess the detectability of FA in preserved specimens of boreal toads for a variety of external morphological features. If variation in FA is detected in museum specimens, it may be possible to compare levels of FA in specimens from both historical and extant populations. Further, definition of a useful subset of traits could be used to assess FA rapidly in field populations.

METHODS

I measured external morphological characters on ten boreal toads (*Bufo boreas*) housed at the University of Colorado Museum (UCM). For each specimen, I recorded specimen number, collection date and locality information, sex (not identified for individuals <50 mm snout-vent length), snout-vent length, snout-urostyle length, total length between beginning and end of middorsal stripe, gap length (if any) in middorsal stripe, and offset distance (if any) of middorsal stripe. Bilateral characters measured included: Parotoid length (measured at longest point), parotoid width (measured at widest point), head length (measured from indentation on upper snout to corner of mouth), eye diameter (measured at inner corners of eyelid), tibia length (maximum distance, including soft tissue), radius length (maximum distance, including soft tissue), and hand length (measured from distal end of second metatarsal to posterior end of palmer wart). For mature males, I measured nuptial pad length (maximum length on male thumbs). Wart number was determined by counting the number of large warts touching a string held between the posterior end of the parotoid gland and the vent.

All measurements were made to the nearest 0.1 mm with calipers. I employed several strategies to reduce unconscious bias in measuring the traits. For each series of measurements, I concealed any prior measurements. The calipers were closed between each measurement and the numbers on the scale turned out of sight while the feature was being measured (Pomory 1997). Snout-vent length, snout-urostyle length, and middorsal stripe length (as well as gap and offset, if any) were measured first. For bilateral characters, I measured all the characters on the right side of the specimen, then measured all the characters on the left side.

For each specimen, I measured each character on each of three different dates to obtain information on measurement error (Ryan et al. 1995).

RESULTS

The toads ranged in snout-vent length from 2.87 to 8.0 cm (mean 5.8 cm, n =10). For a character to exhibit fluctuating asymmetry, the mean (R - L) of the character equals zero and the character has a binomial distribution. Student's t-tests were calculated for all bilateral characters except nuptial pad length (only 5 toads had this feature). For each toad, the mean for each character was calculated as the mean of the measures from the right side minus the mean of the measure for the left side. None of the grand means differed significantly from zero (critical t value 2.228; Table 9).

Because of the small sample sizes, I did not test whether the distribution for each character conformed to expected values for a binomial distribution.

The data for eight characters were tested using two-way ANOVA with repeated measures, with larger values in column 1 and smaller values in column 2 (Pomory 1997, Sokal and Rohlf 1969). "Larger side" and "smaller side" were determined for each character for an individual by comparing the means for particular characters. Results are shown in tables 10 through 17.

Table 9. Characters, means, and t values (critical t value = 2.228).

Character	Mean R-L (n=10)	t value
Parotoid length	-0.2767	-0.9605
Parotoid width	0.0150	0.6881
Head length	0.0137	0.6703
Eye diameter	0.0063	0.6101
Tibia length	-0.0157	-1.1175
Radius length	-0.0133	-0.5969
Hand length	0.0030	0.1482
Wart number	0.2333	0.3771

Table 10. ANOVA for parotoid length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	4.44468			
Large vs. small	1	0.08740	0.08740	21.8010**	5.12
Subjects x Large vs. small	9	0.03608	0.00400		
Repeated measures (rm)	2	0.00211	0.00106	1.8395	3.55
Subjects x rm	18	0.01032	0.00057		
Large vs. small x repeated measures	2	0.00036	0.00018	0.1685	3.55
Subjects x Large vs. small x rm	18	0.01940	0.00108		
Total	59	4.60037			

Table 11. ANOVA for parotoid width

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	1.68257			
Large vs. small	1	0.03408	0.03408	9.174*	5.12
Subjects x Large vs. small	9	0.03344	0.00372		
Repeated measures (rm)	2	0.00025	0.00012	0.284	3.55
Subjects x rm	18	0.00792	0.00044		
Large vs. small x repeated measures	2	0.00086	0.00043	2.805	3.55
Subjects x Large vs. small x rm	18	0.00277	0.00015		
Total	59	1.76189			

Table 12. ANOVA for head length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	10.18652			
Large vs. small	1	0.02282	0.02282	5.688*	5.12
Subjects x Large vs. small	9	0.03610	0.00401		
Repeated measures (rm)	2	0.01381	0.00690	3.171	3.55
Subjects x rm	18	0.03919	0.00218		
Large vs. small x repeated measures	2	0.00607	0.00304	3.202	3.55
Subjects x Large vs. small x rm	18	0.01706	0.00095		
Total	59	10.32156			

Table 13. ANOVA for eye diameter

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	1.08587			
Large vs. small	1	0.01148	0.01148	28.169***	5.12
Subjects x Large vs. small	9	0.00367	0.00041		
Repeated measures (rm)	2	0.00294	0.00147	2.161	3.55
Subjects x rm	18	0.01226	0.00068		
Large vs. small x repeated measures	2	0.00208	0.00104	1.310	3.55
Subjects x Large vs. small x rm	18	0.01432	0.00080		
Total	59	1.13262			

Table 14. ANOVA for tibia length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	27.97655			
Large vs. small	1	0.02860	0.02860	18.024**	5.12
Subjects x Large vs. small	9	0.01428	0.00159		
Repeated measures (rm)	2	0.0000 4	0.00002	0.008	3.55
Subjects x rm	18	0.04773	0.00265		
Large vs. small x repeated measures	2	0.00449	0.00225	0.729	3.55
Subjects x Large vs. small x rm	18	0.05547	0.00308		
Total	59	28.12716			

Table 15. ANOVA for radius length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	16.37347			
Large vs. small	1	0.05281	0.05281	27.589**	5.12
Subjects x Large vs. small	9	0.01723	0.00191		
Repeated measures (rm)	2	0.02585	0.01293	0.653	3.55
Subjects x rm	18	0.35645	0.01980		
Large vs. small x repeated measures	2	0.04217	0.02109	4.842*	3.55
Subjects x Large vs. small x rm	18	0.07839	0.00436		
Total	59	16.94637			

Table 16. ANOVA for hand length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	11.91588			
Large vs. small	1	0.02440	0.02440	7.066*	5.12
Subjects x Large vs. small	9	0.03108	0.00345		
Repeated measures (rm)	2	0.00022	0.00011	0.184	3.55
Subjects x rm	18	0.01094	0.00061		
Large vs. small x repeated measures	2	0.00044	0.00022	1.072	3.55
Subjects x Large vs. small x rm	18	0.00372	0.00021		
Total	59	11.9867			

Table 17. ANOVA for wart number

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	145.41667			
Large vs. small	1	20.41667	20.41667	5.727*	5.12
Subjects x Large vs. small	9	32.08333	3.56481		
Repeated measures (rm)	2	7.03333	3.51667	1.728	3.55
Subjects x rm	18	36.6333	2.03519		
Large vs. small x repeated measures	2	7.63333	3.81667	3.215	3.55
Subjects x Large vs. small x rm	18	21.36667	1.18704		
Total	59	270.58333			

For all bilateral characters tested, the larger side was significantly larger than the smaller side. Only for radius length did the magnitude of the difference between the larger side and smaller side vary significantly with the repeated measures. In all cases, the proportion of the total variance attributable to size differences was quite small, varying from 0.002 for hand length to 0.075 for wart number (mean proportion = 0.016).

For departures from symmetry to meet the criteria for fluctuating asymmetry, they must not show directional asymmetry. The data was tested for directional asymmetry by conducting a two-way ANOVA with the left side and right side measurements in the original columns (Pomory 1997). Results of these tests are shown in tables 18 through 25.

Table 18. ANOVA for parotoid length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	4.44468			
Right vs. Left	1	0.01148	0.01148	0.923	5.12
Subjects x Right vs. Left	9	0.11200	0.01244		
Repeated measures (rm)	2	0.00211	0.00106	1.840	3.55
Subjects x rm	18	0.01032	0.00057		
Right vs. Left x repeated measures	2	0.00224	0.00112	1.152	3.55
Subjects x Right vs. Left x rm	18	0.01752	0.00097		
Total	59	4.60036			

Table 19. ANOVA for parotoid width

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	1.68257			
Right vs. Left	1	0.00338	0.00338	0.474	5.12
Subjects x Right vs. Left	9	0.06414	0.00713		
Repeated measures (rm)	2	0.00025	0.00012	0.284	3.55
Subjects x rm	18	0.00792	0.00044		
Right vs. Left x repeated measures	2	0.00037	0.00018	1.020	3.55
Subjects x Right vs. Left x rm	18	0.00326	0.00018		
Total	59	1.76188			

Table 20. ANOVA for head length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	10.1865			
Right vs. Left	1	0.00280	0.00280	0.449	5.12
Subjects x Right vs. Left	9	0.05612	0.00624		
Repeated measures (rm)	2	0.01381	0.00690	3.171	3.55
Subjects x rm	18	0.03919	0.00218		
Right vs. Left x repeated measures	2	0.00212	0.00106	0.910	3.55
Subjects x Right vs. Left x rm	18	0.02101	0.0012		
Total	59	10.32156			

Table 21. ANOVA for eye diameter

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	1.08587			
Right vs. Left	1	0.00060	0.00060	0.372	5.12
Subjects x Right vs. Left	9	0.01455	0.00162		
Repeated measures (rm)	2	0.00294	0.00147	2.161	3.55
Subjects x rm	18	0.01226	0.00068		
Right vs. Left x repeated measures	2	0.00044	0.00022	0.250	3.55
Subjects x Right vs. Left x rm	18	0.01596	0.00089		
Total	59	1.13262			

Table 22. ANOVA for tibia length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	28.03795			
Right vs. Left	1	0.00368	0.00368	1.249	5.12
Subjects x Right vs. Left	9	0.02654	0.00295		
Repeated measures (rm)	2	0.00137	0.00069	0.390	3.55
Subjects x rm	18	0.03173	0.00176		
Right vs. Left x repeated measures	2	0.00049	0.00025	0.082	3.55
Subjects x Right vs. Left x rm	18	0.05414	0.00301		
Total	59	28.15590			

Table 23. ANOVA for radius length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	16.37347			
Right vs. Left	1	0.00267	0.00267	0.356	5.12
Subjects x Right vs. Left	9	0.06737	0.00748		
Repeated measures (rm)	2	0.02585	0.01293	0.653	3.55
Subjects x rm	18	0.35645	0.01980		
Right vs. Left x repeated measures	2	0.01317	0.00659	1.104	3.55
Subjects x Right vs. Left x rm	18	0.10739	0.00597		
Total	59	16.94637			

Table 24. ANOVA for hand length

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	11.91588			
Right vs. Left	1	0.00228	0.00228	0.386	5.12
Subjects x Right vs. Left	9	0.05320	0.00591		
Repeated measures (rm)	2	0.00022	0.00011	0.184	3.55
Subjects x rm	18	0.01094	0.00061		
Right vs. Left x repeated measures	2	0.00036	0.00018	0.860	3.55
Subjects x Right vs. Left x rm	18	0.00380	0.00021		
Total	59	11.98670			

Table 25. ANOVA for wart number

Source of variation	*df*	*SS*	*MS*	F_s	$F_{0.05}$
Subjects	9	145.41667			
Right vs. Left	1	0.81667	0.81667	0.142	5.12
Subjects x Right vs. Left	9	51.68333	5.74259		
Repeated measures (rm)	2	7.03333	3.51667	1.728	3.55
Subjects x rm	18	36.63333	2.03519		
Right vs. Left x repeated measures	2	2.63333	1.31667	0.899	3.55
Subjects x Right vs. Left x rm	18	26.36667	1.46481		
Total	59	270.58333			

There was no significant difference between left and right sides for any bilateral character tested. At least nine basic types of FA index have been used (Palmer and Strobeck 1986; Richard Jones, pers. comm.). Means and standard deviations for three indices are listed in Table 26 for bilateral characters, with indices for individual specimens calculated as follows:

1) R-L
2) R/L
3) (R-L)/(R+L)

Table 26. FA indices.

Character		R-L	R/L	(R-L)/(R+L)
Parotoid length	Mean	-0.0276667	0.987871	-0.0080538
	SD	0.091084174	0.093399809	0.046204495
Parotoid width	Mean	0.0150004	1.0321527	0.0128911
	SD	0.068929418	0.119557285	0.05542695
Head length	Mean	0.0136664	1.0164746	0.0034208
	SD	0.064472852	0.04923108	0.018138241
Eye diameter	Mean	0.0063331	1.0188562	0.0086992
	SD	0.032828124	0.05398831	0.026620265
Tibia length	Mean	-0.0156663	0.9958227	-0.0021628
	SD	0.044333867	0.017644702	0.008895073
Radius length	Mean	-0.013333	0.9950675	-0.0028462
	SD	0.070640715	0.040849059	0.020287075
Hand length	Mean	0.0090007	0.9985084	-0.0010185
	SD	0.063402557	0.035227835	0.017298939
Wart number	Mean	0.299994	0.0	0.003816
	SD	1.933780536	0.254797135	0.11109537

DISCUSSION

Although a complete suite of statistical tests was not conducted, initial evaluation indicates that detectable levels of fluctuating asymmetry may be present in bilateral characters of museum specimens of *Bufo boreas*. In the ten specimens examined, mean character size did not differ significantly from zero, larger sides of bilateral characters are significantly larger than smaller sides, and when tested with character measurements in the original left-right orientation, there is no indication of directional asymmetry. However, some potential problems with these morphological traits are discussed below.

Some of the results may be due to distortion of the specimens in preservation. Some authors (Swaddle et al. 1994) argue against the use of museum specimens since FA differences observed in the specimens may be the result of natural selection rather than developmental conditions, collection of specimens may be biased (possibly in favor of large and/or symmetrical individuals), and conditions of preservation may result in asymmetries other than those due to development. However, museum specimens are the only available source of information related to time-dependent events for the boreal toad.

Preservation itself is unlikely to differentially alter the left or right side of an animal. However, the position in which the specimen is fixed or stored can alter the amount of soft tissue present for external measurements of the radius and tibia lengths, two measures which I observed to have ambiguous endpoints. In many cases, the vitreous humor was reduced or absent within the eyes, so even though the endpoints

appeared to be well-defined, the measurements of eye diameter may have been of distortions in the eye rather than of the diameter. This may be the source of the highly significant results in the ANOVA to evaluate larger versus smaller sides for these three characters. These three characters would be difficult to measure on living specimens in field conditions, and because of the question concerning their reliability in measurements of preserved specimens, probably should be omitted from the list of measures taken in future efforts.

The remaining bilateral characters (parotoid length, parotoid width, head length, hand length, and wart number) are candidates for use in the field with living animals.

Further evaluations of FA indices need to be made. In the three indices calculated, Index 1 does not correct for any differences in character size, while indices 2 and 3 correct for size at the individual level (Palmer and Strobeck 1986). Samples sizes were judged to be too small at this time (and my statistical tools insufficiently developed) to attempt comparisons between specimens collected from different localities or at different times. However, the potential presence of detectable FA makes such comparisons very desirable in any future studies. The effects of character size on FA levels also needs to be assessed. Further studies should continue to involve multiple measures, since this will provide more opportunity to find a trait that has fitness correlates. In addition, there are some FA indices that allow combination of multiple traits into individual and population indices.

There are several possible refinements in methods that may strengthen any FA "signal," including use of digital calipers (which may reduce any errors in reading the measurements as well as enhance the speed of processing, especially for live specimens).

As described earlier, increased environmental or genetic stress may be related to variation in levels of FA in declining *Bufo boreas* populations. By comparing levels of FA from museum specimens preserved at different times, it may be possible to detect increasing levels of FA as the period of reported toad declines is approached. Also, comparisons of toads collected within the central portion of the elevational range versus marginal (high and low elevation) sites may show biologically meaningful variation in FA.

In the 1997 field season, I measured several toads at boreal toad breeding sites. In future field seasion I will investigate the relationship of FA levels of individuals within populations to various fitness correlates, including whether males found in amplexus have different levels of FA than males not observed in amplexus, exhibit different probabilities of survival over winter, or differences in growth between years.

In summary, this preliminary study indicates that *Bufo boreas* may have detectable levels of FA for several characters. The utility of these characters for interpopulational comparisons of FA remains to be determined, but there are several promising lines of investigation.

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MOLECULAR GENETIC ANALYSES OF THE ENDANGERED BOREAL TOAD IN COLORADO AND SOUTHEAST WYOMING

ABSTRACT

Specimens of *Bufo boreas* collected throughout Colorado and southeast Wyoming were analyzed using two mitochondrial data sets: 1) sequences of the control region identified by single-stranded conformational polymorphisms and restriction-site polymorphisms, and 2) sequence data from the whole mitochondrial DNA (mtDNA) based on restriction-site polymorphisms. Both methods detect divergent mtDNA haplotypes (also called alleles). Results of analyses suggest:

1. All specimens analyzed in Colorado and southeast Wyoming fall into a cluster of closely related haplotypes.

2. The three largest populations in Colorado (Rocky Mountain National Park, the Clear Creek Drainage, and Chaffee County) all have unique mtDNA haplotypes and significantly different frequencies of shared mtDNA haplotypes. Specimens within the Clear Creek drainage have a pattern consistent with a metapopulation structure (all populations represented by few individuals are subsets of the largest population at Henderson Mine).

Previous analyses of mtDNA of the *Bufo boreas* species group are reviewed, because results may impact conservation programs for the Colorado and southeast Wyoming cluster. Relevant findings were:

1. Toads in Colorado and southeast Wyoming are within a previously unrecognized and highly divergent mtDNA clade described here as the Southern Rocky Mountain clade. The mtDNA of toads in Colorado and southeast Wyoming does not form a monophyletic mtDNA clade but divergence is described using parsimony, neighbor-joining and parsimony-network analyses.

2. Mitochondrial data suggest that toads originally migrated into Colorado from northern Utah, because haplotypes very closely related to those in Colorado and southeast Wyoming have been found in northern Utah.

All data combined suggest a four tiered approach to conservation management. Toads in the Southern Rocky Mountain mtDNA clade (Colorado, southeast Wyoming, southeast Idaho, and northern Utah) should be managed together as the most inclusive unit and separate from *Bufo boreas* elsewhere. Toads from northern Utah, Colorado, and southeast Wyoming should be managed with cooperation among states, because toads from these regions appear to be closely related. For example, should toads from Colorado and southeast Wyoming go extinct, northern Utah might provide the best source population for reintroductions. Toads from the geographically disjunct region of Colorado and southeast Wyoming should be managed as a third tier, because populations in these regions have closely related mtDNAs and they are geographically disjunct from toads elsewhere. The smallest management units are represented by three regions within Colorado (Rocky Mountain National Park, the Clear Creek Drainage, and Chaffee County). These populations are differentiated from each other by both unique alleles and significantly different frequencies of shared alleles.

INTRODUCTION

The *Bufo boreas* group, as currently recognized (Blair 1964; Blair 1972a; Schmidt 1953; Feder 1973; Stebbins 1985), comprises four recently evolved and morphologically similar species distributed across western North America (Fig. 1). Three species, *B. canorus* (Camp 1916), *B. exsul* (Myers 1942), and *B. nelsoni* (Stejneger 1893) are thought to be localized relictual isolates resulting from Pleistocene climatic changes and glaciation events (Myers 1942; Karlstrom 1962) and all three occur in California or western Nevada. The nominal species, *B. boreas* (Baird and Girard 1852), encompasses the rest of the range and comprises two subspecies, *B. boreas boreas* (Baird and Girard 1853b) and *B. boreas halophilus* (Baird and Girard 1853a). The latter occurs along the coast from middle California to Baja California, Mexico, and ranges eastward just into Nevada. *Bufo boreas boreas* encompasses the rest of the range from the southern

coast of Alaska to central California and eastward into the Rocky Mountains of Colorado. The current taxonomy is based on previous analyses of the group (Karlstrom 1962; Schuierer 1963, Bogart 1972, Feder 1973, Graybeal 1993). However, these analyses included specimens from a small portion of the range only, or made admittedly tentative phylogenetic hypotheses. Only one of the studies above included specimens from Colorado (Karlstrom 1962) and this study suggested that further analyses of the group were warranted especially those that included toads from the eastern half of the range. An understanding of the phylogenetic relationship of the toads in Colorado to the rest of the group is needed in order to identify conservation management strategies. Is the toad in Colorado the same species as is found across the rest of the range? What are the phylogenetic groups within *Bufo boreas* that should be managed together?

Bufo boreas, found in Colorado, is disjunct from toads in the rest of the range. Populations of toads in Colorado are separated from toads in Utah by at least 200 km and the dry intermountain basin of the Green River. Populations in southeastern Wyoming are separated from those in northwestern Wyoming by the Red Desert and dry plains in southwest and central Wyoming. These dry regions serve as effective barriers to migration today, but the biogeographic history of the toads in Colorado is not clear. When did barriers to dispersal first form and by what route did toads originally disperse into Colorado? Which populations of toads outside of Colorado are most closely related to those within Colorado? Toads at the southern-most end of the range within Colorado (the range extends into northern New Mexico) are currently disjunct from toads elsewhere. What is the relationship of these toads to the rest of the toads in Colorado? Are barriers to dispersal in this region real, or is the disjunct range an artifact of limited sampling in the region?

Bufo b. boreas has declined in the southern Rocky Mountains (Nesler and Goettl 1996; Garber 1994; Corn et al. 1989; Carey 1993; Stuart and Painter 1994), Utah (Ross et al. 1995), and northwestern Wyoming (Garber 1994; Koch and Peterson 1995). Some populations within the Oregon Cascades (Olson 1992; Blaustein and Olson 1991; Blaustein et al., 1994a and 1994b), the Sierra Nevada and central Valley of California (D. L. Martin pers. comm.; Drost and Fellers 1996; Fisher and Shaffer 1996) also have declined locally. Species of the *B. boreas* group with restricted distributions have declined including *B. nelsoni* in Nevada (Altig and Dodd 1987) and *B. canorus* in California (Kagarise Sherman and Morton 1993; Bradford and Gordon 1992; Drost and Fellers 1996; D. L. Martin pers. comm.). The recent declines of amphibians in general, and of groups in the *Bufo boreas* species group in particular, suggest that conservation measures are warranted. Toads with restricted distributions (e. g., toads in Colorado and southeast Wyoming) may be especially vulnerable.

Due to the lack of previous phylogenetic analyses (that included toads from Colorado), the disjunct range of toads in Colorado, and the recent declines of toads in the group, mtDNA phylogenetic analyses were initiated at the University of Colorado in 1991. Results from these analyses (Goebel 1996, Goebel et al., submitted) identified specimens from the eastern portion of the toads range (referred to here as the Southern Rocky Mountain Clade) as a distinct genetic lineage, highly divergent from toads across the rest of the range (Figures 2 and 3). Phylogenetic analyses identify the Southern Rocky Mountain clade as comprising approximately 24% of the diversity within the group (Goebel, 1996; diversity based on branch lengths). Although species are not typically described on the basis of mtDNA divergence alone, the divergence found is consistent with species-level mtDNA divergences in the *Bufo boreas* species group as well as the *Bufo americanus* group (Goebel, unpublished data). Clearly, the current taxonomic classification does not recognize all of the diversity identified with mtDNA. Other monophyletic clusters that correspond to geographic regions but are not recognized taxonomically are in S. Utah, Southern Rocky Mountains and Grouse Creek Mountains, Box Elder County of UT, southern California and Darwin Canyon, CA (Figure 2). Taxonomic revision of the *B. boreas* species group is needed.

Bufo boreas Group

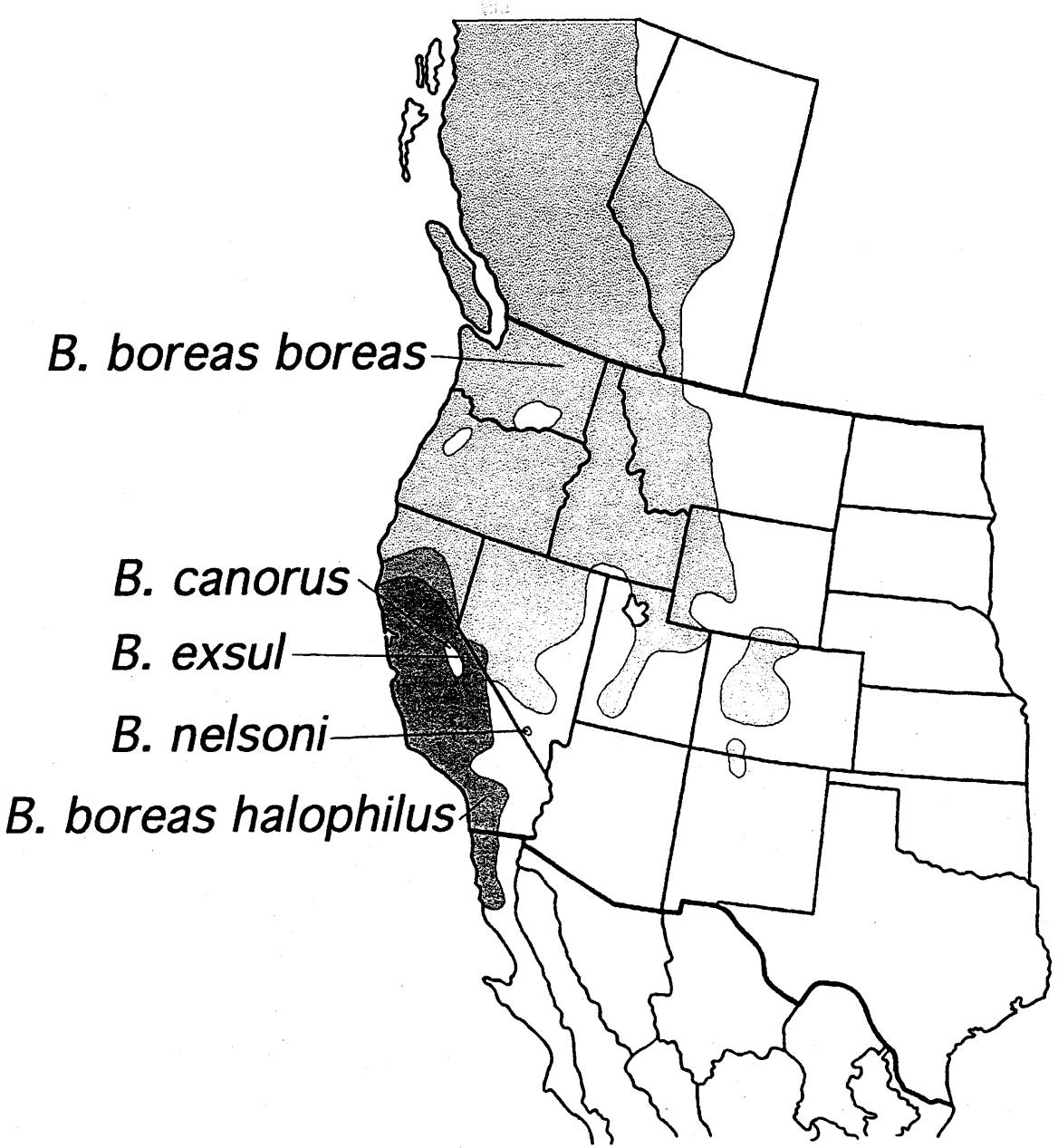

Figure 1. Ranges of taxa in the *Bufo boreas* species group. The range of *Bufo boreas boreas* is indicated by light shading and the range of *Bufo boreas halophilus* by dark shading. A zone of overlap in northern California is indicated by intermediate shading. *Bufo canorus*, *Bufo exsul* and *Bufo nelsoni* have very limited distributions. Ranges were taken from Stebbins (1985).

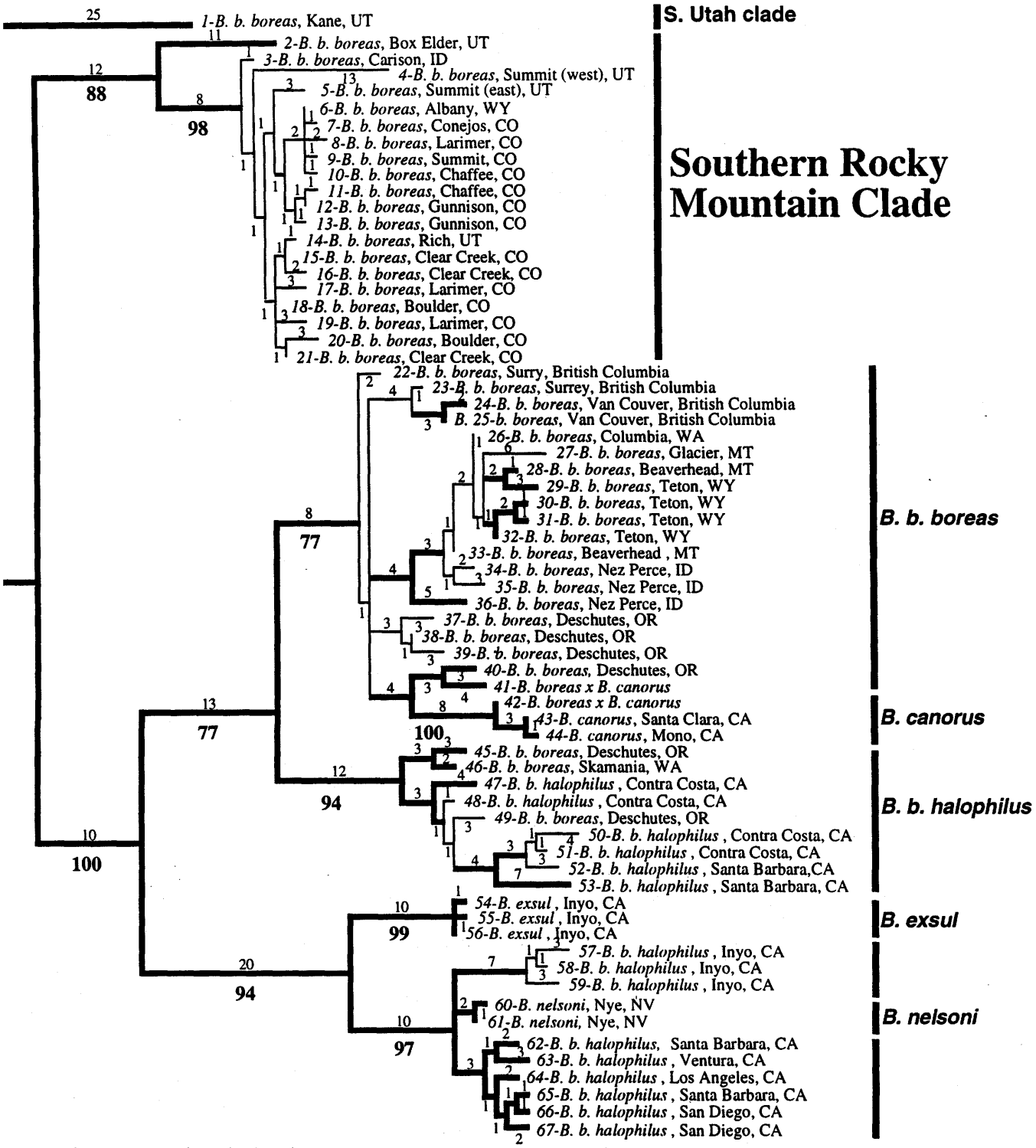

Figure 2. Systematic relationships within the *Bufo boreas* species group based on mitochondrial DNA analyses. Note the high divergence of the previously unrecognized Southern Rocky Mountain clade which includes all specimens from Colorado. Further subdivision is apparent (haplotype 2 from Box Elder Co., UT is divergent from the rest of the clade).

Technical data: This tree is one of the most parsimonius trees based on analysis of mitochondrial control region, 12S ribosomal DNA and cytochrome oxidase I sequences as well as restriction site data from the whole mitochondrial DNA. Large numbers next to bold lines are bootstrap values. Small numbers next to the lines are branchlengths. Branches in bold are present in the strict consensus tree based on all most parsimonious trees (length = 357; based on more than 32700 trees). Tree is unrooted but strongly supported clusters are considered to be clades, because the root probably lies among them. Additional analyses are in progress. Specimens are identified by haplotype number (as in Goebel et al. submitted), taxon name as identified by the collector, and by county and state of collection locality.

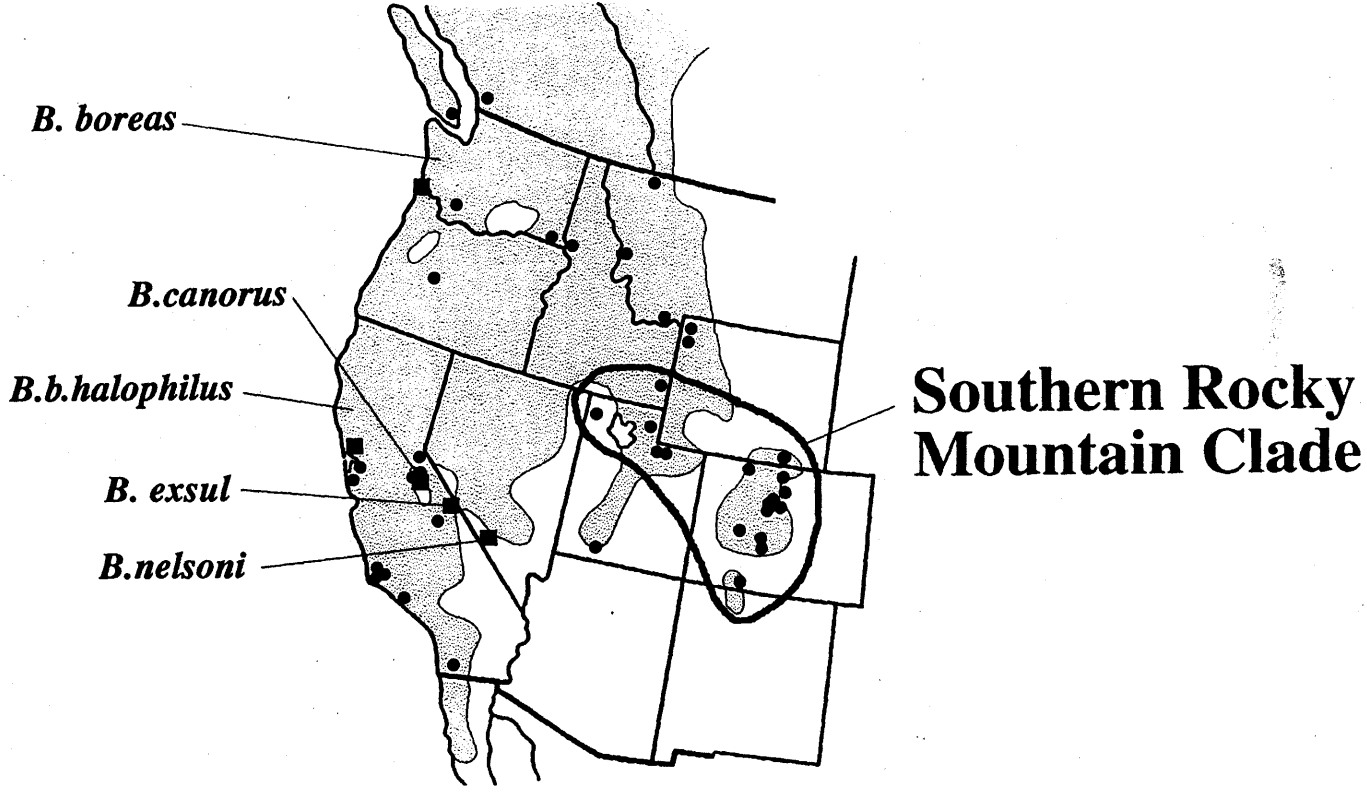

Figure 3. Potential range of the Southern Rocky Mountain Clade as identified by mitochondrial DNA analyses. Localities of specimens analyzed with mitochondrial DNA are identified with circles and localities of type specimens of described taxa are identified with squares. Localities of specimens within the Southern Rocky Mountain Clade (Figure 2) are circumscribed. The current data do not identify the geographically disjunct group in Colorado and southeast Wyoming as a monophyletic group (see discussion for details). However, additional subdivision within the group can be seen in Figure 2 (e. g., haplotype 2 from Box Elder Co., UT is highly differentiated from other specimens).

Due to these findings, the goals of genetic research were discussed in various meetings of the Boreal toad recovery team, and are stated in the Boreal Toad Recovery Plan (Goettl, 1997). Goals are to:

1. Determine phylogenetic relationships among taxa. Specifically to:
   - 1a. Identify the phylogenetic relationship *B. boreas* in the Southern Rocky Mountains to other taxa within the *B. boreas* species group.
   - 1b. Identify the geographic boundaries of the Southern Rocky Mountain lineage.
   - 1c. Identify a sister taxon (closest related lineage) to the lineage in Colorado and southeast Wyoming.

2. Investigate genetic subdivision among populations in Colorado and southeast Wyoming. Specifically to:
   - 2a. Identify genetic relationships within and among populations in Colorado and southeast Wyoming.
   - 2b. Identify geographic ranges for identified subgroups (management units).
   - 2c. Identify populations that might serve as source populations based on pattern of relatedness among populations and degree of variation within populations.

3. Determine levels of phylogenetic diversity in order to set priorities for conservation. Specifically to:
   - 3a. Identify level of phylogenetic diversity within Colorado and southeast Wyoming.
   - 3b. Identify level of phylogenetic diversity between Colorado and other lineages in the species group.
   - 3c. Identify level of diversity between taxa in the *B. boreas* group and other bufonids within North America.

Goals 1a-c are nearing completion. Phylogenetic relationships among named taxa of the *B. boreas* species group and specimens collected from across the range have been hypothesized using mtDNA (goal 1a). Four highly divergent lineages were identified (Figure 2; Goebel et al. submitted) and boreal toads of the Southern Rocky Mountains comprise one of those lineages. Relationships among these four lineages are not clear, because a root for the tree was not identified with confidence. However, the root probably lies among the four lineages.

All specimens analyzed within Colorado and southeast Wyoming lie within the Southern Rocky Mountain clade. However, the geographic boundaries of the Southern Rocky Mountain clade (goal 1b) are not clear at this time. The closest related lineage (sister lineage) to the lineage in Colorado/southeast Wyoming (goal 1c) appears to be within northern Utah. Additional specimens from Idaho, Nevada, Montana, and western Oregon and Washington have been received and are being analyzed. Specimens from Utah, which are most critical for this analysis, are still lacking.

Research to address Goals 2a-c has been funded by the Colorado Division of Wildlife and methods and results are discussed in this report. Specifically, I discuss results of mtDNA analyses using SSCP (single stranded conformational polymorphisms) and restriction-site analyses which were used to address genetic subdivision among populations in Colorado and southeast Wyoming. Additional analyses of restriction-site data from the whole mitochondrial DNA are discussed also. Nuclear genetic analyses (amplified fragment length polymorphisms, AFLPs) are in progress, but results are not reported here because research is still ongoing. However, results from nuclear DNA analyses are critical, because mtDNA is effectively a single locus. A multi-locus approach which includes nuclear DNA analyses should provide the best resolution.

Methods to identify genetic alleles relatively cheaply and quickly are essential in analyzing large numbers of specimens across Colorado and southeast Wyoming. Large numbers of specimens are needed in order to identify the relationships of all newly identified populations (especially those in isolated areas) to those across the rest of Colorado and southeast Wyoming. Analysis of geographically isolated populations is

especially critical because isolated populations of *B. boreas* in Utah, Nevada and California were found to have highly divergent mtDNA haplotypes (which frequently accompanies speciation, e. g., *B. canorus*, *B. exsul* and *B. nelsoni*). Large numbers of specimens are needed also for frequency-based analyses in order to differentiate between the effects of random sampling of small populations and true frequency differences among populations. Approaches using the polymerase chain reaction (PCR) are desirable, because they allow analysis of small or degraded samples (egg tissue, tadpole tails, animals found dead in the field, blood samples from non-destructive field sampling).

Two methods of analysis were chosen, both based on PCR approaches. For the first analysis, single stranded conformational polymorphisms (SSCP) and restriction site data were used to identify variation in the mtDNA control region. SSCP data are useful to detect single (or few) base pair changes in sequences by detecting differential migration of the single-stranded sequence relative to known standard sequences. Sequence data can be obtained from a large number of specimens quickly. The mtDNA control region was chosen for SSCP analyses because it is one of the fastest evolving regions of the mtDNA and because much data from this DNA region is already available for *Bufo boreas* (Goebel et al., submitted). For the second approach, analyses using the method of Amplified Fragment Length Polymorphisms (AFLP; Vos et al. 1995) was chosen as a technique to "fingerprint" genomic DNA. Unlike other PCR-based fingerprinting methods (e. g., RAPD: random amplified polymorphic DNA; DAF:amplification fingerprinting; AP-PCR:arbitrarily primed PCR), AFLP methods are based on ligating known DNA fragments to genomic DNA and using the ligated fragments as primer sites. Thus, primer sites are perfect matches. This reduces sensitivity to reaction conditions, DNA quality, and PCR temperature profiles, all of which limit the utility of other methods (Vos et al. 1995). Reduced sensitivity to PCR reaction conditions is desirable, because specimens for this analysis have been collected over a large number of years and vary in their degree of degradation.

MATERIALS AND METHODS

Analyses of sequence data using SSCP analyses (Single Stranded Conformational Polymorphisms) and restriction-site polymorphisms.

Specimens (N=163) were sampled from across Colorado and southeast Wyoming. Specimens were collected from 22 localities (Appendix 1) and data were combined into 13 regions (Figures 4 and 5). Total DNA was extracted from blood, muscle, liver, or egg tissue, using proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation (Maniatis et al. 1982; Hillis and Davis 1986).

Sequence variation within approximately half of the control region (1063 bp) was determined from banding patterns of single stranded conformations. For SSCP analyses, a large mtDNA fragment was amplified from genomic DNA. A second round of amplification was used to produce six non-overlapping fragments which ranged in size from 198 to 121 base pairs. Detailed methods for SSCP analyses (DNA amplification, gel running conditions) are provided elsewhere (Goebel, 1997; Goebel in prep). Unique SSCP banding patterns were confirmed with sequence data. Sequences of approximately half of the control region were obtained from both DNA strands for at least one accession of all unique control region sequences.

Restriction-site polymorphisms were identified using standard techniques (Southern 1975; Maniatis et al. 1982; Hillis and Moritz 1990). Restriction enzymes with polymorphic cutting sites in the control region were identified from known sequences. The restriction enzyme Dra I was used to confirm sequence within one SSCP fragment, because differential migration of some conformations was difficult to detect with SSCP analyses alone. Restriction site polymorphisms from the whole mtDNA were collected previously (Goebel 1996; Goebel et al., submitted) and results are included here because of their relevance to population analyses in Colorado and southeast Wyoming.

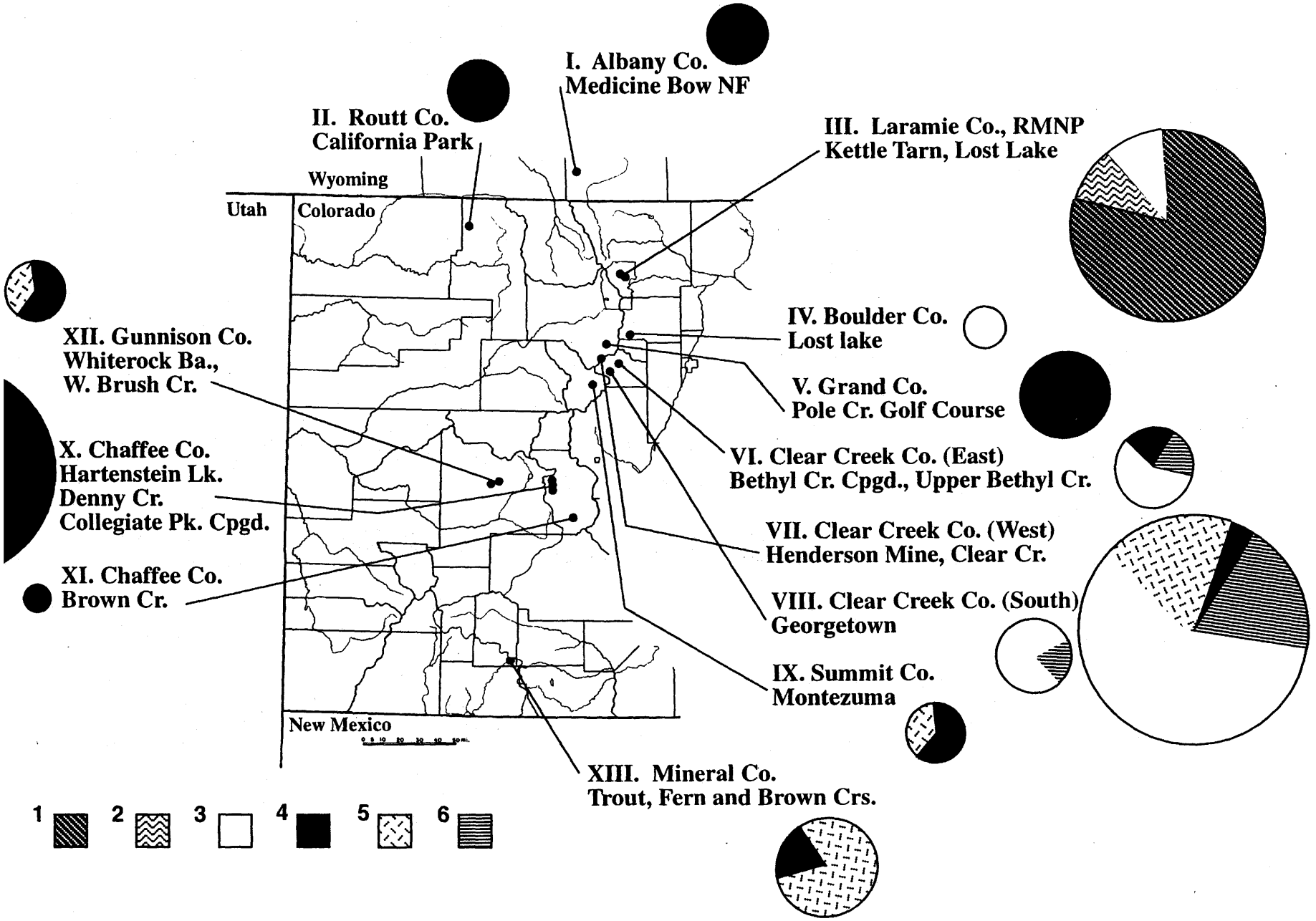

Figure 4. Haplotype frequencies for 13 populations of *B. boreas* in Colorado and southeast Wyoming based on control region sequence data determined by SSCPs. Six unique haplotypes were identified. Size of circles indicates number of specimens examined (number and frequencies given in Tables 1 and 2).

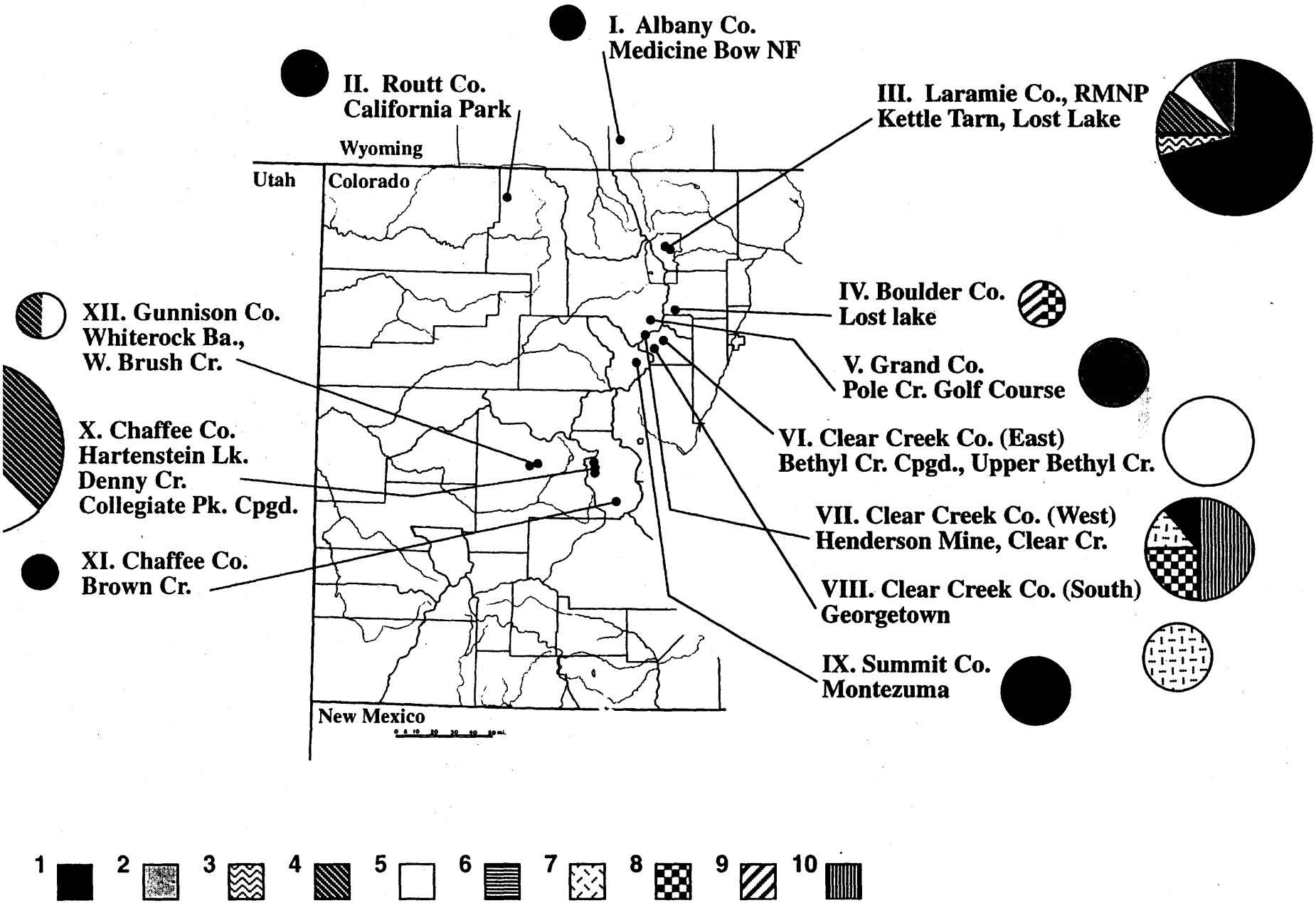

Figure 5. Haplotype frequencies for 13 populations of *B. boreas* in Colorado and southeast Wyoming based on restriction sites from the whole mitochondrial DNA. Ten unique haplotypes were identified. Size of circles indicates number of specimens examined (number and frequencies given in Tables 11 and 12.

Haplotype frequency analyses

Frequencies of haplotypes were calculated for each of 13 populations sampled. Frequencies were calculated independently for control region SSCP, and restriction sites from the whole mtDNA, because each data set included different numbers of specimens. Even though both data sets analyze mtDNA, they represent different characters from the mtDNA genome. Statistically significant frequency differences between populations were identified using the statistical package BIOSYS (Swofford 1989).

Phylogenetic analyses (from Goebel et al., submitted and in prep.).

Phylogenetic analyses from previous analyses (Figures 2, 6-8) were performed using parsimony (PAUP 3.1, Swofford 1993) and the heuristic search option. To identify the set of most parsimonious trees, 500 random addition sequence replicates were performed using the tree-bisection algorithm, saving five trees per replicate. A strict consensus tree of all trees of minimum length was calculated. Even though this procedure did not identify all most-parsimonious trees, it was considered to accurately reflect the strict consensus tree, because further replicates did not result in the collapse of any additional nodes in the strict consensus tree. Bootstrapping (1000 bootstraps, ten replicate per bootstrap, saving only the five most parsimonious trees per replicate; Felsenstein 1985, 1988) were estimated in order to compare degree of support for discovered bufonid clades. Bootstrap values $\geq$70% in this analysis are considered to support clades strongly (Hillis and Bull 1993; Cummings et al. 1995). Phylogenetic relationships were also estimated using neighbor-joining techniques (Saitou and Nei 1987) using PHYLIP (Phylogeny Inference Package 3.5C by J. Felsenstein, PowerPC Macintosh version compiled by D. Gilbert). Minimum p-distances were chosen for the final analyses because distances between all samples were small.

Parsimony networks were constructed manually. Character changes among haplotypes were identified from aligned data sets. Haplotypes differing by unique changes (autapomorphies) were connected first. Character changes shared by two or more haplotypes were identified and haplotypes were connected in a way to minimize the number of character changes (parsimony criterion).

RESULTS

Haplotype frequency analyses

The number and frequency of haplotypes for each of 13 populations were estimated for SSCP data (Table 1) and restriction sites from the whole mtDNA (Table 2). Frequency difference among populations are evident from pie diagrams for both data sets (Figures 4 and 5 respectively). Both data sets have highly significant heterogeneity in allele frequencies among populations. However, only three populations (III-RMNP, VII-Henderson Mine, X-Chaffee County) have enough samples to suggest true genetic differentiation. Genetic differentiation among other smaller populations may be due to small sample sizes.

In both data sets RMNP is differentiated from both Henderson Mine and Chaffee County by both unique alleles and highly significant allele frequency differences among shared alleles. Restriction-site data differentiate Henderson Mine and Chaffee County by both unique alleles and highly significant allele frequency differences among shared alleles. SSCP data differentiate Henderson Mine and Chaffee County by highly significant allele frequencies. The small populations surrounding the three largest populations appear, in general, to be subsets of the large populations. These data suggest that geographically close populations may form a metapopulation structure.

The geographically disjunct population in southern Colorado (Mineral county, CO) has mtDNA haplotypes identical to those found elsewhere in Colorado.

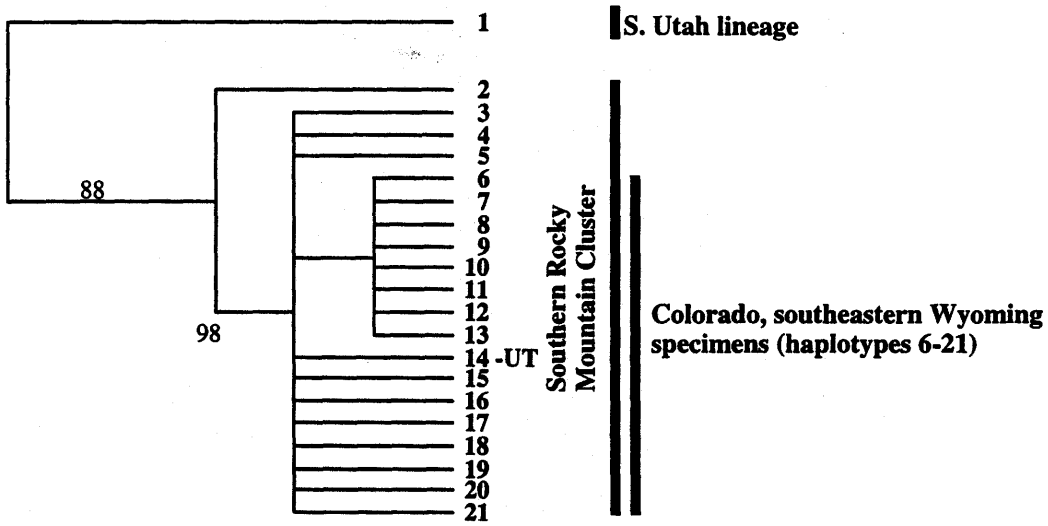

Figure 6. Strict consensus tree of specimens from Colorado/southeastern Wyoming. Specimens numbered as in Figures 6-9. Numbers on branches are bootstrap values. Specimens 1-5 and 14 are from Utah and Idaho.

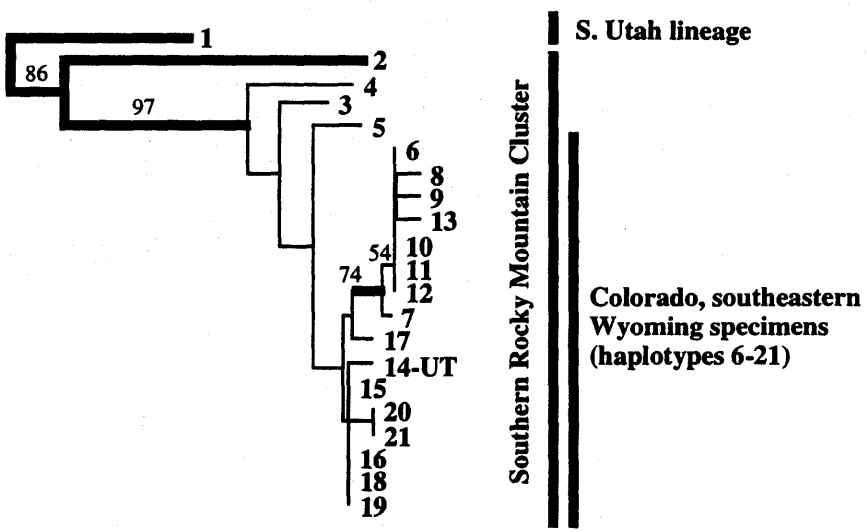

Figure 7. Neighbor-joining tree of specimens from Colorado/southeastern Wyoming. Specimens numbered as in Figures 6-9. Numbers on branches are bootstrap values; branches with high bootstrap values are in bold. Specimens 1-5 and 14 are from Utah and Idaho.

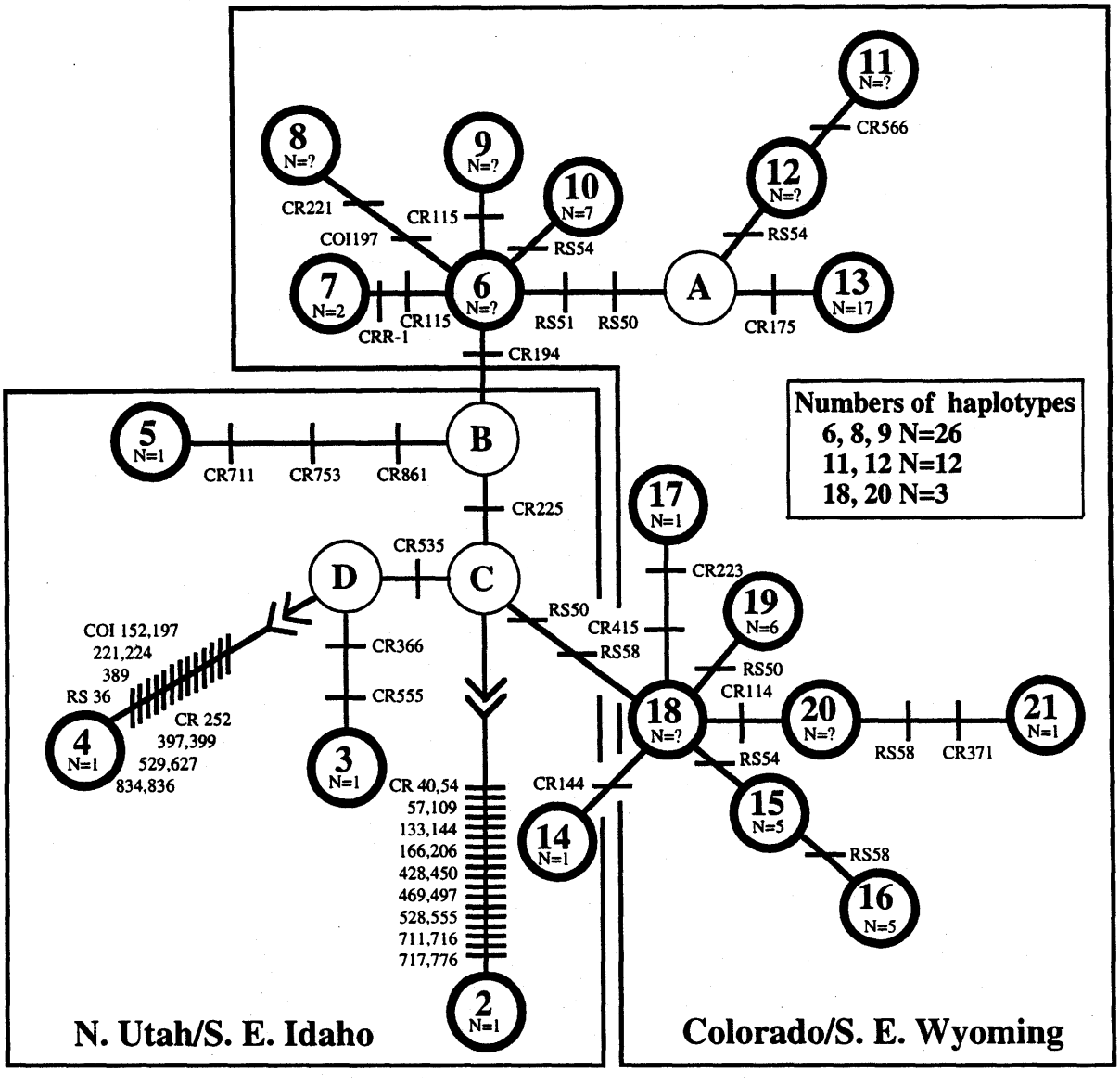

Figure 8. Parsimony network of specimens in the southern Rocky Mountain cluster. Numbers in circles refer to haplotypes (as in Figures 6-9); circles with letters refer to hypothesized haplotypes. Character changes are identified on the branches with the following abbreviations: CR=control region, COI=cytochrome oxidase I, RS-restriction site character data.

DISCUSSION

What are the management units within Colorado?

One goal of genetic analyses (goal 2) was to use SSCP data to identify genetic variation within and among populations in Colorado and southeast Wyoming. When populations have similar genetic make-up (i. e. similar allele identities and frequencies), gene flow between populations is assumed to be high. When populations have unique alleles or significantly different frequencies of alleles, gene flow is assumed to be low. Too few samples were collected from many populations to have high confidence in all results. However, unique alleles and statistically significant differences were found between the three main breeding groups in Rocky Mountain National Park, the Clear Creek Drainage and Chaffee County. More samples (ideally 30 or more per population) will be needed in order to differentiate between effects of random sampling of small populations (e. g., those in Routt County or Wyoming) and true frequency differences among populations.

How significant are the mtDNA differences? A comparative data base for bufonids is still lacking however, inferences can be made from other animals. Some marine mammal taxa, identified from morphological differences, have shown little or no mtDNA differences among them. Mitochondrial DNA is thought to evolve more slowly in poikilotherms than in mammals (Rand 1994; Martin and Palumbi 1993). If

Table 1. Frequencies of mtDNA haplotypes in 13 populations in Colorado and southeast Wyoming. Haplotypes represent DNA sequence variation in the control region identified by SSCP analyses.

Population		Haplotype					
	N	1	2	3	4	5	6
I.	3	-	-	-	1.000	-	-
II.	3	-	-	-	1.000	-	-
III.	35	0.800	0.114	0.086	-	-	-
IV.	2	-	-	1.000	-	-	-
V.	7	-	-	-	1.000	-	-
VI.	5+	-	-	0.600	0.200	-	0.200
VII.	38	-	-	0.579	0.026	0.184	0.210
VIII.	5	-	-	0.800	-	-	0.200
IX.	4	-	-	-	0.500	0.250	0.250
X.	28	-	-	-	0.786	0.214	-
XI.	1	-	-	-	1.000	-	-
XII.	3+	-	-	-	0.667	0.333	-
XIII.	13	-	-	-	0.385	0.615	-
Total	147	0.190	0.027	0.231	0.320	0.156	0.075

Table 2. Frequencies of mtDNA haplotypes in 12 populations in Colorado and southeast Wyoming. Haplotypes were identified using restriction site analyses from the whole mitochondrial DNA (data from Goebel, 1996). No samples were available from population XIII at the time of data collection. Haplotypes 1-10 refer to haplotype numbers 42-51 respectively in Goebel (1996).

Population		Haplotype									
	N	1	2	3	4	5	6	7	8	9	10
I.	1	1.000	-	-	-	-	-	-	-	-	-
II.	2	1.000	-	-	-	-	-	-	-	-	-
III.	20	0.700	0.100	0.050	0.100	0.050	-	-	-	-	-
IV.	2	-	-	-	-	-	-	-	0.500	0.500	-
V.	4	-	1.000	-	-	-	-	-	-	-	-
VI.	7	-	-	-	-	1.000	-	-	-	-	-
VII.	4	-	-	-	-	-	-	1.000	-	-	-
VIII.	8	0.125	-	-	-	-	-	0.125	0.250	-	0.500
IX.	4	1.000	-	-	-	-	-	-	-	-	-
X.	26	0.077	-	-	0.346	0.308	0.269	-	-	-	-
XI.	1	1.00	-	-	-	-	-	-	-	-	-
XII.	2	-	-	-	0.500	0.500	-	-	-	-	-
Total	81	0.309	0.074	0.012	0.148	0.210	0.086	0.062	0.037	0.012	0.049

bufonid taxa (which are poikilotherms) have even less mtDNA variation among species than that found in mammals, then the small differences found in this analysis is significant. These data suggest that the populations with fixed allelic differences and/or significant frequency differences should constitute different management units until further data are available (nuclear data are being generated currently). Specifically, the three regions for which many samples have been analyzed (Rocky Mountain National Park, the Clear Creek Drainage and Chaffee County), should be managed independently. In contrast, populations that are geographically close (e. g., the many populations within the Clear Creek Drainage) share alleles. Frequency differences found among these close populations may be due to limited sampling.

Managing inbreeding

Some concern of low genetic variation within populations is due to the potential complications of inbreeding. Data presented here do not identify any populations with significantly low levels of mtDNA variation. A single haplotype only was identified in a few populations, but this appears to be due to limited sampling. Translocations among populations, specifically to avoid inbreeding, are controversial. If alleles deleterious to a specific environment or region have already been purged, then translocations which reintroduce such deleterious alleles could be harmful. While there may be appropriate reasons to translocate animals, translocations specifically for genetic reasons alone are flawed (Avise 1996).

One goal of conservation should be to restore pre-fragmentation levels of dispersal and gene flow (Avise 1996). Directions and rates of transport should be based on knowledge of the historical pattern of gene flow (e. g., Vrijenhoek 1996). Care should be taken so that translocated animals to not swamp locally

adapted gene complexes as may have been done with fisheries management (Vrijenhoek 1996). Pre-fragmentation levels of gene flow may best be restored by maintaining appropriate habitat between isolated populations rather than by translocations. Direct translocations among geographically distant populations could never have occurred historically. Unique mtDNA alleles and significantly different allele frequencies among the three largest populations (Rocky Mountain National Park, Clear Creek Drainage and Chaffee County) suggest limited gene flow among them. Translocations among these populations are not recommended.

Is the disjunct group of *B. boreas* in Colorado and southeast Wyoming a new species?

The data provided here do not unambiguously identify the geographically disjunct groups of toads in Colorado and southeast Wyoming as a distinct species. Species identification of the group is dependent first and foremost on the particular species concept one chooses to delineate species. One criterion, the ability to interbreed and produce viable offspring (biological species, Mayr 1963) is used rarely in animals with external fertilization and poor mate recognition systems (toads and other frogs are known to breed among species and occasionally with dead conspecifics and clumps of mud). In addition, the ability to interbreed may an ancestral trait (the inability to interbreed may be a recently derived trait in some groups), and therefore not a good measure of phylogenetic divergence. A second criterion for species recognition is monophyly, which is inferred by the identification of a trait (e. g., morphological or genetic characters) present in all members of the species, but absent in all members outside of the species (phylogenetic species, Cracraft 1989). The mitochondrial DNA lineages within Colorado are not monophyletic (Figures 6 and 7). However, other faster evolving nuclear genes may be. Some biologists might conclude that toads in Colorado are a separate species because they are a monophyletic lineage of populations, even though mtDNA is not yet monophyletic. However, this definition would require all disjunct populations to be recognized as independent species and is therefore controversial.

Even though toads in Colorado cannot be defined as a species on the available data, they are an evolutionarily significant unit or management unit. If toads in Colorado should go extinct, they could not repopulate by natural migration due to geographic barriers. Toads in Colorado are not a monophyletic mtDNA lineage, but they probably have significantly different frequencies of mtDNA haplotypes from populations outside the region. Clearly, toads in Colorado should be managed independently of those elsewhere, but in cooperation with managers in Utah and other regions with closely related toads (toads in the southern Rocky Mountain Clade).

Parsimony networks: frequency of haplotypes and inference of speciation and biogeography

Phylogenetic relationships among closely related haplotypes may lack resolution in parsimony analyses due to single-step changes that are autapomorphic (Figure 6). In this case, evolutionary history may be inferred by parsimony networks (also called allele or haplotype trees) which use the frequency alleles and number of autapomorphic changes among alleles for phylogenetic inference. Geographical associations of haplotypes combined with patterns of haplotype trees can be used to examine gene flow (Slatkin 1989; Slatkin and Maddison 1989 and 1990) historical fragmentation or range expansion (e. g., Lavery et al. 1996) or to discriminate between gene flow and historical events (e. g., Templeton 1993).

Within populations, mutations accumulate by a poisson process (Templeton 1993), depending on the number of individuals through both space and time. Parsimony networks typically depict relationships as radiations of haplotypes or "stars". In general and in the absence of selection for particular mtDNA haplotypes, old and common haplotypes are least affected by bottlenecks (either real, due to population fluctuations, or artifactual, due to sampling few individuals) and are at star centers. Rare alleles occur at tips of radiations and differ from star centers by few (or single) mutations. Thus, position in the haplotype tree provides hypotheses of relative age of haplotypes (Castelloe and Templeton 1994) as do frequencies (Ewens 1990; Hudson 1990; Crandall and Templeton, 1993).

Three star-radiations can be seen among the closely related mtDNA haplotypes of the Southern Rocky Mountain clade (Figure 8). Haplotypes 6, 18 and A are at the centers of stars. Haplotypes 2-5 represent single samples from more extensive populations; further sampling may identify multiple star phylogenies in these populations. Rare alleles (e. g., haplotypes 7, 21) occur at tips of radiations and differ from centrally located alleles, by few mutations. Within Colorado/ S. E. Wyoming the number of specimens with centrally located haplotypes (6, 18, A) is not known exactly, due to the limited number of specimens for which sequence data are available. However, the large number of specimens whose haplotype is inferred from restriction-site data alone (e. g., 26 haplotypes of 6, 8, or 9; 17 haplotypes of 13), suggest that there may be many specimens at centrally located haplotypes 6 and A, and additional star-points may be present also. Haplotype networks in populations that have expanded recently may produce a pattern of radiations with many haplotypes but few character changes between haplotypes (e. g., coconut crabs of various Pacific island; Lavery et al. 1996). In contrast, the pattern seen in small populations that have persisted through evolutionary time will have stochastic fluctuations which disintegrate haplotype networks into multiple inter-connected stars (e. g., coconut crabs isolated on Christmas Island; Lavery et al. 1996). If the multiply-starred network pattern currently found in Colorado/ S. E. Wyoming (Fig. 5, star centers at 6, 18, A) reflects a historically small but stable population, then the recent (1970-present) and severe declines of toads in the region are an evolutionary anomaly and strong conservation measures are warranted.

The very low diversity between haplotype 14 in northern Utah and haplotype 18 in Colorado was unexpected, but can be explained by different population histories. First, toads may have been abundant across the whole southern Rocky Mountain region and have been recently (in evolutionary time) isolated as disjunct ranges. If haplotype 18 is old (and was common) across the southern Rocky Mountains, then a star phylogeny within Utah with 18 at its center, and radiations unique to Utah (e. g., haplotype 14), would be expected. The geographically isolated populations in Colorado/S. E. Wyoming cannot be separated from N. Utah on the basis of reciprocal mtDNA monophyly. However, if both northern Utah and Colorado/S.E. Wyoming have sets of uniquely derived mtDNA haplotypes, then hypotheses of ancestral haplotypes retained in both localities would be supported. In this case, the parsimony network (Figure 8) may depict gene lineage sorting expected among closely related taxa or populations (e. g., Ball et al. 1990; Takahata 1989). Other species in the *B. boreas* group (e. g. *B. canorus, B. exsul*, and *B. nelsoni*) have been defined on the basis of unique frequencies of allozyme alleles even though no fixed differences were found between species. Similarly, *B. boreas* in Colorado and southeast Wyoming can be described as a distinct evolutionary unit based on unique mtDNA alleles even though mtDNA-fixed differences are not present.

Lack of monophyly may also be due to gene flow. A second hypothesis of population history suggests that immigration of toads into Colorado/S. E. Wyoming and Utah is evolutionarily recent. Haplotypes 6 and 18 (which are old and were common) may have immigrated into Colorado/S. E. Wyoming and unique radiations have evolved forming the star phylogenies in Fig. 9. Again, if 6 and 18 are old (and were common) they would have a high probability of entering Colorado/S. E. Wyoming within any migration event. A recent invasion would also explain the apparent lack of highly divergent lineages within Colorado/ S. E. Wyoming. Third, an evolutionarily recent migration of toads from Colorado into Utah could explain the low divergence between haplotypes 14 and 18. Migration could have been mediated by humans; translocation of boreal toads is suspected for *B. boreas* in Utah and Nevada (Hovingh in press) and *B. exsul* in California (Schuierer 1962). Translocations of tadpoles, which are used as fishing bait, may not be uncommon.

Further analyses needed

Specimens currently are being collected to increase sample sizes from small populations. Specimens from northern Utah are especially critical, because they may be most closely related to toads in Colorado will be valuable in accessing the species status of toads in Colorado. Variation in nuclear DNA within and among

populations is currently being examined also using DNA fingerprint techniques. The kinds of data currently generated are limited by the availability of funding. Ideally, funding would be committed for multiple-year projects so that the best data to address the question of interest can be generated, rather than data that can be generated within a single budget-year only. Genetic analyses have been slow not because an unlimited amount of genetic data needs to be generated but because such limited amount of funding has been allocated to genetic analyses.

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Appendix 1. Voucher specimens, age class, tissue type and localities of specimens examined in the *Bufo boreas* group. Missing data are indicated with "?". Toads indicated as juveniles were metamorphs at the time of collection. All specimens collected as adults were found dead in the field. Blood was collected from some adults who were left in the field after a photo identification.

	Species/ Specimen	Voucher Specimen	Age Class	Tissue Type	Locality
Specimens examined within Colorado and southern Wyoming					
B. boreas boreas - Colorado					
1.	AMG154-KT5	PT2305825	Adult	RBC	Larimer County, CO N=10. Kettle Tarn, Rocky Mountain National Park. June and 30 July 93. Collectors: M. Jennings, A. M. Goebel, P. S. Corn.
2.	AMG155-KT6	PT1333876	Adult	RBC	"
3.	AMG156-KT7	PT1828787	Adult	RBC	"
4.	AMG157-KT8	PT1830627	Adult	RBC	"
5.	AMG158-KT9	PT1826799	Adult	RBC	"
6.	AMG159-KT10	PT1317118	Adult	RBC	"
7.	AMG141-KT23E	--	--	Eggs	"
8.	AMG140-KT24E	--	--	Eggs	"
9.	AMG142-KT22E	--	--	Eggs	"
10.	AMG143-KT13E	--	--	Eggs	"
11.	AMG115-EKT	--	--	Eggs	"
12.	AMG-138LLAD	USNM	Adult	Muscle	Larimer County, N=10. Lost Lake, Rocky Mountain National Park. 31 July 1993. Collectors: P. S. Corn, A. M. Goebel, June 1993
13.	AMG139-LL1E	--	--	Eggs	June 1993
14.	AMG257-LL14	--	Tadpole	Tail	September, 1992
15.	AMG258-LL15	--	Tadpole	Tail	"
16.	AMG280-LL23	--	Tadpole	Tail	"
17.	AMG259-LL16	--	Tadpole	Tail	"
18.	AMG281-LL24	--	Tadpole	Tail	"
19.	AMG260-LL17	--	Tadpole	Tail	"
20.	AMG278-LL21	--	Tadpole	Tail	"
21.	AMG267-LL18M	--	Metamorph	Whole leg	"
22.	AMG160-L1	--	Adult	Blood	
23.	AMG161-L2	--	Adult	Blood	
24.	AMG162-L3	--	Adult	Blood	
25.	AMG163-L4	--	Adult	Blood	
26.	AMG164-L5	--	Adult	Blood	
27.	AMG165-L6	--	Adult	Blood	
28.	AMG114-LLA	--	Adult	Blood	
29.	AMG41-LLT2	--	Tadpole	Tail	

30.	AMG43-LLT4	--	Tadpole	Tail	
31.	AMG44-LLT5	--	Tadpole	Tail	
32.	AMG47-LLT8	--	Tadpole	Tail	
33.	AMG55-LLT16	--	Tadpole	Tail	
34.	AMG57-LLT18	--	Tadpole	Tail	
35.	AMG66-LLT27	--	Tadpole	Tail	
36.	AMG26-Dillon1	--	Juvenile	Muscle	Summit County, CO N=4. Near Montezuma. September, 1992. Collector:
37.	AMG28-Dillon3	--	Juvenile	Muscle	"
38.	AMG29-Dillon4	--	Juvenile	Muscle	"
39.	AMG30-Dillon5	--	Juvenile	Muscle	"
40.	AMG397-BC1	--	Adult	RBC	Chaffee County, CO N=1. Brown's Creek. 20 miles S Buena Vista. 15 June, 1995. Collector: P. S. Corn.
41.	AMG87-CPC49	--	Juvenile	Muscle	Chaffee County, CO N=10. 100 ft. E of Cottonwood Creek Campground (Collegiate Peaks Campground), off Cottonwood Pass Rd. Collectors: P. S. Corn, A. M. Goebel.
42.	AMG87-CPC48	--	Juvenile	Muslce	"
43.	AMG89-CPC50	--	Juvenile	Muscle	"
44.	AMG90-CPC51	--	Juvenile	Muscle	"
45.	AMG91-CPC52	--	Juvenile	Muscle	"
46.	AMG92-CPC53	--	Juvenile	Muscle	"
47.	AMG188-CPC54	USNM64330	Adult	Muscle	25 August 1993
48.	AMG189-CPC55	Photo ID Adult	RBC	"	
49.	AMG190-CPC56	Photo ID Adult	RBC	"	
50.	AMG191-CPC57	Photo ID Adult	RBC	"	
51.	AMG192-CPC58	Photo ID Adult	RBC	"	
52.	AMG215-DCT1	--	Tadpole	Tail	Chaffee Co, CO N=7. Denny Creek, 0.6 miles N of Cottonwood Pass Road. 26 August, 1993. Collectors: P. S. Corn, A. M. Goebel.
53.	AMG217-DCT3	--	Tadpole	Tail	"
54.	AMG218-DCT4	--	Tadpole	Tail	"
55.	AMG219-DCT5	--	Tadpole	Tail	"
56.	AMG223-DCT9	--	Tadpole	Tail	"
57.	AMG224-DCT10	--	Tadpole	Tail	"
58.	AMG226-DCT12	--	Tadpole	Tail	"
59.	AMG199-HL1NW	Photo ID Adult	RBC	Chaffee Co, CO N=9. Hartenstein Lake, 2.0 miles N from Cottonwood pass Rd. on Denny Creek Trail. 26 August, 1993. Collectors: P. S. Corn, A. M. Goebel.	
60.	AMG00-HL2NW	Photo ID Adult	RBC	"	
61.	AMG201-HL3NW	Photo ID Adult	RBC	"	
62.	AMG202-HL4S	Photo ID Adult	RBC	"	
63.	AMG203-HL5	Photo ID Adult	RBC	"	
64.	AMG204-HL6S	Photo ID Adult	RBC	"	
65.	AMG207-HLT3S	--	Tadpole	Tail	"
66.	AMG210-HLT6NW	--	Tadpole	Tail	"
67.	AMG212-HLT8NW	--	Tadpole	Tail	"

68.	AMG209-HLT5	--	Tadpole	Tail	"	
69.	AMG392-Wyo1	PhotoID	Adult	RBC	Albany County, WY N=2. Collector: A. M. Goebel and J. D. Wortman.	
70.	AMG392-Wyo3	PhotoID	Adult	RBC	Collector: P. S. Corn.	
71.	AMG484-Wyo4	PhotoID	Adult	RBC		
72.	AMG309-GT1	PhotoID	Adult	RBC	Clear Creek County, CO N=4. Clear Creek, 1.6 miles N of Georgetown. 9 July 1994. Collectors: A. M. Goebel, J. D. Wortman, D. Wortman.	
73.	AMG310-GT2	PhotoID	Adult	RBC	"	
74.	AMG311-GT3	PhotoID	Adult	RBC	"	
75.	AMG312-GT4	PhotoID	Adult	RBC	"	
76.	AMG313-GT5	PhotoID	Adult	RBC	"	
77.	AMG228-WRB1	PhotoID	Adult	RBC	Gunnison County, CO N=6. White Rock Basin, off Copper Creek, 2 km from Gothic. 27 August, 1993. Collectors: P. S. Corn, A. M. Goebel.	
78.	AMG227-WBC1	PhotoID	Adult	RBC	Gunnison County, CO N=1. West Brush Creek, 12 km NE Crested Butte, T13S R85W S15. 26 August, 1995. Collectors: P. S. Corn, A. M. Goebel.	
79.	AMG229-WRBT1	--	Tadpole	Tail	"	
80.	AMG230-WRBT2	--	Tadpole	Tail	"	
81.	AMG231-WRBT3	--	Tadpole	Tail	"	
82.	AMG233-WRBT5	--	Tadpole	Tail	"	
83.	AMG235-WRBT7	--	Tadpole	Tail	"	
84.	AMG186-UBethM1	--	Metamorph	Whole leg	Clear Creek County, CO N=2. Off of I-70 August, 1993. Collectors: P. S. Corn and A. M. Goebel	
85.	AMG187-UBethM2	--	Metamorph	Whole leg	"	
86.	AMG187-UBethM3	--	Metamorph	Whole leg	"	
87.	AMG402(BC6)-96-3	PhotoID	Adult	Blood	"	
88.	AMG181-Bethyl1	Photo ID	Adult	RBC	"	
89.	AMG182-Bethyl2	Photo ID	Adult	RBC	"	
90.	AMG183-Bethyl3	Photo ID	Adult	RBC	"	
91.	AMG184-Bethyl4	Photo ID	Adult	RBC	"	
92.	AMG177-Bethyl5					
93.	AMG178-BethylM1	--	Metamorph	Whole leg	"	
94.	AMG179-BethylM2	--	Metamorph	Whole leg	"	
95.	AMG180-BethylM3	--	Metamorph	Whole leg	"	
96.	AMG97-IPA	--	Metamorph	Whole leg	Boulder County, CO N=7. Lost Lake, Indian Peaks Wilderness, 2.6 miles W of Eldora. September, 1991. Collectors: P.S.Corn, A.M. Goebel	
97.	AMG-98-IPB	--	Metamorph	Whole leg	"	
98.	AMG-99-IPC	--	Metamorph	Whole leg	"	
99.	AMG-100-IPD	--	Metamorph	Whole leg	"	
100.	AMG-101-IPE	--	Metamorph	Whole leg	"	
101.	AMG-102-IPF	--	Metamorph	Whole leg	"	

102.	AMG-314-HM1	PhotoID	Adult	RBC	Clear Creek County, CO N=6. Settling Ponds, Henderson Mine Rd. Collectors: A. M. Goebel, J. D. Wortman.
103.	AMG-315-HM2	PhotoID	Adult	RBC	"
104.	AMG-316-HM3	PhotoID	Adult	RBC	"
105.	AMG-317-HM4	PhotoID	Adult	RBC	"
106.	AMG-318-HM5	PhotoID	Adult	RBC	"
107.	AMG-319-HM6	PhotoID	Adult	RBC	"
108.	AMG-319-HM7	PhotoID	Adult	RBC	"
109.	AMG-319-HM8	PhotoID	Adult	RBC	"
110.	AMG-319-HM9	PhotoID	Adult	RBC	"
111.	AMG-315-HM10	PhotoID	Adult	RBC	"
112.	AMG-316-HM11	PhotoID	Adult	RBC	"
113.	AMG-317-HM12	PhotoID	Adult	RBC	"
114.	AMG-318-HM13	PhotoID	Adult	RBC	"
115.	AMG-319-HM14	PhotoID	Adult	RBC	"
116.	AMG-319-HM15	PhotoID	Adult	RBC	"
117.	AMG-319-HM16	PhotoID	Adult	RBC	"
118.	AMG-319-HM17	PhotoID	Adult	RBC	"
119.	AMG-319-HM18	PhotoID	Adult	RBC	"
120.	AMG-319-HM19	PhotoID	Adult	RBC	"
121.	AMG-482-96-83	USNM	Juvenile	Muscle	toad from captive breeding program
122.	AMG-504-HMH1	--	Eggs		Henderson Mine, "Hezbo". Collector: Lauren Levo
123.	AMG-505-HMH2	--	Eggs		"
124.	AMG-506-HMH3	--	Eggs		"
125.	AMG-507-HMH4	--	Eggs		"
126.	AMG-508-HMH5	--	Eggs		"
127.	AMG-509-HG6	--	Eggs		Henderson Mine, "Herman Gulch". Collector: Lauren Levo.
128.	AMG-510-HG7	--	Eggs		"
129.	AMG-511-HG8	--	Eggs		"
130.	AMG-512-HG9	--	Eggs		"
131.	AMG-513-HG10	--	Eggs		"
132.	AMG-514-HG11	--	Eggs		"
133.	AMG-515-HG12	--	Eggs		"
134.	AMG-516-HG13	--	Eggs		"
135.	AMG-517-HG14	--	Eggs		"
136.	AMG-518-HG15	--	Eggs		"
137.	AMG-320-CLCR7	PhotoID	Adult	RBC	Clear Creek County, CO N=3. Clear Creek, 0.95 miles below Clear Creek Campground, off Hwy 40. Collectors: A. M. Goebel, J. D. Wortman.
138.	AMG-321-CLCR8	PhotoID	Adult	RBC	"
139.	AMG-322-CLCR9	PhotoID	Adult	RBC	"
140.	AMG393-PCGC1	PhotoID	Adult	RBC	Grand County, CO. N=4. Pole Creek Golf Course, Winter Park, CO. 3 June 1995. Collectors: A. M. Goebel, J. D. Wortman, G. Horstman.

141.	AMG394-PCGC2	PhotoID	Adult	RBC	"
142.	AMG395-PCGC3	PhotoID	Adult	RBC	"
143.	AMG396-PCGC4	PhotoID	Adult	RBC	"
144.	AMG463-96-64	--	--		Gore Creek Beaver Pond above Golf Course. Collector: Toby Mourning.
145.	AMG-480-96-81	--	--		North Ten Mile Creek. Collector: Greg Horstman.
146.	AMG-481-96-82				
147.	AMG-398-CalP1	PhotoID	Juvenile	RBC	Routt County, CO N=2. First Creek, California Park, Routt National Forest, 100 ft W of FR150. Collectors:
148.	AMG-399-CalP2	PhotoID	Juvenile	RBC	"
149.	AMG-483-96-84				
150.	AMG-351-STEVE1		Adult		Love Lake, San Juan Mts. Collector: Steve Corn
151.	AMG-419-96-20	PhotoID	Adult	RBC	Mineral County, CO N= Trout/Fern Creeks. Collector: John Goettl, 6 June, 1996.
152.	AMG-422-96-23	PhotoID	Adult	RBC	"
153.	AMG-420-96-21	PhotoID	Adult	RBC	"
154.	AMG-421-96-22	PhotoID	Adult	RBC	"
155.	AMG-418-96-19	PhotoID	Adult	RBC	"
156.	AMG-472-96-72	--	Tadpole	tail	"
157.	AMG-473-96-73	--	Tadpole	tail	"
158.	AMG-474-96-74	--	Tadpole	tail	"
159.	AMG-475-96-75	--	Tadpole	tail	"
160.	AMG-467-96-68	--	Tadpole	tail	"
161.	AMG-521-97-31	--	Eggs		Jumper Creek, Mineral Co. Collector: Craig Fetkavich
162.	AMG-522-97-32	--	Eggs		"
163.	AMG-524-97-34	--	Eggs		"

UCM - University of Colorado Museum, Boulder, CO
USNM - U.S. National Museum, Smithsonian Institution, Washington, D.C.
PhotoID - Photographic Identification
PIT - Passive Internal Transponder (PIT tag) Number