Colorado Division of Wildlife

Boreal Toad Research Progress Report

1999

Mark S. Jones (Editor)

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Preface

The Boreal Toad Recovery Team was formed in 1994, in response to reports of significant declines in boreal toad distributions in the Southern Rocky Mountains. These apparent declines resulted in an "Endangered" listing by Colorado and a "Status 2" species designation federally. The boreal toad is currently considered "warranted but precluded" for federal listing under the Endangered Species Act. The first Boreal Toad Recovery Plan was completed in 1997 under the direction of John Goettl; the Recovery Plan and Conservation Agreement have now been combined into one working document (Loeffler [ed.] 1998). Currently, the Recovery Team is coordinated by Chuck Loeffler, the Colorado Division of Wildlife (CDOW) Manager for Reptiles, Amphibians, Mollusks, and Crustaceans.

This report covers boreal toad research sponsored by the CDOW in 1999 by several researchers and has been consolidated 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 results of :

- 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. This work was confounded in 1999 by a disease outbreak identified as chytridiomycosis; this topic will be covered also. Mark Jones 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.

- Research conducted under a CDOW MOU with Colorado State University to develop a statistical/spatial habitat model for the boreal toad. The principal investigator is Andy Holland.

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

HENDERSON/URAD BOREAL TOAD STUDIES

SITE DESCRIPTION AND BACKGROUND

Research on population size, stability, movement, and habitat use has been conducted at the Henderson/Urad Mine since 1995. The Henderson Mine breeding locality consists of numerous ponds and wetlands in an area which is heavily disturbed due to molybdenum mining by the Climax Molybdenum Company. The mine is located west of Empire, Colorado at an elevational range of 10,000 to 10,500 feet. The specific breeding sites have been designated as follows: Power Alley, Hesbo, Treatment Pond, Donut, Anne's Pond, 2-Pond, and Upper Urad (Figure 1). In 1999, egg masses were located at two additional sites at the mine, with survival to metamorphosis at both.

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

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

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

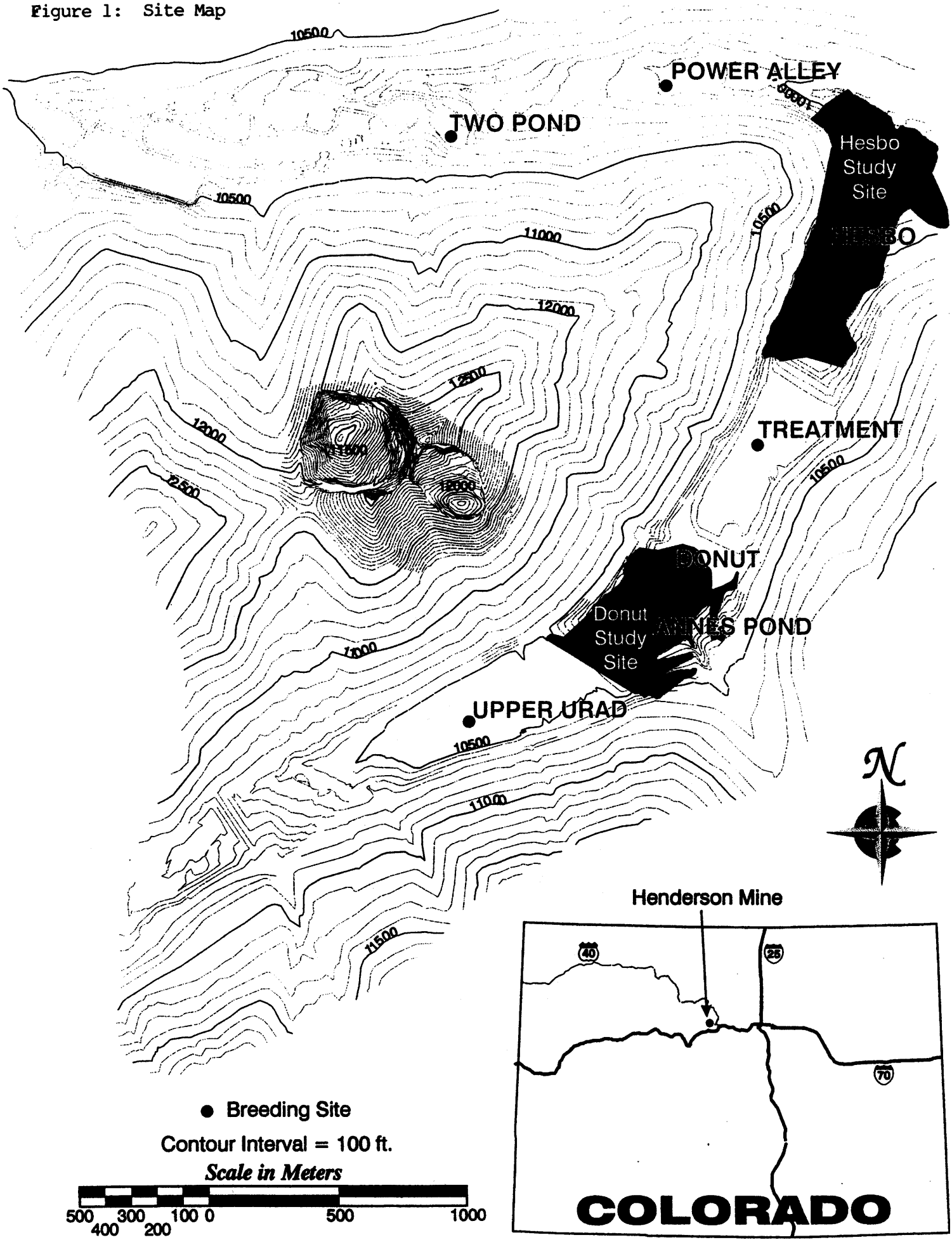
Donut is a newer pond above the water treatment facility which serves as a catch basin for some of the upstream runoff. This site typically has 5-6 egg masses but because it is higher in elevation than the other sites, breeding occurs later making weather conditions post

metamorphosis critical to toadlet survival and dispersal. In addition, there are few suitable hibernaculum close to this site. All toadlets froze in 1995 and 1996. We believe some toadlets survived in 1997. Survival of toadlets was good in 1998 and 1999, presumably a result of increased vegetation and small mammal burrows on the islands.

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

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

Figure 1: Site Map



MATERIALS AND METHODS

The Henderson/Urad breeding population was 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 for radio telemetry 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 Conservation Plan and Agreement (Loeffler [ed.] 1998). Each toad captured during the night surveys was sexed, weighed (±0.1g), and measured (snout to urostyle length, ± 0.01mm). Each individual was then scanned for a PIT tag and if one was not found, a tag (AVID ITI-125S) was inserted dorsally. The tags were inserted by pinching the skin on the toads back (slightly off center and anterior), making a small incision using sterile scissors, inserting the sterile tag in a posterior direction using forceps, and closing the incision using surgical adhesive. All PIT tag numbers were recorded along with the other pertinent data on individuals and site. 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.

Twenty-nine toads (fifteen males and fourteen females) were radio tagged in May and June 1999 at Hesbo, Donut, and Anne's Pond with Holohil BD-2G radio transmitters weighing 2g each, with an expected battery life of six months. The radios were fixed to the toads using a waist harness constructed of plastic coated fishing leader material fastened with crimp collars inside 2mm vinyl tubing. An additional five toads (all males) were tagged during the summer as replacements for individuals killed by various predators (Jones et al 1999), disease, or which lost their transmitters (Table 1).

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

Central to the study of boreal toad biology is their use of various habitats and our ability to define their habitat requirements and preferences. This process involves defining the availability of individual habitat types within the study area and then determining the usage of these habitats. We can determine individual use of various habitats through our radio tracking activities; defining what is available is not as easy, and in almost all studies involving habitat selection, this is a subjective decision based on the researchers knowledge of the animals movement. By changing the spatial scale of what an investigator deems to be available to an animal or if habitat types are arranged in an aggregated pattern (Porter and Church 1997), the resultant conclusion about selection or preference for individual habitat types will also change.

TABLE 1: Contact Statistics for Radio-Tagged Boreal Toads in the Henderson/Urad Study Area in 1999

Toad ID	Start Date	Sex	Contacts	Days Monitored	Comments
041	05/26/99	M	9	63	#589 from 1998 - found dead 7/27/99 NE island Donut
042	05/26/99	F	16	98	Through August - still tracking
043	06/15/99	F	15	70	Through August - still tracking
044	05/26/99	F	12	70	8/23/99 - last date radio heard
045	05/26/99	F	9	56	Radio fell off - found 7/27/99
046	05/26/99	M	8	56	Radio fell off - found #585 last year
047	05/26/99	F	4	28	Radio faded out - could not hear after 6/22/99
048	05/27/99	F	6	48	Tracked to Red-Tailed hawk's nest
049	05/26/99	F	7	41	Found dead at treatment
050	05/26/99	F	14	106	Radio fell off 9/8/99
051	05/27/99	M	6	43	Found dead 7/5/99
052	05/27/99	F	19	118	Found dead 9/21/99 in Donut
053	05/27/99	M	2	20	Radio found - across S. Urad Lake - suspected hawk kill
053-588	06/15/99	M	5	24	Looked sick - sent to Pathologist
548	07/9/99	M	11	54	Thru Aug. - still tracking winter radio
054	05/31/99	M	2	16	Radio found across So. Urad Lake - suspected hawk or raven
055	05/31/99	M	6	38	Found dead in Hesbo 7/7/99
056	05/31/99	M	4	30	Found dead in Hesbo ditch 6/29/99
057	05/31/99	M	4	30	Found dead 6/29/99 SW and S. Urad Lake
671	06/29/99	M	9	62	Dug up - found radio & M toad. Put radio on him use #672
672	08/30/99	M	3	2	Through August - still tracking
059	05/31/99	M	5	36	Found dead - spring seep 7/5/99
060	05/31/99	M	14	93	Through August - still tracking
061	05/31/99	M	7	44	Found dead in Hesbo sedge 7/13/99
969	07/14/99	M	10	47	Still tracking winter #924
062	05/31/99	M	4	30	Found dead in So. Urad Lake 6/29/99
584	05/31/99	M	14	69	Found radio - dug out of hole under willow
586	05/31/99	F	10	64	Found dead in Ann's Pond 8/2/99
592	05/31/99	F	4	30	Found dead SW corner So. Urad Lake 6/29/99
590	05/31/99	M	5	36	Found dead 7/5/99 on hill North of Donut
588	05/31/99	F	11	72	Found dead 8/10/99 between Ann's Pond and Donut
574	05/31/99	F	16	93	Through August - Found dead 10/27/99
587	05/31/99	F	10	72	Could not hear 8/10/99 or after
337	07/13/99		3	22	Eaten by Red-Tailed Hawk. Tracked to nest
752	07/13/99		4	29	Could not hear from 8/10/99 on

For this reason, we used two different spatial scales to define habitat availability. First, we combined all three study areas (Hesbo, Donut, and Upper Urad) because we know from our telemetry work during the last three years that toads can move from one end of the valley to the other and we have seen some interaction between study areas. For the second analysis, we defined availability of habitat types for each study area (because toads generally stayed within their respective area) by drawing a 300 m buffer around the pooled toad locations for each study site and calculating the availability of each habitat type within that polygon (Figure 2).

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 assign habitat types to each location. The habitat categories were defined as aspen/conifer, road, spring, stream, lentic water (lake, reservoir, pond), and rock/grass. In addition, a photograph was taken at each toad location each week to verify the habitat classifications assigned in ARC/INFO. Only toads which had six or more habitat locations were included in the analysis. 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).

Home range analysis was conducted to gain insight into the areas used by boreal toads. This allowed the quantification of differences in home range size between individuals, years, and breeding sites. Home range estimates were produced on two temporal scales for each boreal toad. One estimate is based on all the radio-tracking locations. The other estimate includes only locations observed after 10 June to represent post-breeding home range size. The area used by all toads associated with a breeding site was also approximated by pooling all individual locations for that site. Minimum convex polygon and fixed kernel (Worton 1989) methods were used to estimate the home ranges for all individuals that had six or more radio-tracking locations. Minimum convex polygon estimates were calculated with program CALHOME (Kie et al.1994) and fixed kernel estimates were produced with the Home Ranger, v. 1.5, (Hovey 1999).

Minimum convex polygon (MCP) is a common home range estimation method that assumes a uniform utilization distribution (Samuel and Garton 1985). With the MCP method, any area inside the polygon has an equal probability of containing a location. MCP is calculated by drawing a polygon around a specified percentage of the radio-tracking locations. Ninety-five percent of the locations were used here. MCP has the disadvantages of increasing the size of the home range estimate as the number of locations increases and not allowing for a precision estimate (White and Garrott 1990). MCP is useful, however, to get a general idea about the size and shape of use areas and their relation to the breeding site. Many of the utilization distributions are linear between two core areas and MCP includes these corridors in the estimate.

The fixed kernel method (FK) is a nonparametric method that uses point percentage contours to estimate a utilization distribution (a frequency distribution) for the sample locations (Worton 1989). Ninety-five percent volume contours were used for these fixed kernel home range estimates. This method was chosen because it does not place constraints on the form of the utilization distribution (Worton 1989). This attribute was attractive because the radio-tracking locations for many individuals

have several core areas of use and therefore exhibit multi-modal distributions. The fixed kernel approach was found by Seaman and Powell (1996) to be the most accurate estimator of simulated home ranges. The fixed kernel approach also incorporates uncertainty by including areas where locations were not observed. It is therefore a better approach for estimating home range size when all movements by an animal are not monitored. Incorporation of uncertainty does have the drawback of overestimating home range size when sample sizes are small (Seaman and Powell 1996).

The methods for FK home range estimation employed here included an estimation of the optimum smoothing parameter and estimation of standard errors by bootstrapping. The smoothing parameter controls the width of the kernel (a probability density). Kernel width determines how much influence specific points have on the density estimate (Seaman and Powell 1996). This determines if activity areas will be encompassed by one large polygon or several smaller ones. Least-squares cross-validation is the preferred method for estimating the smoothing parameter (Worton 1989, Seaman and Powell 1996). Program Home Ranger allows calculation of an optimum smoothing parameter, through least-squares cross-validation, for each individual. This method permitted the identification of core activity areas even in relatively small home ranges.

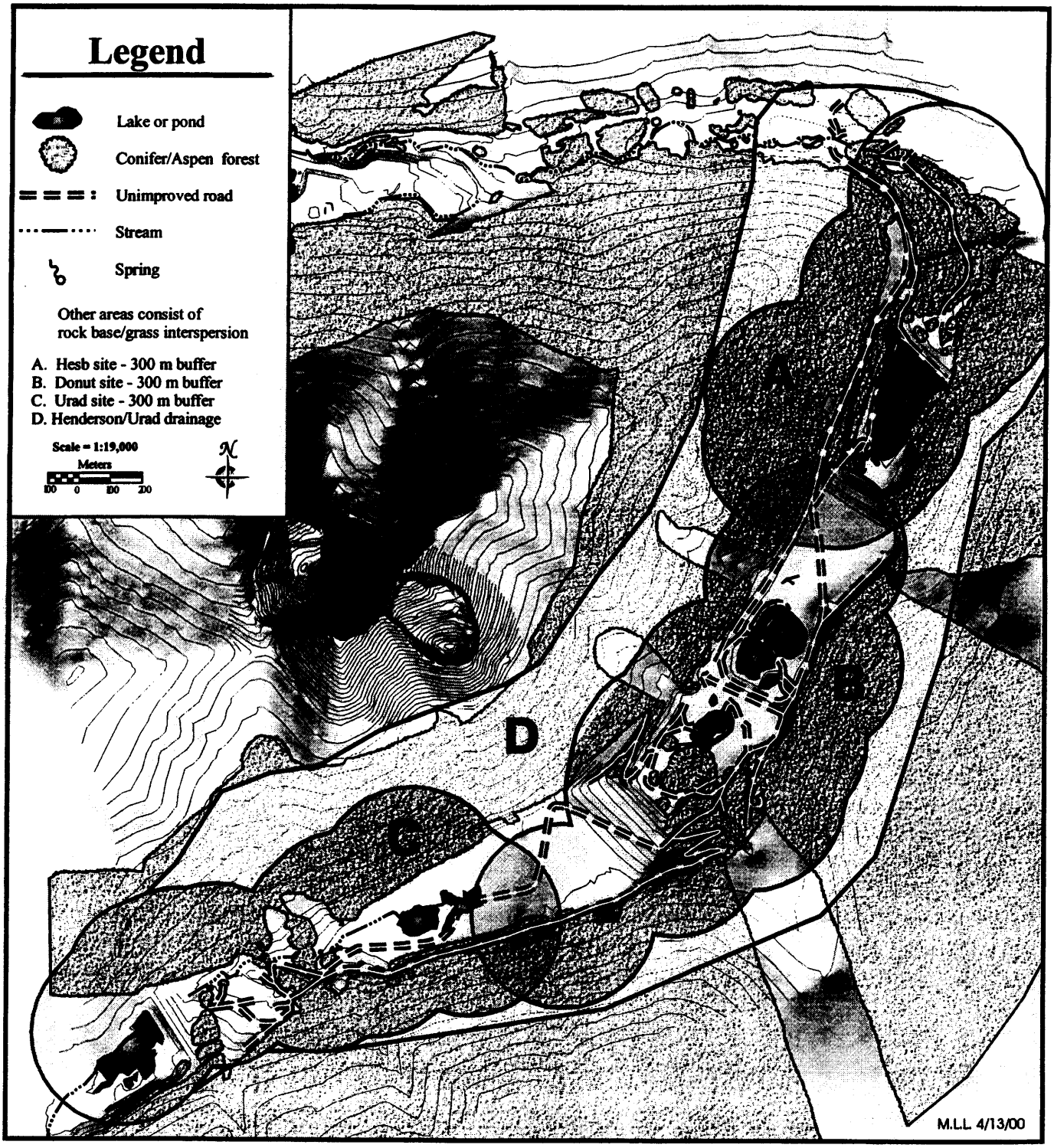
Fixed kernal home range estimates were examined for differences related to year, breeding site, and sex effects. All points for boreal toads with at least six radio tracking locations were used in this analysis. Because a plot of the studentized residuals versus their predicted values revealed increasing variance of the residuals (which violates assumptions of ANOVA model), the nonparametric Kruskal-Wallis Test was used (SAS 1994).

Capture-recapture methods were used to estimate population numbers of males at each breeding site from 1995 to 1999. 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 a t-test (α=0.05).

Pathology work in 1999 was performed by Dr. David Green of the USGS National Wildlife Health Center in Madison Wisconsin, Dr. Joyce Longcore of the University of Maine at Orono, and Dr. Allan Pessier, Pathology Fellow with the Zoological Society of San Diego. Specimens were either sent live on ice packs, preserved in formalin, or frozen with dry ice depending on their condition and the anticipated tests/procedures to be done.

Figure 2: Map of designated study areas in the Henderson/Urad area, 1999



RESULTS and DISCUSSION

Breeding Site Monitoring: 1999

Hesbo- Hesbo was monitored at night weekly from May 18 to June 15, 1999. Additional biweekly daylight surveys were conducted throughout the summer. The peak of breeding activity occurred on May 25 with 72 adults observed (65 male, 7 female). Night surveys were discontinued because all of the adults handled had been previously handled in 1999. Twenty-three egg masses were deposited, resulting in approximately 20,000 tadpoles. During 1999, Lauren Livo continued dytiscid beetle larvae predation studies at this site.

Power Alley- Power Alley was night monitored weekly from May 18 to June 15, 1999. The most adults observed at this site was 33 males, no females were seen during monitoring. One egg mass was laid at this site which later desiccated.

Upper Urad- Upper Urad was night monitored weekly from June 22 to June 29, 1999. Seven adults including one gravid female was the highest number of toads observed on any occasion. One egg mass was deposited which fungused and died. No successful reproduction in 1999.

Donut- Donut was night monitored weekly from May 31 to June 22, 1999. Seventeen egg masses were deposited at this site, several died from fungus. Lauren Livo conducted tadpole ecology experiments at this site. Although some toadlets died from desiccation and exposure at this site, we believe survival was better than in previous years because many metamorphosed onto the islands, which are thickly vegetated and have suitable hibernaculum close to the edge of the water.

Treatment- Treatment was night monitored from June 15 to June 29, 1999. The greatest number of adults observed in one night was four. No egg masses were observed, but based on the groups of tadpoles observed on June 23, we suspect two egg masses were present. Monitoring was continued at this site throughout the summer with good survival to metamorphosis. It is still not known whether many survive the winter at this site as there are few suitable hibernacula and juveniles are not typically seen the following spring .

Anne's Pond- Anne's Pond was monitored from May 25 to June 29, 1999. The most adults observed in one night was 21, we checked a total of 7 females during the course of the active breeding period. Nineteen egg masses were laid, all of which dessicated because of our inability to keep water levels stable.

Other Breeding Sites

1- Pond- Boreal toads were first observed breeding in 1-Pond in 1998. Many juveniles were observed in June, 1999 indicating good over winter survival. In 1999, tadpoles were again observed at this site, probably from two egg masses. Most of the tadpoles metamorphosed and dispersed by September 29.

John's Pond- John's Pond is a small catch basin by the domestic water treatment plant on the Henderson side of the mine. Breeding was first observed at this site in 1998. In 1999, tadpoles from one egg mass were observed on June 30. Most metamorphosed and dispersed by September 29.

Lower Urad Lake- This was the second year we observed breeding in Lower Urad Lake. On June 23, two egg masses were observed in the north west cove. On subsequent visits it appeared that one of these disappeared and the other produced approximately 100 tadpoles. These tadpoles gradually disappeared and it is believed that none survived to metamorphosis.

Climatic conditions each year have a major impact on survival and recruitment. Spring storms frequently kill egg masses and early fall freezing conditions either directly kill toadlets or negatively impact dispersal to suitable hibernaculum. On may 25, 1999 two male and one female adult toads were found dead following a snowstorm. Summer drought can dry breeding ponds before metamorphosis can occur (Anne's Pond 1999). In 1999, several egg masses deposited late, succumbed to fungus, presumably the result of elevated water temperature. 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 for timely metamorphosis and dispersal. Water level fluctuation resulting in desiccation of egg masses is also very common. In 1997, 1998, and 1999 all 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 levels 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. Long term research is needed to define possible long term fluctuations and to distinguish between natural and anthropogenically caused declines (Pechmann et al. 1991).

Habitat Use and Movement

Locational data was collected on a total of 24 radio tagged boreal toads (Table 1) which had six or more contacts and was used to calculate movement and habitat use. A total of 102 locations were recorded for male toads and 134 locations for females. It should be noted that major heterogeniety between individual toads was observed in both habitat use and movement data. Maps of individual radio telemetered toad movements and habitat use may be found in Appendix 1. As defined earlier, habitat availability was defined using two spatial scales. First, preference was determined using the entire Upper Urad drainage as available habitat. Next, preference was based on available habitat using a 300 m buffer around the pooled toad locations at each of the three study areas (Figure 2).

The habitat areas were defined as conifer/aspen, river, spring seep, lake (lentic water), road, and rock/grass. For the combined study areas (N=24), conifer/aspen contained 19.6% of the toad locations and represented 59.0% of the available habitat showing avoidance of this habitat ($P<0.00$). The spring seep category contained 0.8% of the toad locations and represented 0.5% of the available habitat; this use was not significantly out of proportion with availability. The lake category had 16.9% of the

locations and represented 2.6% of the habitat, showing significant selection (P<0.00). Areas defined as road contained 4.7% of the locations and represented 1.8% of the habitat and therefore were selected (P<0.03). Rocky areas were selected for (P<0.00) since they contained 57.3% of the locations and only represented 35.9% of the habitat (Figure 3).

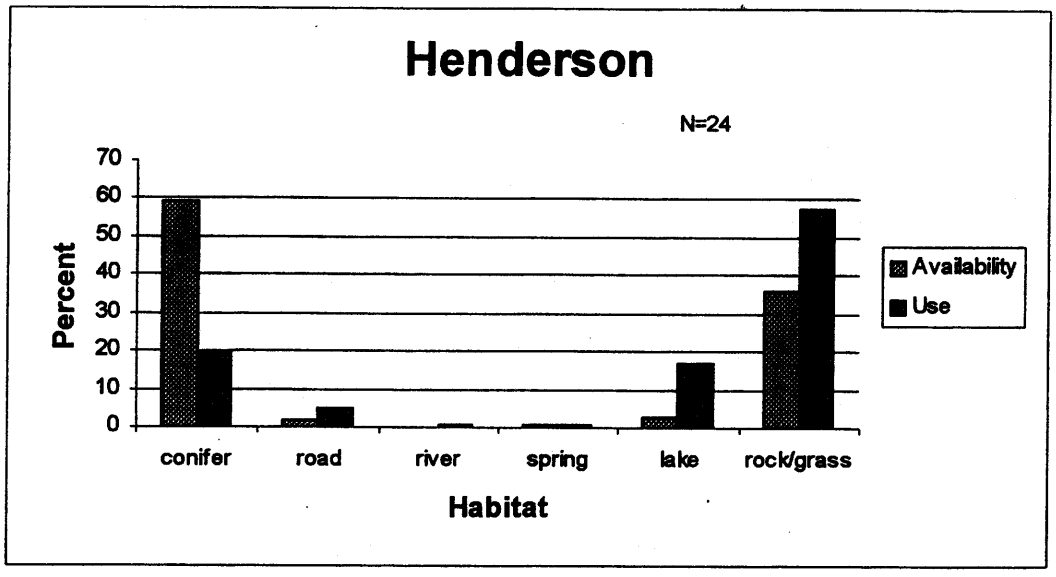


Figure 3. Use of habitat categories in 1998 by boreal toads using the entire Urad valley as available habitat.

Habitat use in each study area was then analyzed separately by defining the available habitat as everything within a 300 m buffer drawn around the pooled toad locations for each site. For the Hesbo study area (N = 16), the conifer/aspen category contained 23.1% of the toad locations and represented 59.1% of the available habitat showing avoidance of this habitat (P<0.00). The spring seep category contained 1.4% of the toad locations and represented 1.7% of the available habitat; this use was not significantly out of proportion with availability. The lake category had 21.1% of the locations and represented 4.3% of the habitat, showing significant selection (P<0.003). Areas defined as road contained 4.8% of the locations and represented 2.1% of the habitat and therefore this category was used randomly. Rocky areas were selected for (P<0.02) since they contained 49.7% of the locations and only represented 32.5% of the habitat (Figure 4). It should be noted, however, that the majority of the rocky areas in the Hesbo study site were actually rock outcroppings within the upland conifer/aspen habitat type.

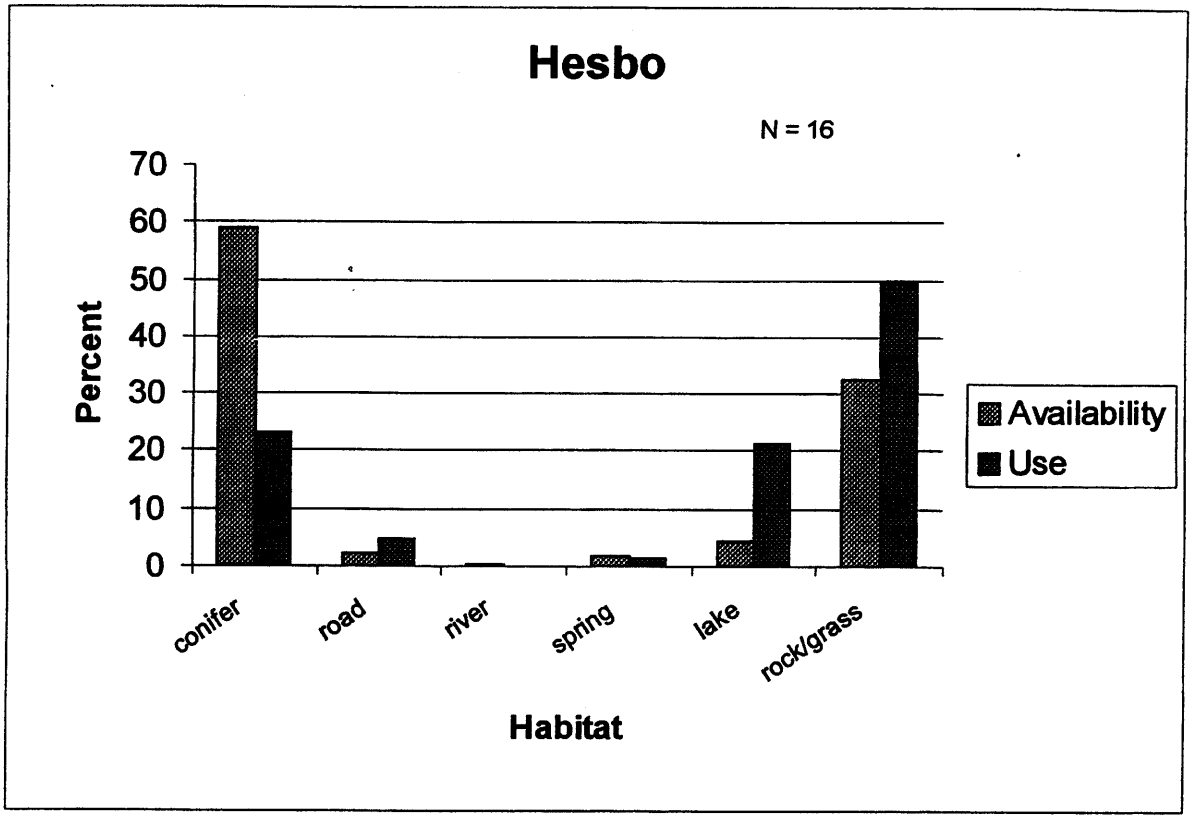


Figure 4. Use of habitat categories in 1998 by boreal toads using the entire Urad valley as available habitat.

For the Donut study area (N=9), the conifer/aspen category contained 18.6% of the toad locations and represented 47.5% of the available habitat showing avoidance of this habitat ($P<0.00$). The spring seep category contained no toad locations and represented 0.1% of the available habitat; this use was not significantly out of proportion with availability. The lake category had 14.0% of the locations and represented 2.8% of the habitat and therefore was used in a proportion greater than available although not significant ($P=0.08$). Areas defined as road contained 5.8% of the locations and represented 3.6% of the habitat and therefore this category was used in proportion to availability. Rocky areas were also used in proportion to their availability, they contained 60.5% of the locations and represented 45.9% of the habitat (Figure 5).

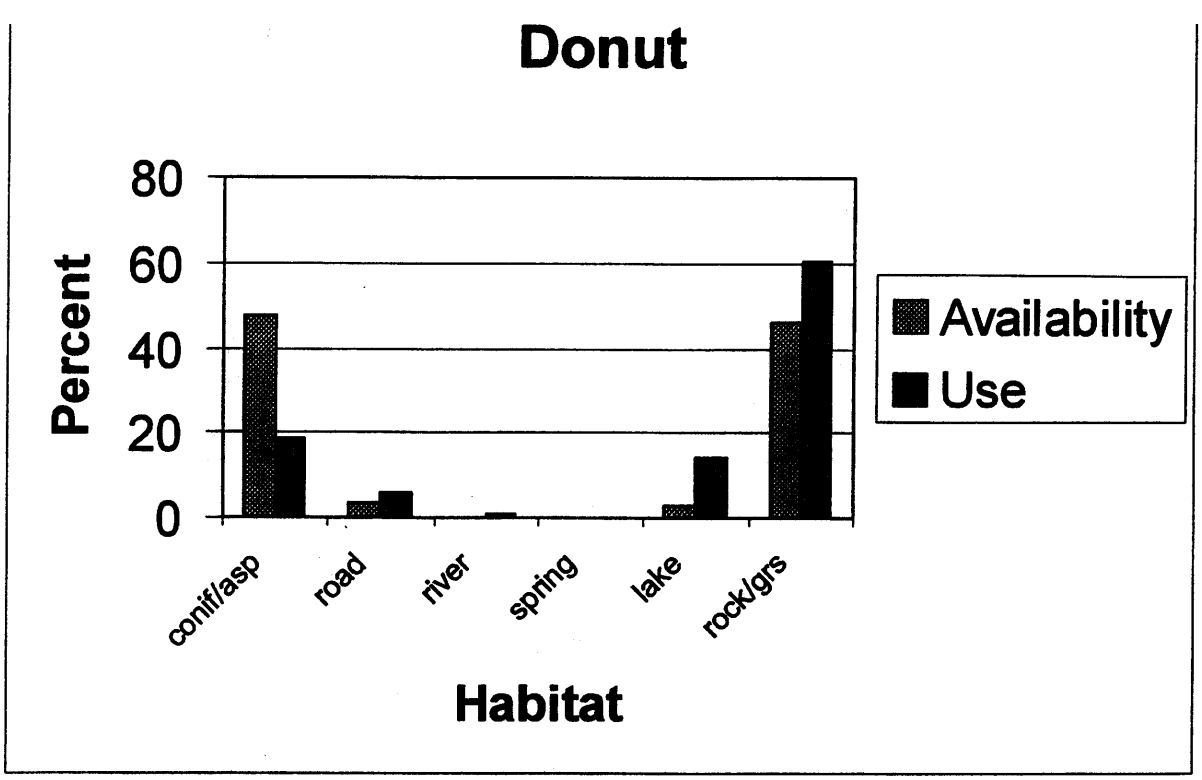


Figure 5. Use of habitat categories in 1999 by boreal toads using a 300 m buffer around the pooled locations in the Donut study area as available habitat.

The only habitat categories which were used in the Urad study area were river and rocky areas, although all other habitat types were present. The river category contained 4.5% of the toad locations and represented 0.3% of available habitat, showing use in proportion to availability. Rocky areas contained 95.5% of the total toad locations but only represented 52.3% of the available habitat showing significant ($P<0.02$) selection for these areas (Figure 6). It should be noted that rocky areas in the Upper Urad study were found both around the breeding site and as rock outcroppings in upland aspen/conifer areas.

Our data for the last three years shows that the toads do indeed use a wide variety of habitat types and that there is 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. Bartelt and and Peterson (1994) found boreal toad habitat selection highly correlated with the presence of shrubs which they were using for both osmo- and thermal regulation. It appears Henderson Mine toads frequently make use of ground squirrel burrows for this purpose.

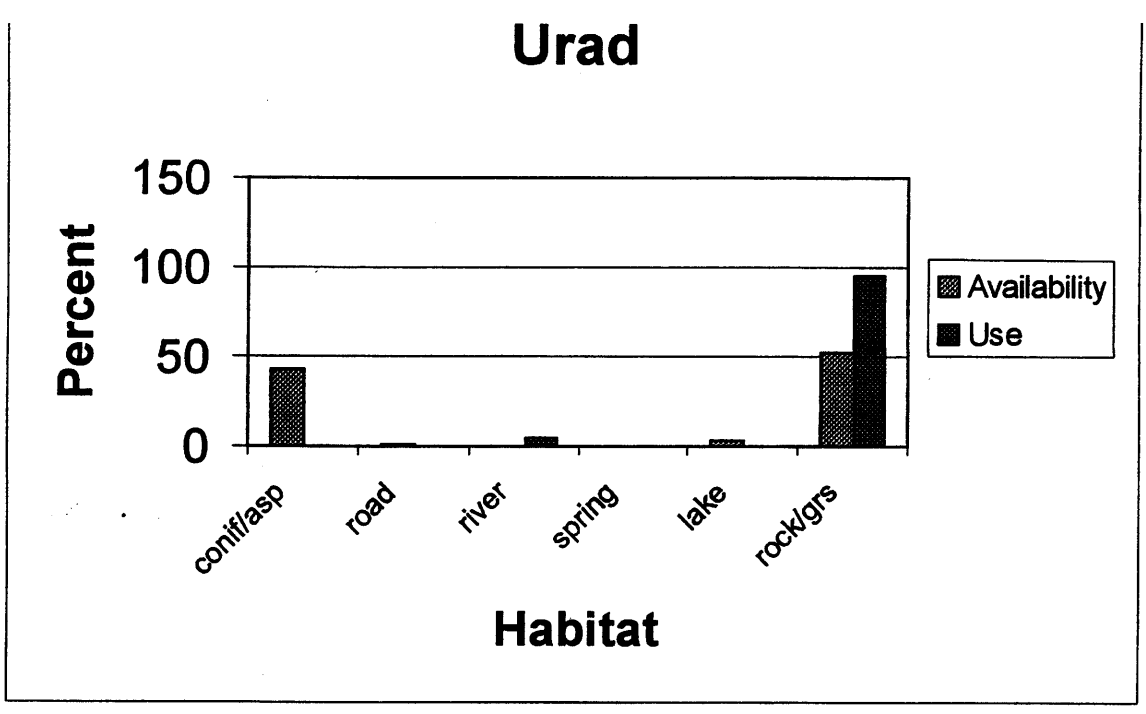


Figure 6. Use of habitat categories in 1999 by boreal toads using a 300 m buffer around the pooled locations in the Urad study area as available habitat.

Movement was calculated for each toad weekly on a 3 m^2 digital elevation model in ARC/INFO as previously described. Two hundred thirty five individual weekly movement measurements were calculated for 24 toads. The average distance moved per day for all telemetered toads was 22.5 m (SD=46.6, N=235). Male toads moved an average of 15.9 m per day (SD=28.2, N=133) and females moved an average of 31.0 m per day (SD=62.2, N=102). There was much greater variability between female average daily movement increments than male, Figure 7. This was due to females moving long distances away from the breeding sites after depositing eggs. The minimum average distance moved per day was 0.6 m by a female, which was tracked for 70 days, and the maximum average daily movement was 211.1 m by a female monitored for a total of 118 days. The maximum distance traveled by any telemetered toad during the summer of 1999 was 6,484.8 m by a female monitored 106 days. Average daily movement increments for telemetered toads were significantly greater for females than males ($p = 0.01$, $t = -2.47$, $df = 233$).

Boreal toad movement patterns are highly variable between individuals. 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 further into upland habitats.

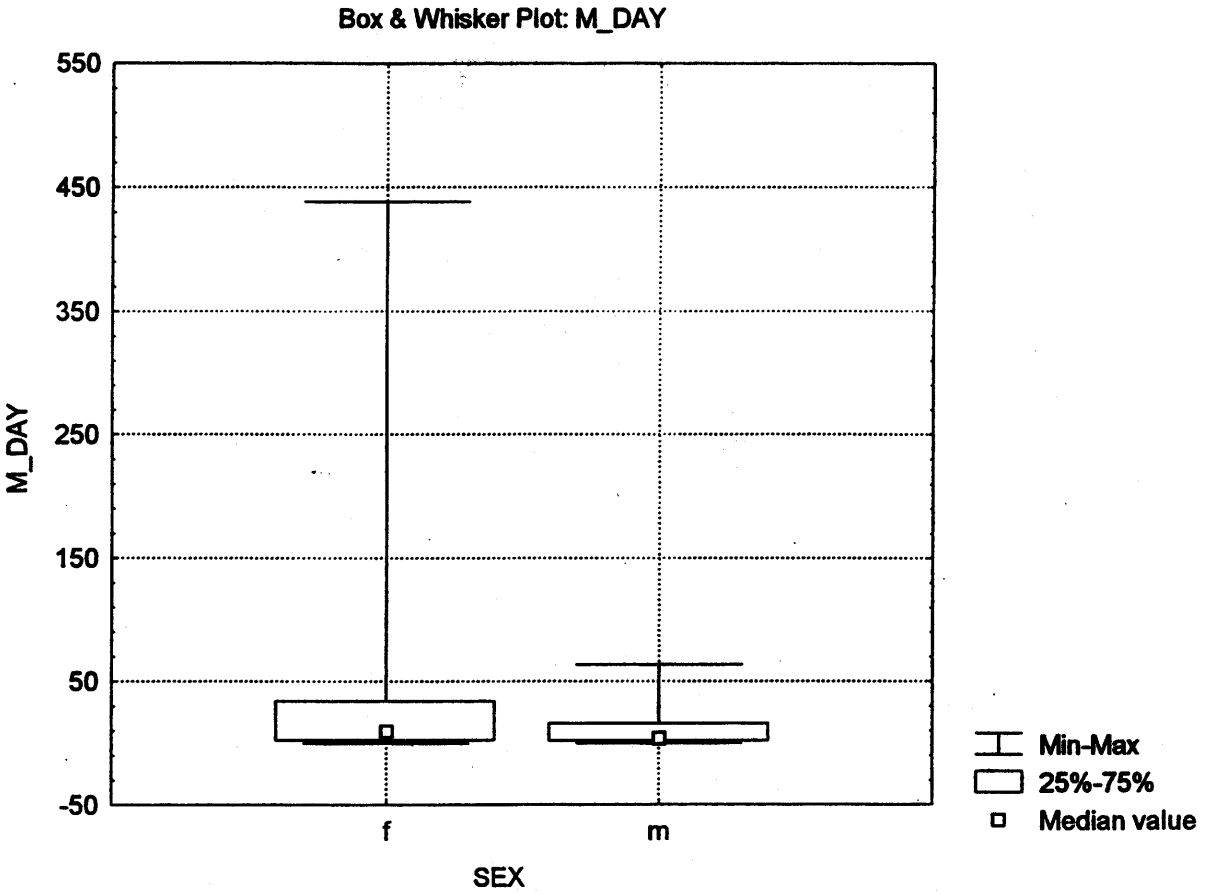


Figure 7. Comparison of male and female boreal toad daily movement increments (male=133, female=102) at the Henderson/Urad study site in 1999.

Home Range Estimates

As with movement, there were major differences (heterogeneity) between individual toad home range sizes. We can probably assume that we are missing movements when we only locate individuals once per week. Therefore, I feel that the FK method is probably more useful in terms of recommending construction setbacks or designating critical habitat. If a toad has two core use areas, the FK method will result in a smaller home range size because it will draw circles around each use area, whereas the MCP will use one area that encompasses all the points. Therefore the FK method better represents habitat that is commonly used, ie. it doesn't include the path the toad took to get to the second area. The mean home range area in 1999 for telemetered boreal toads in the Henderson population was 12.2 ha using the FK method and 13.8 ha with the MCP (Table 2). The mean area used by males was 7.1 ha using the FK method and 0.9 using the MCP approach. The mean area used by females was 16.9 ha using the FK method and 25.6 ha using the MCP approach. The mean home range area excluding the breeding site was 18.8 ha using the FK method and 13.0 ha with the MCP. The mean area used by males was 1.3 ha using the FK method and 1.0 ha using the MCP approach. The mean area used by females was 26.0 ha using the FK method and 21.4 using the MCP approach. Cumulative home range estimates were also calculated for all telemetered toads at each site for 1997, 1998, and 1999 (Table 4).

The Kruskal-Wallis Test revealed highly significant year effects when comparing 1998 home range sizes to 1999 (chi-square = 21.5, df = 1, $P < 0.001$). There were no significant site (chi-square = 0.47, df = 1, $P = 0.49$) or sex effects (chi-square = 0.36, df = 1, $P = 0.55$) and little evidence to support them. The differences in home range size between years may be the result of climatic conditions. During cooler or wetter summers the telemetered toads have larger home ranges that encompass areas further away from the breeding site.

I feel there are some general tendencies which warrant discussion. In general, females move further from the breeding site after breeding (possibly because they do not return to breed each year) and set up a fairly discreet home range. This increases the variability in female home range size when the breeding site is included (Figure 8). In general, males don't go as far from their breeding site (possibly because they return each year) but may move around quite a bit in a fairly well defined area. Males often return to the breeding site or other wetland in the vicinity several times during the summer and then return to upland habitats. Females seem to be more inclined to take up residence in an upland area which contains a spring or wet area and seldom return to the breeding area during the summer. The same toads (both sexes) were observed repeatedly 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. Other authors have also noted distinct home range areas in anuran populations (Brattstrom 1962; Campbell 1976; Parker and Gittins 1979; Bartelt and Peterson 1994).

Table 2. Home range estimates for radio telemetered toads (all points) in the Henderson Mine area in 1999.

Year	Site	Toad Number	Sex	Locations	Fixed kernel (ha)	Se (fixed kernel)	MCP (ha)
1999	Donut	41	M	8	0.91	0.098	0.82
1999	Hesbo	42	F	16	1.27	0.11	2.73
1999	Hesbo	43	F	18	1.22	0.09	3.55
1999	Donut	44	F	12	46.69	4.47	85.80
1999	Hesbo	45	F	9	3.69	0.38	6.02
1999	Donut	46	M	10	3.40	0.48	1.14
1999	Hesbo	48	F	7	4.71	0.70	0.06
1999	Hesbo	49	F	7	22.11	3.11	7.51
1999	Donut	50	F	14	85.17	6.47	139.90
1999	Hesbo	51	M	6	57.77	13.42	1.22
1999	Donut	52	F	17	15.98	1.78	25.68
1999	Hesbo	53	M	7	1.36	0.25	0.41
1999	Hesbo	55	M	6	1.80	0.30	0.16
1999	Hesbo	60	M	14	1.86	0.20	2.89
1999	Hesbo	61	M	7	6.85	1.42	0.16
1999	Hesbo	358	M	8	1.43	0.27	0.03
1999	Hesbo	548	M	11	2.05	0.30	2.36
1999	Donut	574	F	19	1.17	0.06	4.91
1999	Hesbo	584	M	15	0.32	0.04	0.19
1999	Donut	586	F	8	5.15	0.47	12.22
1999	Donut	587	F	9	14.62	1.66	16.18
1999	Donut	588	F	11	1.77	0.16	2.36
1999	Hesbo	969	M	11	0.21	0.02	0.47
Mean Home Range					12.24		13.77
Mean	Male				7.09		0.90
	Female				16.96		25.57

Table 3. Home range estimates for radio telemetered toads in the Henderson Mine area in 1999, breeding site locations deleted.

Year	Site	Toad Number	Sex	Locations	MCP (ha)	Fixed kernel (ha)	Se (fixed kernel)
1999	Donut	41	M	7	0.27	0.30	0.04
1999	Hesbo	42	F	14	1.20	0.83	0.09
1999	Hesbo	43	F	15	1.72	0.40	0.03
1999	Donut	44	F	10	17.11	131.55	18.82
1999	Hesbo	45	F	6	2.68	54.79	14.61
1999	Donut	46	M	8	1.00	3.18	0.45
1999	Donut	50	F	12	139.90	94.50	6.71
1999	Donut	52	F	16	25.68	15.56	1.91
1999	Hesbo	60	M	12	2.78	1.87	0.16
1999	Hesbo	358	M	8	0.03	1.43	0.30
1999	Hesbo	548	M	11	2.36	2.05	0.25
1999	Donut	574	F	17	4.82	1.05	0.05
1999	Hesbo	584	M	13	0.15	0.25	0.03
1999	Donut	586	F	7	12.22	5.94	0.53
1999	Donut	587	F	8	6.74	4.84	0.43
1999	Donut	588	F	10	2.36	1.61	0.15
1999	Hesbo	969	M	11	0.47	0.21	0.02
Mean home range					13.01	18.84	
Mean	Male				1.01	1.33	
	Female				21.44	25.96	

Table 4. Cumulative boreal toad home range estimates by site from the Henderson study area.

Year	Site	Tag Number	Sex	Locations	Fixed Kernal	SE fixed kernal	Minimum Convex Polygon
1999	Hesbo	all	both	142	10.06	0.35	12.09
1999	Donut	all	both	108	52.16	2.73	57.40
1998	Hesbo	all*	both	196	18.25	1.04	9.83
1998	Donut	all	both	115	22.21	1.35	2.52
1997	Hesbo	all	both	114	2.96	0.14	4.45
1997	Donut	915 and 919	both	23	1.01	0.07	1.50

* Excludes toad 587– Radio harness injury.

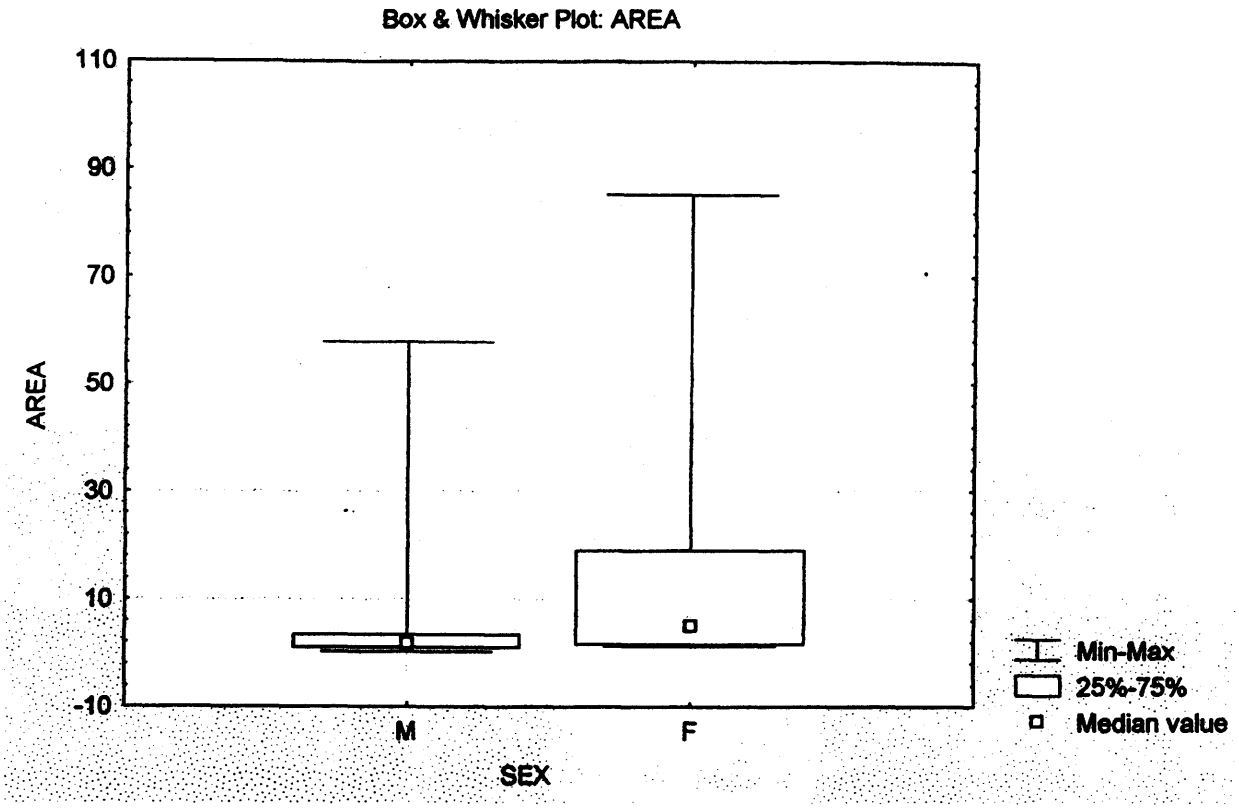


Figure 8. Comparison of home range sizes for male and female telemetered toads at the Henderson Mine in 1999, area in square meters.

Breeding Site Population Estimates

Boreal toads at theUrad/Hendersonbreeding sites were PIT tagged during 1995 to 1999 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 5. Population estimates for male boreal toads at the breeding sites in the Urad/Henderson area from 1995 to 1999.

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

In all cases, the estimate derived from the Capture model (Table 5.) was nearly the same as the total number handled at each site indicating we had PIT tagged and handled close to the entire breeding population of males each year at each site. Based on the 1996 estimates, the male breeding population in the Henderson/Urad metapopulation was approximately 227, 233 in 1997, 306 in 1998, and 188 in 1999. There were not enough tags implanted at all sites to calculate estimates, especially in 1998 when breeding occurred on a small scale at several new locations. This type of work is critical in defining natural fluctuation in breeding numbers over time due to dominant year classes and identifying declines due to other causes such as disease.

Female Returns

As stated earlier, the number of female boreal toads in the Henderson/Urad area is difficult to estimate because they were never recaptured again in the same year, and only rarely in subsequent years. Table 6 lists all females which returned to breed from 1995 to 1999. During this time we pit tagged a total of 86 unique females and had 10 returns. This is fairly conclusive evidence that it is

unusual for females to breed in consecutive years, although it does occur. Six hundred nineteen unique males were tagged during this same time period .

Other evidence which indicates females generally don't breed every year is breeding site sex ratios. Our data, and work conducted by Campbell in 1976 indicate that male:female capture rates during breeding are skewed toward male dominance, even though sex ratios observed after breeding approximate 50:50. From 1995 to 1999 during breeding site surveys, a total of 86 individual females (all years and all sites combined) were handled in comparison to 221 males in 1995, 223 males in 1996, 209 males in 1997, 306 males in 1998, and 177 males in 1999 (all sites combined). The yearly male:female sex ratios were 20:1 in 1995, 32:1 in 1996, 10:1 in 1997, 8:1 in 1998, and 9:1 in 1999. More research needs to be conducted on the biology, physiology, and population dynamics of female boreal toads as this information may be a key link in recovery efforts. Trends in population size and breeding success at all known boreal toad breeding sites is being monitored on an ongoing basis. It is obvious 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.

Females also show the high degree of breeding site fidelity exhibited by males as only one female returned to a different site than originally tagged even though they routinely traveled the length of the Urad valley as documented by our radio tracking activities. There is a substantial amount of evidence that adult male boreal toads exhibit a high degree of breeding site fidelity and rarely change breeding sites unless their natal site is destroyed. Data from 1995 to 1997 is summarized in Jones et al. 1998. From 1995 to 1999, 94% of all males handled during breeding site surveys never changed sites, only 6% of the males handled visited a different site at least once during those years.

Table 6. Returns of PIT tagged female boreal toads from 1995 to 1999 at the Henderson Mine study area.

Tag Number	Site(s)	Year	SV length (mm)	Weight (g)
17325635	Hesbo	1996	85.00	43
	Hesbo	1997	78.83	52
17579122	Annes Pond	1996	85.00	79
	Annes Pond	1998	83.91	82
28365802	Hesbo	1998	70.86	45
	Hesbo	1999	76.52	55
11098104	Hesbo	1995	85.00	75
	Hesbo	1996	75.00	65
11360034	Annes Pond	1998	78.97	71
	Annes Pond	1999	76.41	68
14893850	Hesbo	1997	75.60	49
	Hesbo	1998	76.46	54
15585598	Hesbo	1996	87.00	72
	Hesbo	1999	85.00	65
16298110	Hesbo	1997	77.91	65
	Hesbo	1999	79.60	81
16576774	Donut	1996	80.00	64
	Upper Urad	1997	82.90	67
15520298	Power Alley	1995	70.00	40
	Power Alley	1997	75.41	41

An Overview of Colorado Population Declines and Implications of Chytrid Fungus

Declines of amphibians both in Colorado and world wide have been attributed to a variety of causes including disease (Carey 1992), habitat alterations (Altig and Dodd 1987), predators (Jones et al. 1999), UV radiation (Blaustein et al. 1994), and climatic events (Corn and Fogleman 1984). Other researchers believed that declines reflected little more than long term natural population fluctuations (Pechmann et al. 1991).

There is a growing mass of evidence that implicates a pathogenic fungus as one of the primary causes of declines of certain amphibians in Colorado from the mid 1970's to present. A newly identified chytrid fungus, *Batrachochytrium dendrobatitis* (Longcore et al. 1999) that infects and can kill amphibians has been found in Australia, Central America, and North America (Daszak et al. 1999).

On June 29, 1999 four radio telemetered boreal toads in the Hesbo breeding site area were found dead with no outward signs of the cause of death. This occurrence was alarming since we had experienced minimal mortality of radio telemetered toads in the previous three years of radio tracking, and when we did lose a toad, the cause of death was usually apparent such as predation by a raccoon. Subsequent to finding the first mortalities, more extensive searches were conducted. Eight more toads were found dead during the week of July 5th, two of which were not telemetered which indicated the mortality was not associated with the radio tracking activities. Observed toad mortality seemed to spread from the Hesbo breeding site up the valley to Treatment and then Donut and Anne's Pond. This sequence of mortality may be temperature related and more research will be conducted in 2000 to investigate this potential relationship.

The cause of death of the previously mentioned toads was attributed to chytridiomycosis due to *Batrachochytrium dendrobatidis* by Dr. David Green (USGS National Wildlife Heath Center) on July 14, 1999. This diagnosis confirmed our fears that this pathogenic fungal species was present at the Henderson/Urad location. Since the first diagnosis of chytridiomycosis at the Henderson Mine, there have been a total of 24 toad deaths at this site which were confirmed cases of chytrid infection (Table 7). The radio tracking project allowed early detection of the die-off and enabled us to collect specimens which were useful for histology, isolation, and culture of the fungus. During our radio tracking activities in previous years it was common to observe six to eight non-telemetered toads per day. Toward the end of the 1999 field season, non-telemetered toads were rarely seen which is a good indication that the die-off may be extensive in this metapopulation. We will be able to assess the extent of the mortality when we conduct breeding site surveys in spring 2000 and calculate subsequent population estimates from this data.

Table 7. List of toads found dead at the Henderson Mine in 1999

Toad ID	Start Date	Sex	Where Radioed	Comments
592	06/29/99	F	DONUT	SE corner of L. Urad Lake - grass/willow
057	06/29/99	M	HESBO	SW area of L. Urad Lake - grass/willow
056	06/29/99	M	HESBO	In borrow pit SW of Hesbo - grass/water
062	06/29/99	M	HESBO	4 feet out in L. Urad Lake - SW area
059	07/05/99	M	HESBO	Spring seep NE of Hesbo - 50 feet below U. L.
051	07/05/99	M	HESBO	30 feet SW of Hesbo Dam
049	07/05/99	F	HESBO	NE end of treatment - 60 feet from road
	07/05/99	M		About 2 feet from 049 above
	07/05/99	M		About 100 feet from to NW in edge of pond
*	05/25/99	M		Found dead in Hesbo breeding pond after snowstorm
*	05/25/99	M		Found dead in Hesbo breeding pond after snowstorm
*	05/28/99	F		Found dead in Hesbo breeding pond after snowstorm
590	07/05/99	M	DONUT	At base of cliffs West of Donut
053	07/09/99	M	HESBO	Sent to pathologist live
055	07/07/99	M	HESBO	Lauren found dead in Hesbo near shed
*048	07/14/99	M	HESBO	In Red Tailed Hawk's nest up hill
041	07/27/99	M	ANN'S POND	SE corner of East island Donut
*337	08/03/99	M	HESBO	Eaten by Red Tailed Hawk
	08/03/99	M	ANN'S POND	Found dried up on S shore Ann's Pond
588	08/10/99	F	DONUT	Half way between Ann's Pond & Donut west hill
586	08/02/99	F	ANN'S POND	North edge Ann's Pond. Lauren found
	08/13/99	M	HESBO	
061	07/13/99	M	HESBO	In sedge SE part of Hesbo
052	09/21/99	F	DONUT	In water on NW side of Donut
574	05/31/99	F		Found dead 10/27/99 - Chytrid

KEY

* Not related to die off
- 9/8/99 Lauren found sick toad Pit tag #028556830, male. Sent to David Green (sent live)
- 9/4-6/99 Sent three live tads to Joyce Langcore in Maine (one from Lauren, one radioed with tongue out (358) and one male found down hill from Hesbo

It appears that this chytrid fungus has been in Colorado for quite a few years but it's origin is still unknown. All of the histologic samples collected in 1995 from boreal toad mortalities in the Urad valley as well as captive specimens which were reared at the Fort Collins Research Hatchery were reexamined by Dr. Allan Pessier (San Diego Zoological Society) and chytrid was found to be present. Dr. Pesssier also reexamined specimens collected in Rocky Mountain National Park in 1998, chytridiomycosis was determined to be the cause of death. Previous diagnoses of these specimens had implicated the fungus *Basidiobolus* which is very similar morphologically but seldom pathogetic (J. E. Longcore, pers. Comm.). Although no boreal toads were collected during the die-offs reported in the 1970's and 1980's, a northern leopard frog (Rana pipiens) collected during a die-off in the 1970's was found to have been infected with *B. dendrobatitis* (Carey et al. 1999).

At this time there is growing evidence that chytridiomycosis may have been the primary cause of declines experienced by the boreal toad in Colorado in the late 1970's and early 1980's. Although all of the evidence is circumstantial, the earlier reports of die-offs are consistent with mortality associated with the chytrid fungus, *B. dendrobatitis*.

As very little is currently known about this pathogen, substantial resources will be devoted in 2000 to identify the extent of the fungus in Colorado and to develop methods to deal with this problem. During breeding site monitoring we will collect ventral smears from a representative sample of adult boreal toads at each site to look for the presence of chytrid fungus. We are currently funding the development of a PCR (polymerase chain reaction) test which should be much more sensitive to screen for chytrid. In addition, we would like to expand the capabilities of the PCR testing procedure to test soil and water samples from potential reintroduction sites. At this time there is no known procedure for treating chytrid infections in the wild and very limited success in treating captive populations of amphibians.

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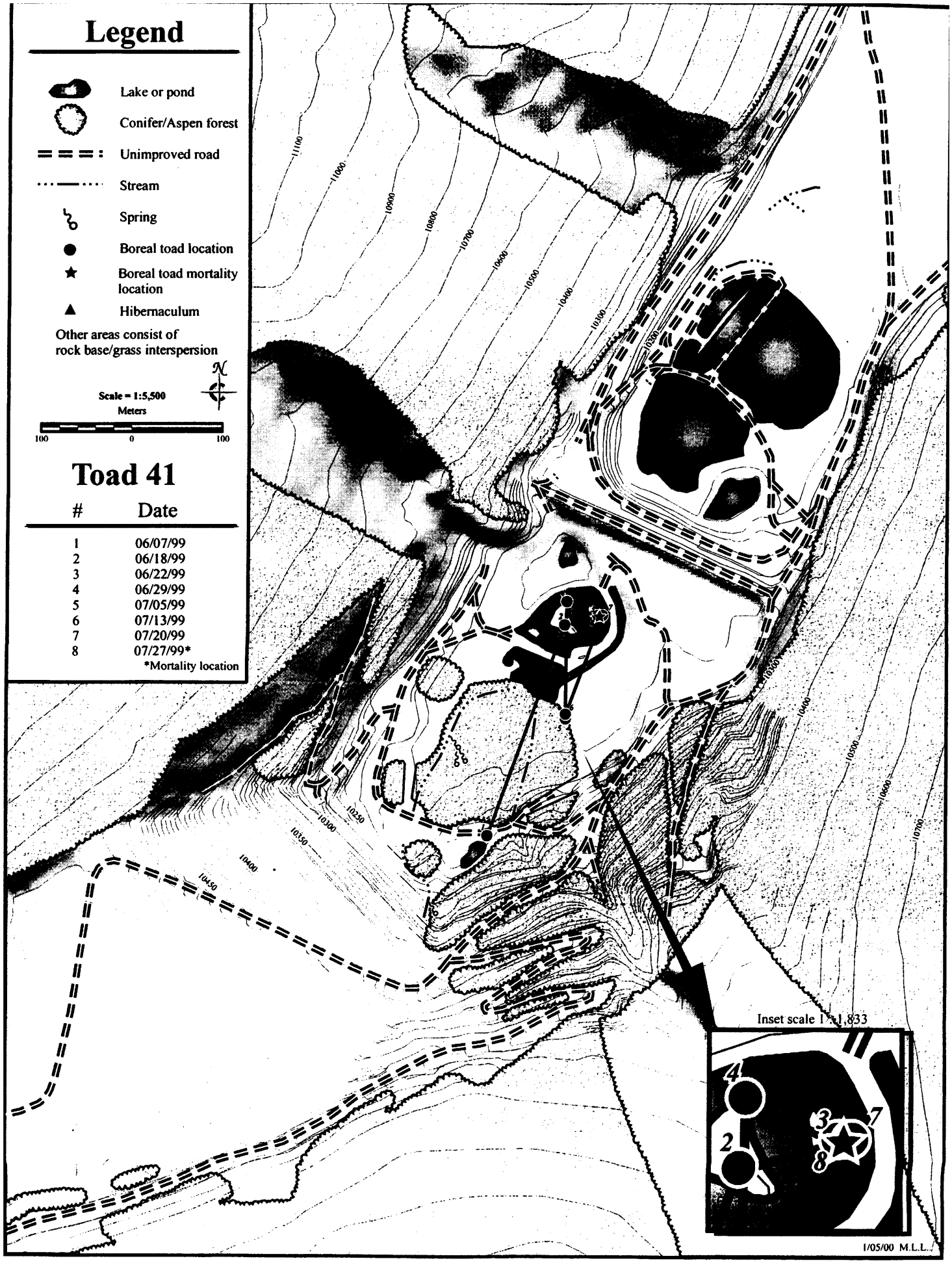
APPENDIX 1

Boreal toad radio telemetry contact locations in the Henderson/Urad study area, 1999.


Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 41
Date
1 06/07/99
2 06/18/99
3 06/22/99
4 06/29/99
5 07/05/99
6 07/13/99
7 07/20/99
8 07/27/99*
*Mortality location
11100
11000
10900
10800
10700
10600
10500
10400
10300
10250
10350
10400
10450
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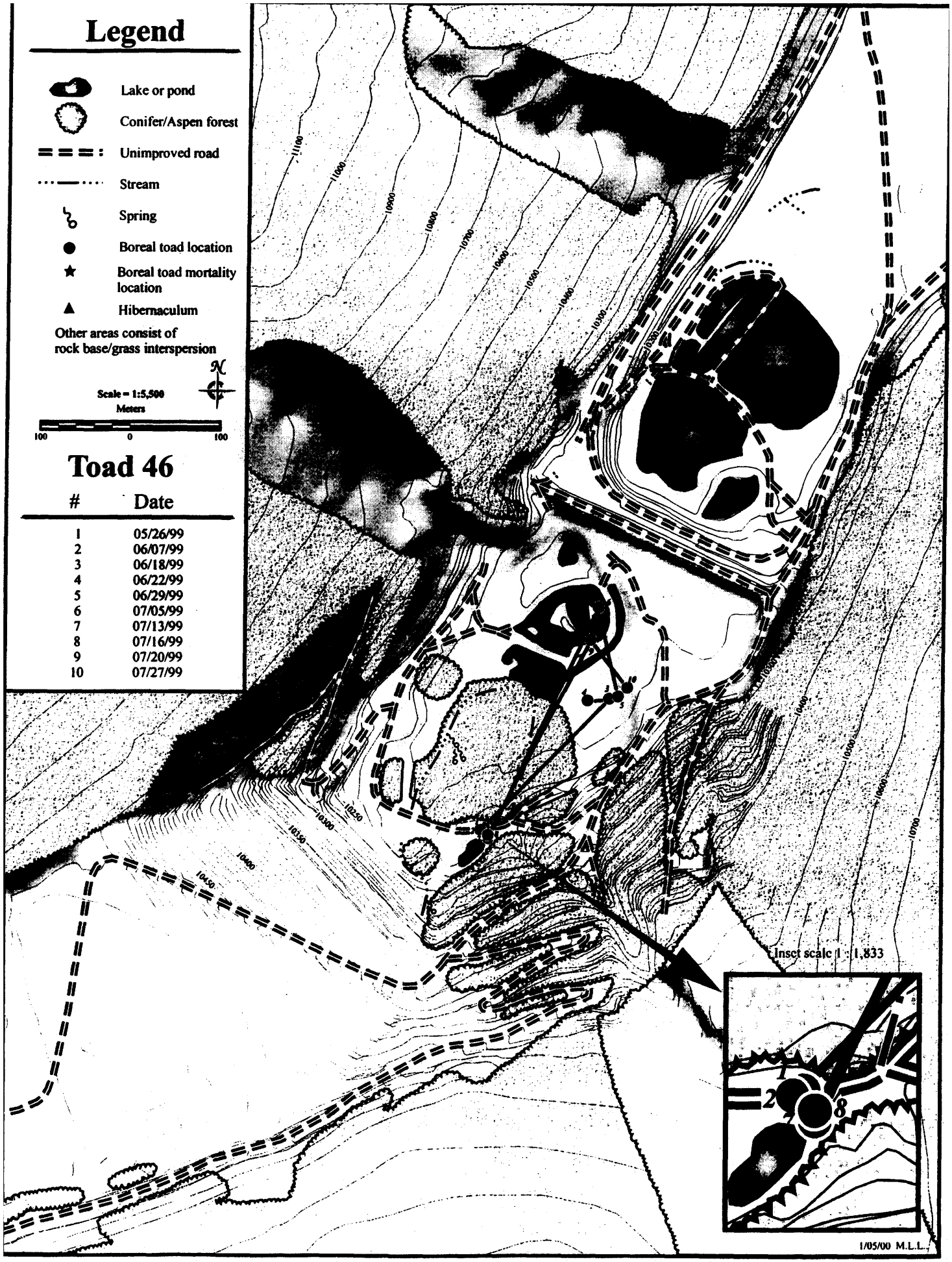
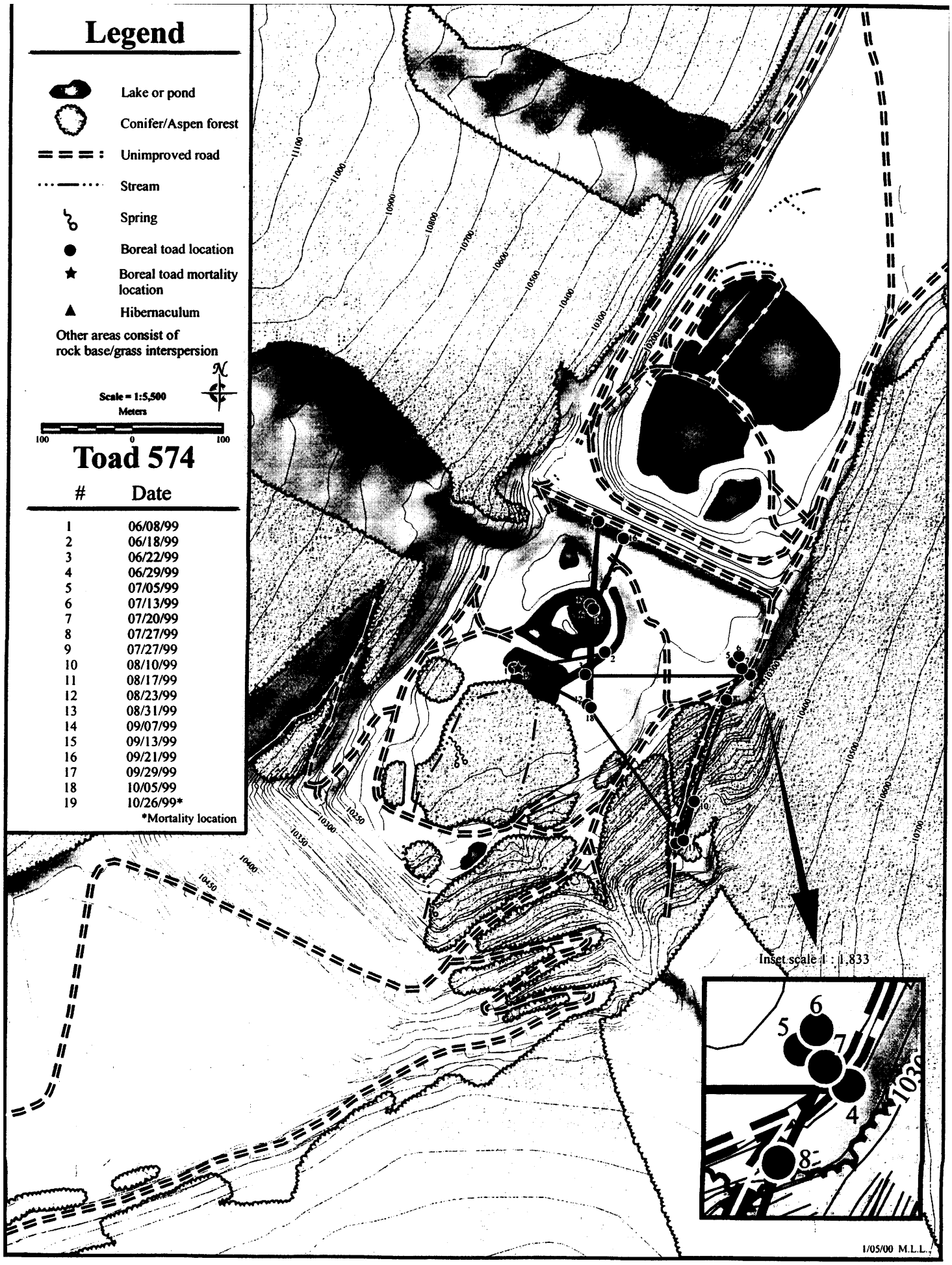
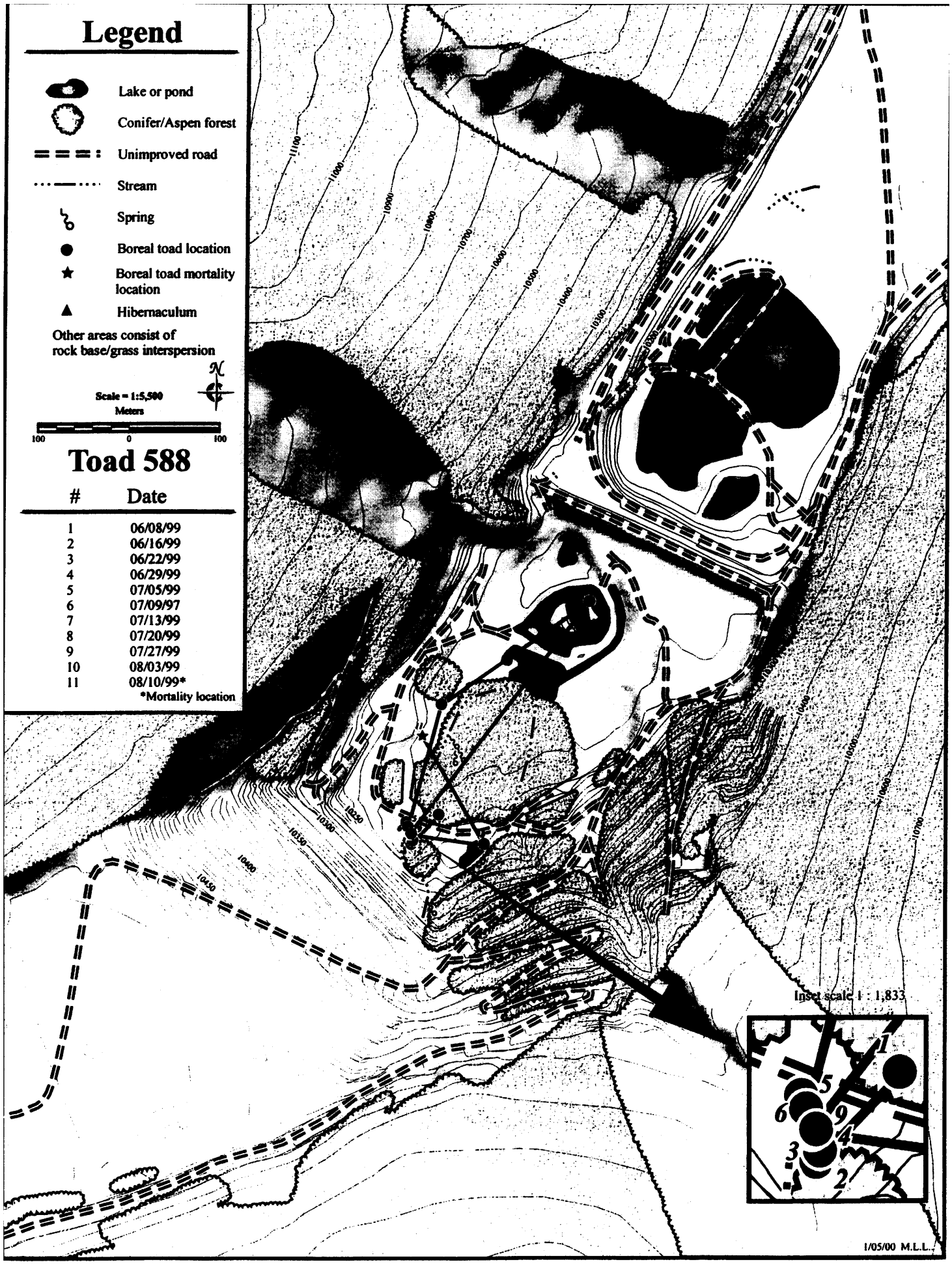
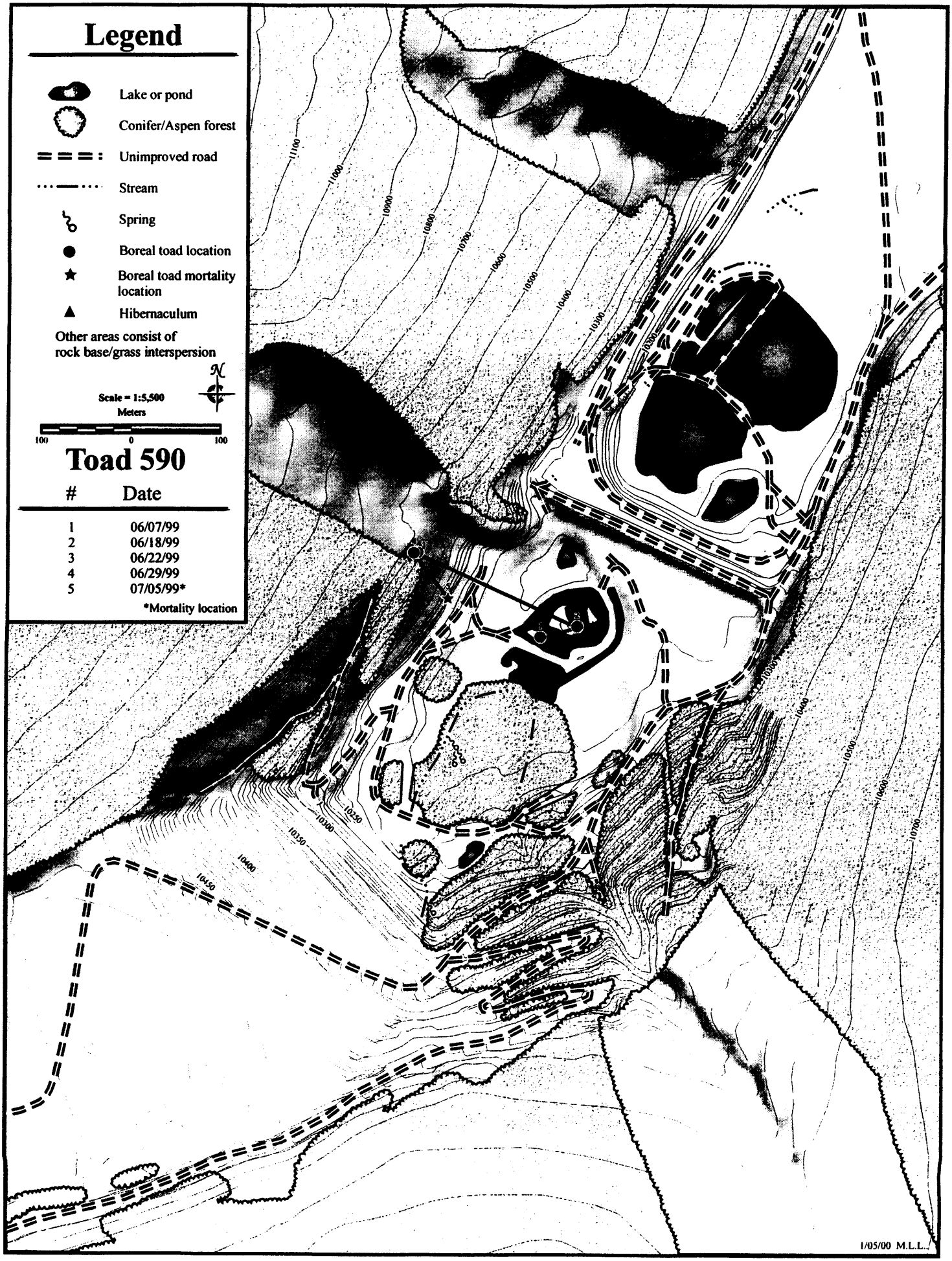
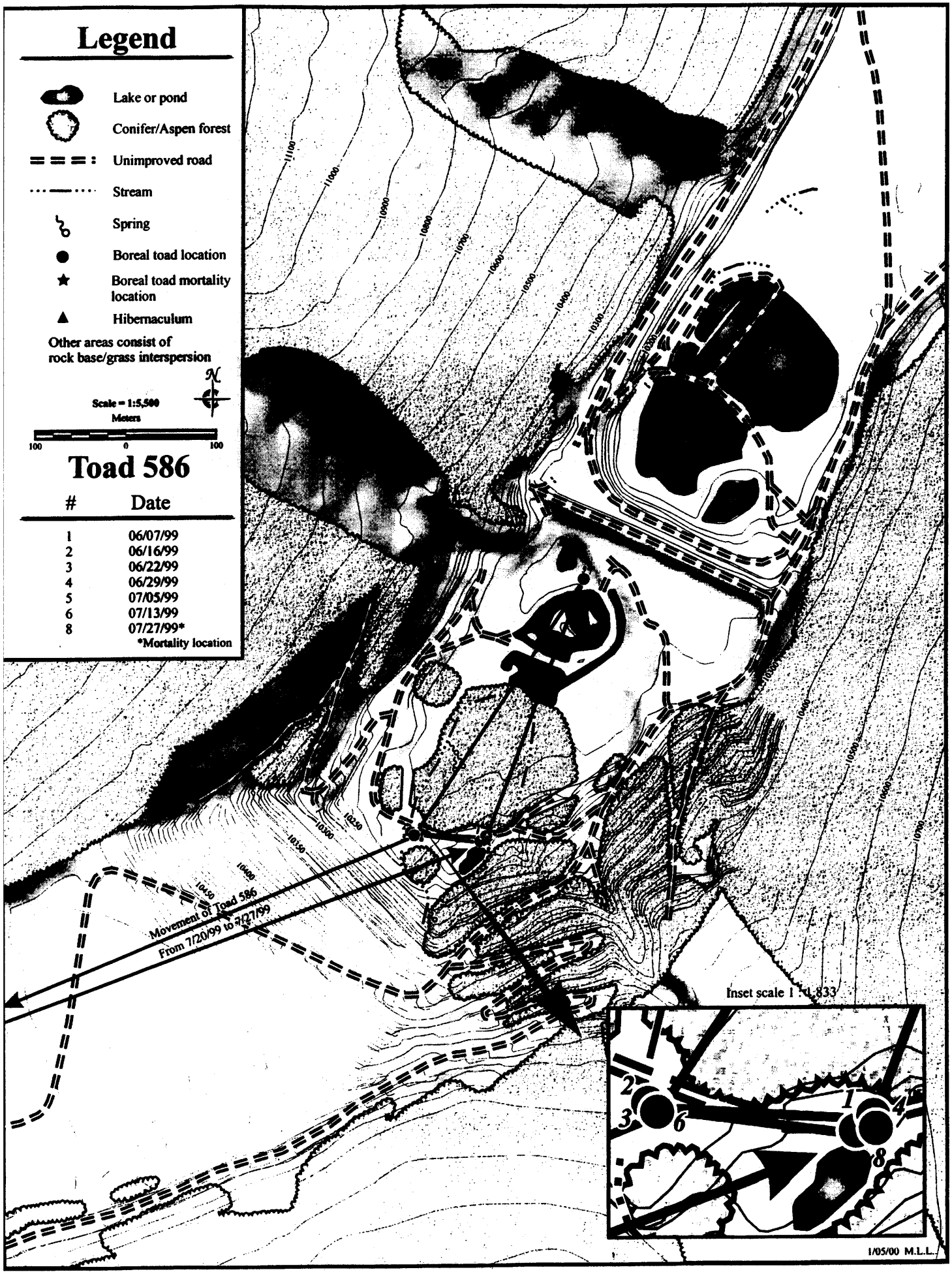
4
2
3
7
8
1/05/00 M.L.L.

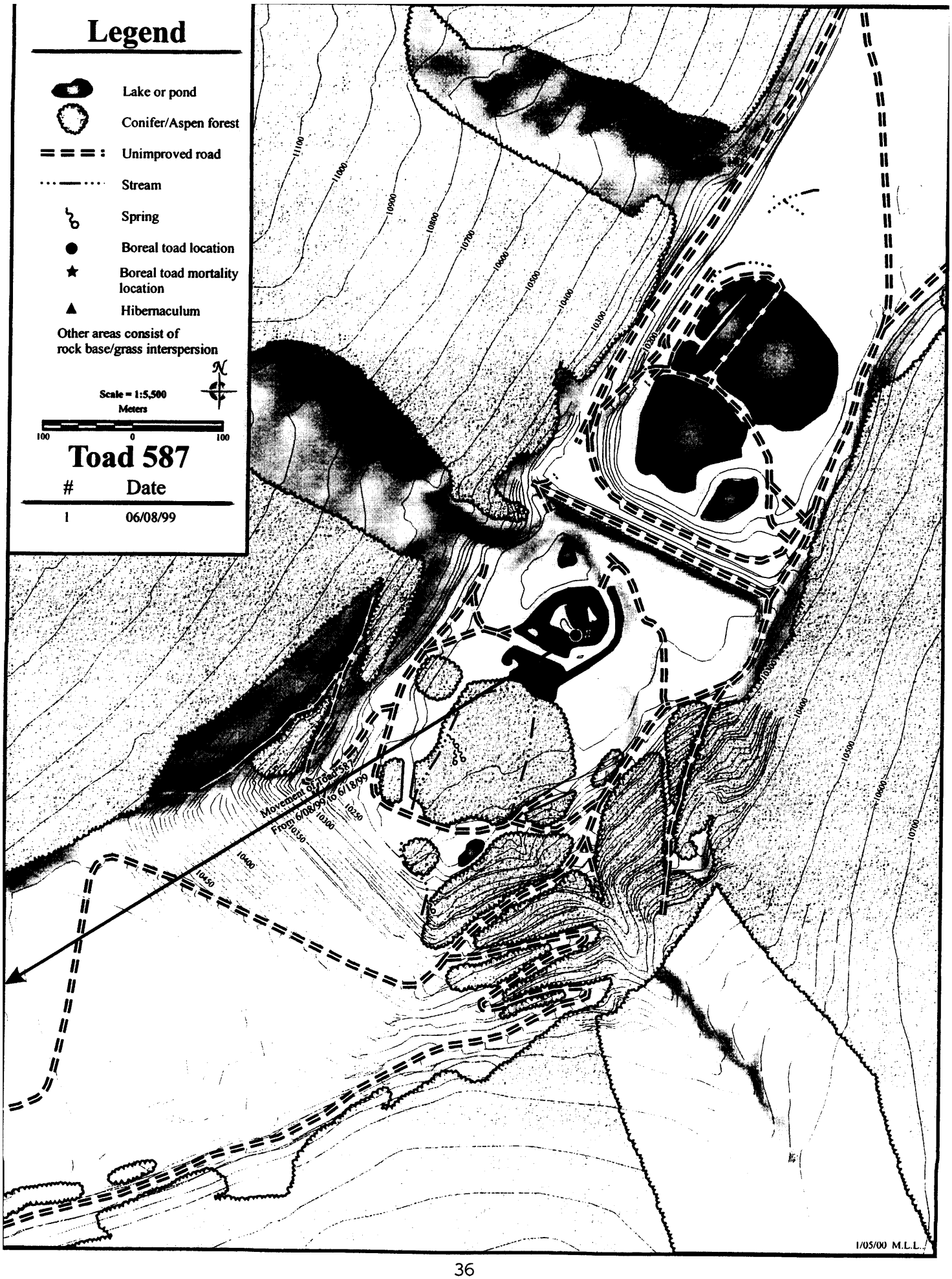

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 46
Date
1 05/26/99
2 06/07/99
3 06/18/99
4 06/22/99
5 06/29/99
6 07/05/99
7 07/13/99
8 07/16/99
9 07/20/99
10 07/27/99
11100
11000
10900
10800
10700
10600
10500
10400
10300
10250
10350
10400
10450
Inset scale 1 : 1,833
1
2
8
1/05/00 M.L.L.

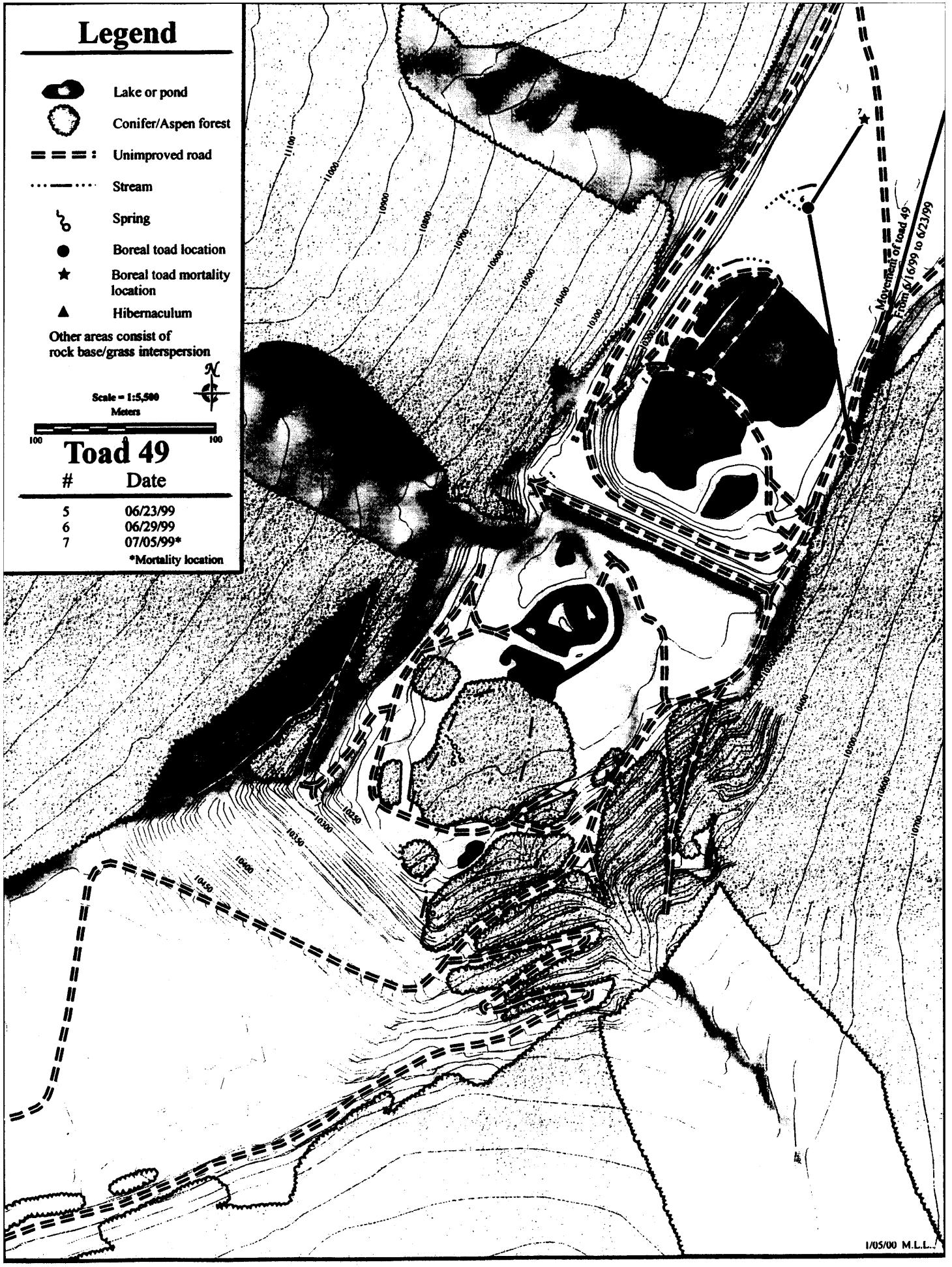

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 574
Date
1 06/08/99
2 06/18/99
3 06/22/99
4 06/29/99
5 07/05/99
6 07/13/99
7 07/20/99
8 07/27/99
9 07/27/99
10 08/10/99
11 08/17/99
12 08/23/99
13 08/31/99
14 09/07/99
15 09/13/99
16 09/21/99
17 09/29/99
18 10/05/99
19 10/26/99*
*Mortality location
11100
11000
10900
10800
10700
10600
10500
10400
10300
10200
10250
10350
10450
Inset scale 1 : 1,833
1/05/00 M.L.L.

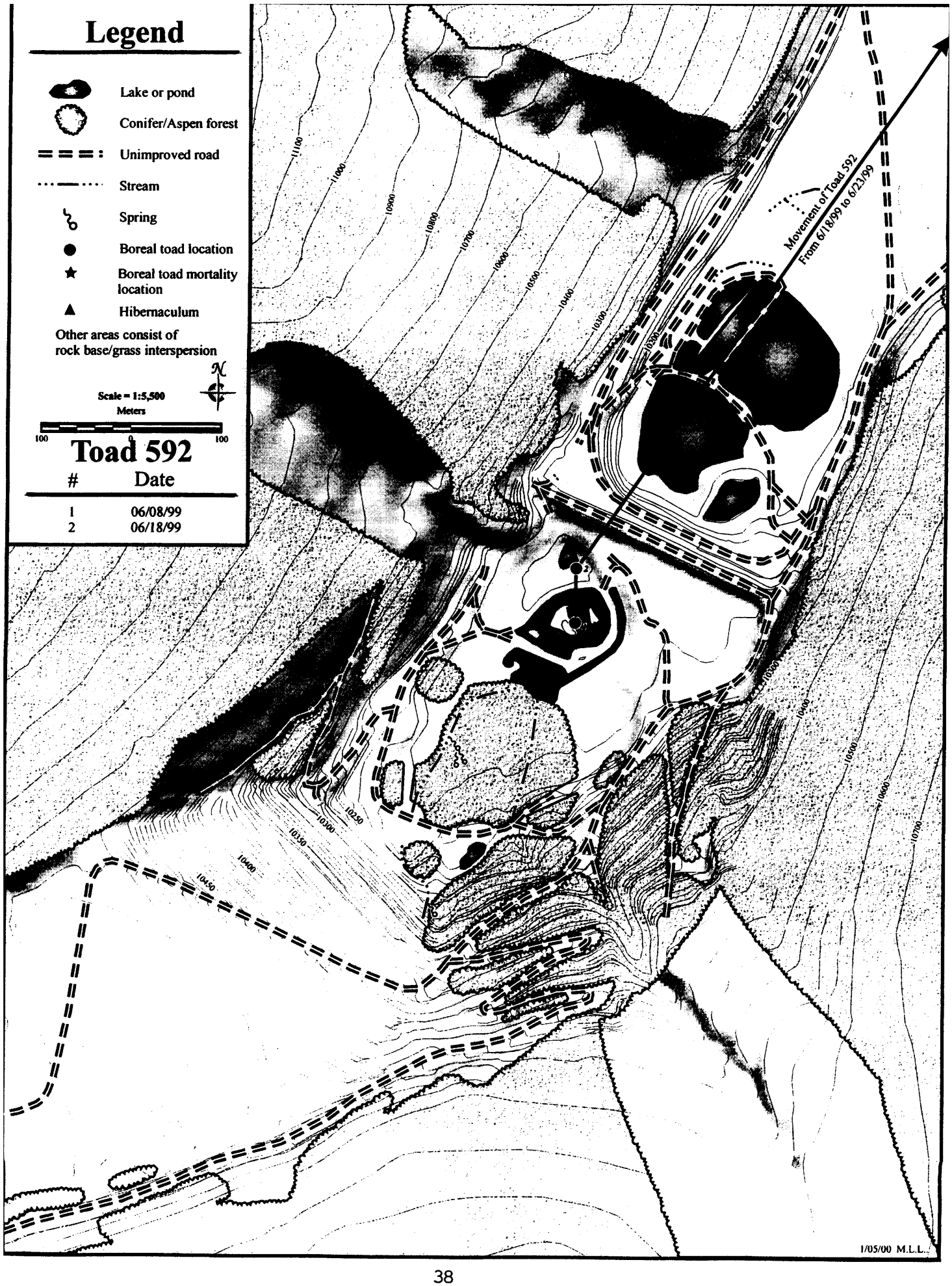

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 588
Date
1 06/08/99
2 06/16/99
3 06/22/99
4 06/29/99
5 07/05/99
6 07/09/97
7 07/13/99
8 07/20/99
9 07/27/99
10 08/03/99
11 08/10/99*
*Mortality location
11100
11000
10900
10800
10700
10600
10500
10400
10300
10250
10300
10350
10400
10450
10400
10500
10600
10700
Inset scale 1 : 1,833
1
5
6
9
4
3
2
1/05/00 M.L.L.

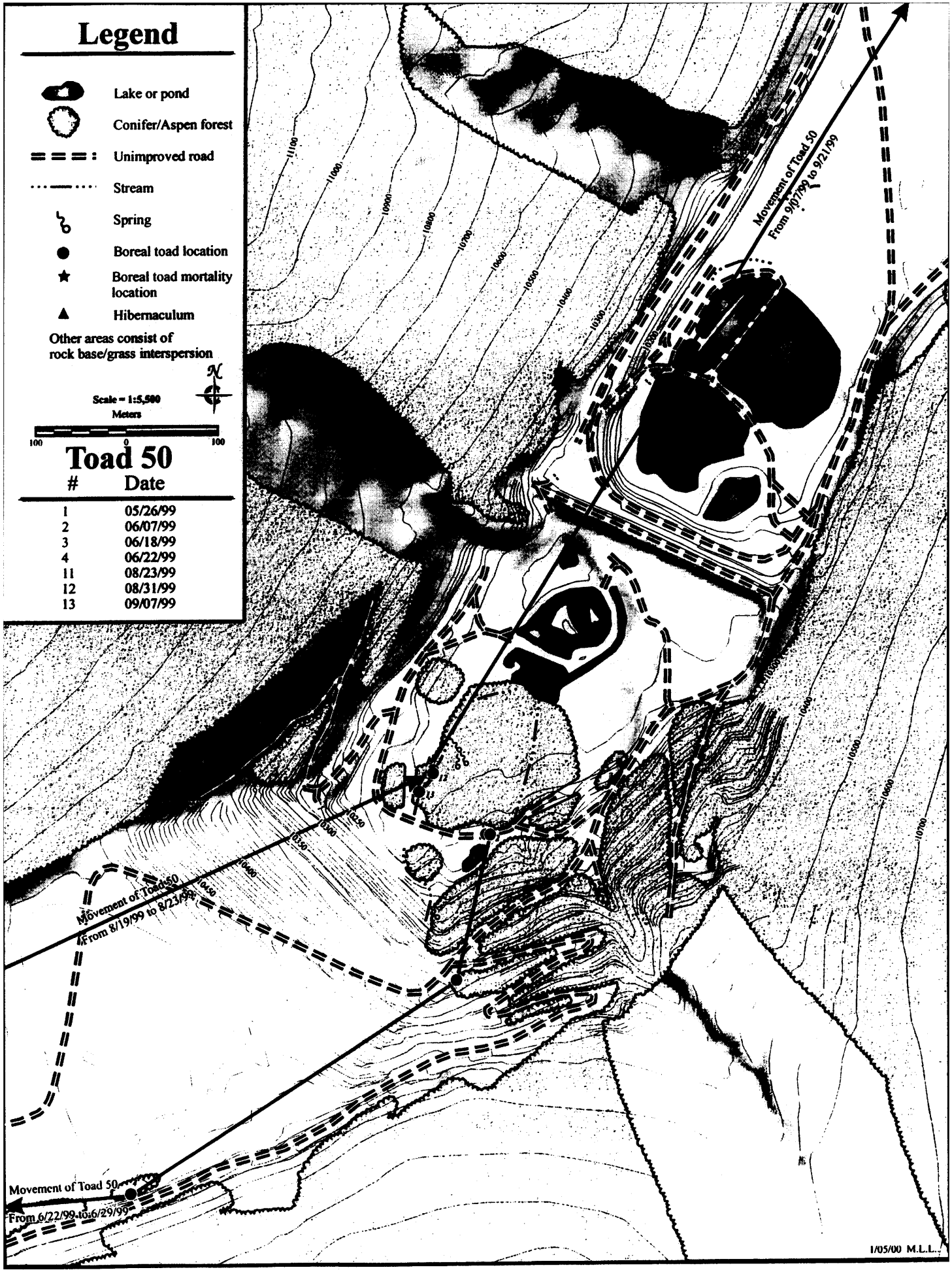

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 590
Date
1 06/07/99
2 06/18/99
3 06/22/99
4 06/29/99
5 07/05/99*
*Mortality location
11100
11000
10900
10800
10700
10600
10500
10400
10300
10200
10250
10350
10450
10550
10650
10750
1/05/00 M.L.L.

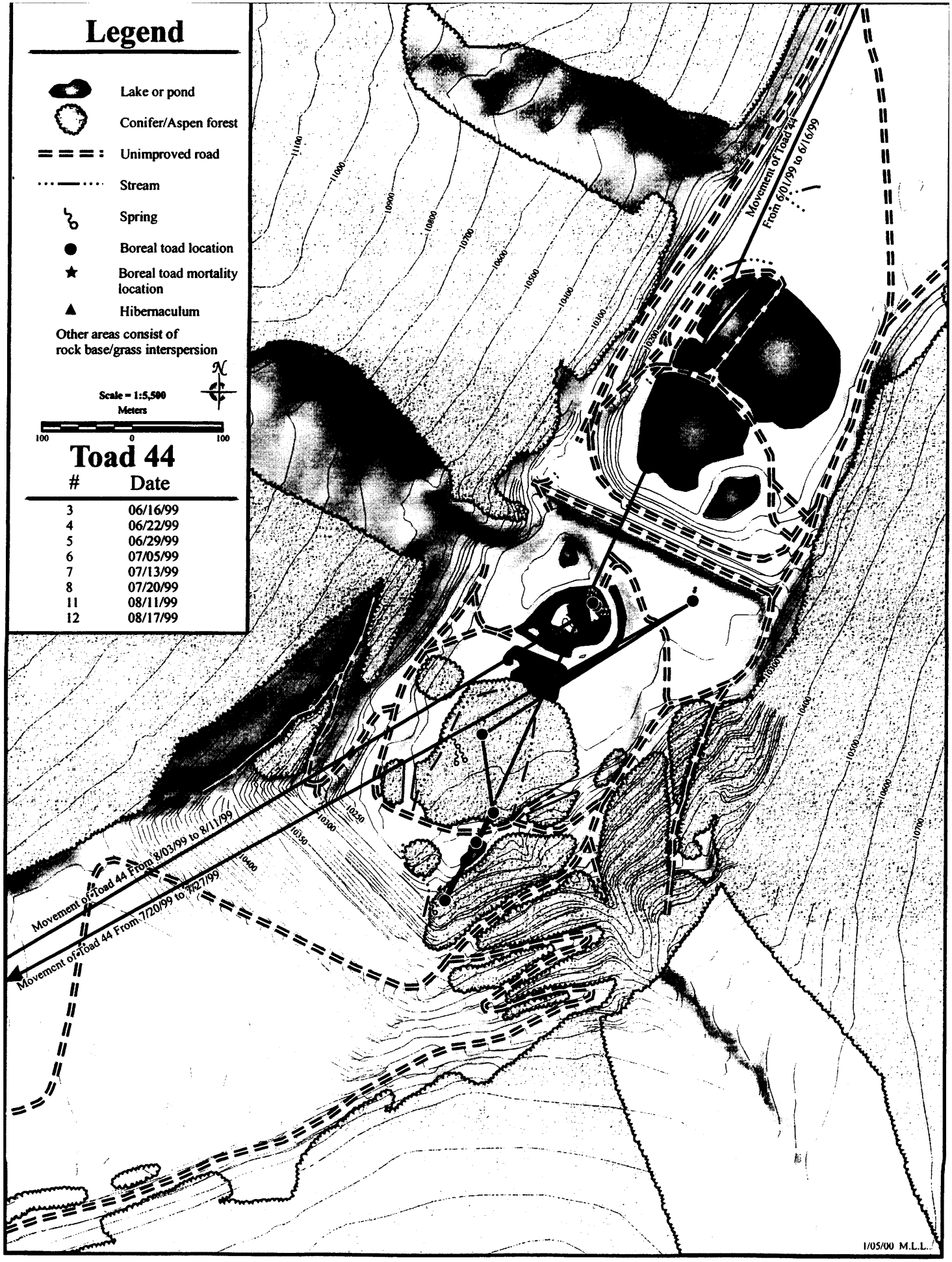

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 586
Date
1 06/07/99
2 06/16/99
3 06/22/99
4 06/29/99
5 07/05/99
6 07/13/99
8 07/27/99*
*Mortality location
11100
11000
10900
10800
10700
10600
10500
10400
10300
10200
10100
10000
10050
10150
10250
10350
10400
10450
10500
10550
10600
10650
10700
Movement of Toad 586
From 7/20/99 to 7/27/99
Inset scale 1 : 1,833
1
2
3
4
6
8
1/05/00 M.L.L.

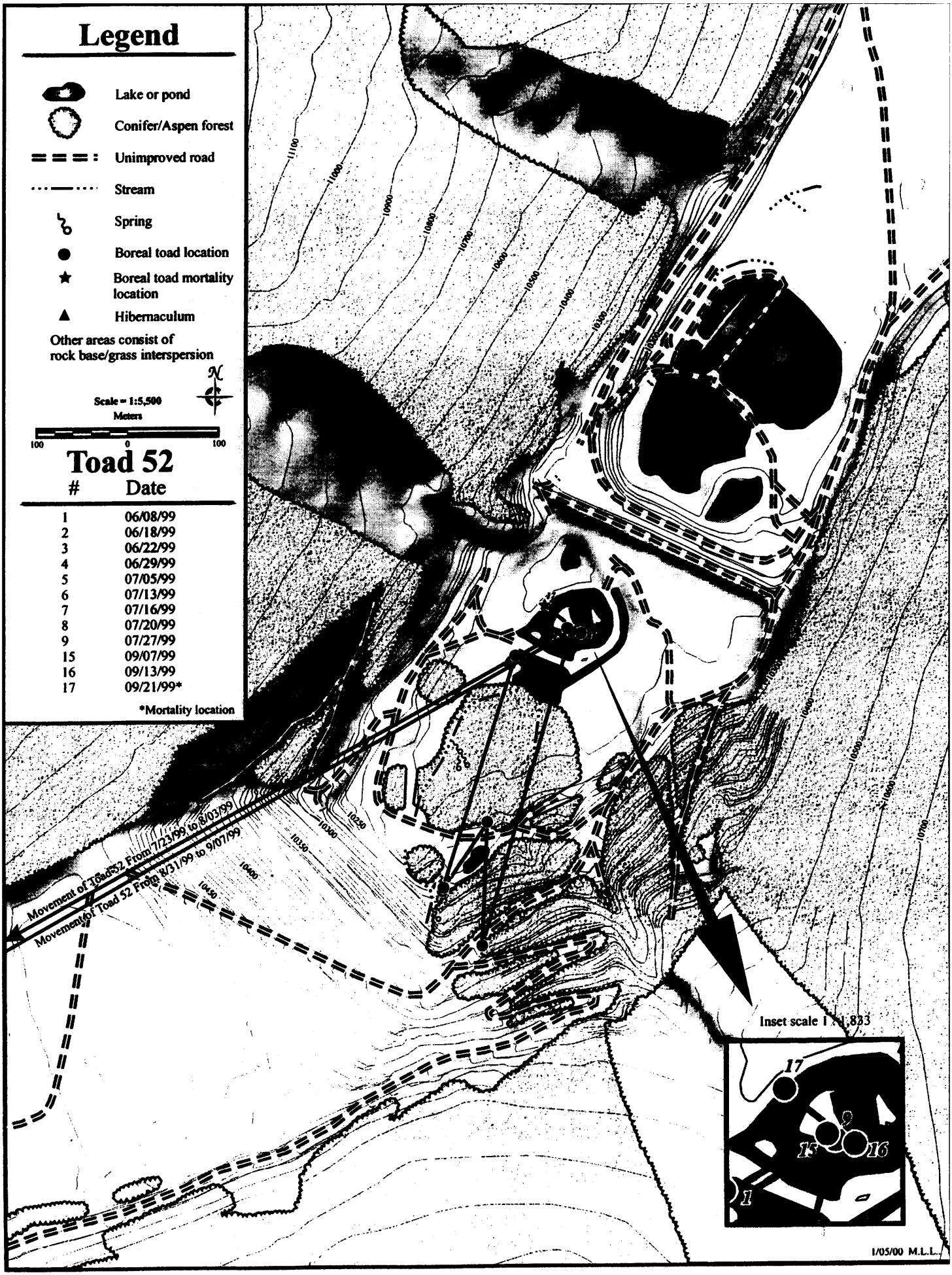

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 587
Date
1 06/08/99
Movement of Toad 587 From 6/08/99 to 6/18/99
11100
11000
10900
10800
10700
10600
10500
10400
10300
10200
10250
10350
10450
1/05/00 M.L.L.

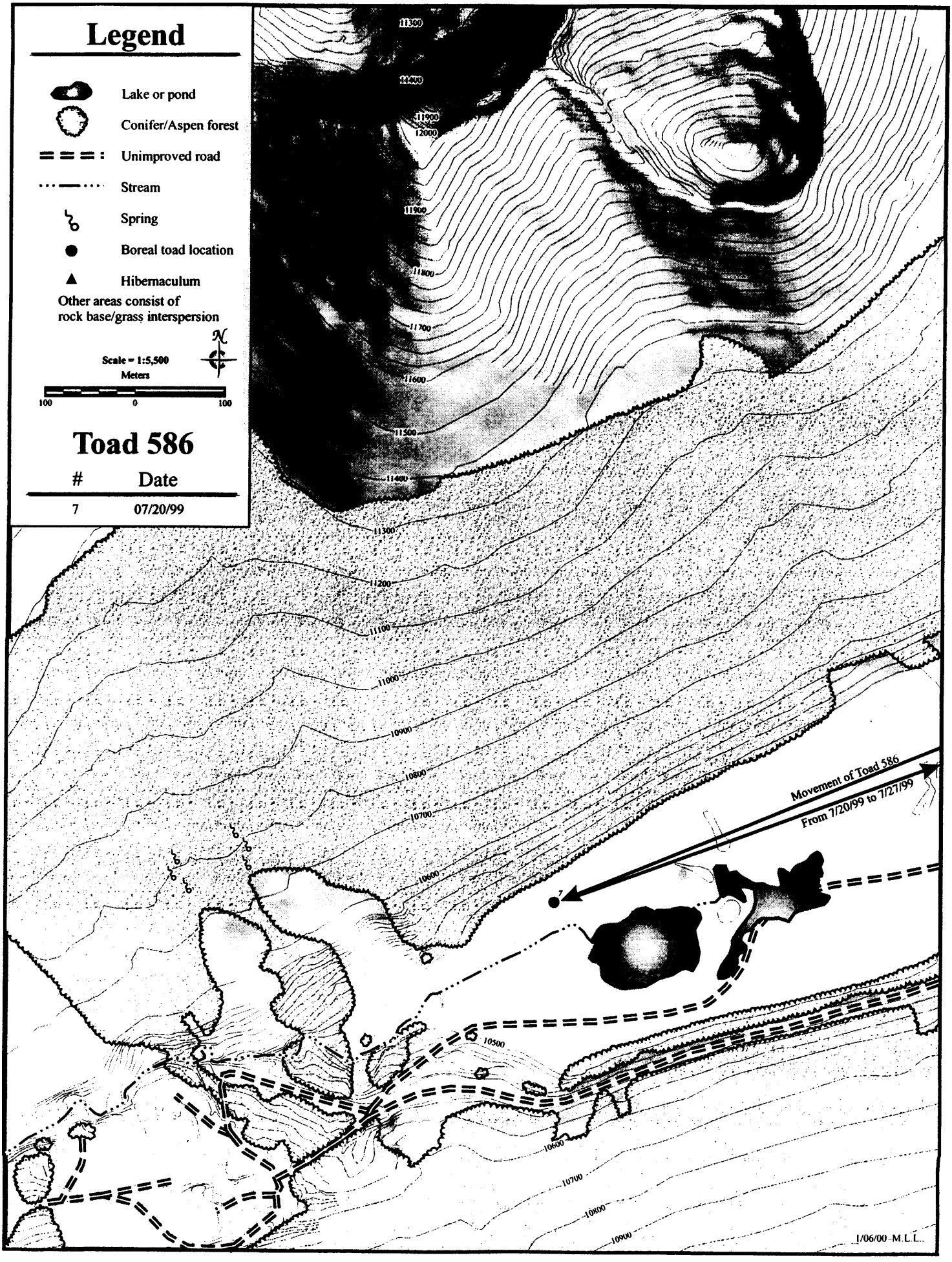

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 49
Date
5 06/23/99
6 06/29/99
7 07/05/99*
*Mortality location
Movement of toad 49 From 6/16/99 to 6/23/99
1/05/00 M.L.L.

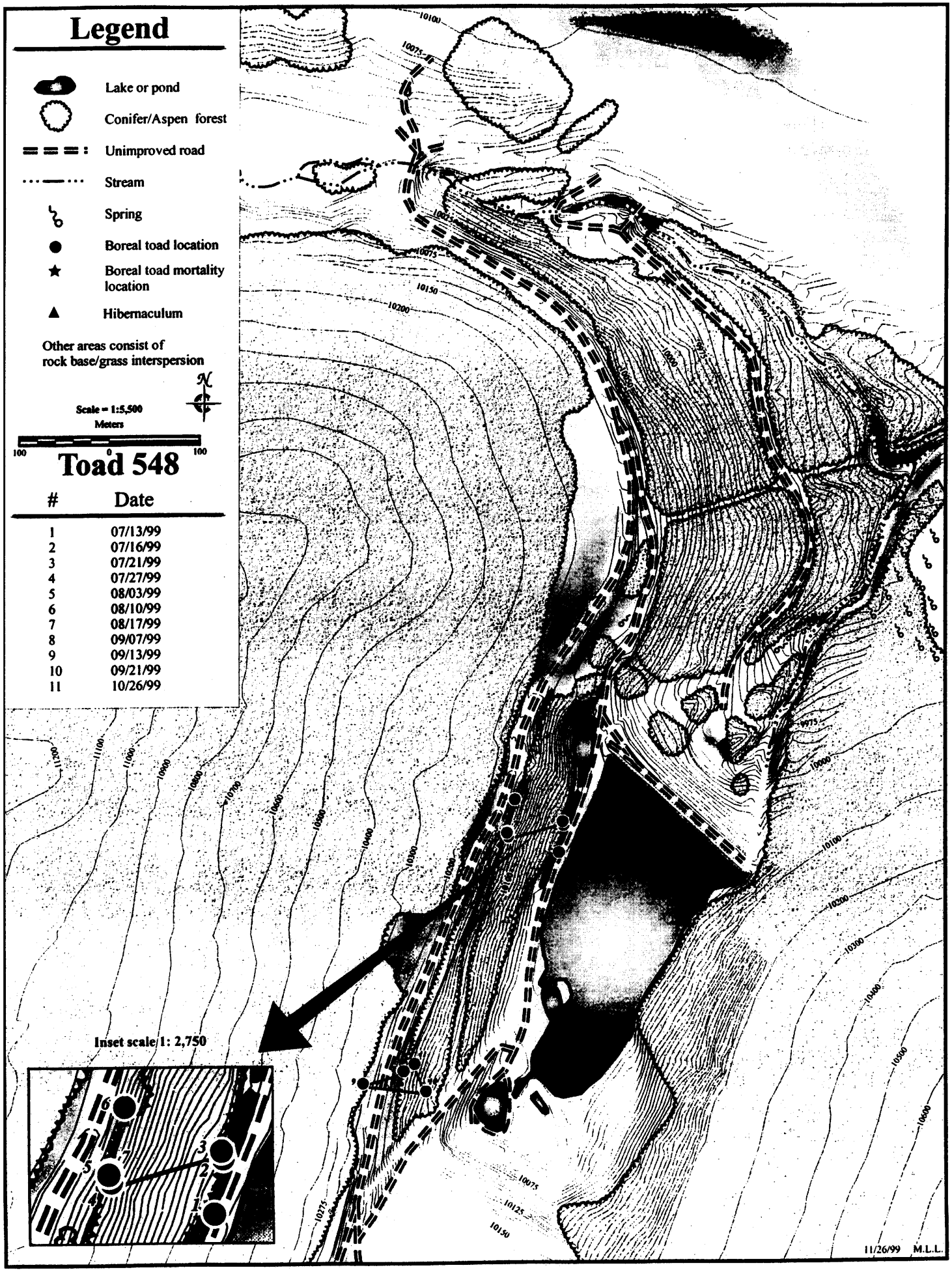

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 592
Date
1 06/08/99
2 06/18/99
Movement of Toad 592 From 6/18/99 to 6/23/99
11100
11000
10900
10800
10700
10600
10500
10400
10300
10250
10200
10150
10100
10050
1/05/00 M.L.L.

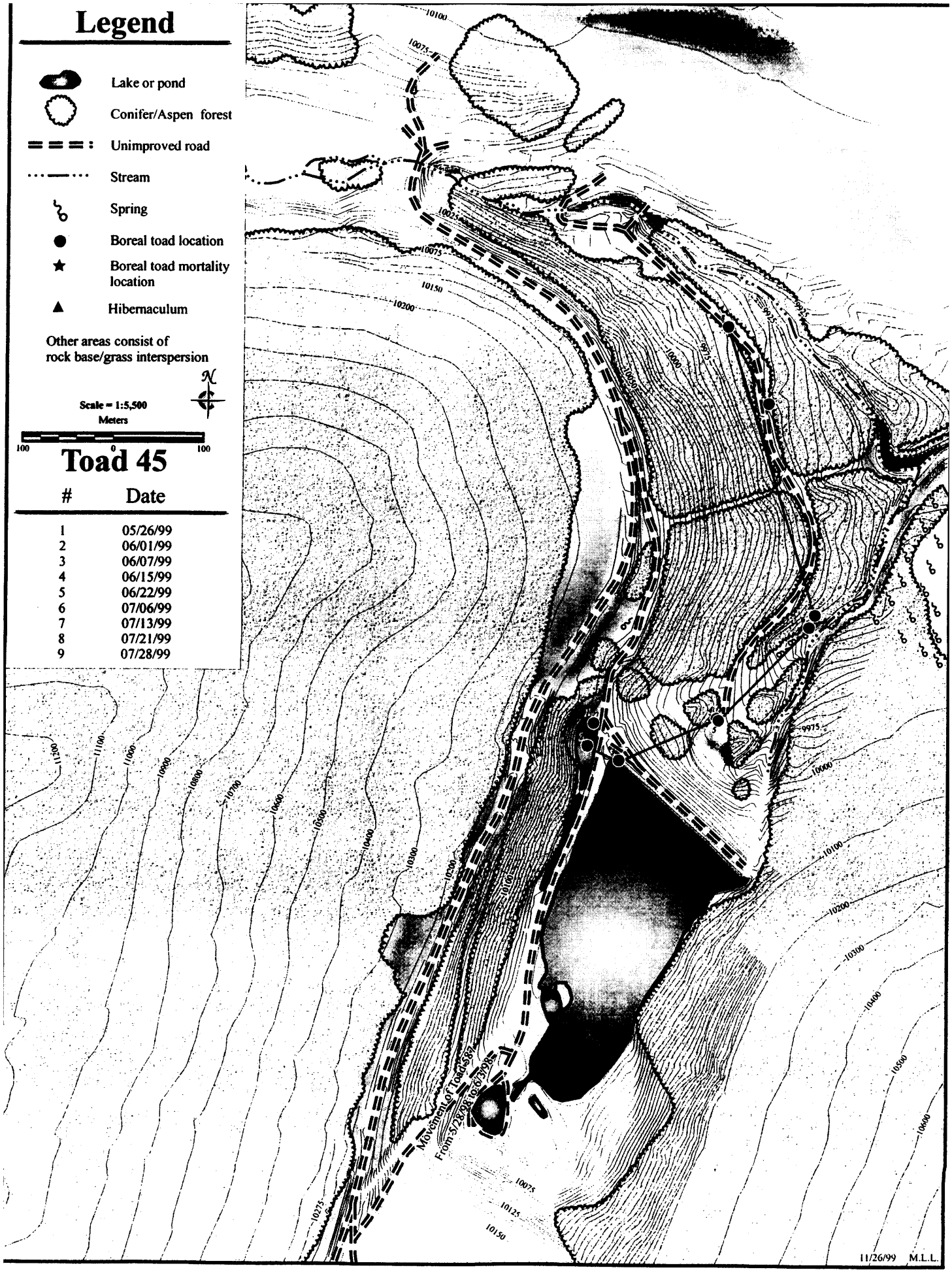

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 50
Date
1 05/26/99
2 06/07/99
3 06/18/99
4 06/22/99
11 08/23/99
12 08/31/99
13 09/07/99
Movement of Toad 50 From 9/07/99 to 9/21/99
Movement of Toad 50 From 8/19/99 to 8/23/99
Movement of Toad 50 From 6/22/99 to 6/29/99
1/05/00 M.L.L.

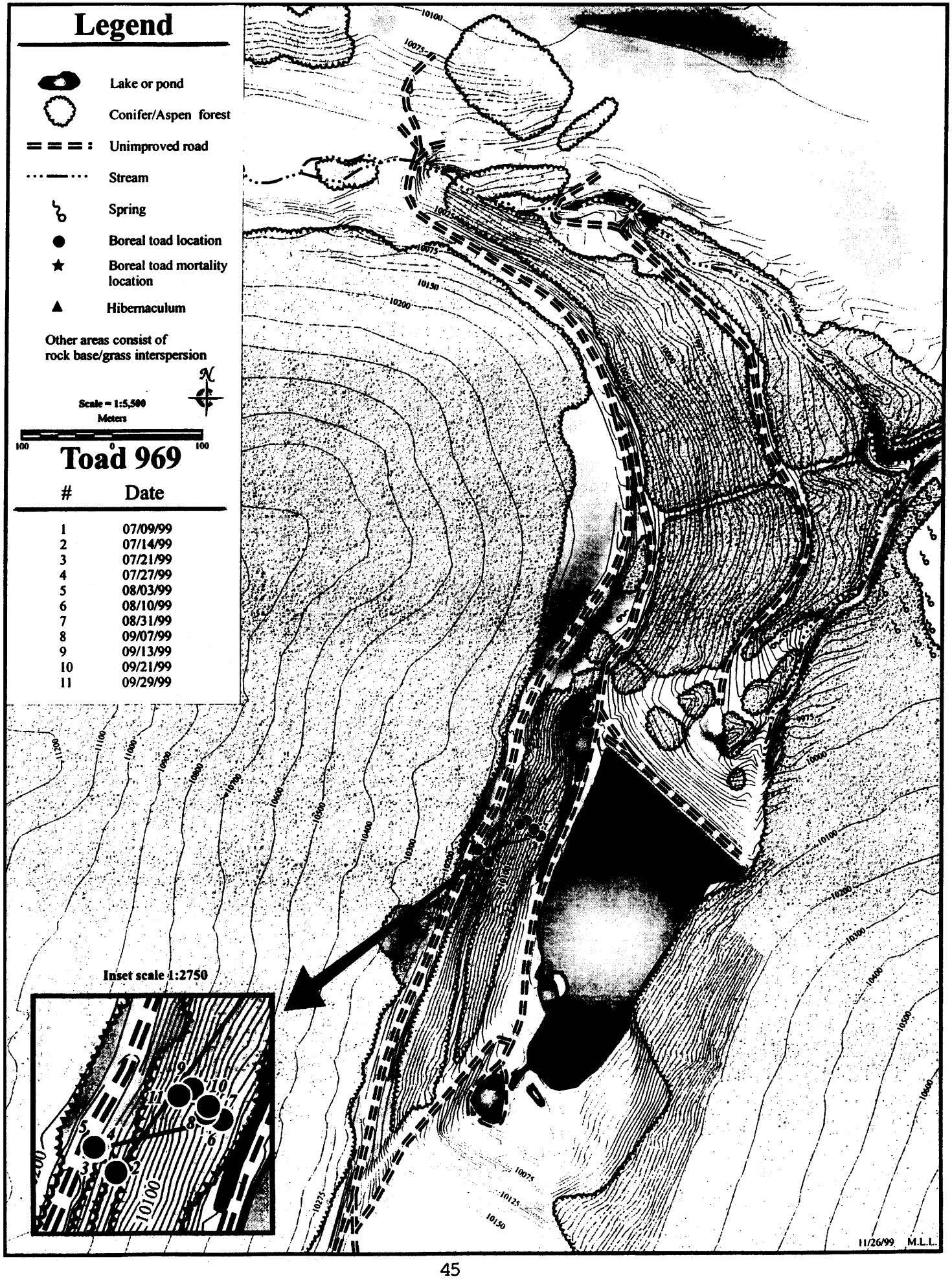

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 44
Date
3 06/16/99
4 06/22/99
5 06/29/99
6 07/05/99
7 07/13/99
8 07/20/99
11 08/11/99
12 08/17/99
Movement of Toad 44 From 6/01/99 to 6/16/99
Movement of Toad 44 From 8/03/99 to 8/11/99
Movement of Toad 44 From 7/20/99 to 7/27/99
11100
11000
10900
10800
10700
10600
10500
10400
10300
10250
10350
10400
10300
10400
10500
10600
10700
1/05/00 M.L.L.

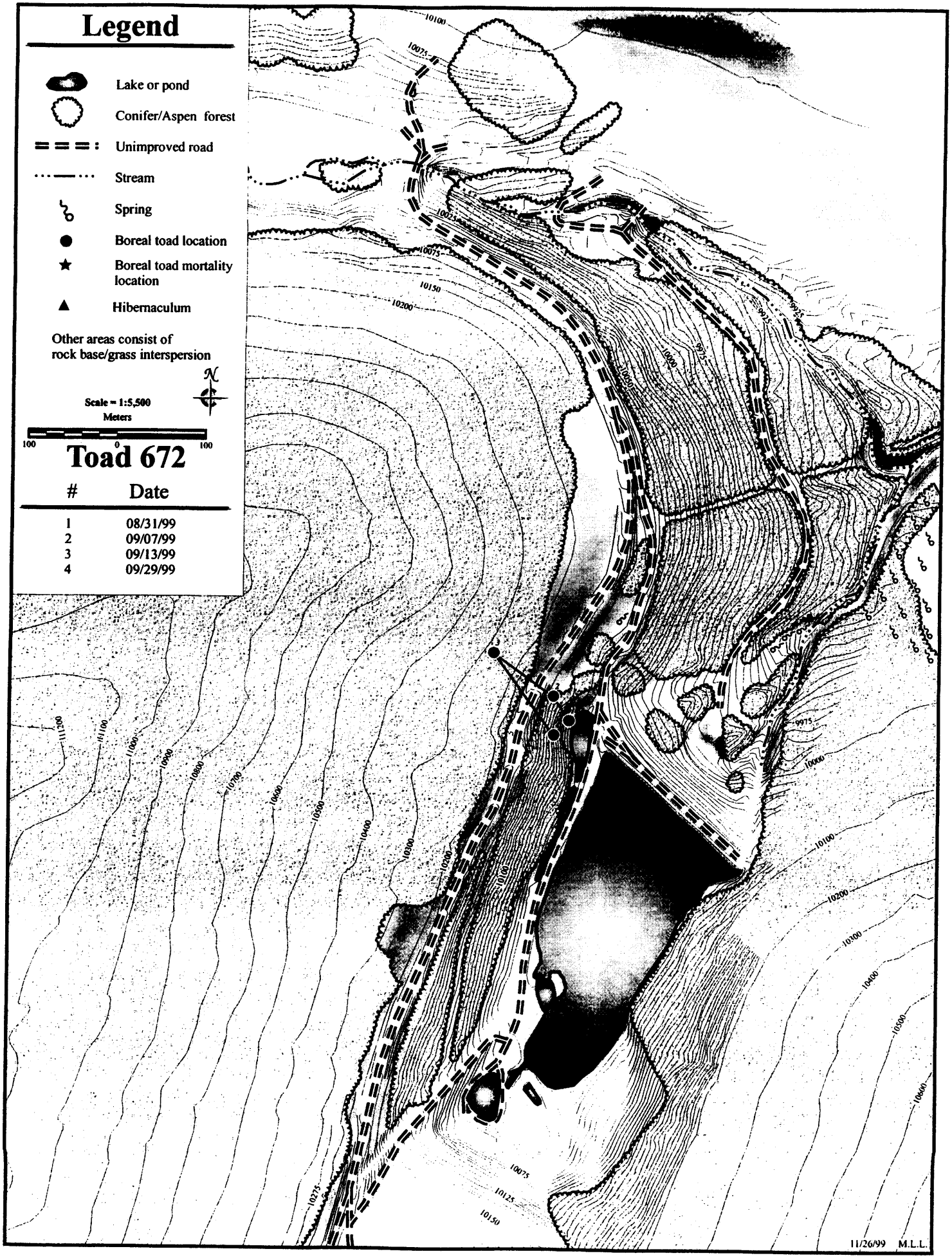

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 52
Date
1 06/08/99
2 06/18/99
3 06/22/99
4 06/29/99
5 07/05/99
6 07/13/99
7 07/16/99
8 07/20/99
9 07/27/99
15 09/07/99
16 09/13/99
17 09/21/99*
*Mortality location
Movement of Toad 52 From 7/23/99 to 8/03/99
Movement of Toad 52 From 8/31/99 to 9/07/99
Inset scale 1 : 1,833
17
9
15
16
1
1/05/00 M.L.L.

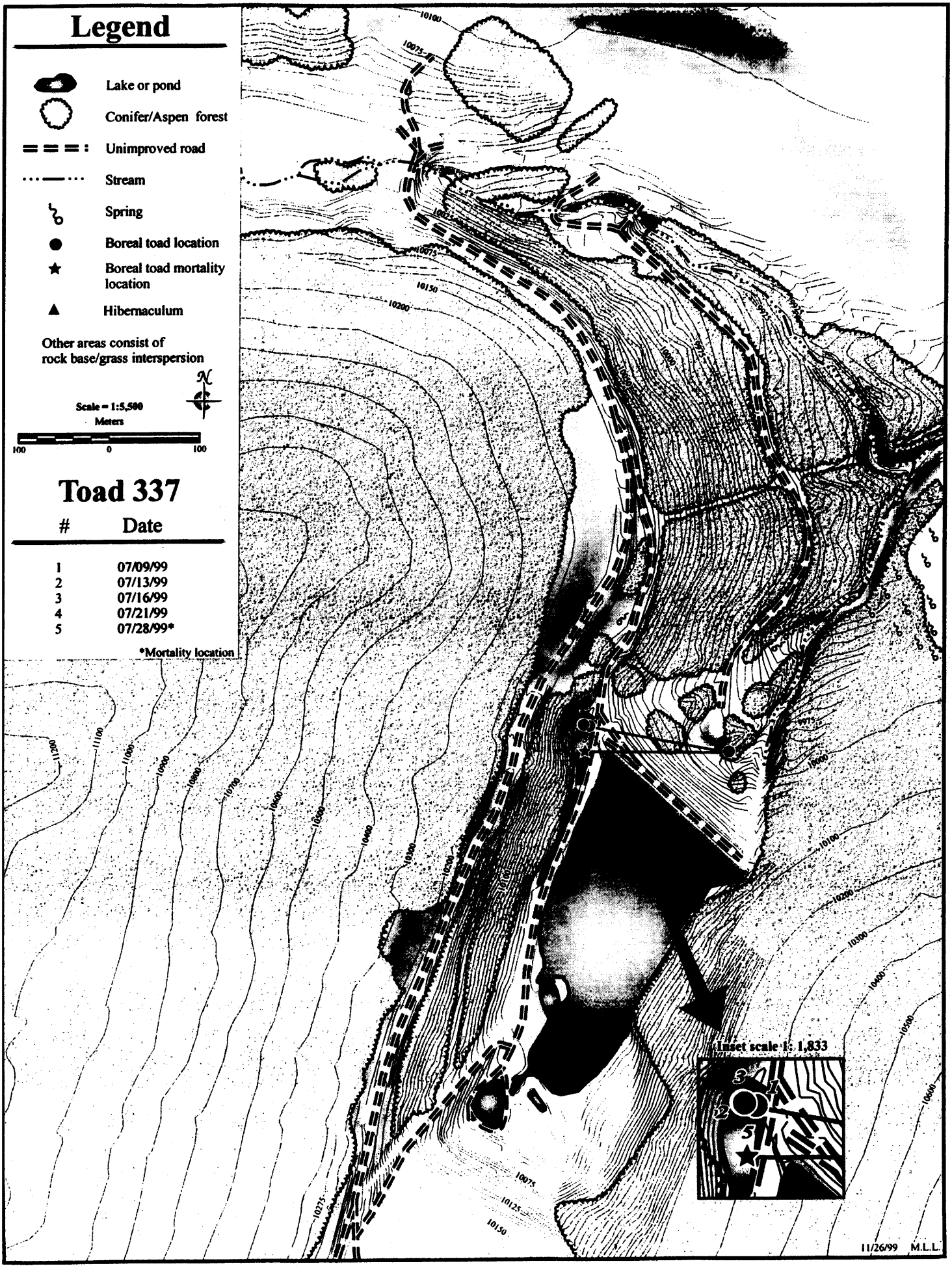

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 586
Date
7 07/20/99
11300
11400
11500
11600
11700
11800
11900
12000
11900
11800
11700
11600
11500
11400
11300
11200
11100
11000
10900
10800
10700
10600
10500
Movement of Toad 586
From 7/20/99 to 7/27/99
7
10500
10600
10700
10800
10900
1/06/00 M.L.L.

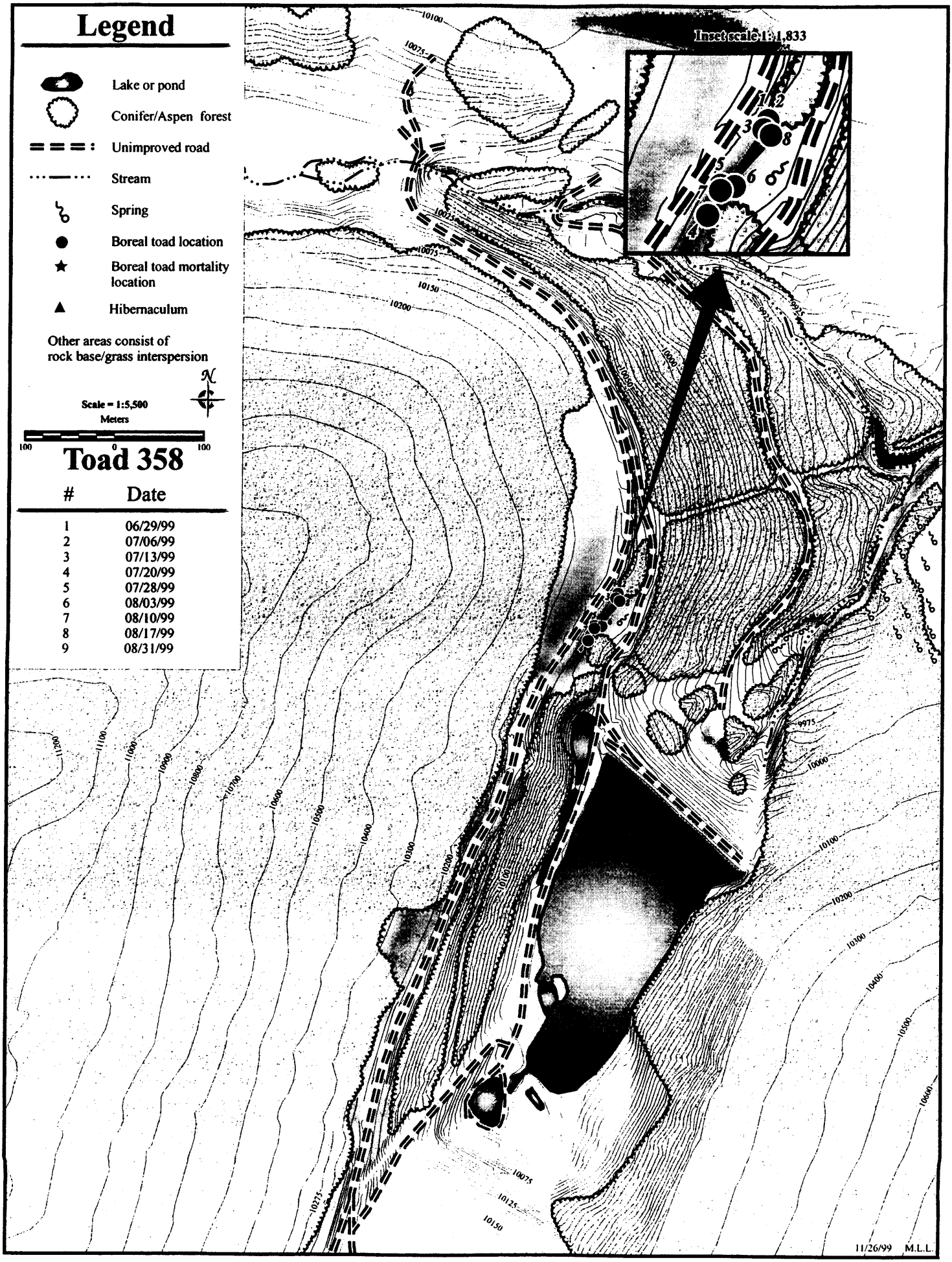

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 548
Date
1 07/13/99
2 07/16/99
3 07/21/99
4 07/27/99
5 08/03/99
6 08/10/99
7 08/17/99
8 09/07/99
9 09/13/99
10 09/21/99
11 10/26/99
Inset scale 1: 2,750
11/26/99 M.L.L.

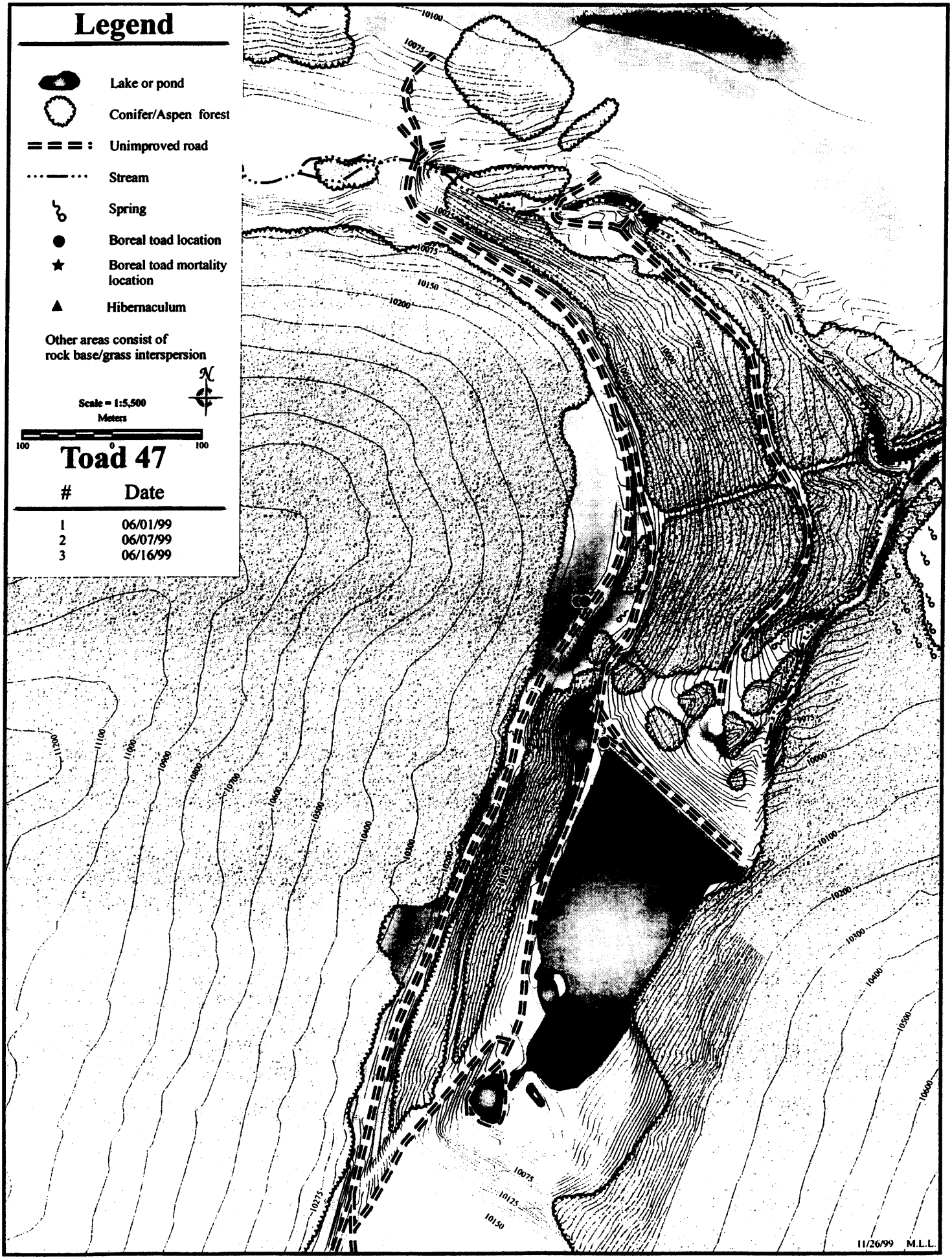

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 45
Date
1 05/26/99
2 06/01/99
3 06/07/99
4 06/15/99
5 06/22/99
6 07/06/99
7 07/13/99
8 07/21/99
9 07/28/99
Movement of Toad 58 From 5/28/98 to 7/28/98
11/26/99 M.L.L.

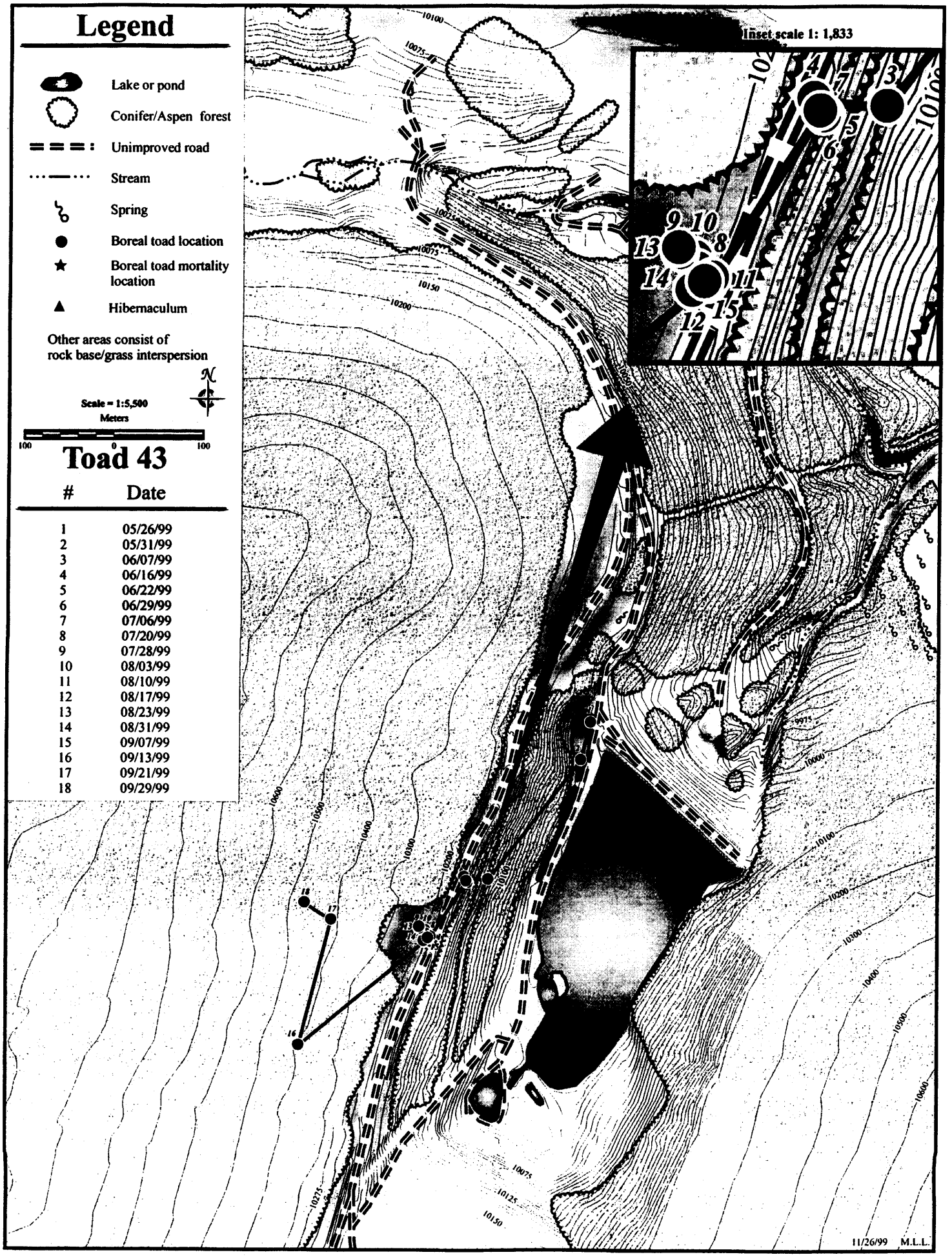

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 969
Date
1 07/09/99
2 07/14/99
3 07/21/99
4 07/27/99
5 08/03/99
6 08/10/99
7 08/31/99
8 09/07/99
9 09/13/99
10 09/21/99
11 09/29/99
Inset scale 1:2750
11/26/99 M.L.L.

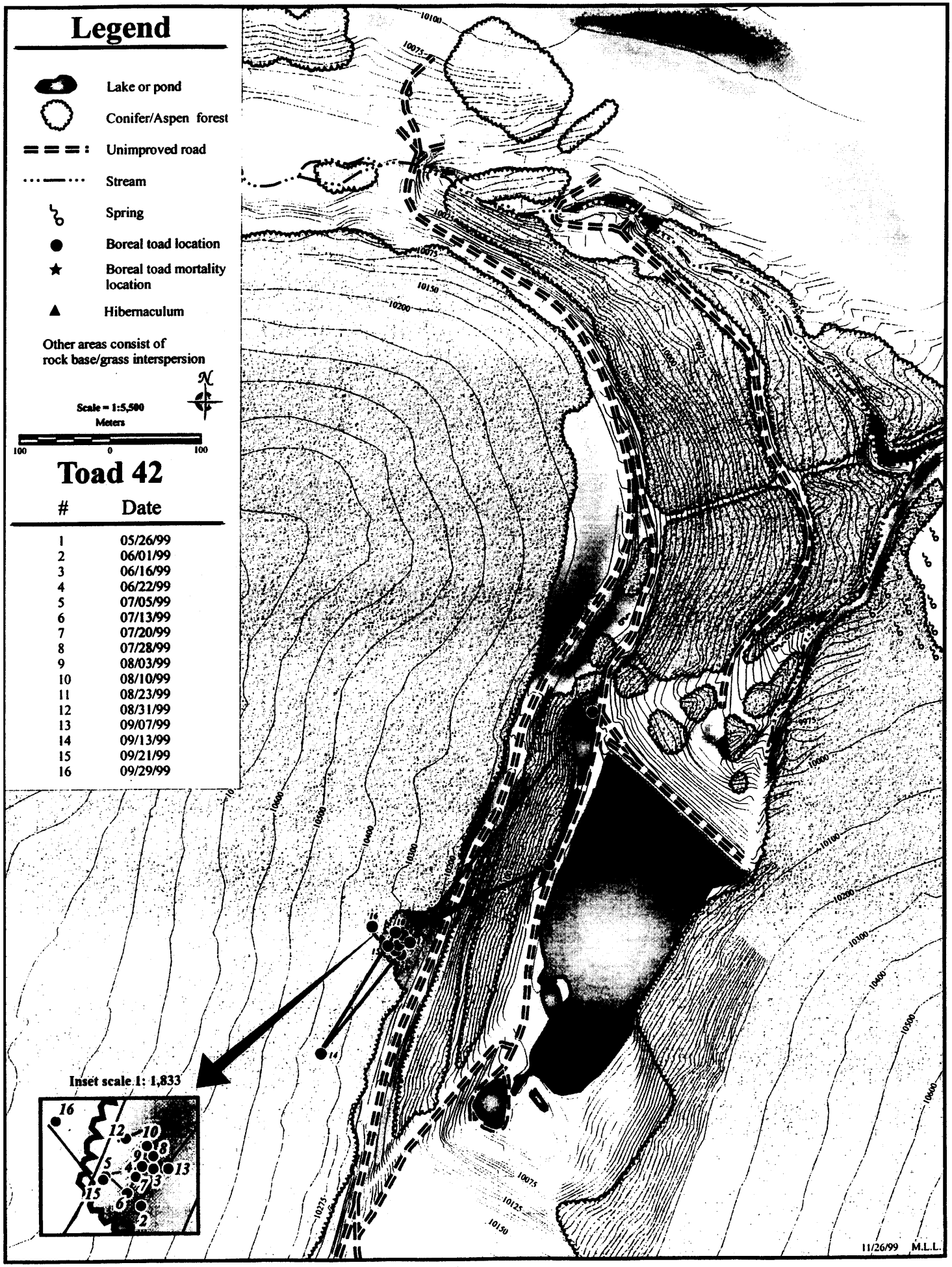

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 672
Date
1 08/31/99
2 09/07/99
3 09/13/99
4 09/29/99
11/26/99 M.L.L.

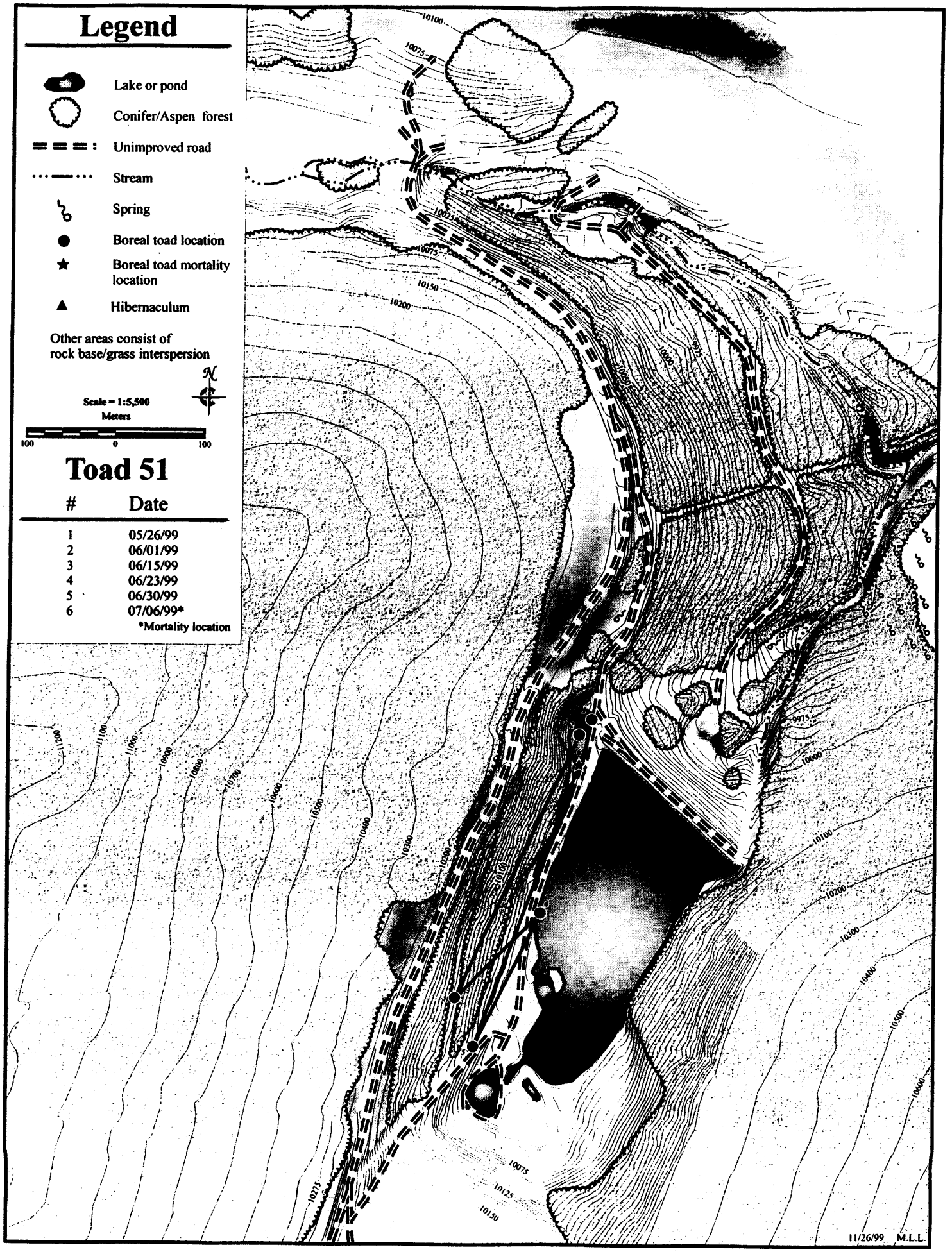

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 337
Date
1 07/09/99
2 07/13/99
3 07/16/99
4 07/21/99
5 07/28/99*
*Mortality location
Inset scale 1: 1,833
11/26/99 M.L.L.

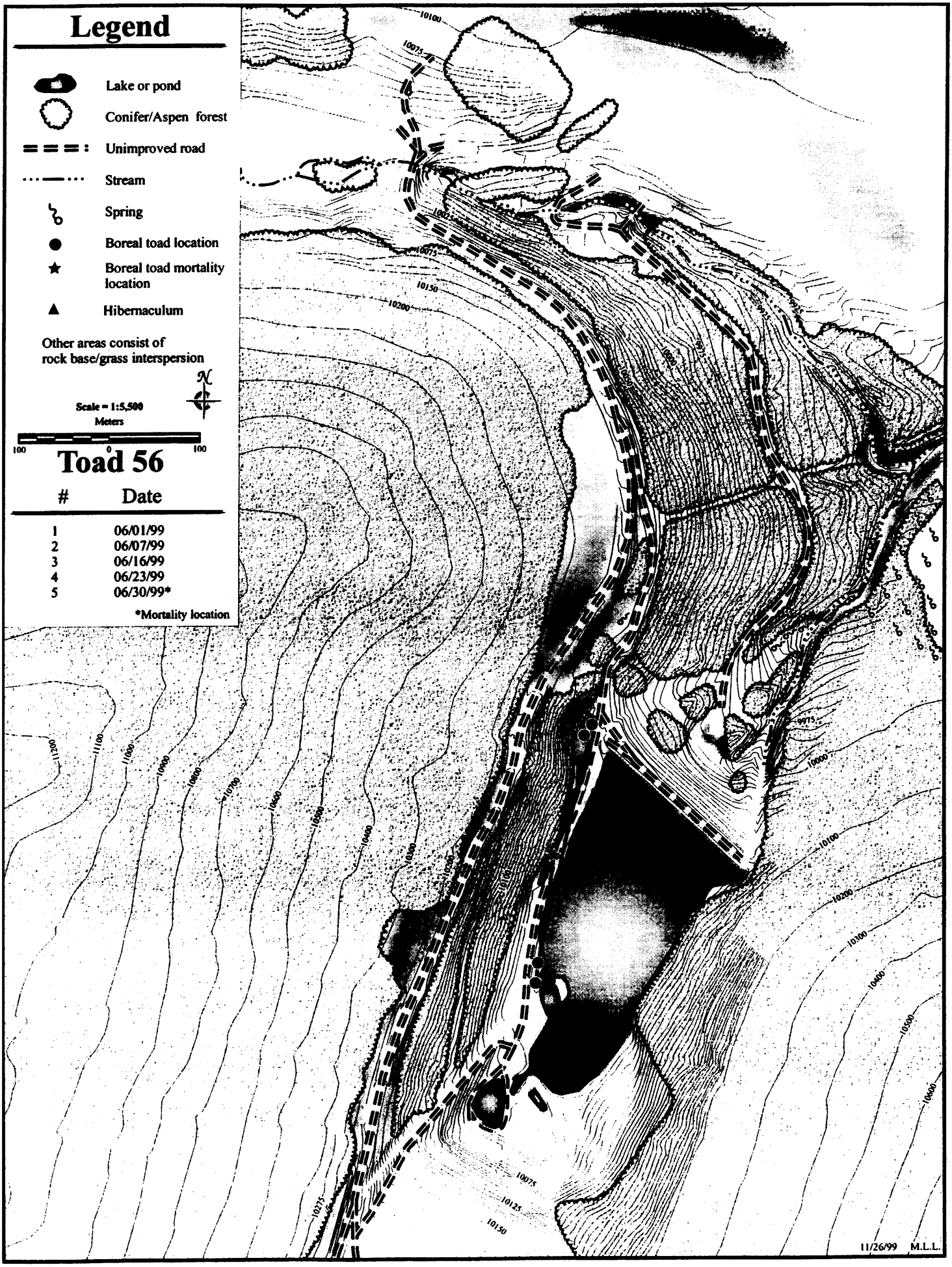

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 358
Date
1 06/29/99
2 07/06/99
3 07/13/99
4 07/20/99
5 07/28/99
6 08/03/99
7 08/10/99
8 08/17/99
9 08/31/99
Inset scale 1: 1,833
11/26/99 M.L.L.

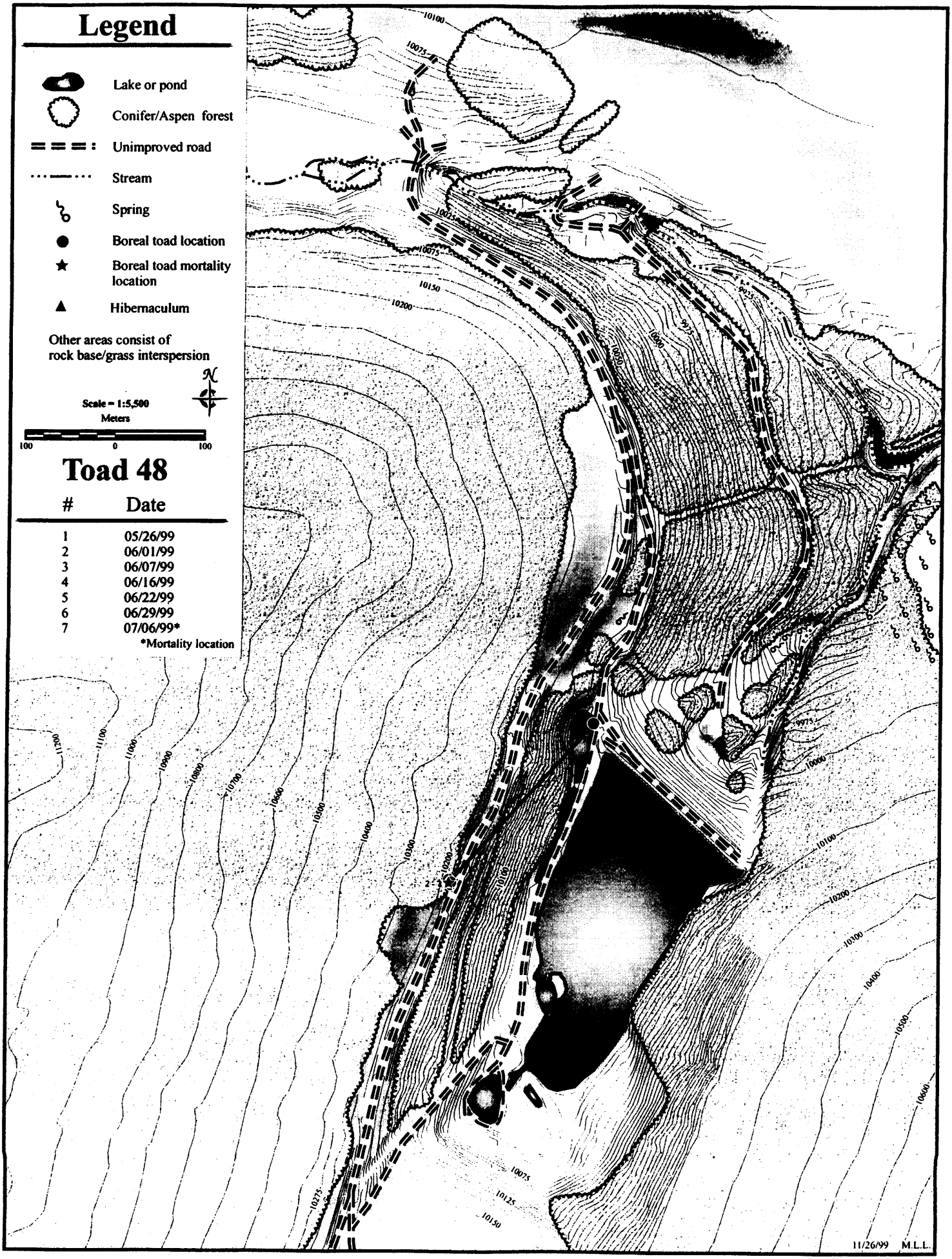

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 47
Date
1 06/01/99
2 06/07/99
3 06/16/99
11/26/99 M.L.L.

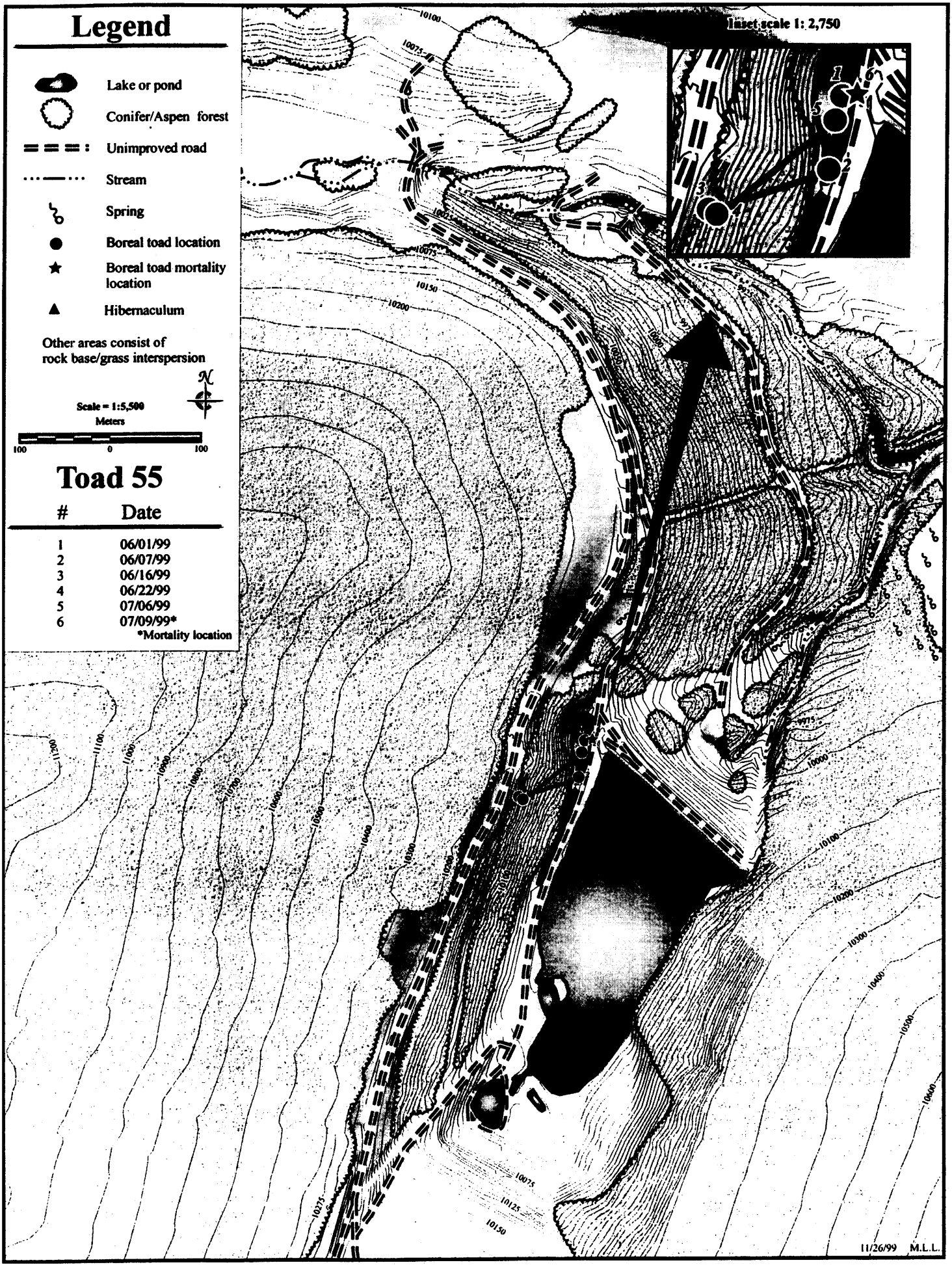

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 43
Date
1 05/26/99
2 05/31/99
3 06/07/99
4 06/16/99
5 06/22/99
6 06/29/99
7 07/06/99
8 07/20/99
9 07/28/99
10 08/03/99
11 08/10/99
12 08/17/99
13 08/23/99
14 08/31/99
15 09/07/99
16 09/13/99
17 09/21/99
18 09/29/99
Inset scale 1: 1,833
11/26/99 M.L.L.

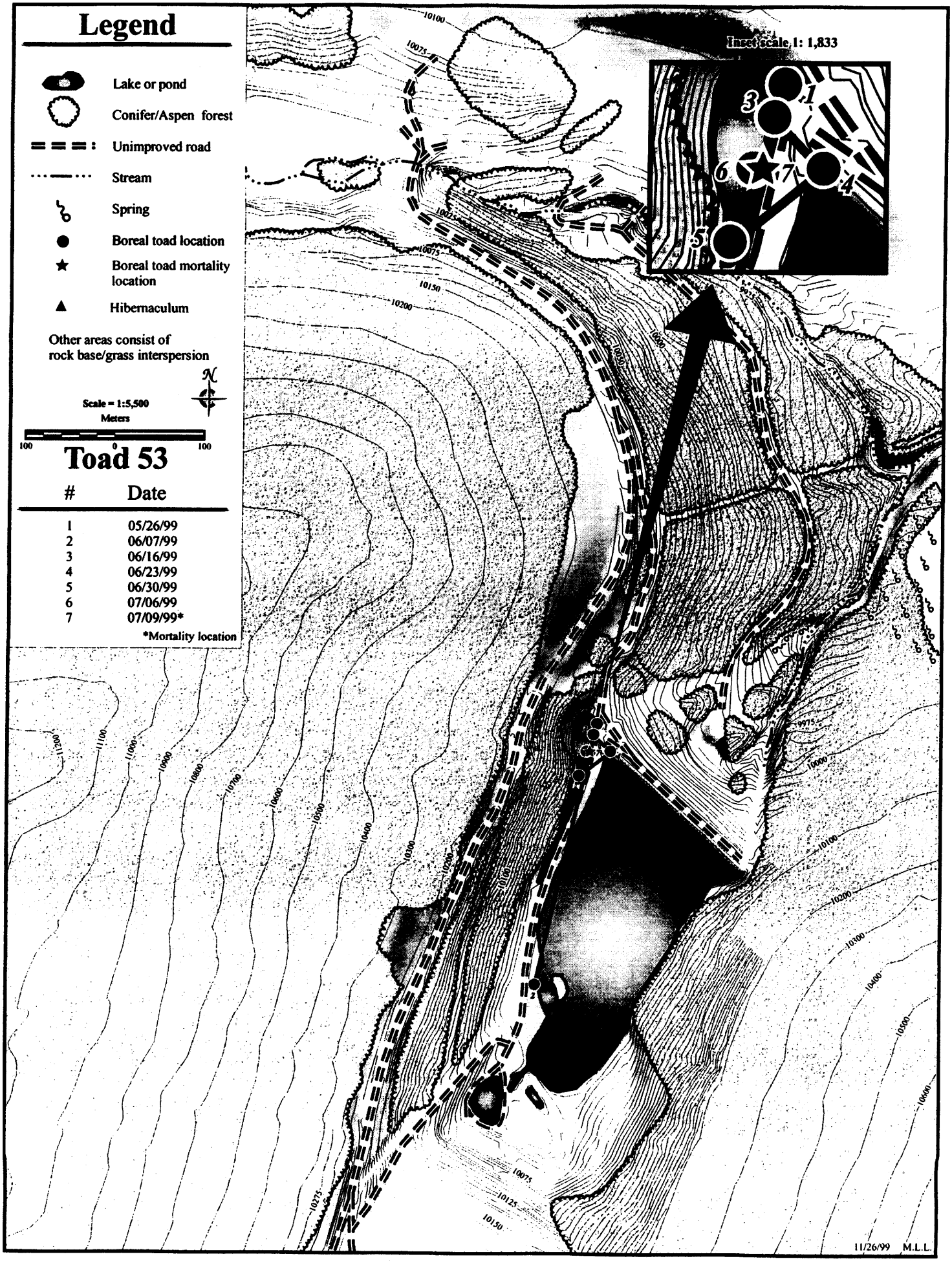

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 42
Date
1 05/26/99
2 06/01/99
3 06/16/99
4 06/22/99
5 07/05/99
6 07/13/99
7 07/20/99
8 07/28/99
9 08/03/99
10 08/10/99
11 08/23/99
12 08/31/99
13 09/07/99
14 09/13/99
15 09/21/99
16 09/29/99
Inset scale 1: 1,833
11/26/99 M.L.L.

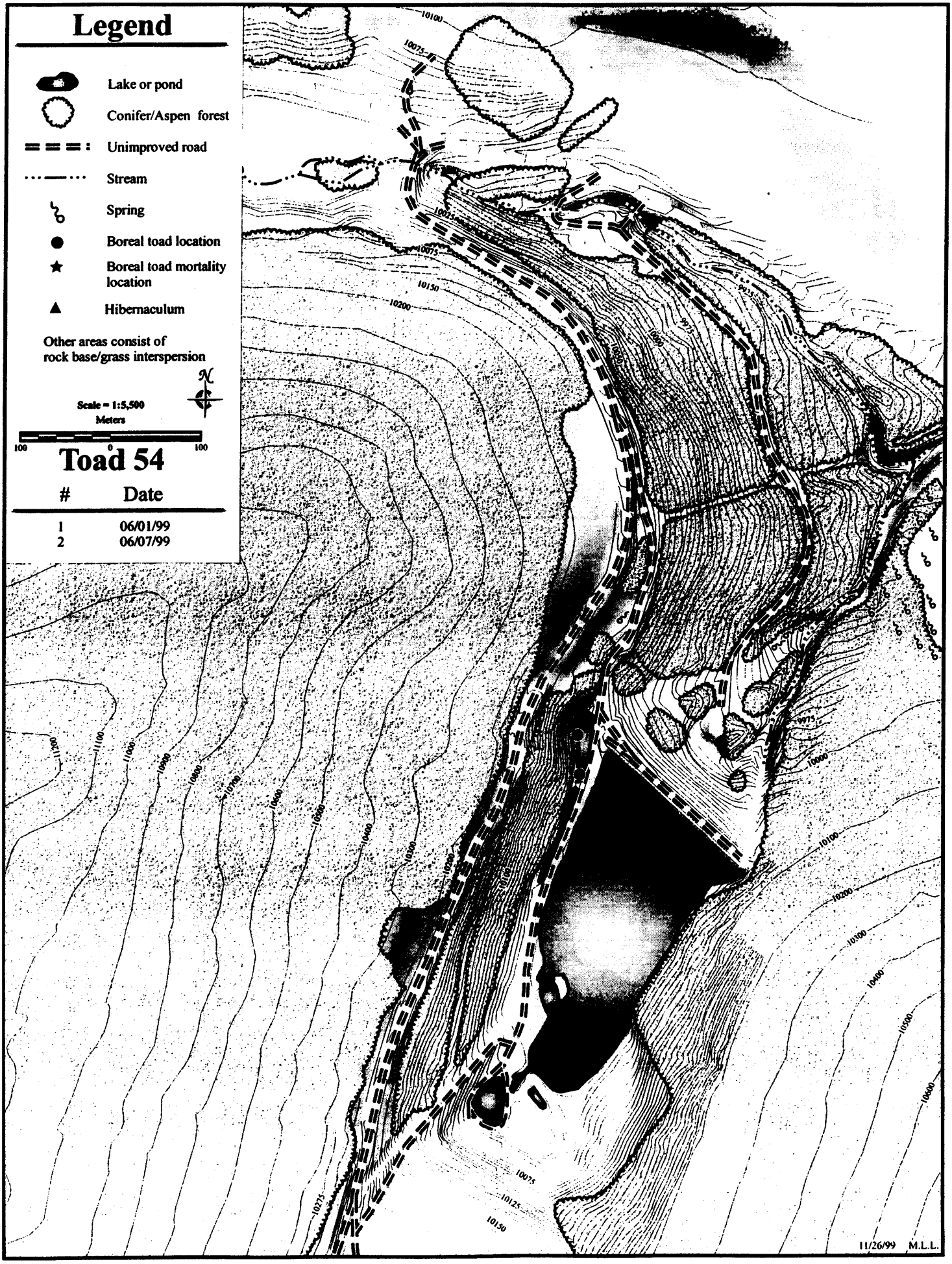

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 51
Date
1 05/26/99
2 06/01/99
3 06/15/99
4 06/23/99
5 06/30/99
6 07/06/99*
*Mortality location
11/26/99 M.L.L.

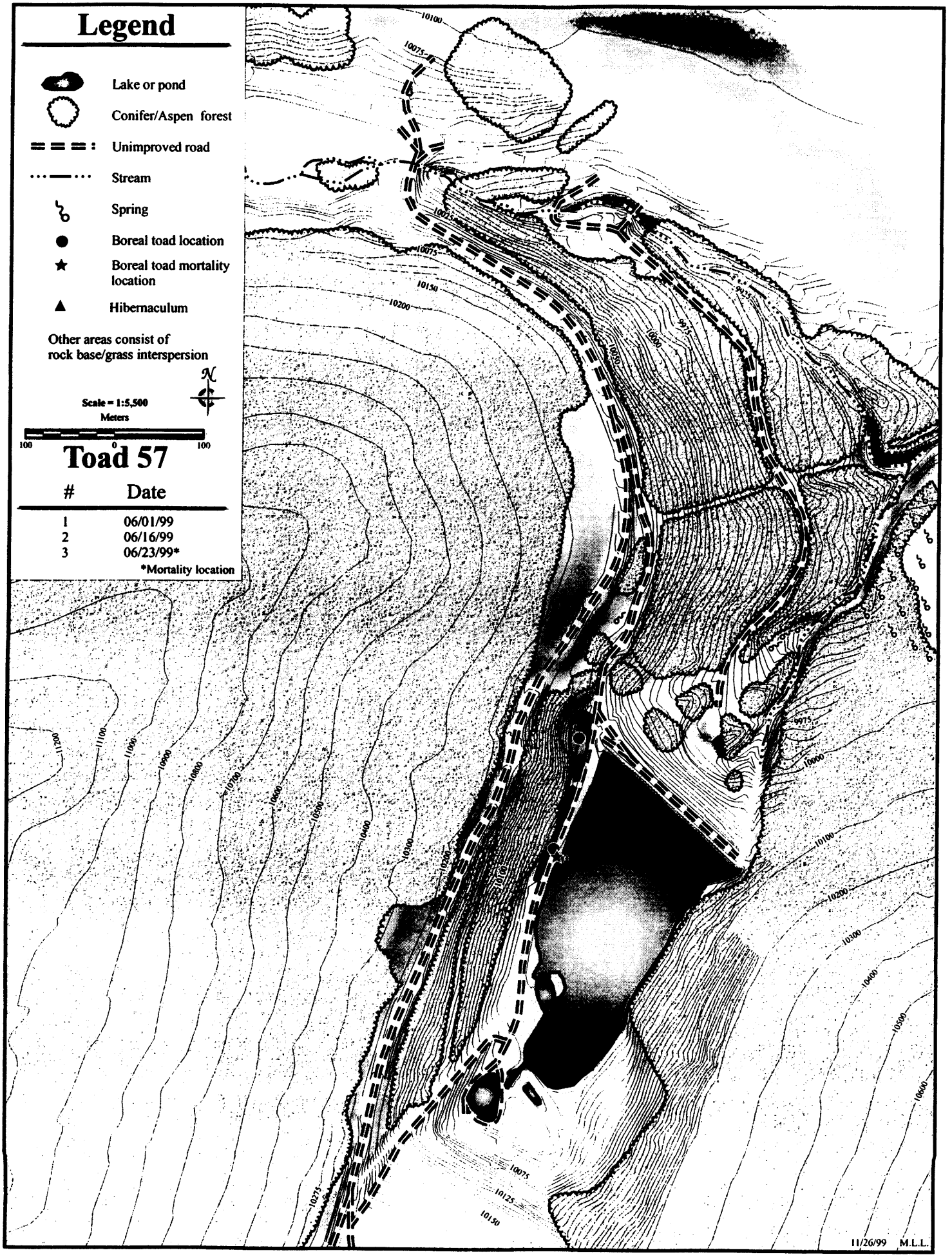

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 56
Date
1 06/01/99
2 06/07/99
3 06/16/99
4 06/23/99
5 06/30/99*
*Mortality location
11/26/99 M.L.L.

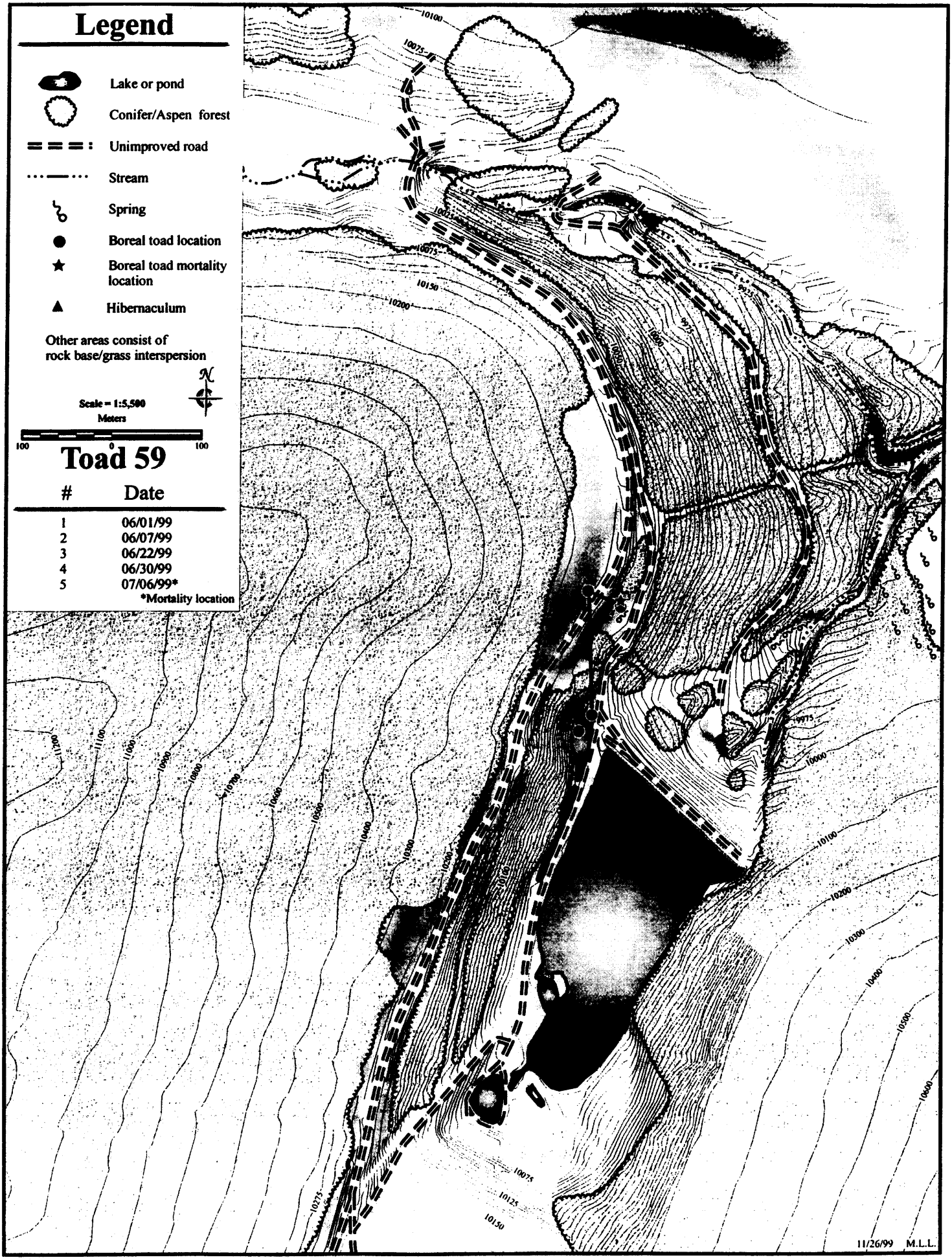

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 48
Date
1 05/26/99
2 06/01/99
3 06/07/99
4 06/16/99
5 06/22/99
6 06/29/99
7 07/06/99*
*Mortality location
11/26/99 M.L.L.

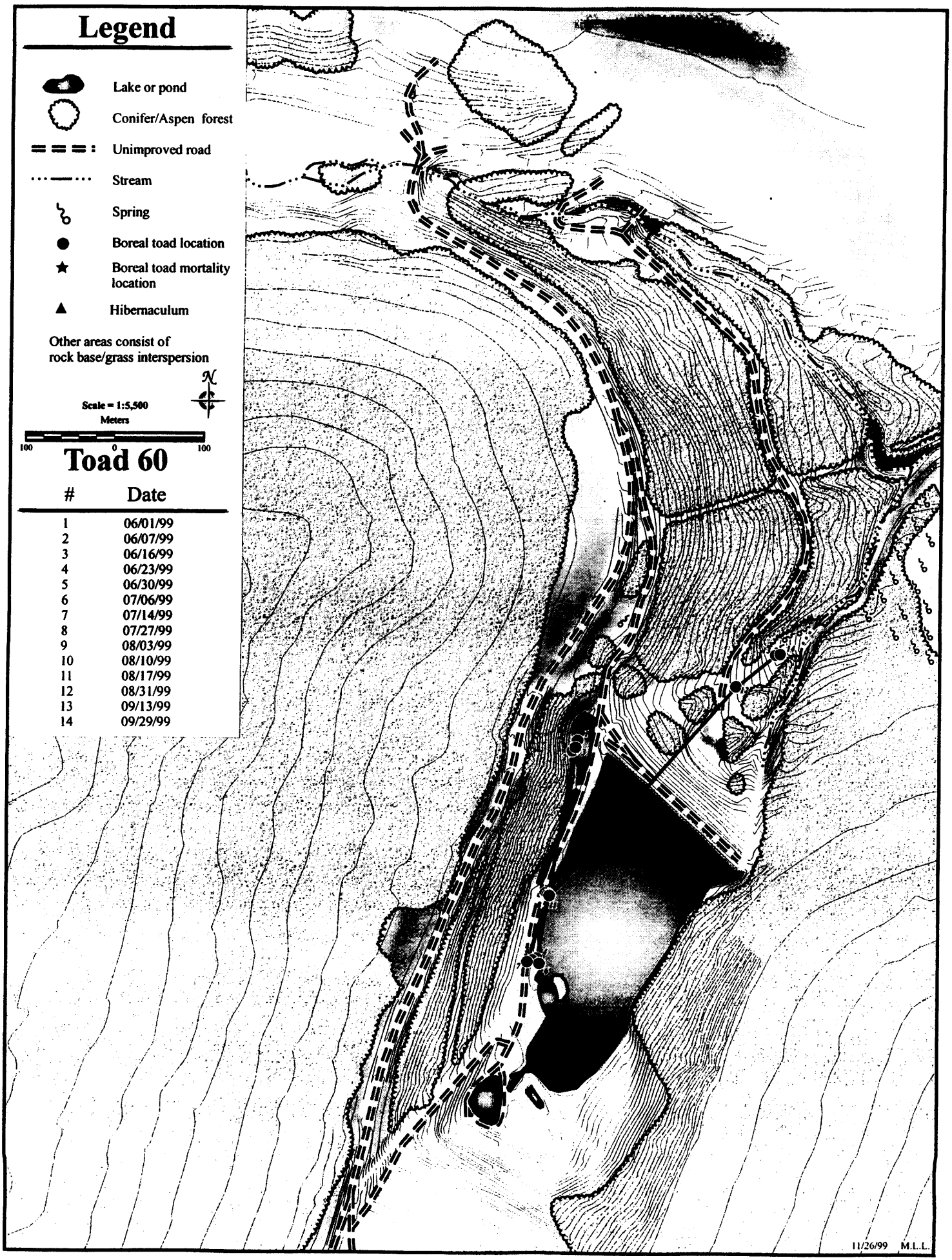

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 55
Date
1 06/01/99
2 06/07/99
3 06/16/99
4 06/22/99
5 07/06/99
6 07/09/99*
*Mortality location
Inset scale 1: 2,750
1
2
3
4
5
6
10100
10075
10150
10200
10125
10275
11/26/99 M.L.L.

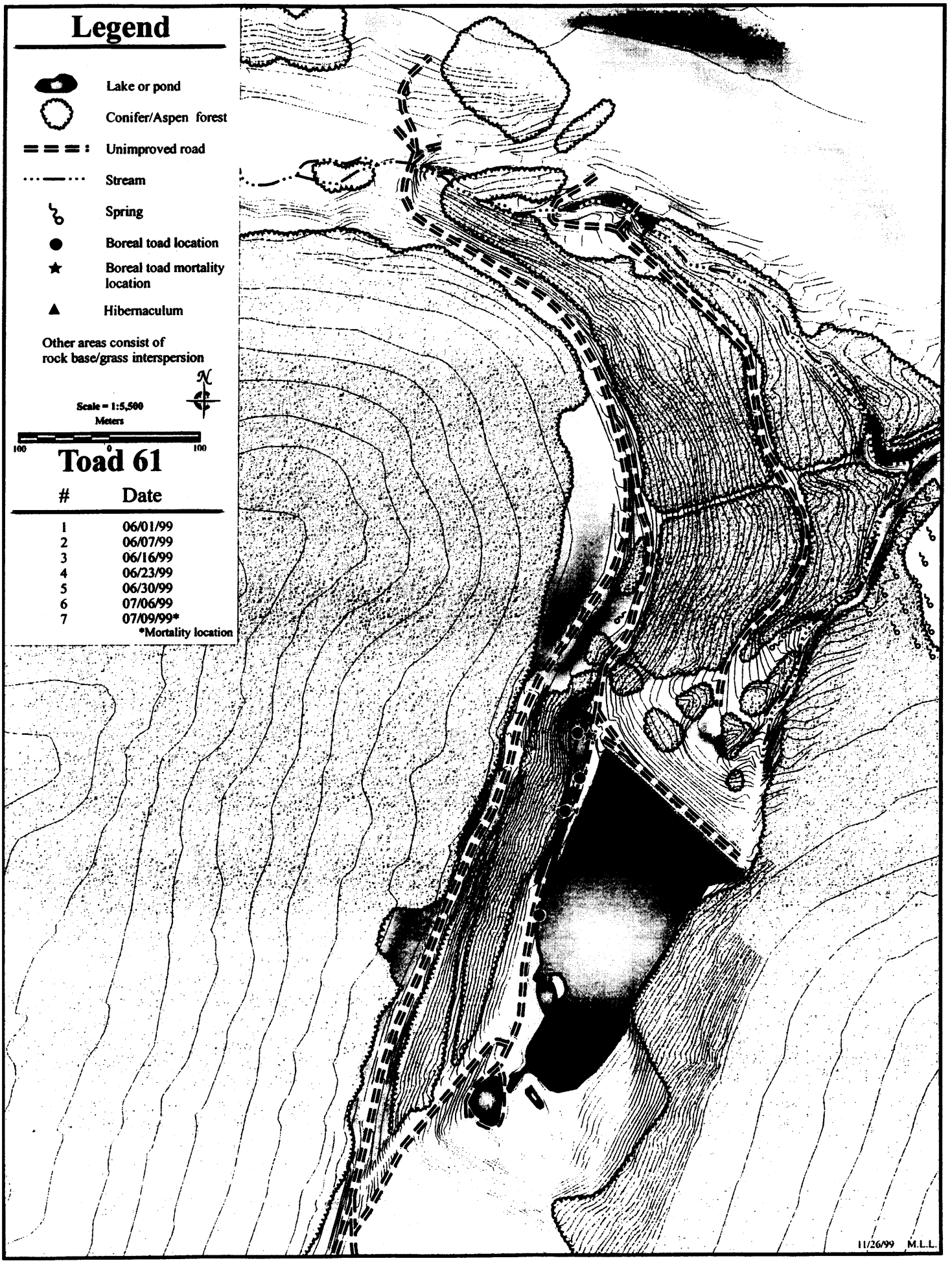

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 53
Date
1 05/26/99
2 06/07/99
3 06/16/99
4 06/23/99
5 06/30/99
6 07/06/99
7 07/09/99*
*Mortality location
Inset scale 1: 1,833
11/26/99 M.L.L.

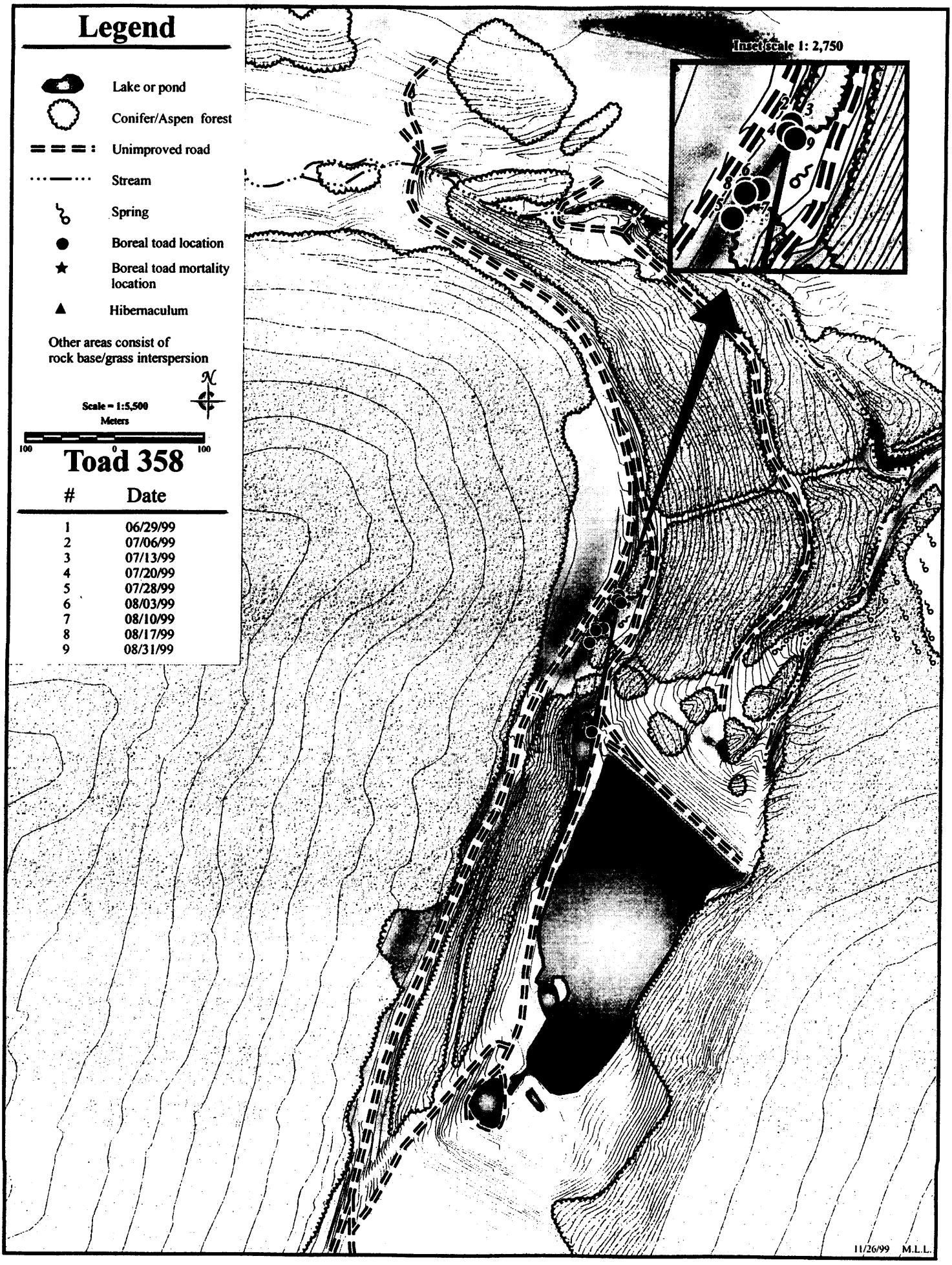

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 54
Date
1 06/01/99
2 06/07/99
11/26/99 M.L.L.

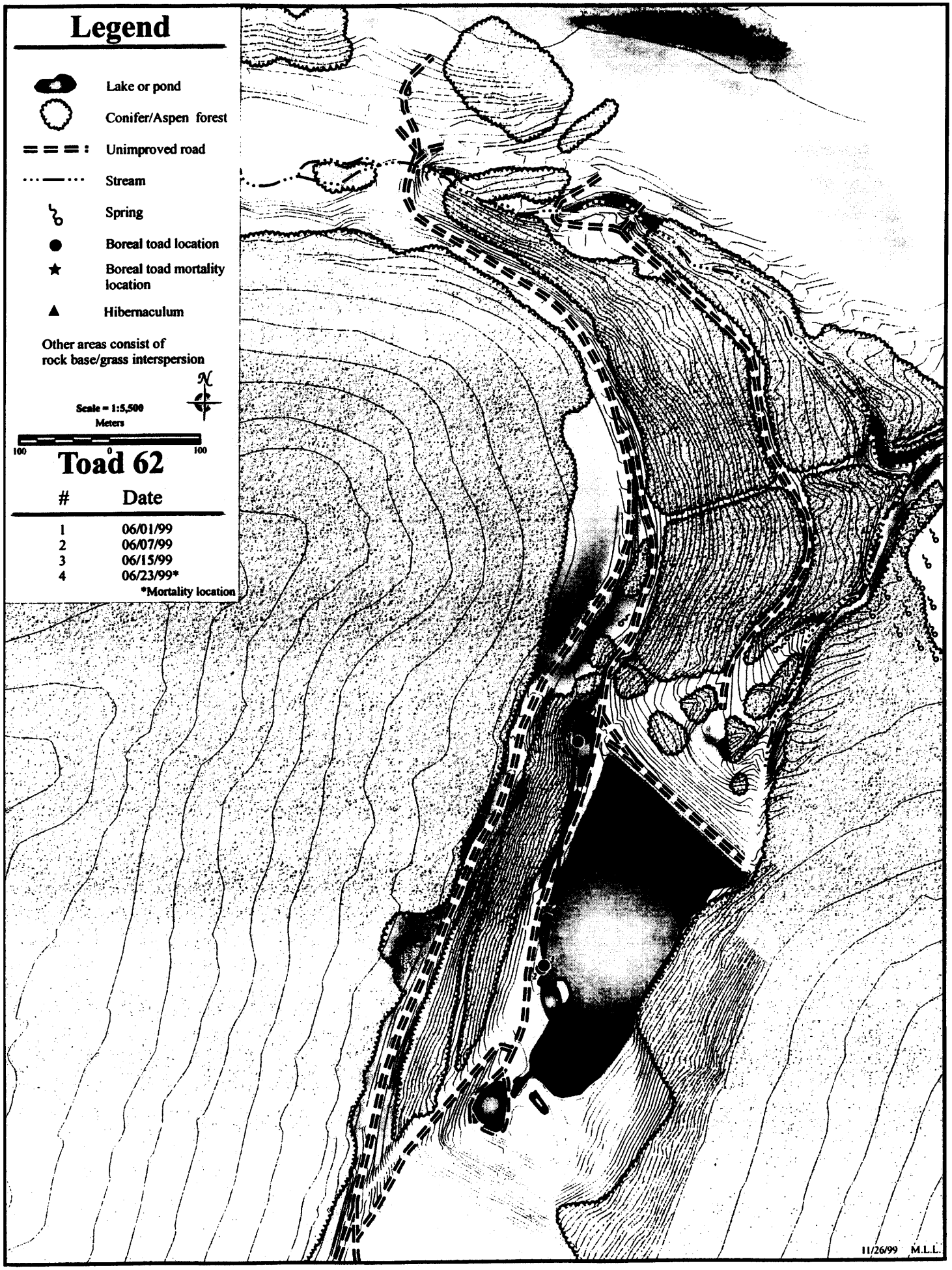

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 57
Date
1 06/01/99
2 06/16/99
3 06/23/99*
*Mortality location
11/26/99 M.L.L.

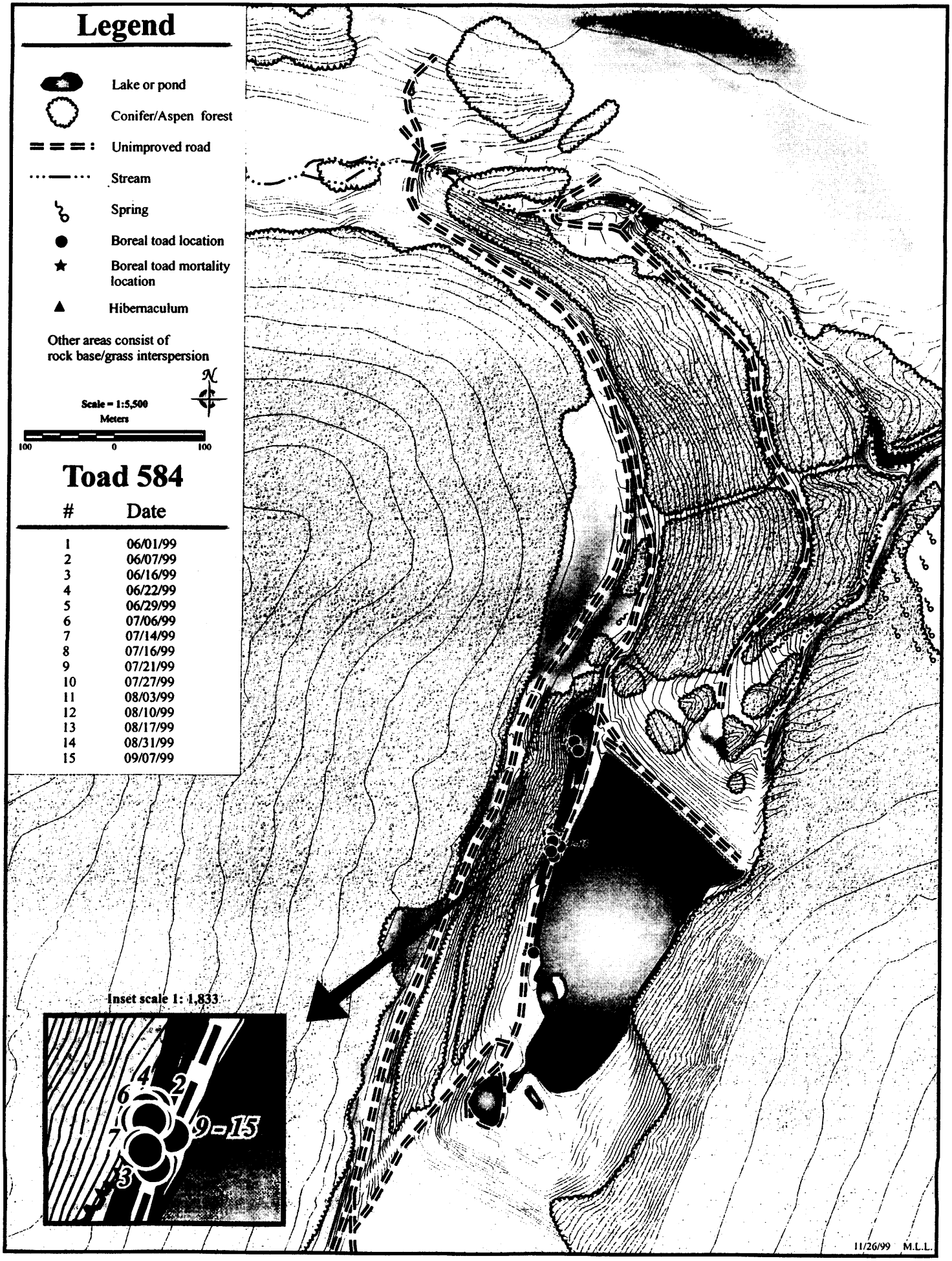

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 59
Date
1 06/01/99
2 06/07/99
3 06/22/99
4 06/30/99
5 07/06/99*
*Mortality location
11/26/99 M.L.L.

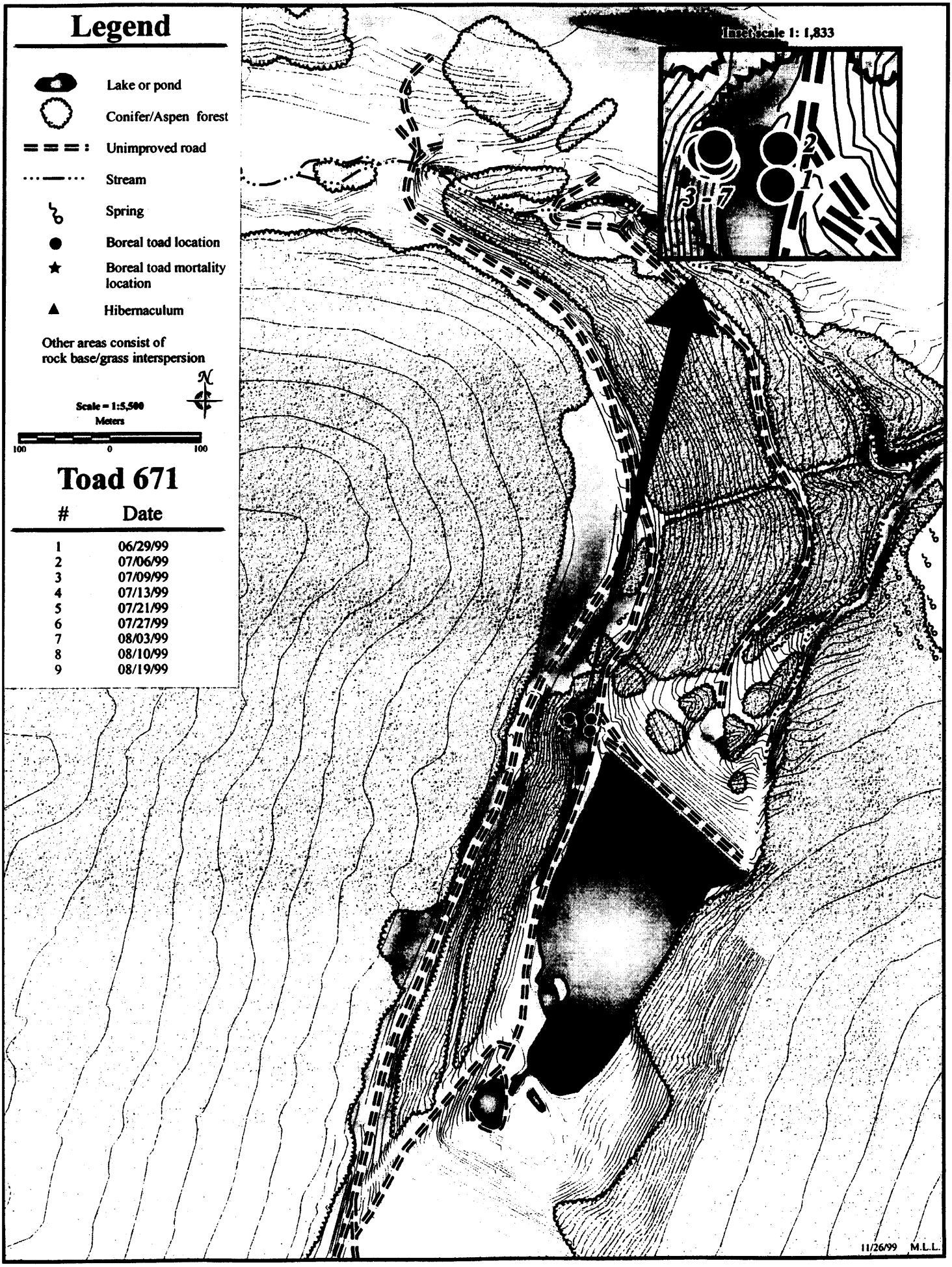

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 60
Date
1 06/01/99
2 06/07/99
3 06/16/99
4 06/23/99
5 06/30/99
6 07/06/99
7 07/14/99
8 07/27/99
9 08/03/99
10 08/10/99
11 08/17/99
12 08/31/99
13 09/13/99
14 09/29/99
11/26/99 M.L.L.

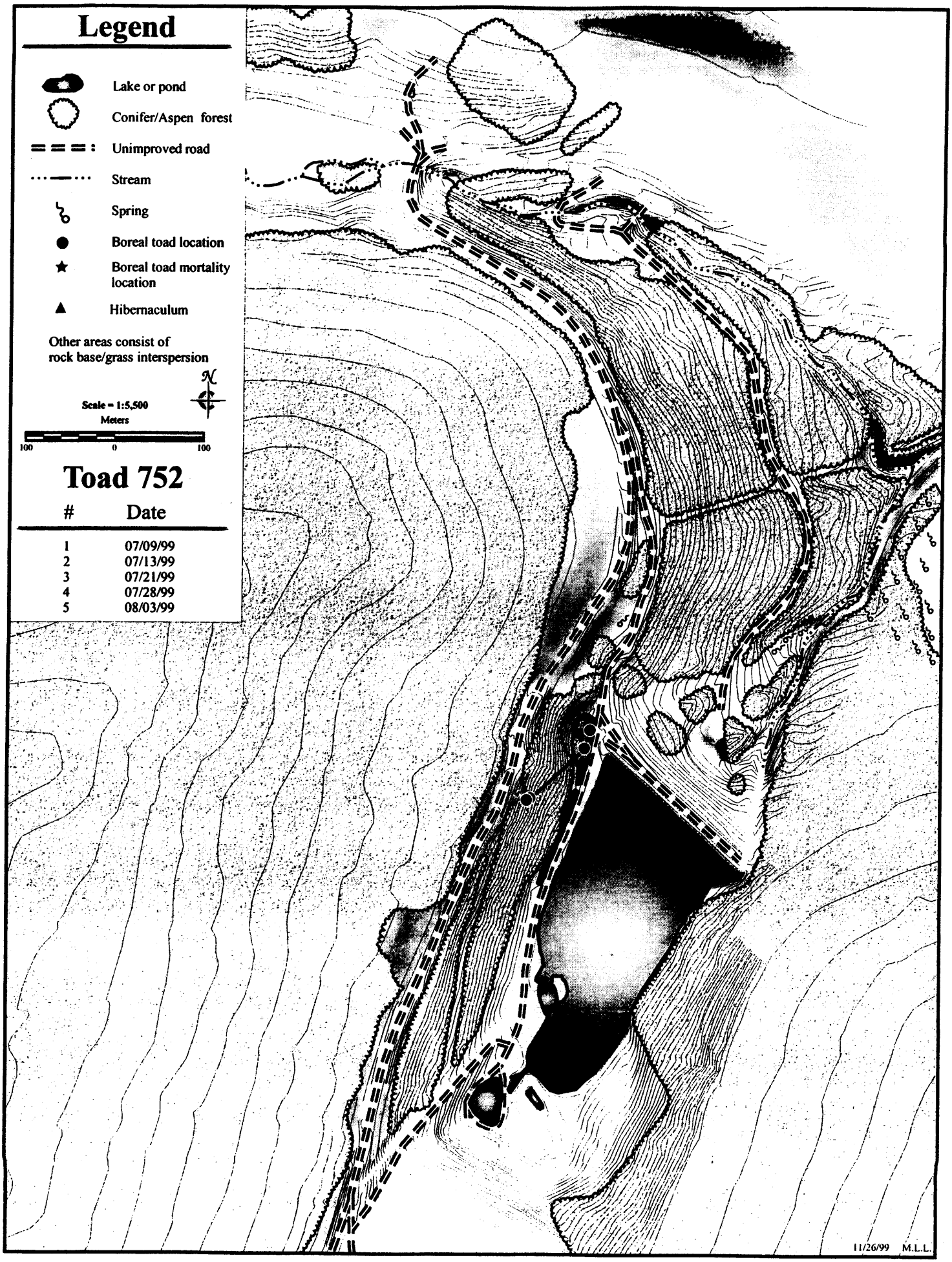

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 61
Date
1 06/01/99
2 06/07/99
3 06/16/99
4 06/23/99
5 06/30/99
6 07/06/99
7 07/09/99*
*Mortality location
11/26/99 M.L.L.

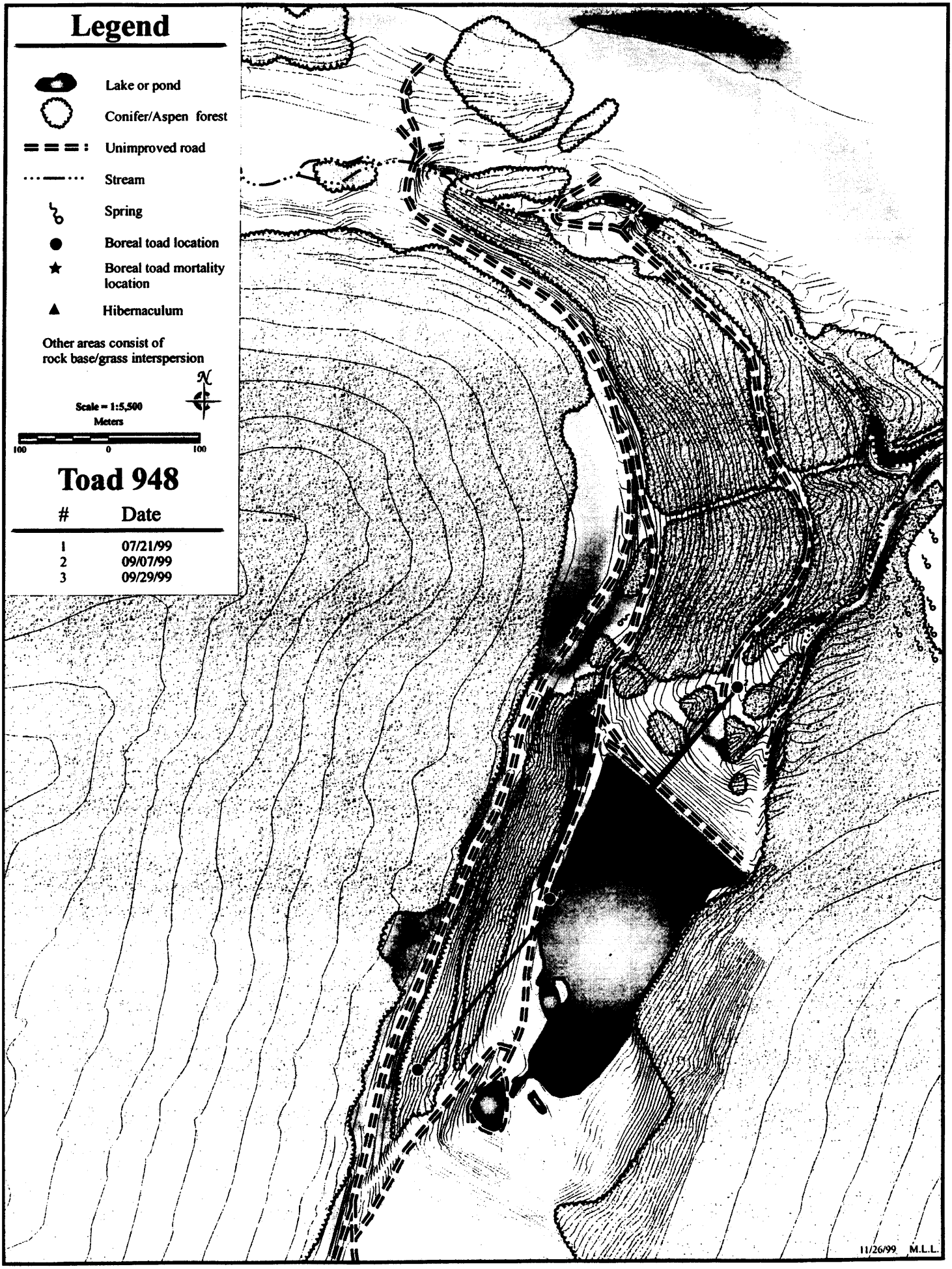

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 358
Date
1 06/29/99
2 07/06/99
3 07/13/99
4 07/20/99
5 07/28/99
6 08/03/99
7 08/10/99
8 08/17/99
9 08/31/99
Inset scale 1: 2,750
11/26/99 M.L.L.

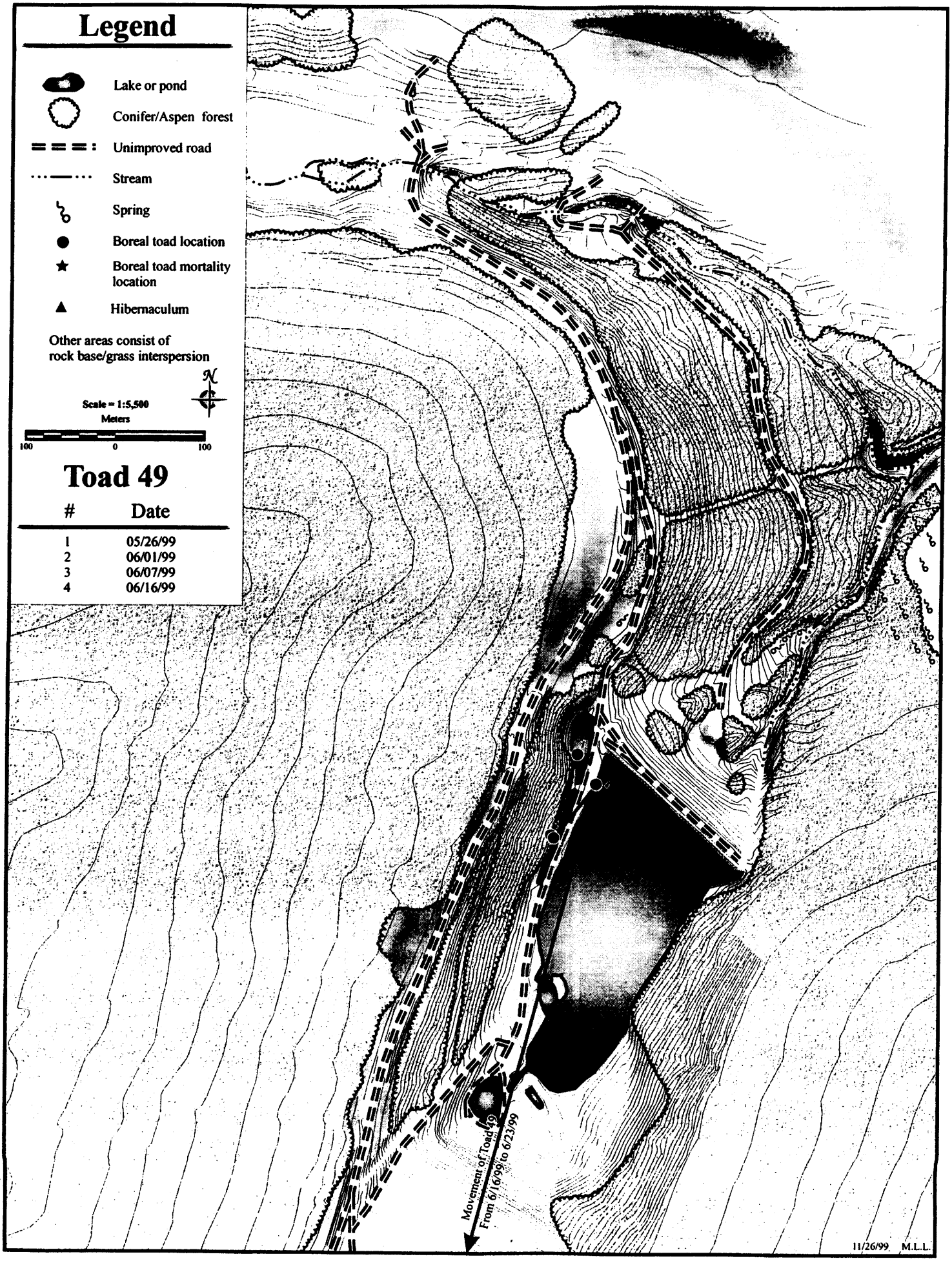

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 62
Date
1 06/01/99
2 06/07/99
3 06/15/99
4 06/23/99*
*Mortality location
11/26/99 M.L.L.

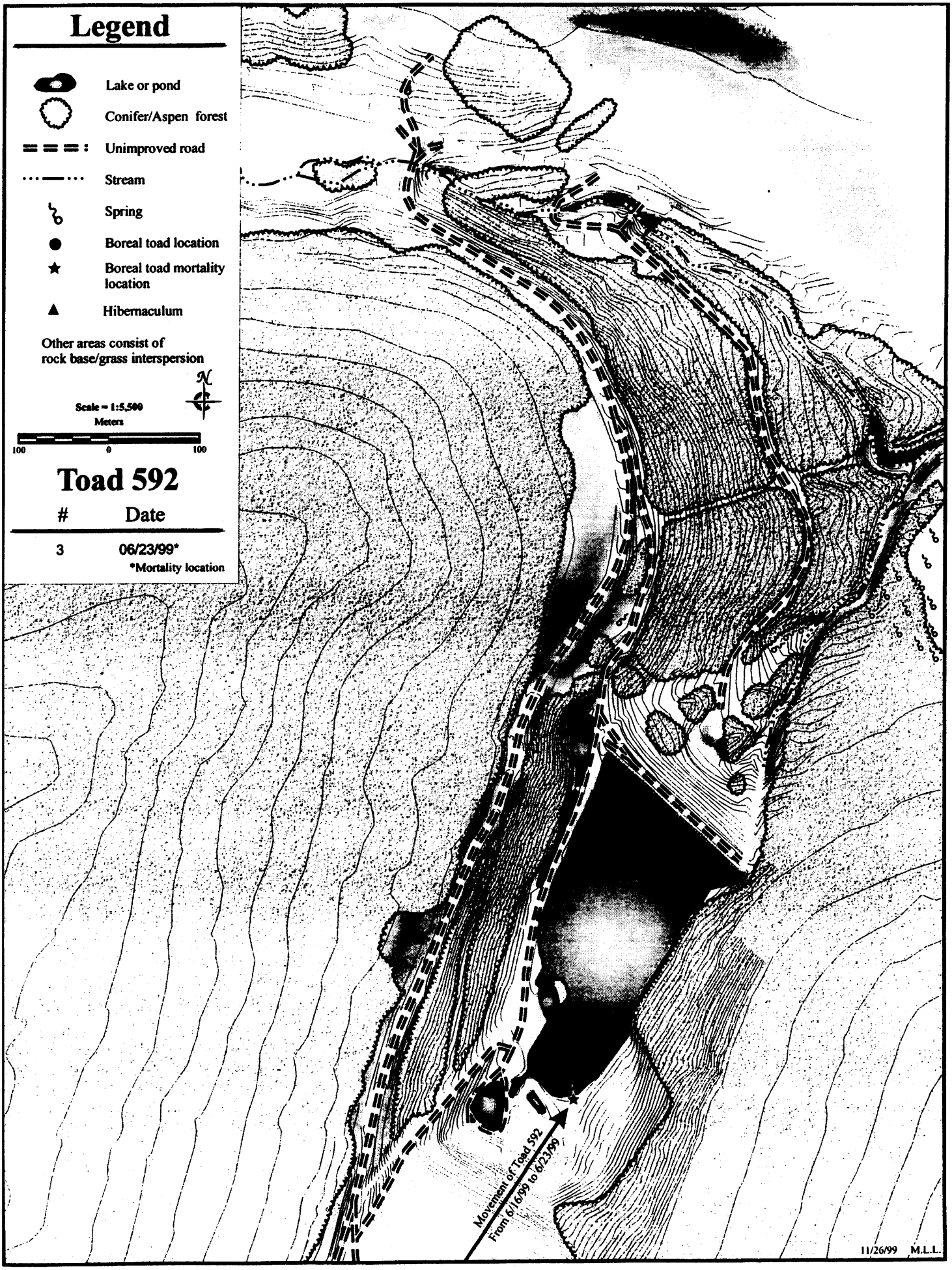

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 584
Date
1 06/01/99
2 06/07/99
3 06/16/99
4 06/22/99
5 06/29/99
6 07/06/99
7 07/14/99
8 07/16/99
9 07/21/99
10 07/27/99
11 08/03/99
12 08/10/99
13 08/17/99
14 08/31/99
15 09/07/99
Inset scale 1: 1,833
9-15
11/26/99 M.L.L.

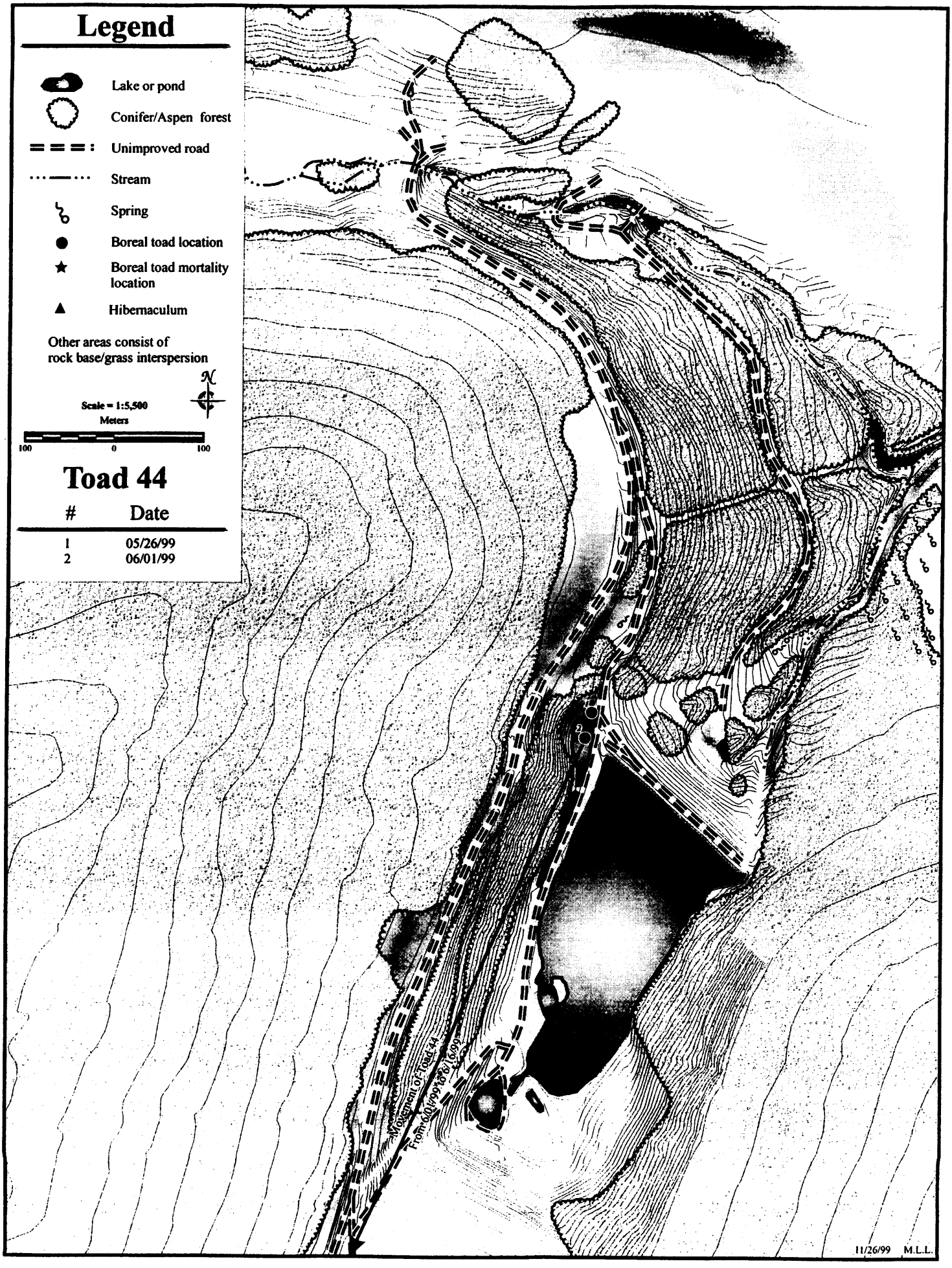

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 671
Date
1 06/29/99
2 07/06/99
3 07/09/99
4 07/13/99
5 07/21/99
6 07/27/99
7 08/03/99
8 08/10/99
9 08/19/99
Inset scale 1: 1,833
2
1
3 - 7
11/26/99 M.L.L.

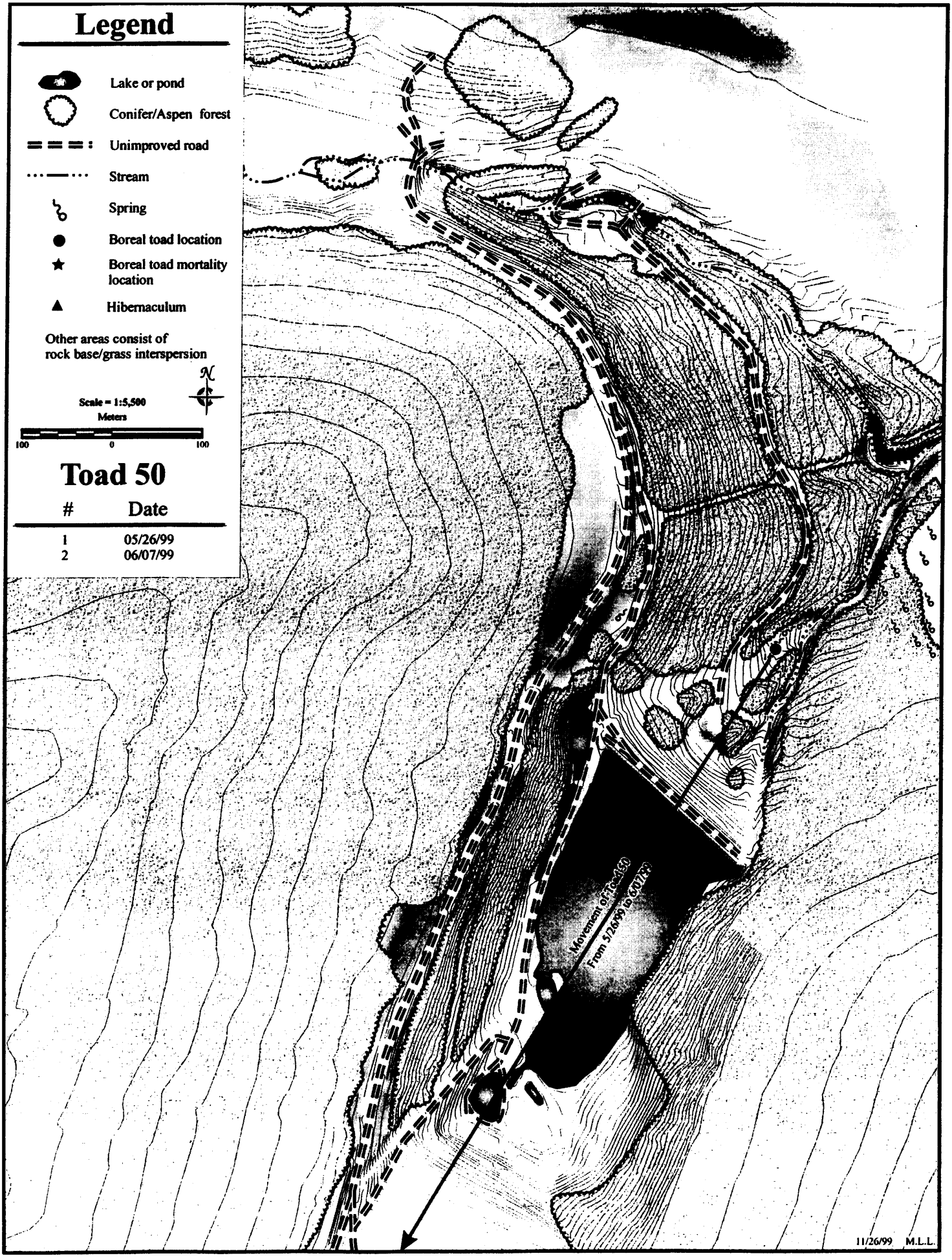

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 752
Date
1 07/09/99
2 07/13/99
3 07/21/99
4 07/28/99
5 08/03/99
11/26/99 M.L.L.

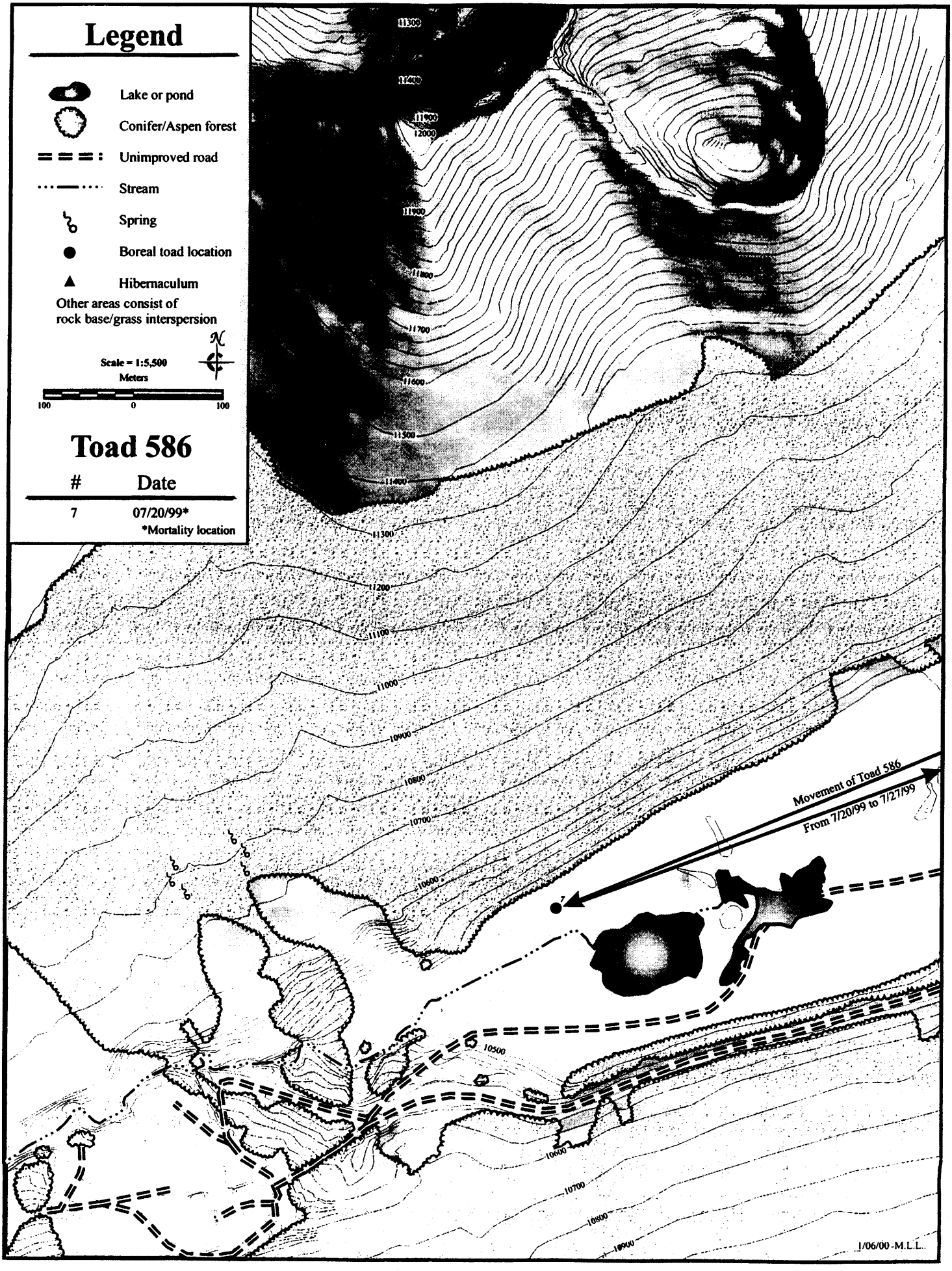

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 948
Date
1 07/21/99
2 09/07/99
3 09/29/99
11/26/99 M.L.L.

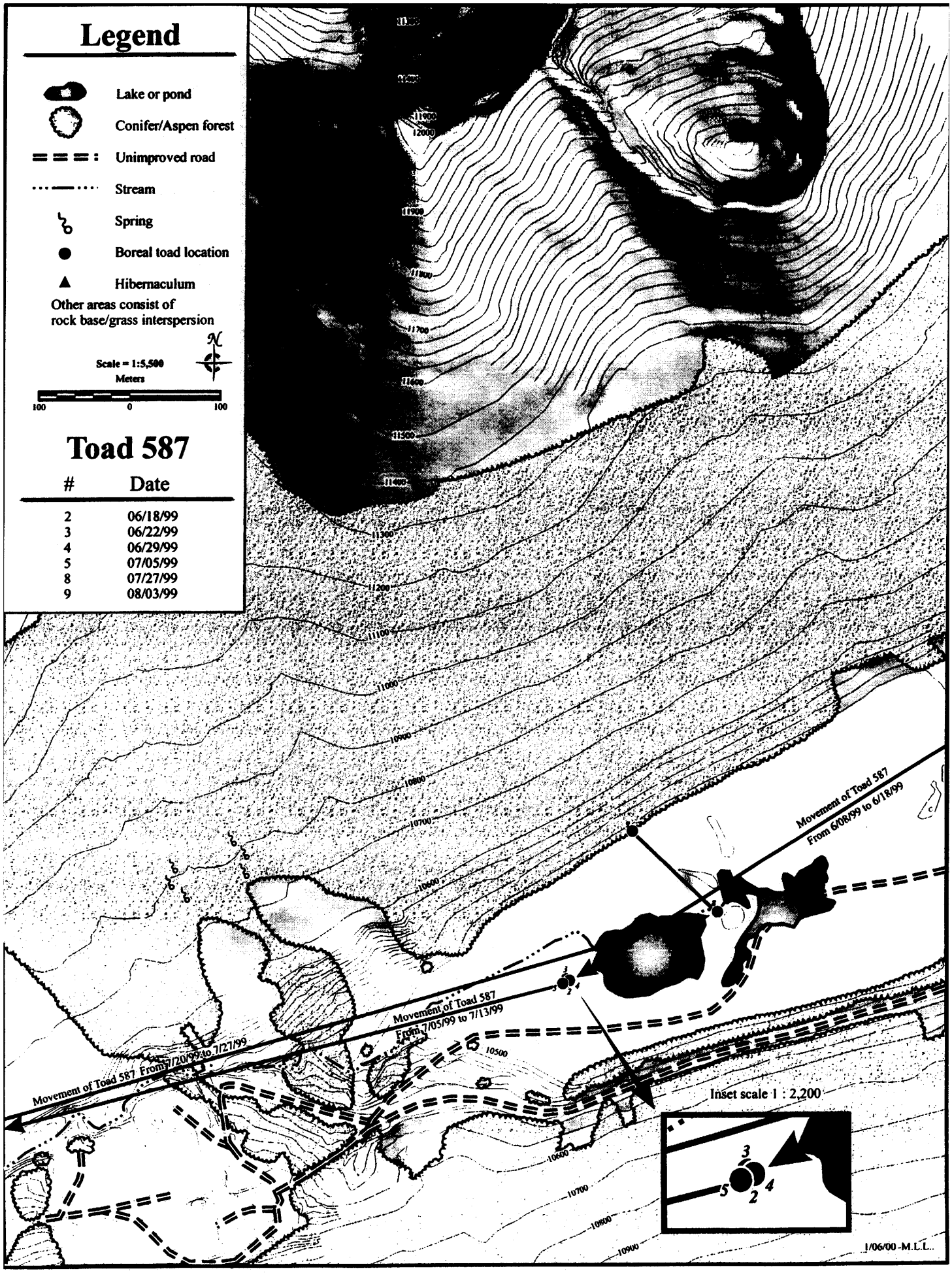

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 49
Date
1 05/26/99
2 06/01/99
3 06/07/99
4 06/16/99
Movement of Toad 49 From 6/16/99 to 6/23/99
11/26/99 M.L.L.

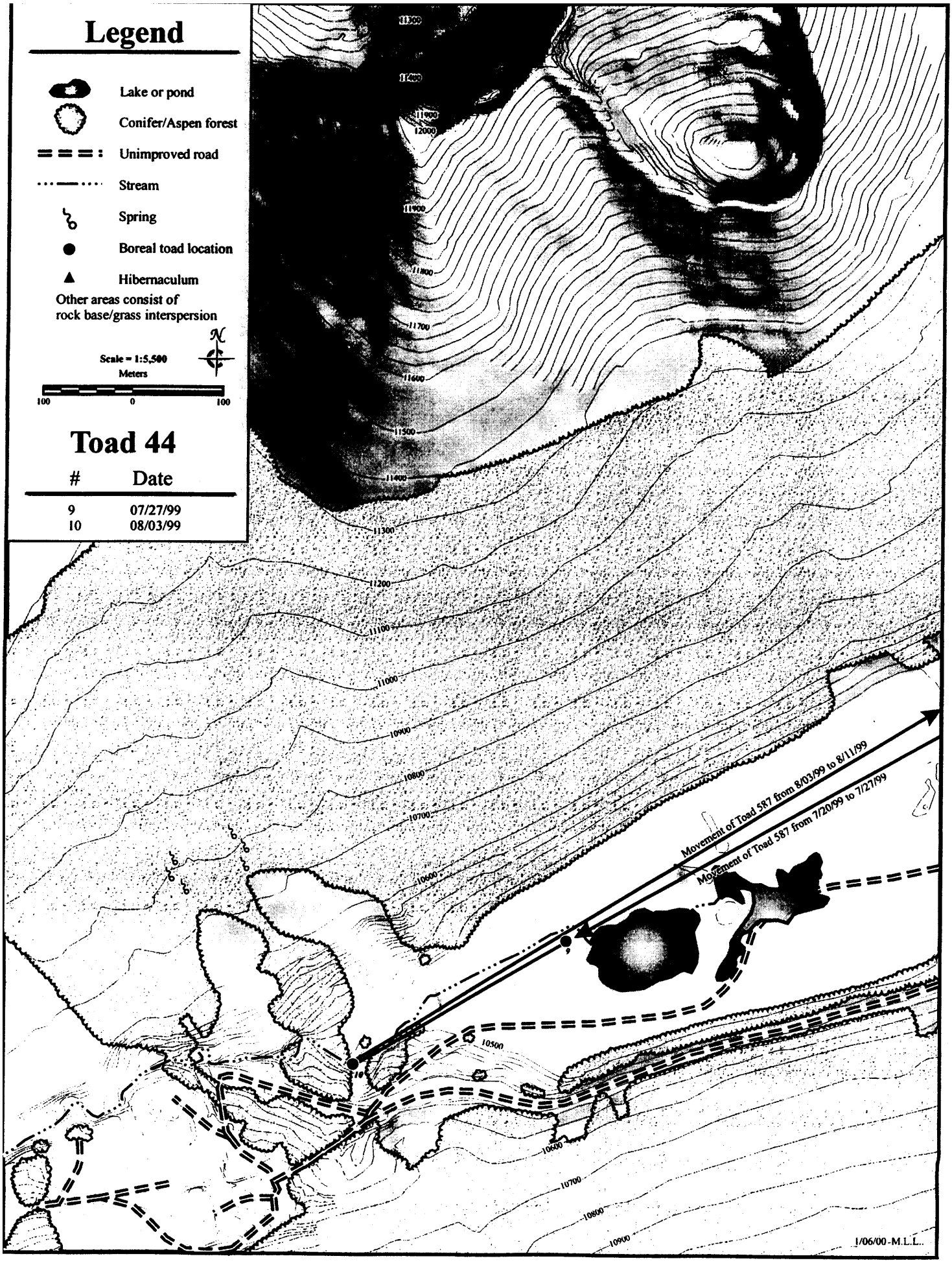

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 592
Date
3 06/23/99*
*Mortality location
Movement of Toad 592 From 6/16/99 to 6/23/99
11/26/99 M.L.L.

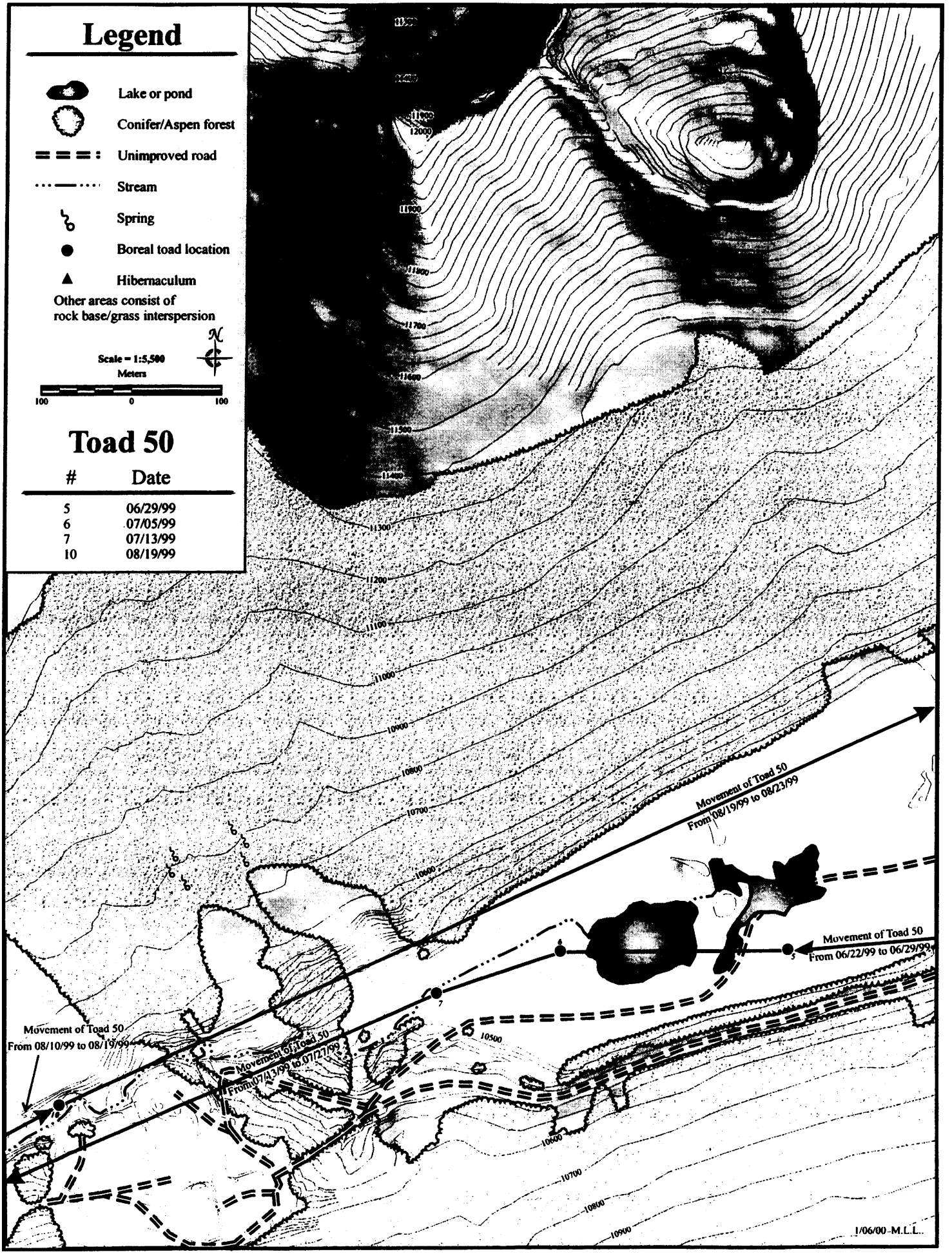

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 44
Date
1 05/26/99
2 06/01/99
Movement of Toad 44
From 06/01/99 to 10/6/99
11/26/99 M.L.L.

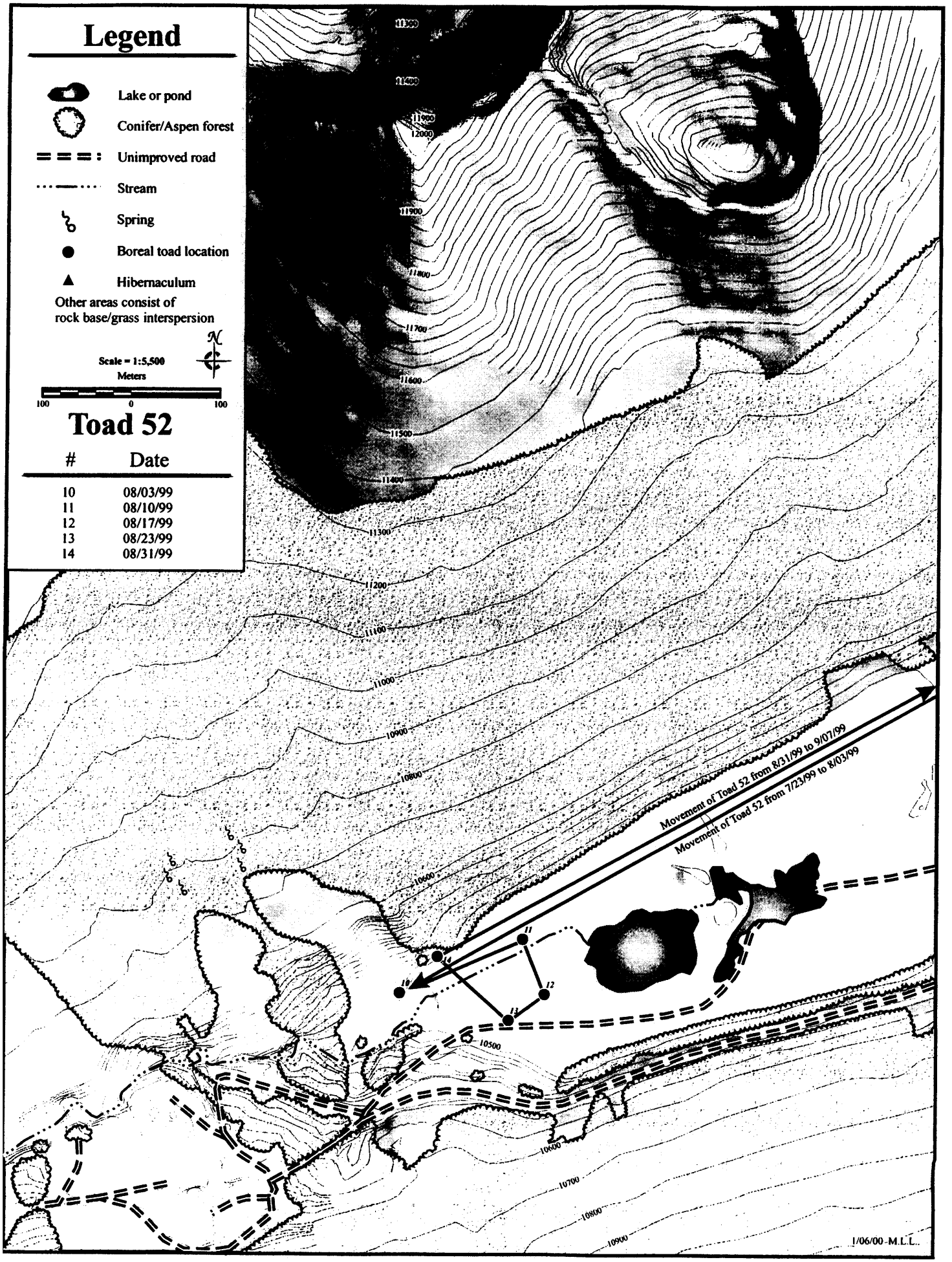

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Boreal toad mortality location
Hibernaculum
Other areas consist of rock base/grass interspersion
N
Scale = 1:5,500
Meters
100
0
100
Toad 50
Date
1 05/26/99
2 06/07/99
Movement of TC-450
From 5/26/99 to 6/07/99
11/26/99 M.L.L.

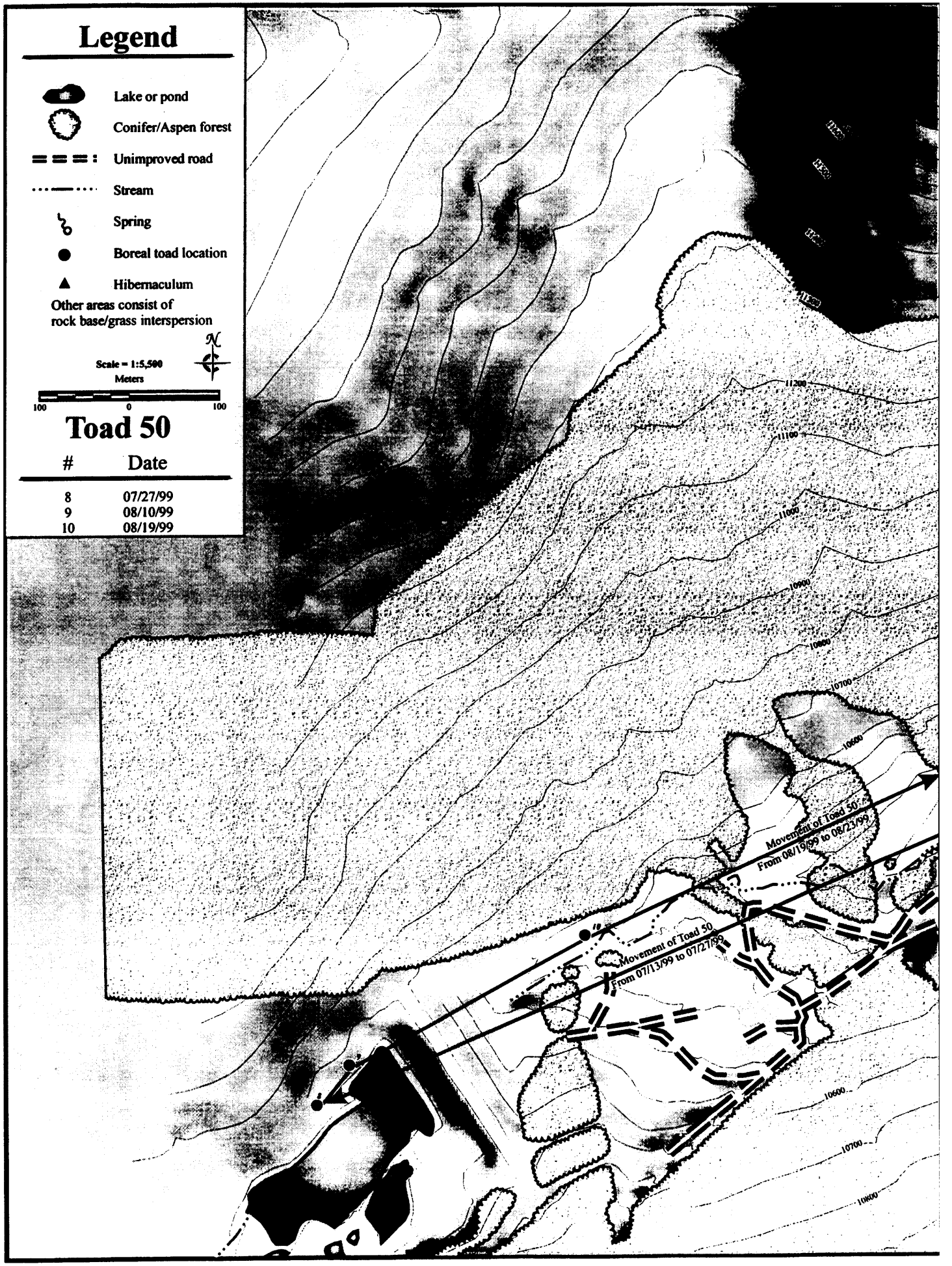

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 586
Date
7 07/20/99*
*Mortality location
11300
11400
11900
12000
11800
11700
11600
11500
11400
11300
11200
11100
11000
10900
10800
10700
10600
10500
10600
10700
10800
10900
Movement of Toad 586
From 7/20/99 to 7/27/99
1/06/00 - M.L.L.

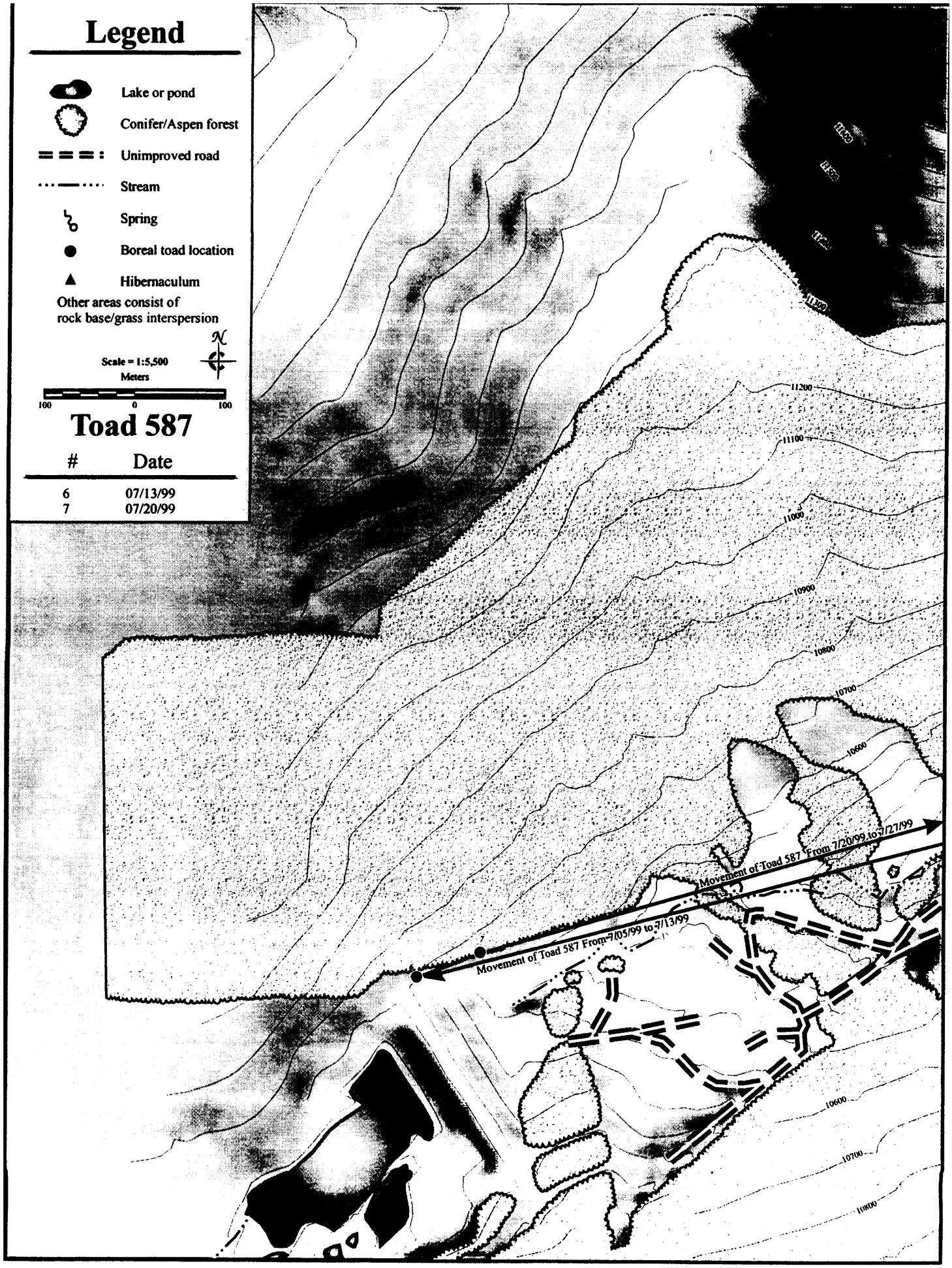

Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 587
Date
2 06/18/99
3 06/22/99
4 06/29/99
5 07/05/99
8 07/27/99
9 08/03/99
Movement of Toad 587 From 6/08/99 to 6/18/99
Movement of Toad 587 From 7/05/99 to 7/13/99
Movement of Toad 587 From 7/20/99 to 7/27/99
Inset scale 1 : 2,200
1/06/00 - M.L.L.


Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of
rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 44
Date
9 07/27/99
10 08/03/99
11300
11400
11900
12000
11900
11800
11700
11600
11500
11400
11300
11200
11100
11000
10900
10800
10700
10600
10500
10600
10700
10800
10900
Movement of Toad 587 from 8/03/99 to 8/11/99
Movement of Toad 587 from 7/20/99 to 7/27/99
1/06/00-M.L.L.


Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of
rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 50
Date
5 06/29/99
6 07/05/99
7 07/13/99
10 08/19/99
11900
12000
11800
11700
11600
11500
11400
11300
11200
11100
11000
10900
10800
10700
10600
10500
Movement of Toad 50
From 08/19/99 to 08/23/99
Movement of Toad 50
From 06/22/99 to 06/29/99
Movement of Toad 50
From 08/10/99 to 08/19/99
Movement of Toad 50
From 07/13/99 to 07/27/99
1/06/00 M.L.L.


Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 52
Date
10 08/03/99
11 08/10/99
12 08/17/99
13 08/23/99
14 08/31/99
Movement of Toad 52 from 8/31/99 to 9/07/99
Movement of Toad 52 from 7/23/99 to 8/03/99
1/06/00-M.L.L.


Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of
rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 50
Date
8 07/27/99
9 08/10/99
10 08/19/99
Movement of Toad 50
From 08/19/99 to 08/23/99
Movement of Toad 50
From 07/13/99 to 07/27/99
11200
11100
11000
10900
10800
10700
10600
10600
10700
10800


Legend
Lake or pond
Conifer/Aspen forest
Unimproved road
Stream
Spring
Boreal toad location
Hibernaculum
Other areas consist of
rock base/grass interspersion
Scale = 1:5,500
Meters
100
0
100
Toad 587
Date
6 07/13/99
7 07/20/99
11200
11100
11000
10900
10800
10700
10600
Movement of Toad 587 From 7/20/99 to 7/27/99
Movement of Toad 587 From 7/05/99 to 7/13/99

APPENDIX 2

Breeding site water quality results for 1997, 1998 and 1999.

SITE	DATE	TEMP	COND	PH	ALKALINITY	Al	As	Cd	Cu	Fe	Mn	Pb	Se	Zn
ABOVE TRICKLE PARK RES 215GM1	08/03/98	12.3	50.5	7.82	28.0	132	<10	<0.20	4.2	1710	26.2	<5.0	<5.0	<5.0
BEAVER POND 215 GM2	08/03/98	12.4	127.5	8.04	80.0	223	<10	<0.20	4.5	1096	287.0	<5.0	<5.0	5.2
BROWN'S CREEK	08/22/97	10.1	41	7.45	21.0	32	<10	<0.20	2.3	448	56.7	<5.0	<5.0	<5.0
BROWN'S CREEK	06/29/99		044	7.69	20.6	365	<15	<0.15	<1.0	227	<10	<2.0	<2.0	<10
BROWN'S CREEK	08/29/99					34	<15	<0.15	<1.0	54	<10	<2.0	<2.0	<10
CALIFORNIA PARK ROUTT N. FORES	05/26/99		065	5.5	41.2	943	<15	<0.15	1.4	2330	96	<2.0	<2.0	<10
COLL. PEAKS CAMPGROUND	07/28/99		091	7.50	58.0	67	<15	<0.15	1.0	929	17	<2.0	<2.0	<10
COLLEGIATE PEAKS	08/23/97	9.2	67	7.63	37.8	80	<10	<0.20	1.4	369	17.8	<5.0	<5.0	5.6
COLLEGIATE PEAKS	05/22/99					1300	<15	<0.15	18.7	23474	93	2.4	<2.0	11
COLLEGIATE PEAKS CHAFFEE CO	08/28/99		097	7.55	60.8	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS LOWER POND	05/28/98		83.8		46.0	137	<10	<0.20	1.1	760	21.5	<5.0	<5.0	<5.0
COLLEGIATE PEAKS MIDDLE POND	05/28/98		71.6		34.8	66	<10	0.21	1.5	395	17.7	<5.0	<5.0	<5.0
CRAIG'S POND FETKAVICH	06/08/99					54	<15	<0.15	1.1	196	<10	<2.0	<2.0	<10
CUCUMBER GULCH	06/18/97	21.8	44	7.08	14.2	83	<10	<0.20	1.4	368	17.7	<5.0	<5.0	5.5
CUCUMBER GULCH	06/24/98					59	<10	<0.20	1.6	192	12.5	<5.0	<5.0	<5.0
DENNY CREEK	08/23/97	8.8	38	7.51	23.0	224	<10	0.33	8.4	2280	45.1	5.7	<5.0	26.7
DENNY CREEK	05/28/98					104	<10	<0.20	<1.0	262	13.1	<5.0	<5.0	<5.0
DENNY CREEK	05/27/99					236	<15	<0.15	<1.0	604	28	<2.0	<2.0	<10
DENNY CREEK	09/04/99		034	7.71	18.8	55	<15	<0.15	<1.0	316	14	3.0	<2.0	<10
DENNY CREEK	09/04/99		045	7.72	29.0	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
DIAMOND PARK	06/27/97	24.0	73	7.53	30.6	184	<10	<0.20	3.3	886	82.4	<5.0	<5.0	8.0
DIAMOND PARK	06/02/99		057		34.8	95	<15	<0.15	2.3	309	10	<2.0	<2.0	<10
DIAMOND PARK	07/07/99					126	<15	<0.15	3.6	267	<10	<2.0	<2.0	<10
FOUR MILE CREEK CHAFFEE, CO	08/19/97	9.6	47	8.12	21.5	442	<10	<0.20	1.6	1558	106.0	<5.0	<5.0	<5.0
FOUR MILE CREEK CHAFFEE, CO	08/04/98		53.2		22.6	145	<10	<0.20	83.3	1302	110.6	<5.0	<5.0	10.1
FOUR MILE CREEK CHAFFEE, CO	09/19/98		63.8		27.6	254	<10	<0.20	1.3	2188	122.3	<5.0	<5.0	<5.0
FOUR MILE CREEK CHAFFEE, CO	06/09/99					381	<15	<0.15	<1.0	754	64	<2.0	<2.0	<10
GORE CREEK SOUTH SIDE	07/22/99		166	6.65	91.2	52	<15	<0.15	<1.0	239	20	<2.0	<2.0	<10
GUNNISON TRIANGLE PASS	06/19/98	14.4	11.9	6.5	8.6	176	<10	<0.20	1.2	406	15.9	<5.0	<5.0	5.4
HARTENSTEIN LAKE	08/30/97	10.1	18	6.93	10.6	72	<10	<0.20	<1.0	162	3.7	<5.0	<5.0	<5.0
HARTENSTEIN LAKE	09/07/98		27.4		13.8	74	<10	<0.20	1.8	470	16.5	<5.0	<5.0	6.2
HARTENSTEIN LAKE	06/24/99					56	<15	<0.15	<1.0	87	<10	<2.0	<2.0	<10
HARTENSTEIN LAKE	07/13/99		15.1		8.2	66	<10	<0.20	1.2	95	3.4	<5.0	<5.0	<5.0
HERMAN GULCH	05/28/97					2471	<10	<0.20	7.1	1441	38.2	<5.0	<5.0	21.2
HERMAN GULCH	06/24/97		92	7.21	19.8	181	<10	0.26	3.1	665	264.5	<5.0	<5.0	<5.0
HERMAN GULCH	05/20/98	12.1	100.0	7.23	34.2	179	<10	0.30	2.4	1415	621.8	<5.0	<5.0	<5.0
HERMAN GULCH	05/20/98	12.1	100.0	7.23	34.2	159	<10	0.26	1.9	1047	286.4	<5.0	<5.0	<5.0
HERMAN GULCH	07/23/98	12.5	100.0	8.10	48.6	175	<10	<0.20	2.2	102	4.7	<5.0	<5.0	5.6
HERMAN GULCH	08/22/98		589		59.4	103	<10	<0.20	<1.0	265	13.2	<5.0	<5.0	<5.0
HERMAN GULCH (RUT)	06/24/97		711	7.30	61.6	419	<10	0.33	4.3	1400	225.4	<5.0	<5.0	7.2
HOLY CROSS S UPPER POOL EAGLE	07/31/98		16.1		8.2	127	<10	<0.20	1.1	26	2.1	<5.0	<5.0	6.4
JL2 SUMMIT CO	06/29/98					36	<10	<0.20	2.4	33	5.8	<5.0	<5.0	<5.0
JUMPER CREEK	05/21/98	12.5	51.6	6.75	29.8	611	<10	<0.20	1.5	323	8.2	<5.0	<5.0	<5.0
JUMPER CREEK	08/18/98		81.2		42.6	21	<10	<0.20	1.0	121	2.4	<5.0	<5.0	<5.0
JUMPER CREEK	05/31/99					491	<15	<0.15	<1.0	259	<10	<2.0	3.7	<10
JUMPER CREEK	07/05/99					527	<15	<0.15	3.0	440	45	<2.0	<2.0	<10
KROENKE LAKE	08/29/97	10.5	22	6.93	10.4	150	<10	0.22	4.0	861	14.3	<5.0	<5.0	8.2
KROENKE LAKE	06/30/98					63	<10	<0.20	1.6	142	10.1	<5.0	<5.0	<5.0
KROENKE LAKE	06/30/99					48	<15	<0.15	1.5	561	18	<2.0	<2.0	<10
LAKE OWEN BEAVER POND	08/20/99					70	<15	<0.15	2.5	837	68	<2.0	<2.0	12
LILY PAD LAKES TRAIL	07/01/98					87	<10	<0.20	1.7	1355	94.4	<5.0	<5.0	7.3

SITE	DATE	TEMP	COND	PH	ALKALINITY	Al	As	Cd	Cu	Fe	Mn	Pb	Se	Zn
LOST LAKE BOULDER, CO	08/22/97	20.8	46	6.98	17.4	290	<10	<0.20	3.6	437	17.9	<5.0	<5.0	11.0
LOST LAKE BOULDER, CO	08/18/98		48.3		24.2	38	<10	<0.20	1.2	253	12.2	<5.0	<5.0	6.9
MAGDALENE GULCH	09/01/99		038	7.67	22.4	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
MIDDLE COTTONWOOD CHAFFEE	07/28/99		042	7.60	25.8	19	<15	<0.15	<1.0	66	<10	<2.0	<2.0	<10
MIDDLE COTTONWOOD CHAFFEE CO	09/11/99		049	7.90	28.4	<15	<15	<0.15	<1.0	77	<10	<2.0	<2.0	<10
MORGAN'S GULCH CHAFFEE CO	07/12/99		045	7.39	26.0	104	<15	<0.15	1.3	1215	<10	<2.0	<2.0	<10
MORGAN'S GULCH CHAFFEE CO	09/11/99		040	7.62	21.2	208	<15	<0.15	3.2	2058	14	<2.0	<2.0	<10
MORGANS GULCH CHAFFEE, CO	09/06/97	12.1	52	6.98	28.2	52	<10	<0.20	2.7	2442	243.7	<5.0	<5.0	6.0
MORGANS GULCH CHAFFEE, CO	06/15/98		32.9		17.4	73	<10	<0.20	1.0	204	2.4	<5.0	<5.0	<5.0
MOUNT BETHEL	06/02/97					343	<10	<0.20	2.8	208	6.3	<5.0	<5.0	<5.0
MOUNT BETHEL	06/16/97					204	<10	<0.20	7.2	131	10.0	17.5	<5.0	5.0
MOUNT BETHEL	05/20/98	11.8	39.7	7.51	22.8	200	<10	<0.20	1.5	151	3.8	<5.0	<5.0	<5.0
MOUNT BETHEL	07/23/98	14.4	67.4	8.66	38.4	20	<10	<0.20	<1.0	37	4.1	<5.0	<5.0	<5.0
MOUNT BETHEL	08/22/98		83.5		44.8	24	<10	<0.20	<1.0	67	6.4	<5.0	<5.0	<5.0
N. TEN MILE CREEK NT6	06/22/98					27	<10	<0.20	2.7	115	13.3	<5.0	<5.0	<5.0
N. TEN MILE HIGHEST	06/23/97	22.0	70	7.55	35.0	28	<10	<0.20	<1.0	103	7.1	<5.0	<5.0	<5.0
N. TEN MILE RELOCATION POND	06/23/97	22.6	56	7.22	19.8	79	<10	<0.20	2.1	227	13.3	<5.0	<5.0	8.2
N. TEN MILE UPPER	06/23/97	21.9	63	7.42	30.4	132	<10	<0.20	1.5	583	48.9	<5.0	<5.0	16.2
N. TEN MILE UPPER LITTLE POND	06/23/97	21.8	105	7.5	58.2	80	<10	<0.20	<1.0	1356	122.2	<5.0	<5.0	42.8
N. TENMILE CREEK NT4,5,&6	06/22/98					34	<10	<0.20	1.6	120	9.2	<5.0	<5.0	<5.0
N. TENMILE CREEK NT5	06/22/98					18	<10	<0.20	<1.0	151	30.2	<5.0	<5.0	<5.0
NFS3	07/16/99		6.95	109	41.8	37	<15	<0.15	2.5	880	37	<2.0	<2.0	<10
NORTH WILLOW CREEK	07/09/98	16.0	25.1	6.5	13.0	152	<10	<0.20	1.9	299	15.3	<5.0	<5.0	<5.0
NT2	07/02/99		74	7.12	40.4	109	<15	<0.15	3.3	2907	213	2.4	2.8	<10
NT4	05/28/99		53	6.91	26.2	143	<15	<0.15	5.6	341	19	2.0	<2.0	<10
NT4	06/11/99		59	6.97	29.2	43	<15	<0.15	2.2	55	<10	<2.0	<2.0	<10
NT4	07/02/99		59	7.08	36.6	132	<15	<0.15	1.5	179	13	<2.0	<2.0	<10
PC1	06/09/99		63	6.78	15.8	68	<15	0.66	7.4	696	95	<2.0	<2.0	424
PERU CREEK	07/15/97	22.3	80	7.12	18.2	58	<10	0.41	10.3	410	61.0	5.3	<5.0	242.5
PERU CREEK	06/25/98					30	<10	0.41	6.8	500	42.0	<5.0	<5.0	343.5
PINGREE PARK TWIN LAKES U POND	07/24/98	13.0	23.6	7.81	15.4	1704	<10	<0.20	3.6	1212	19.3	<5.0	<5.0	10.9
POLE CREEK #15	06/15/97					225	<10	<0.20	1.5	286	24.5	<5.0	<5.0	<5.0
POLE CREEK #4	06/09/97	21.2	61	7.10	28.6	306	<10	<0.20	1.1	301	15.0	<5.0	<5.0	<5.0
POND AT EGGELSTON	08/03/98	12.5	74.2	8.27	42.4	117	<10	<0.20	1.0	100	10.8	<5.0	<5.0	<5.0
POND S OF MESA/DELTA LINE E65	08/04/98	12.5	17.1	7.87	8.8	67	<10	<0.20	2.0	122	11.4	<5.0	<5.0	6.6
RAINBOW LAKE	05/22/99					<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
ROCK CREEK PARK	08/05/99					18807	28	0.79	19.8	28041	540	14.6	4.6	57
SAYRES GULCH CHAFFEE CO	09/06/97	10.5	92	7.1	57.4	68	<10	<0.20	<1.0	45	1.7	<5.0	<5.0	<5.0
SAYRES GULCH CHAFFEE CO	09/13/98		112.1		59.2	56	<10	<0.20	1.8	203	19.2	<5.0	<5.0	<5.0
SAYRES GULCH CHAFFEE CO	06/17/99					30	<15	<0.15	4.1	507	26	<2.0	<2.0	<10
SECOND POND ABOVE TRICK215GMI2	08/03/98	13.2	38.5	7.66	17.6	40	<10	<0.20	1.3	208	21.1	<5.0	<5.0	<5.0
SNAKE RIVER SR2	06/25/98					13	<10	<0.20	1.5	75	18.0	<5.0	<5.0	10.8
SNAKE RIVER SR3	06/25/98					14	<10	<0.20	2.4	18	15.9	<5.0	<5.0	110.9
SODA CREEK	06/09/97	21.5	20	7.17	7.4	174	<10	<0.20	2.1	110	9.5	<5.0	<5.0	7.4
SODA CREEK	05/24/99		018	4.5	8.4	132	<15	<0.15	<1.0	70	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD CHAFFEE CO	09/04/99		152	7.78	99.4	32	<15	<0.15	1.9	218	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD CHAFFEE, CO	06/01/98		109.7		57.6	66	<10	<0.20	1.4	221	5.7	<5.0	<5.0	<5.0
SOUTH COTTONWOOD CHAFFEE, CO	09/07/98		106.1		55.6	84	<10	<0.20	2.6	463	7.6	<5.0	<5.0	<5.0
SOUTH COTTONWOOD CHAFFEE, CO	06/03/99		63	6.99	28.2	48	<15	<0.15	5.1	129	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD CHAFFEE, CO	06/07/99					41	<15	<0.15	2.5	1094	43	<2.0	<2.0	<10
SOUTH COTTONWOOD WEST CHAFFEE	08/30/98					24	<10	<0.20	<1.0	1800	109.5	<5.0	<5.0	<5.0

SITE	DATE	TEMP	COND	PH	ALKALINITY	Al	As	Cd	Cu	Fe	Mn	Pb	Se	Zn
SOUTH COTTONWOOD WEST CHAFFEE	09/04/99		136	7.59	75.8	26	<15	<0.15	1.6	887	52	<2.0	<2.0	<10
SOUTH FORK BIRD CREEK	07/02/99					91	<15	<0.15	2.4	799	120	<2.0	<2.0	13
SR6	06/08/99					414	<15	2.55	1.5	515	428	8.7	<2.0	2021
SR7	07/16/99		102	7.05	7.2	197	<15	0.72	3.5	875	265	3.3	<2.0	211
STAIRWAY ROUTT N. FOREST	08/04/99		020	4.5	10.6	48	<15	<0.15	1.5	120	<10	<2.0	<2.0	<10
STAIRWAY ROUTT N. FOREST	08/23/99		018	5.0	9.4	49	<15	<0.15	1.9	184	<10	<2.0	<2.0	<10
STRAIT CREEK	06/26/98					38	<10	<0.20	3.7	81	10.3	<5.0	<5.0	<5.0
TA BRAGG	08/05/99					97	<15	<0.15	3.0	649	44	<2.0	<2.0	21
TEXAS CREEK GUNNISON CO	08/21/99		042	7.65	23.8	88	<15	<0.15	1.1	364	13	<2.0	<2.0	<10
TRIANGLE PASS	06/29/98	11.2	23.4	8.39	5.0	54	<10	<0.20	<1.0	217	4.7	<5.0	<5.0	<5.0
TRIANGLE PASS	07/27/98	12.0	29.0	7.56	3.6	137	<10	<0.20	1.1	373	19.3	<5.0	<5.0	<5.0
TRIANGLE PASS	08/24/98		40.5		19.8	83	<10	<0.20	1.6	287	23.2	<5.0	<5.0	<5.0
TRIANGLE PASS	09/30/98		55.1		28.6	67	<10	<0.20	1.6	464	17.3	<5.0	<5.0	6.9
TRIANGLE PASS	06/23/99		72	7.74	46.4	78	<15	0.23	4.1	341	25	<2.0	<2.0	<10
TRIANGLE PASS	08/12/99		031	7.75	15.4	108	<15	<0.15	1.6	463	30	<2.0	<2.0	<10
TRIANGLE PASS	09/02/99		021	7.61	34.8	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
URAD-HENDERSON ANN'S POND	06/10/97					2553	<10	2.21	13.2	1706	1264.6	57.8	<5.0	412.5
URAD-HENDERSON 1-POND	08/19/99		200	7.18	70.8	99	<15	0.15	3.3	101	879	<2.0	<2.0	65
URAD-HENDERSON 2 POND	05/14/97		1128	7.68	268.0	3111	<10	1.89	5.4	1055	<1.0	7.7	6.3	1284.6
URAD-HENDERSON 2 POND	07/22/98	14.2	181.3	7.20	51.2	306	<10	0.78	3.1	44	1484.2	<5.0	<5.0	225.8
URAD-HENDERSON ANN'S POND	05/27/98	10.9	112.6	5.8	5.6	2856	<10	7.28	20.2	292	4344.5	14.6	<5.0	1225.5
URAD-HENDERSON ANN'S POND	07/08/98	12.3	39.7	7.04	14.0	537	<10	0.26	8.0	545	278.8	20.5	<5.0	48.5
URAD-HENDERSON ANN'S POND	07/13/99		44	7.40	13.4	337	<15	0.16	4.9	182	103	3.7	<2.0	37
URAD-HENDERSON DONUT	05/27/98	10.8	57.1	7.28	4.8	372	<10	0.72	3.2	139	1549.6	6.1	<5.0	185.3
URAD-HENDERSON DONUT	07/07/98	13.3	57.1	7.19	15.8	212	<10	<0.20	2.9	84	148.1	<5.0	<5.0	41.0
URAD-HENDERSON DONUT	07/13/99		51	7.48	15.8	239	<15	<0.15	3.0	45	112	<2.0	<2.0	17
URAD-HENDERSON DONUT	08/19/99		55	7.42	17.8	1155	<15	0.21	5.8	923	346	7.4	<2.0	61
URAD-HENDERSON ECLAIR	10/02/97	11.5	1154	3.2		34710	18	13.38	31.9	<10	8850.7	55.0	5.9	<5.0
URAD-HENDERSON ERIN'S POND	05/15/97		307	7.04	14.0	1492	<10	0.93	9.8	937	2612.6	12.0	<5.0	330.5
URAD-HENDERSON HESBO	05/15/97		80	7.72	22.8	421	<10	<0.20	1.5	221	244.4	<5.0	<5.0	8.2
URAD-HENDERSON HESBO	05/28/98	11.5	102.6	7.45	44.4	106	<10	<0.20	1.8	75	258.1	<5.0	<5.0	9.7
URAD-HENDERSON HESBO	07/21/98	14.3	100.0	6.94	99.4	284	<10	<0.20	3.6	250	2397.9	<5.0	<5.0	38.4
URAD-HENDERSON HESBO	07/09/99		179	7.18	68.8	50	<15	<0.15	1.1	46	672	<2.0	<2.0	<10
URAD-HENDERSON HESBO	08/19/99		184	7.04	71.2	31	<15	<0.15	1.8	29	401	<2.0	<2.0	<10
URAD-HENDERSON JS POND	08/19/99		169	7.09	72.4	122	<15	<0.15	7.2	866	3044	<2.0	2.0	15
URAD-HENDERSON JS POND (STMNT)	07/22/98	14.2	181.3	7.20	51.2	262	<10	0.33	17.5	601	3437.9	5.7	<5.0	32.1
URAD-HENDERSON POWER ALLEY	05/15/97		64	7.79	2.04	1112	<10	<0.20	2.4	606	49.0	<5.0	<5.0	28.6
URAD-HENDERSON POWER ALLEY	05/28/98	11.1	102.6	7.40	20.6	142	<10	<0.20	2.4	129	55.7	<5.0	<5.0	30.3
URAD-HENDERSON POWER ALLEY	07/08/98	13.1	58.8	6.98	25.6	23	<10	<0.20	2.0	236	150.3	<5.0	<5.0	32.1
URAD-HENDERSON TREATMENT	05/28/98	11.7	100.0	7.23	35.8	176	<10	0.35	5.6	77	279.0	<5.0	<5.0	24.9
URAD-HENDERSON TREATMENT	07/08/98	13.2	100.0	6.75	97.2	29	<10	1.27	8.6	160	2662.7	<5.0	<5.0	52.3
URAD-HENDERSON TREATMENT	07/09/99		1087	7.26	60.0	74	<15	0.88	6.1	45	357	<2.0	<2.0	50
URAD-HENDERSON TREATMENT	08/19/99		836	7.16	93.4	132	<15	0.50	7.3	194	294	<2.0	<2.0	54
URAD-HENDERSON UPPER URAD	06/25/97					553	<10	1.14	6.1	239	<1.0	<5.0	<5.0	468.2
URAD-HENDERSON UPPER URAD	07/08/98	15.4	100.0	6.66	11.8	390	<10	0.62	5.9	143	<1.0	5.9	10.7	426.9
URAD-HENDERSON UPPER URAD	07/13/99		546	6.75	12.0	559	<15	1.99	16.8	133	33800	3.1	4.3	788
VINTAGE BELOW HEND CLEAR CREEK	05/15/97		135	7.41	20.2	267	<10	0.43	3.5	209	218.9	<5.0	<5.0	144.8
WEST BRUSH CREEK	06/24/99		32	6.58	9.8	104	<15	<0.15	1.7	433	17	<2.0	<2.0	<10
WEST BRUSH CREEK	08/31/99		185	7.87	133.8	17	<15	<0.15	1.2	558	45	<2.0	<2.0	<10

SITE	DATE	HARDNESS	EXCEEDS METAL STANDARDS
ABOVE TRICKLE PARK RES 215GM1	08/03/98	61	Fe
BEAVER POND 215 GM2	08/03/98	74	Al,Fe
BROWN'S CREEK	06/29/99	24	Al
CALIFORNIA PARK ROUTT N. FORES	05/26/99	36	Al
COLLEGIATE PEAKS	05/22/99	49	Al
DENNY CREEK	08/23/97	23	Al,Cu,Fe,Pb
DENNY CREEK	05/27/99	21	Al
DIAMOND PARK	06/27/97	27	Al
DIAMOND PARK	06/02/99	36	Al
DIAMOND PARK	07/07/99	29	Al
FOUR MILE CREEK CHAFFEE, CO	08/19/97	21	Al,Fe
FOUR MILE CREEK CHAFFEE, CO	08/04/98	20	Cu,Fe
FOUR MILE CREEK CHAFFEE, CO	09/19/98	24	Al,Fe
FOUR MILE CREEK CHAFFEE, CO	06/09/99	13	Al
GUNNISON TRIANGLE PASS	06/19/98	6	Al,Cu
HARTENSTEIN LAKE	07/13/99	6	Cu
HERMAN GULCH	05/28/97	18	Al,Cu,Fe
HERMAN GULCH	06/24/97	200	Al
HERMAN GULCH	05/20/98	116	Al,Fe
HERMAN GULCH	05/20/98	179	Al,Fe
HERMAN GULCH	07/23/98	6	Al,Cu
HERMAN GULCH (RUT)	06/24/97	168	Al,Fe
HOLY CROSS S UPPER POOL EAGLE	07/31/98	5	Cu
JUMPER CREEK	05/21/98	25	Al
JUMPER CREEK	05/31/99	34	Al
JUMPER CREEK	07/05/99	36	Al
KROENKE LAKE	08/29/97	12	Cd,Cu
LILY PAD LAKES TRAIL	07/01/98	35	Fe
LOST LAKE BOULDER, CO	08/22/97	20	Al,Cu
MORGAN'S GULCH CHAFFEE CO	07/12/99	21	Al
MORGAN'S GULCH CHAFFEE CO	09/11/99	27	Al
MORGANS GULCH CHAFFEE, CO	09/06/97	58	Fe
MOUNT BETHEL	06/02/97	19	Al
MOUNT BETHEL	06/16/97	22	Al,Cu,Pb
MOUNT BETHEL	05/20/98	24	Al
N. TEN MILE UPPER LITTLE POND	06/23/97	50	Fe
NORTH WILLOW CREEK	07/09/98	11	Al,Cu
NT2	07/02/99	49	Al
NT4	07/02/99	36	Al
PERU CREEK	07/15/97	28	Cu,Pb,Zn
PERU CREEK	06/25/98	31	Cu,Zn
PINGREE PARK TWIN LAKES U POND	07/24/98	10	Al,Cu,Fe
POLE CREEK #15	06/15/97	28	Al
POLE CREEK #4	06/09/97	26	Al
POND S OF MESA/DELTA LINE E65	08/04/98	7	Cu
ROCK CREEK PARK	08/05/99	100	Al
SNAKE RIVER SR3	06/25/98	43	Zn
SODA CREEK	06/09/97	6	Al,Cu
SODA CREEK	05/24/99	8	Al
SOUTH COTTONWOOD WEST CHAFFEE	08/30/98	106	Fe
SOUTH FORK BIRD CREEK	07/02/99	15	Al
SR6	06/08/99	44	Al
SR7	07/16/99	53	Al
TA BRAGG	08/05/99	32	Al

SITE	DATE	HARDNESS	EXCEEDS METAL STANDARDS
TEXAS CREEK GUNNISON CO	08/21/99	24	Al
TRIANGLE PASS	08/12/99	21	Al
URAD-HENDERSON ANN'S POND	06/10/97	30	Al,Cd,Cu,Fe,Pb,Zn,Mn
URAD-HENDERSON 1-POND	08/19/99	135	Al
URAD-HENDERSON 2 POND	05/14/97	313	Al,Fe,Zn
URAD-HENDERSON 2 POND	07/22/98	93	Al,Zn,Mn
URAD-HENDERSON ANN'S POND	05/27/98	44	Al,Cd,Cu,Pb,Zn,Mn
URAD-HENDERSON ANN'S POND	07/08/98	19	Al,Cu,Pb,Zn
URAD-HENDERSON ANN'S POND	07/13/99	22	Al
URAD-HENDERSON DONUT	05/27/98	27	Al,Cd,Pb,Zn,Mn
URAD-HENDERSON DONUT	07/07/98	27	Al
URAD-HENDERSON DONUT	07/13/99	28	Al
URAD-HENDERSON DONUT	08/19/99	34	Al
URAD-HENDERSON ECLAIR	10/02/97	104	Al,Cd,Cu,Pb,Mn
URAD-HENDERSON ERIN'S POND	05/15/97	173	Al,Pb,Zn,Mn
URAD-HENDERSON HESBO	05/15/97	38	Al
URAD-HENDERSON HESBO	07/21/98	143	Al,Mn
URAD-HENDERSON JS POND	08/19/99	82	Al
URAD-HENDERSON JS POND (STMNT)	07/22/98	180	Al,Mn
URAD-HENDERSON POWER ALLEY	05/15/97	27	Al
URAD-HENDERSON TREATMENT	05/28/98	254	Al
URAD-HENDERSON TREATMENT	08/19/99	427	Al
URAD-HENDERSON UPPER URAD	06/25/97	218	Al,Zn
URAD-HENDERSON UPPER URAD	07/08/98	450	Al,Zn
URAD-HENDERSON UPPER URAD	07/13/99	358	Al
VINTAGE BELOW HEND CLEAR CREEK	05/15/97	42	Al,Zn
WEST BRUSH CREEK	06/24/99	12	Al

The above samples exceed Colorado's aquatic life water quality standards for the listed metals.
This does not imply that tadpoles are affected by metals at these sites.
These standards are applied to water samples filtered through a 0.4 micron filter.
Samples containing suspended solids are likely to cause elevated levels of metals.
Furthermore, these standards are designed to protect the most sensitive species.
Tadpoles may be able tolerate much higher levels of metals without harm.

The Effect of Predators on the Growth and Development of *Bufo boreas* Tadpoles

by Lauren J. Livo

INTRODUCTION

Boreal toads (*B. boreas*) breed in a variety of montane habitats in Colorado, from temporary pools to permanent mountain lakes (Campbell, 1970; Hammerson, 1999). Thus, *B. boreas* tadpoles can develop in vastly differing circumstances in terms of the aquatic predator communities to which they are exposed.

When tadpoles occupy the same habitat as a predator, the tadpoles may experience conflicts between behaviors that maximize growth versus those that minimize encounters with predators. Tadpoles of many species, including *B. boreas*, can detect chemical cues from predators (Hews and Blaustein, 1985; Hokit and Blaustein, 1995; Kiesecker et al., 1999; Lefcort, 1998; Lefcort, 1996; Manteifel, 1995; Petranka and Hayes, 1998; Petranka, 1989). Acute responses of tadpoles to predators often include a decrease in activity, avoidance of chemical cues associated with predators, an increase in aggregation, and an increase in refuge use (Bridges and Gutzke, 1977; Feminella and Hawkins, 1994; Griffiths et al., 1998; Petranka and Hayes, 1998; Semlitsch and Gavasso, 1992; Skelly, 1995; Stauffer and Semlitsch, 1993). Long term responses by tadpoles can include alterations of growth and development rates and development of predator-induced phenotypes (Lardner, 1998; McCollum and Leimberger, 1997; McCollum and Van Buskirk, 1996; Relyea, 1998; Relyea and Werner, 1999; Skelly and Werner, 1990; Smith and Van Buskirk, 1995; Van Buskirk and McCollum, 1999; Van Buskirk et al., 1997; Van Buskirk and Relyea, 1998).

In the Front Range of Colorado, larvae of predaceous diving beetles (*Dytiscus* sp.) are important predators of boreal toad tadpoles (Livo, 1998). This study investigates the role that the nonlethal presence of *Dytiscus dauricus* larvae may have in altering tadpole behavior, the degree of induced morphological plasticity, and the size of toadlets at metamorphosis. If tadpoles respond to the presence of predators with adaptive morphological changes, these changes should be associated with increased swimming speed or other feature that increases the probability of tadpole survival in an actual encounter with the predator. Such a response is expected to have a cost, so tadpoles exposed to predators are likely to have a smaller mass at metamorphosis than tadpoles not reared in the presence of a predator. If the nonlethal presence of predators contributes to "stress" for the tadpoles, tadpoles may also show increased levels of fluctuating asymmetry at metamorphosis.

Boreal toads are among the many amphibian species in the western United States that have undergone dramatic population declines since the 1970s (Carey, 1993; Corn et al., 1989; Livo and Yackley, 1997). Although once common in high-elevation wetlands in the Southern Rocky Mountains, many boreal toad populations have become extinct or reduced to small numbers of adult individuals. Because of the reduction in boreal toad numbers, even natural predation events have the potential to threaten remaining populations of this endangered species. Understanding the role that predators play in the early life history of *B. boreas* may aid in the recovery of this species, especially in identification of optimal sites for restoration efforts.

METHODS

Morphology

I planned a randomized block design with three treatment levels and two sibships. The treatments varied in the intensity of chemical cues from *Dytiscus* larvae: a control treatment in which tadpoles were not exposed to *Dytiscus* larvae, a "Low-cue" treatment in which tadpoles were exposed to *Dytiscus* larvae that were maintained on invertebrates, and a "High-cue" treatment in which tadpoles were exposed to *Dytiscus* that fed on *B. boreas* tadpoles. I used six 1-m diameter plastic wading pools in the Urad Valley, Colorado. In late June, I collected 300 hatching tadpoles from each of two separate boreal toad clutches in the Urad Valley. I maintained them in plastic containers until they were too large to have any risk of being able to get through the mesh on the *Dytiscus* containers. On June 30, each of the six wading pools received 100 tadpoles, all Gosner stage ≤26. Over the course of the season, tadpoles from one sibship became affected by a fungal infection; the three wading pools containing these tadpoles were excluded from analysis.

Each wading pool had a temperature logger that recorded water temperature at 15-minute intervals between June 21 and September 12. Water used to fill the pools came from an adjacent creek and was augmented with rainfall. Tadpoles were fed ad libitum a mixture of Mazuri tadpole chow and fish food, supplemented by frozen Romaine lettuce. I removed excess food and cleaned the wading pools three times per week. Despite efforts to keep the water clean, it quickly grew turbid. Although I wanted to maintain the strength of the predator cues by retaining the same water throughout as much of the experiment as possible, on several occasions I cleaned all of the pools and completely replaced their water. I placed netting over the pools to exclude birds and mammals.

Containers used to restrain *Dytiscus* larvae within the pools had diameters of 11-cm, were 20-cm tall, and filled 4-cm deep with white aquarium gravel. Three 5 x 9-cm opening were cut in the sides of the containers, covered with fiberglass window screening, and sealed to permit chemical cues from the *Dytiscus* larvae to move freely into the water of the wading pool. In the control treatment, the containers were present but lacked *Dytiscus* larvae. In the other treatments, one *Dytiscus* larvae was placed in each of three containers. *Dytiscus* larvae in the Low-cue treatment were fed invertebrates throughout the course of the experiment. *Dytiscus* larvae in the High-cue treatment were maintained on boreal toad tadpoles brought to the pools from a nearby site (informally named Donut). Thus, the High-cue treatment contained both predator cues and cues from injured conspecifics. I placed *Dytiscus* larvae in the wading pools beginning June 23.

I also set up a separate pool for reserve *Dytiscus* larvae, which were fed invertebrates When *Dytiscus* larvae in the experimental pools died or matured, I replaced them when possible, ensuring that approximately equal numbers of larvae were in all predator treatment pools. To minimize any cues associated with the consumption of tadpoles by *Dytiscus* larvae, a larva had to be in the reserve pool for at least two days before it could be used as a replacement in the Low-cue treatment. As the season progressed, the number of available *Dytiscus* larvae declined. I removed all remaining *Dytiscus* larvae from the pools on September 1.

I made weekly counts of the tadpoles. On July 7, July 14, July 21, and August 6, I removed five tadpoles per pool to simulate predation (Van Buskirk and Yurewicz, 1998). On July 30 I removed tadpoles as necessary in order that all pools contained the same number of tadpoles so that differences between groups would not be due to differences in densities. I removed ten tadpoles per pool on July 27 and again on August 12 for other experiments. After the experiment on August 12, I preserved the tadpoles and weighed and measured each. Dimensions measured were body length, body width, body depth, tail muscle width, tail muscle depth, and tail length. I used the SAS Canonical Discriminant Analysis procedure to determine whether morphological proportions varied among treatment groups.

When the remaining tadpoles reached Gosner stage 42 (emergence of front limbs), I removed them from the wading pool and transferred them to the lab to complete metamorphosis. At the conclusion of the trials, the toadlets were released.

Swimming performance

On July 9 and August 6, I estimated swimming performance for tadpoles in the wading pools. For each swimming trial, I located a tadpole that was facing away from the edge of the pool and used a thin wooden dowel to tap the tadpole near the base of its tail. I recorded the time (in seconds) and distance (in cm) that the tadpole swam before coming to rest. Trials in which the tadpole was stopped by the wall of the pool were discarded. To minimize disturbance of the tadpoles, I conducted two trials per pool before moving to the next pool, for a total of 14 trials per pool on July 9 and 10 trials per pool on August 6.

Behavior

I videotaped tadpoles in the wading pools several times throughout the summer. For the analysis of tadpoles in each pool, I placed a clear sheet of plastic over a video monitor and marked the placement of tadpoles at a particular videotape counter location. From the same starting point for a pool, I used a custom computer program that allowed me to tally the amount of time individual tadpoles spent at various activity levels. I scored activity levels as follows:

1. Resting or with occasional tail twitches ("resting")
2. Continuous movement of tail with or without forward movement, and with snout usually in contact with substrate ("feeding")
3. Forward movement with snout not in contact with substrate ("swimming")

Use of a 10-second trial period minimized "loss" of tadpoles from swimming out of the field of view. No tadpole was measured more than once.

Aggregation

Throughout the course of the experiment, I photographed each pool using a 35-mm camera equipped with a 24-mm wide angle lens and color slide film. Letters identifying each pool were on 2 x 2-cm white squares laminated in plastic and scattered along the bottoms of the pools. I compared aggregation levels of tadpoles using slides taken on July 5, August 2, and August 13.

I projected slides onto white drawing paper and adjusted the image to 0.25-life size using the white squares and pool margins as size references. After diagraming the distribution of the tadpoles in each pool, I used digital calipers to measure the distance between each tadpole and its nearest neighbor (Clark and Evans, 1954).

Food passage rates

On August 27, three groups of 10 tadpoles collected the previous day from a site in the Urad Valley were assigned randomly to one of three treatments: Group 1, control/no carbon treatment; Group 2, control/carbon treatment; and Group 3, *Dytiscus* exposure/carbon treatment. Each group of tadpoles was placed in a 9-cm diameter plastic jar containing either 400 ml pond water (Group 1) or a dilute carbon solution (groups 2 and 3: 300 ml pond water plus 100 ml water from a stock solution of 1 g carbon suspended in 600 ml water). Tadpoles remained in the containers for 30 minutes, then were transferred into individual plastic cups. Cups for groups 1 and 2 contained 200-ml pond water. Cups for Group 3 contained 200-ml pond water conditioned by the presence of a single *Dytiscus* larva. All cups contained small pieces of tadpole chow. Water temperatures during the experiment varied between 21.6 and 22.6°C.

Material was suctioned out of the bottoms of the cups and checked every 2 hours for the presence of carbon marks. Carbon marks were scored as follows: F (filamentous, loose material as from passage of carbon through gills), S (spots or specks of carbon), C (carbon in formed feces), or R (rodlike or cylindrical carbon not in association with obvious feces). Water levels were restored with the addition of pond water (groups 1 and 2) or water conditioned by the *Dytiscus* larvae (Group 3). After 8 hours, the Gosner stage, snout-vent length, and tail lengths were determined for each tadpole.

Metamorphic traits

The emergence of the forelimbs at Gosner stage 42 is an unambiguous indication that metamorphosis has begun. When examining the tadpoles in the wading pools, I removed and tallied all that had reached this stage. To test the null hypothesis that there was no difference in date of metamorphosis among treatments, I used the Kruskal-Wallis single factor analysis of variance by ranks with date of metamorphosis as the variable (Zar, 1999).

Tadpoles in the process of metamorphosing were removed from the study pools and brought to the lab. Here they were maintained with other tadpoles from their group in tilted plastic containers with pond water at one end. Tadpoles usually do not feed during metamorphosis, or if they do, food intake declines dramatically by Gosner stage 42 (Kuzmin, 1997). Consequently, I did not provide food to transforming tadpoles. The room was illuminated by incandescent lighting on a 12L:12D schedule which approximated the natural photoperiod at the time. I checked tadpoles daily and weighed and measured them when the tail bud appeared to have been absorbed.

At this time, I also tested their rate of movement across a 44-cm long plastic container (30-cm wide and 25-cm tall). To induce voluntary movement, I piled a 50-g mass of black aquarium gravel at one end of the container and illuminated the gravel with a 40-watt incandescent bulb placed even with

the upper edge of the container. I scored the time at which toadlets crossed 12-cm, 24-cm, and 36-cm lines. If a toadlet had not crossed the 12-cm line within 18 minutes, I terminated the trial.

I also assessed predation ability by placing each toadlet in a 15 x 15-cm plastic container (10 cm tall) in which there were three pinhead crickets. I tallied the number of successful and unsuccessful strikes made by the toadlets during a 3-minute trial. The first group of feeding trials was discarded because the smallest available crickets were too large to elicit any predatory behaviors by the toadlets.

To assess levels of fluctuating asymmetry, each toadlet was briefly immobilized with MS-222 (Robinson and Scadding, 1983), positioned on a small plastic dish against a ruler, then photographed with both a ventral and a dorsal view using a camera on a copy stand. From the resulting photographs, I measured tibia length for right and left legs in both toadlet views. For each photograph I also measured a standard distance on the ruler; this measurement was used to make a correction factor to compensate for slight variations in magnification and enlargement.

RESULTS

Mean wading pool temperatures were within 0.1 °C of one another over the course of the summer (Table 1).

Table 1. Mean wading pool temperatures, June 21 to September 12, 1999.

Treatment	Mean temperature ± SD
Control	14.0 ± 5.8°C
Low-cue treatment	13.9 ± 5.6°
High-cue treatment	13.9 ± 5.8°

Morphology

For each treatment group, morphological measurements are summarized in Table 2. Tadpole morphology varied between groups, with significant differences between the Low-cue and High-cue treatments and between Control and High-cue treatments (Table 3). The first discriminant function of the SAS Canonical Discriminant Analysis procedure was highly significant ($F = 4.5681$, $df = 14$, $p = 0.0001$). Tadpoles in the Low-cue treatment were intermediate between Control and High-cue treatments, but not significantly different from the High-cue treatment.

When the effects of size are removed, in proportion to their lengths the High-cue tadpoles have wider bodies compared to the Control or Low-cue groups. Control tadpoles had larger mass, larger body lengths, and somewhat larger body depths, tail lengths, and tail muscle depths in proportion to their widths (Figure 1). Class means on Canonical variables are shown in Table 4.

Table 2. Means (±SD) of variables (for each group, n = 10)

Variable	Control	Low-cue	High-cue
Mass	1.15 ± 0.17	1.07 ± 0.22	0.64 ± 0.11
Body length	17.94 ± 1.00	17.22 ± 0.95	14.59 ± 1.23
Body width	11.30 ± 0.68	10.69 ± 1.10	9.12 ± 0.88
Body depth	8.49 ± 0.37	8.17 ±0.73	6.95 ± 0.37
Tail length	29.50 ± 1.86	28.40 ± 3.01	23.52 ± 1.62
Tail muscle width	3.16 ± 0.35	3.12 ± 0.30	2.60 ± 0.22
Tail muscle depth	4.89 ± 0.50	4.31 ± 0.52	3.95 ± 0.32

Table 3. F statistics (squared distances); probability level in parentheses.

Treatment	Control	Low-cue treatment	High-cue treatment
Control		2.182 (0.0787)	8.303 (0.0010)
Low-cue treatment			5.581 (0.0010)
High-cue treatment			

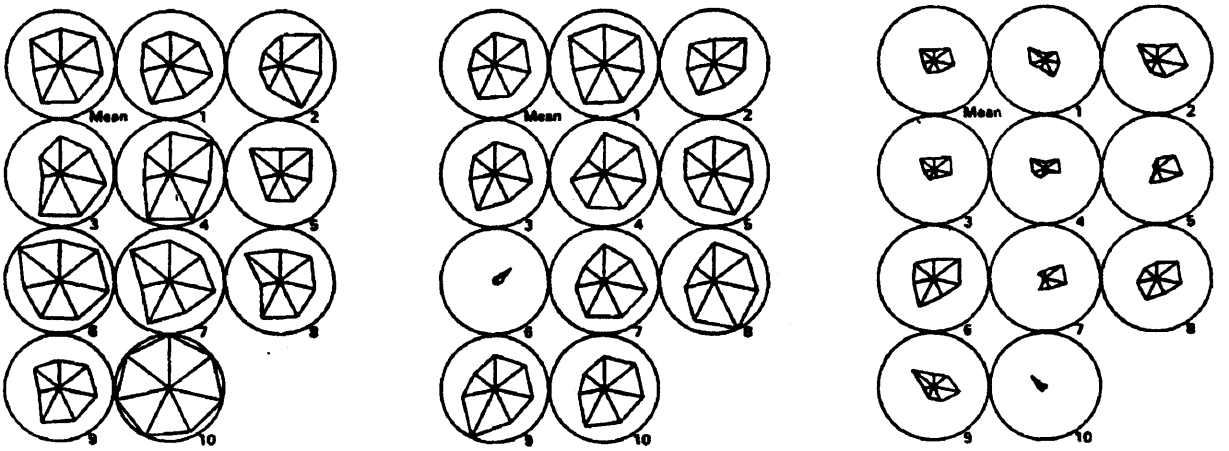


Figure 1. Starplot summary of tadpole morphology by treatment. From left to right, blocks represent Control, Low-cue, and High-cue treatments. The first plot in each block shows the mean values for the treatment. Each circle represents a single tadpole. Clockwise from the vertical in each circle, axes indicate the mass, body length, body width, body depth, tail length, tail muscle width, and tail muscle depth. Length of each axis varies between 0 and100 percent of the plot radius and is proportional to the individual score.

Table 4. Class means on Canonical variables.

Treatment	Canonical variable
Control	1.581
Low-cue	0.669
High-cue	-2.250

Swimming performance

I tested the null hypothesis that there were no differences between groups in swimming performance. Means by treatment group for the July and August trials are shown in Table 5. There was no difference between groups in swimming speed in July ($F = 0.1972$, $df = 2,39$, $p = 0.197$, Table 6). However, in August trials, the High-cue group had significantly faster swimming speeds than the Control or Low-cue groups ($F=4.266$, $df = 2, 27$, $p < 0.05$, Table 7). Figure 2 compares mean swimming speeds for July and August trials by treatments.

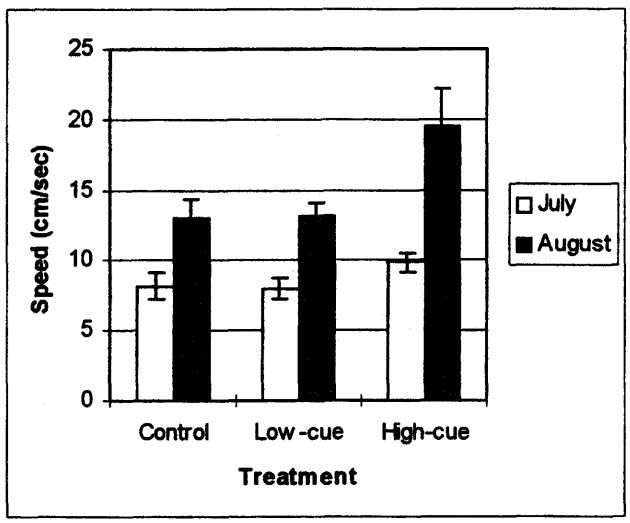


Figure 2. Comparison of swimming speeds between treatment groups.

Table 5. Comparisons of mean swimming speeds (cm/sec) for July and August trials by treatment group.

Group	Mean speed (± SD) July (n=14/treatment)	Mean speed (± SD) August (n= 10/treatment)
Control	8.17 (± 3.65)	13.01 (± 4.14)
Low-cue	7.98 (± 2.77	13.20 (± 2.59)
High-cue	9.88 (± 2.47)	19.47 (± 8.44)

Table 6. ANOVA for swimming speed (cm/sec) by treatment group (July trials).

Source of variation	SS	df	MS	F	p
Between groups	30.519	2	15.259	0.1972	0.197
Within groups	351.527	39	9.0135		
Total	382.046	41			

Table 7. ANOVA for swimming speed (cm/sec) by treatment group (August trials).

Source of variation	SS	df	MS	F	p
Between groups	270.284	2	135.142	4.266*	0.024
Within groups	855.280	27	31.677		
Total	1125.564	29			

Behavior

Small tadpole size at the beginning of the season and clouded water throughout much of July made analysis of these tapes prohibitively difficult. On August 13, I changed the water in the pools, added food to the pools for the tadpoles, fed the *Dytiscus* larvae in the enclosures within the pools, and videotaped the behavior of the tadpoles. For videotape made on this date, I tallied the total amount of time (in seconds) spent by tadpoles resting, feeding, or swimming. There were highly significant differences between treatments in total amounts of time spent in resting, feeding, and swimming ($\chi^2 = 16.763$, $df = 4$, $p < 0.01$) (Table 8, Figure 3).

Table 8. Total amount time in seconds spent at three activity levels by treatment (number in parentheses indicates number of tadpoles per group).

Treatment	Control (26)	Low-cue treatment (27)	High-cue treatment (28)
Resting	139	152.7	182.4
Feeding	102.9	107.2	94.3
Swimming	18.1	10.1	3.3

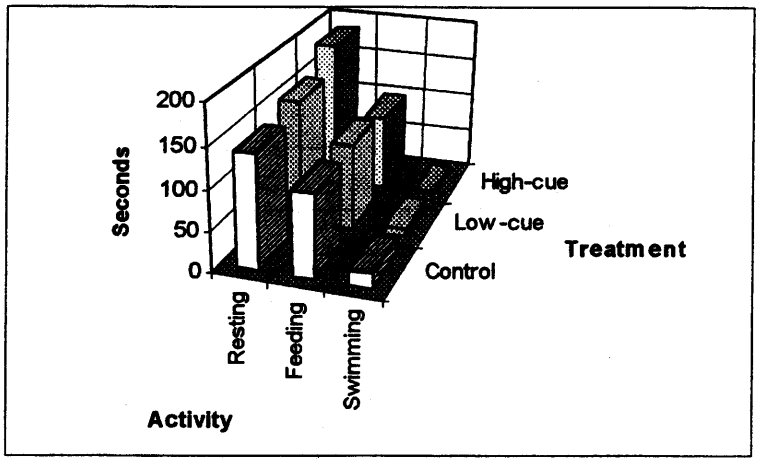


Figure 3. Comparison of total amount of time spent at three activity levels by tadpoles in Control, Low-cue, and High-cue treatments.

Aggregation

R, the coefficient of aggregation developed by Clark and Evans (1954), varies between 0 (complete aggregation) and 2.1491 (a distribution in which individuals have 6 equally-spaced nearest neighbors in a hexagon pattern). A coefficient of 1 indicates a random distribution. All tadpole distributions had aggregation coefficients of <1 (Table 9), indicating a tendency toward aggregation (Figure 4). I used ANOVA to test the null hypothesis that there was no difference between nearest-neighbor distances with respect to treatments. Table 10 compares the mean nearest neighbor distances for the three treatments on July 5, August 2, and August 13. ANOVA results are presented in tables 11-13.

Table 9. Nearest neighbor R coefficients for each wading pool. Sample sizes in parentheses.

Group	5 July 1999 (Julian 186)	2 August 1999 (Julian 214)	13 August 1999 (Julian 225)
Control	0.459051 (81)	0.875236 (53)	0.531764 (42)
Low-cue	0.469125 (73)	0.646239 (81)	0.651553 (37)
High-cue	0.438917 (84)	0.555311 (56)	0.885705 (34)

Table 10. Comparisons of mean nearest neighbor distances for July 5, August 2, and August 13 trials by treatment group.

Group	Mean distance (±SD) July 5	Mean distance (±SD) August 2	Mean distance (±SD) August 13
Control	2.26 ± 2.62, n = 81	5.33 ± 5.29, n = 53	3.63 ± 2.92, n = 42
Low-cue	2.43 ± 3.18, n = 73	3.18 ± 3.38, n = 81	4.74 ± 3.80, n = 37
High-cue	2.12 ± 2.06, n = 84	3.28 ± 4.17, n = 56	6.73 ± 8.55, n = 34

Table 11. ANOVA for nearest neighbor distances (July 5 trials).

Source of variation	SS	df	MS	F	p
Between groups	3.777	2	1.888	0.272	0.762
Within groups	1633.301	235	6.950		
Total	1637.078	237			

Table 12. ANOVA for nearest neighbor distances (August 2 trials).

Source of variation	SS	df	MS	F	p
Between groups	169.139	2	84.570	4.747*	0.009
Within groups	3331.345	187	17.815		
Total	3500.484	189			

Table 13. ANOVA for nearest neighbor distances (August 13 trials).

Source of variation	SS	df	MS	F	p
Between groups	181.749	2	90.874	3.047	0.051
Within groups	3280.551	110	29.823		
Total	3462.299	112			

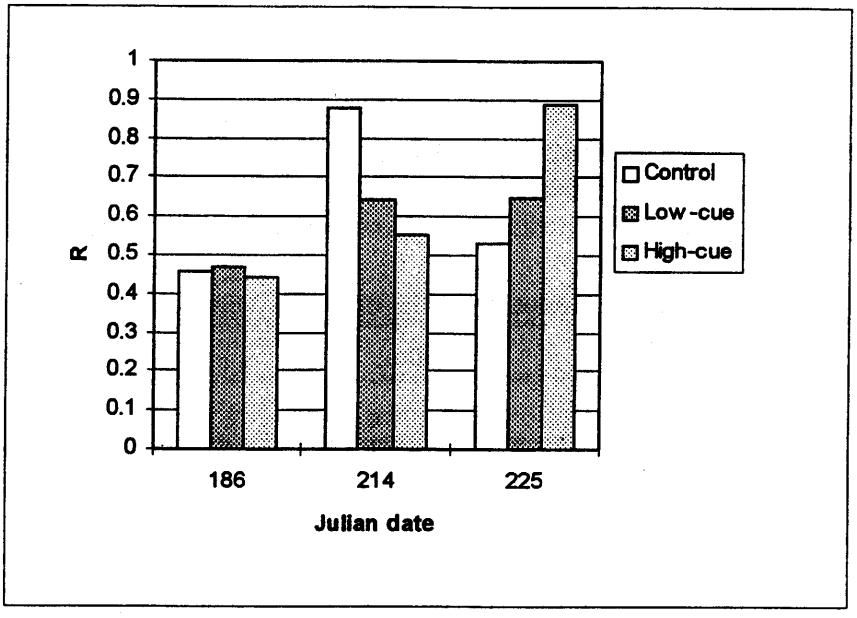


Figure 4. Nearest neighbor scores (R) for treatments on three dates.

Food passage rates

All tadpoles used in this experiment were pre-metamorphic (≤Gosner stage 40; Table 14). In a comparison of the number of scores indicating no carbon (0), carbon not clearly in feces (F, R, and S), and carbon in feces (C), as expected, there were no carbon marks in Group 1 (control/no carbon treatment), while both carbon-treated groups scored with one or more types of carbon, resulting in a highly significant difference ($\chi^2 = 70.00$, $df = 8$, $p < 0.01$). When the comparison was between the two carbon-treated groups, there were highly significant differences between Group 2 and Group 3 ($\chi^2 = 11.12$, $df = 2$, $p < 0.01$). The largest differences involved the number of instances in which carbon was associated with feces (13 times for tadpoles in *Dytiscus*/carbon treatment versus 2 times for the control/carbon treatment).

Table 14. Means (± SD) of tadpole developmental stages, SV lengths, and total lengths of *B. boreas* tadpoles used in carbon passage trials (n = 10 for each group).

	Tadpole development		
Group	Gosner stage	SV (mm)	Total length (mm)
1: Control/no carbon	39.4 ± 1.1	16.1 ± 1.5	42.5 ± 2.9
2: Control/carbon	39.3 ± 1.3	15.4 ± 1.0	41.3 ± 4.3
3: *Dytiscus*/carbon	39.3 ± 0.8	15.6 ± 1.2	41.3 ± 3.3

Metamorphic traits

I used August 31, the date that forelimbs emerged on the first tadpole, as day 1 for determinations of time to metamorphosis (Table 15). There was a highly significant difference between groups for date of metamorphosis (Kruskal-Wallace test, $H = 31.385$, $df = 2$, $p < 0.001$), with the control group having the smallest mean time to metamorphosis (Figure 5).

Table 15. Mean time to metamorphosis (days ± SD); sample sizes in parentheses.

	Control	Low-cue	High-cue
Time to metamorphosis	9.2 ± 3.4 (39)	14.5 ± 3.9 (35)	13.8 ± 4.4 (35)

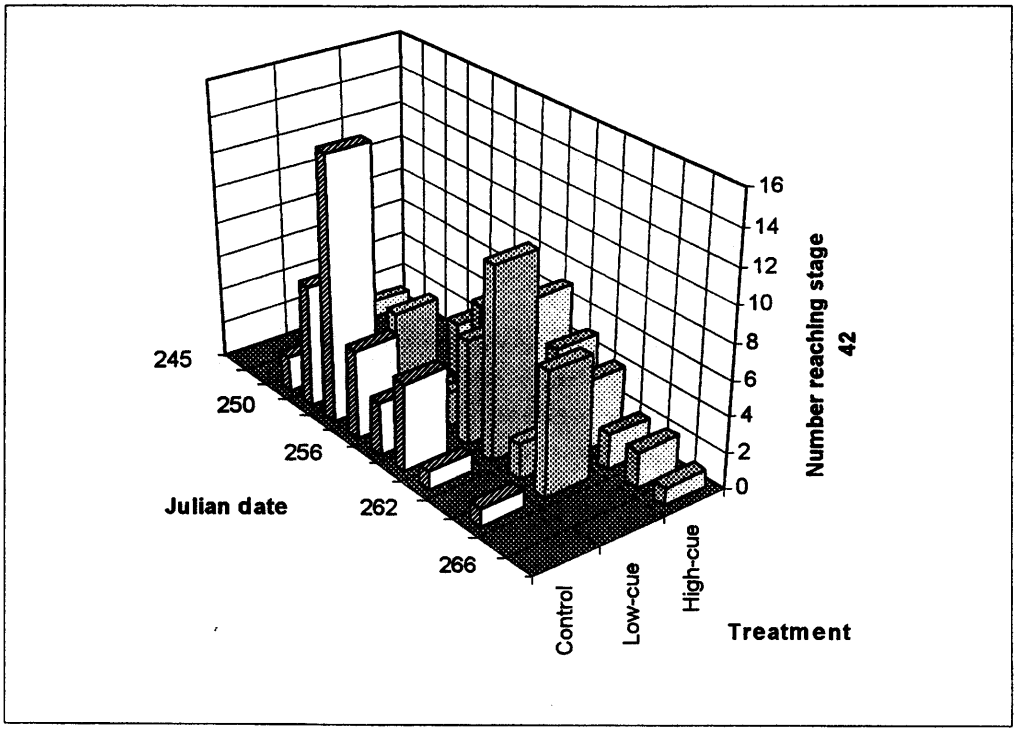


Figure 5. Timing of metamorphosis by treatment.

Table 16 lists mean mass at metamorphosis by treatment. There were highly significant differences in mass at metamorphosis between treatments (Table 17). Both the Control and Low-cue toadlets were significantly different from the High-cue toadlets, but not from one another (Newman-Keuls multiple range test, Zar, 1999).

Table 16. Mean mass at metamorphosis (g ± SD); sample sizes in parentheses.

Control	Low-cue	High-cue
0.64 ± 0.09 (20)	0.62 ± 0.08 (34)	0.48 ± 0.04 (30)

Table 17. ANOVA for mass at metamorphosis.

Source of variation	SS	df	MS	F	p
Between groups	0.411	2	0.205	41.79	< 0.0001
Within groups	0.398	81	0.00492		
Total	0.809	83			

Rate of movement

There was significant heteroscedasticity of variances in the shortest time required for toadlets to move 12 cm, precluding use of ANOVA to make comparisons between groups. Means for the three treatments are shown in Table 18.

I compared the Control and High-cue treatments using the Kruskal-Wallis test (Zar, 1999); there was a significant difference between these two treatments (Kruskal-Wallace test, $H = 4.000$, $df = 1$, $p < 0.05$), with the Control toadlets requiring less time to move 12 cm than the High-cue toadlets.

Table 18. Shortest time to move 12 cm (sec ± SD); sample sizes in parentheses.

Control	Low-cue	High-cue
31.8 ± 25.6 (20)	42.8 ± 26.4 (34)	60.3 ± 56.8 (30)

Predatory ability

I scored a toadlet as reacting to prey either if it captured a pinhead cricket or if it directed one or more unsuccessful strikes toward a cricket. In comparing "Reaction" vs. "No reaction," there was a significant difference among treatments ($\chi^2 = 7.733$, $df = 2$, $p < 0.05$). The control treatment had somewhat fewer than expected toadlets having no reaction, while the Low-cue and High-cue treatments had somewhat higher than expected numbers of toadlets with no reaction.

Fluctuating asymmetry

For each toadlet, the mean for tibia length was calculated as the mean of the corrected dorsal plus ventral measures from the right side minus the mean of the corrected dorsal plus ventral measures from the left side. The mean signed difference between left and right tibias was significantly greater than zero (mean R-L = 0.1229, $t = 2.603$, n = 83). Consequently, this measure does not meet the criterion for fluctuating asymmetry, which requires that the mean difference between left and right characters is not significantly different from zero. When absolute values were compared, there were no significant differences among groups in the amount of asymmetry of tibia length (Tables 19 and 20). There was no significant correlation between the absolute values for individual levels of asymmetry and mass at metamorphosis (Table 21).

Table 19. Mean R-L asymmetry in tibia length (absolute value in mm ± SD); sample sizes in parentheses.

Control	Low-cue	High-cue
0.324 ± 0.292 (20)	0.346 ± 0.263 (34)	0.363 ± 0.302 (29)

Table 20. ANOVA for the absolute values of asymmetry between treatments.

Source of variation	SS	df	MS	F	p
Between groups	0.0187	2	0.009334	0.115855	>0.05
Within groups	6.4453	80	0.08057		
Total	6.4639	82			

Table 21. Correlation coefficients for mass at metamorphosis and mean left-right asymmetry (sample sizes in parentheses).

Control	Low-cue	High-cue
$r = 0.313$ (20)	$r = 0.186$ (34)	$r = 0.011$ (29)

DISCUSSION

Boreal toad tadpoles reared without the presence of predators (Control tadpoles) fared better on nearly all measures compared to tadpoles exposed to chemical cues from *Dytiscus* larvae and injured conspecific tadpoles (High-cue tadpoles). Control tadpoles grew more rapidly, reached metamorphosis earlier and more synchronously, and fared better on tests of predatory ability and dispersal ability than High-cue tadpoles. Low-cue tadpoles reared in the non-lethal presence of *Dytiscus* larvae that were fed invertebrates had intermediate scores for most of their measured responses, although these scores were not always significantly different from either the Control or High-cue treatments.

Compared to Control and Low-cue tadpoles, High-cue tadpoles had altered morphologies, and tended to be wider in proportion to their lengths than tadpoles in the other treatment groups. The single measure on which High-cue tadpoles performed better was in swimming speed measured in August, in which the High-cue tadpoles swam more rapidly than the Control or Low-cue tadpoles. These finding are consistent with a predator-induced phenotype in which morphological changes are associated with an increased ability to survive encounters with predators compared to conspecifics without the altered morphologies. In anuran larvae, improved swimming performance is often associated with development of predator-induced phenotypes (McCollum and Leimberger, 1997; Relyea, 1998).

In some studies, tadpoles respond more strongly to predators that have consumed tadpoles of the same species (Anholt et al., 1996; Laurila et al., 1997; Wilson and Lefcort, 1993). However, this is not a uniform response, as Petranka and Hayes (Petranka and Hayes, 1998) observed no difference in behavior of *B. americanus* when tadpoles were exposed to starved odonate larva versus those fed conspecific tadpoles. In this study, boreal toad tadpoles responded much more strongly to cues from predators fed other boreal toad tadpoles than to cues from predators fed invertebrates.

Responses of anuran tadpoles to predators depend both on anuran identity and predator identity (Relyea, 1998). Anuran species and even populations within species differ in the degree of plasticity that is induced by particular predators. Relyea (1998) reported that *Bufo americanus* tadpoles did not alter their phenotype when reared in the presence of *Dytiscus* larvae. In Colorado, *B. boreas* tadpoles may be selected to respond more strongly to *Dytiscus* larvae if the degree of temporal overlap is greater in this predator-prey system than in the *B. americanus-Dytiscus* system.

Timing and synchrony of metamorphosis may vary depending on predator identity. Some anuran larvae have a reduced time to metamorphosis or metamorphose more synchronously when in the presence of predators (DeVito et al., 1998; Van Buskirk, 1988). These tactics may allow the anurans to escape the risk of predation (as when dragonflies are predators that remain in the aquatic environment) or as a "selfish herd" strategy in which by metamorphosing synchronously, the individual risk of predation is minimized (as when garter snakes are the predators). In the experiments reported here, tadpoles reared in the presence of predators metamorphosed later and less synchronously than Control tadpoles. This may be a result of the temporal relationship between *Dytiscus* larval development and that of *B. boreas*, in which the beetle larvae usually pupate around the time the first tadpoles metamorphose. Consequently, the risk of predation by these insects has declined well before small tadpoles can metamorphose, and as long as food is not limiting in the aquatic habitat, there may be little selective advantage to metamorphose into a terrestrial environment with uncertain resource availability.

Mass at metamorphosis is an important correlate of fitness in amphibians (Amézquita and Lüddecke, 1999; Berven, 1990; Blouin, 1992; Goater et al., 1993; Harris, 1999; Newman, 1992; Newman and Dunham, 1994). Larger toadlets probably begin post-metamorphic life with greater energy reserves and have a greater size range of prey items available to them. In this study, the largest toadlets resulted from the Control treatment; these toadlets also had the highest rates of voluntary movement and of striking and capturing prey. *Bufo boreas* toadlets often must disperse from the breeding site to reach suitable overwintering sites, and mass relates directly to the ability to withstand desiccation (Livo, in prep.).

Tadpoles that are less active feed less and have lower growth rates (Skelly, 1992; Skelly and Werner, 1990). Tadpoles immersed in a dilute carbon solution and then exposed to water conditioned by the presence of a *Dytiscus* larvae had more frequent presence of carbon in their feces than similarly treated tadpoles exposed to pond water. Why would tadpoles exposed to cues from predators appear to hasten the passage of material through their guts? In tadpoles, the gut takes up a considerable volume of the body, and material in the gut probably contributes substantially to the mass of the tadpole (Altig and Kelly, 1974). Cogãlniceanu (1997) found that the dry weight of feces represented nearly 20 percent of the dry weight of *Pelobates fuscus* tadpoles. Given the physical relationship between mass and motion in terms of energy expenditure, for a given tadpole body dimension, more rapid clearance of food might reduce mass sufficiently to allow an initially faster escape during an encounter with a predator. Chronic increase in food passage rates from exposure to predators could affect assimilation efficiency. A reduced ability to assimilate food would compound the effects of lower activity levels and feeding that are observed in tadpoles exposed to predators, contributing to their small size relative to tadpoles not exposed to predators.

Bufo tadpoles, including those of *B. boreas*, respond both to thermal and to light intensity gradients (Beiswenger, 1977; Beiswenger, 1978). They also can differentiate kin from non-kin and prefer to associate with the former (O'Hara and Blaustein, 1982). Tadpoles can also concentrate in patches with favorable levels of resources (Kupferberg, 1998). In all trials, boreal toad tadpoles tended to be aggregated, but degree of aggregation did not appear to vary in any systematic way.

Fluctuating asymmetry (FA) has been suggested as a way to measure developmental stress (Clarke, 1992; Leary and Allendorf, 1989; Leung and Forbes, 1997; Leung and Forbes, 1996; Møller, 1997; Møller and Thornhill, 1997). There are four major patterns of asymmetry in paired structures in bilateral organisms: 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, in which there is no tendency for one side to be larger than the other (Palmer and Strobeck, 1986). 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 biological cycles (Manning et al., 1996). The asymmetry in tibia length observed in these experiments did not conform to the criterion for fluctuating asymmetry, but appeared to be a form of directional symmetry. In this study, asymmetry in tibia length did not appear to correlate with measures, such as toadlet mass, that might correlate with fitness.

This study demonstrates that *B. boreas* tadpoles respond in an apparently adaptive way to the presence of *Dytiscus* larvae, an important predator of tadpoles. Further, the strength of the chemical cues is associated with the degree of tadpole response. Although tadpole response to predators is adaptive, it negatively affects parameters associated with fitness compared to conspecifics not exposed to predators.

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Genetic Analyses of the Southern Rocky Mountain Group of *Bufo boreas* Based on Mitochondrial DNA Sequence and Nuclear AFLP Restriction Site Data

Final report to the Colorado Division of Wildlife

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ABSTRACT

Previous analyses of mitochondrial DNA (mtDNA) of *Bufo boreas* identified substantial and previously unrecognized phylogeographic subdivision, particularly in the southwest and southeast portions of the toad's range. Recognizing genetic subdivision within the Southern Rocky Mountains (SRM; central Colorado, Albany Co., Wyoming, and northern New Mexico) was of special concern, due to recent declines. Both mtDNA sequence data and nuclear Amplified Fragment Length Polymorphisms (AFLP) restriction site data were analyzed from the SRMs to identify genetic variation within and among populations.

All toads from the SRMs have mtDNAs that are very closely related; no divergent haplotypes were found from newly sampled populations. MtDNA data from all previous projects was combined in a phylogenetic analysis. All previously identified major clades (Northwest, Southwest and Southeast) were strongly supported. Within the Southeast region of the toad's range four clades/groups were evident: 1) Kane Co., UT, 2) Box Elder Co., UT, 3) Caribou Co., ID, and 4) A clade that included all animals from the Southern Rocky Mountains, as well as toads from northern Utah.

Parsimony, Neighbor-joining and principal coordinate analyses of AFLP data identified similar groups (Kane Co., UT, Caribou Co., ID and the SRM). Samples from Utah and southeast Wyoming were not analyzed with AFLPs. Relationships within the SRMs were not strongly supported, however, a general pattern of a north/south cline was evident. All populations in the northern portion of Colorado were in the basal portion of the tree, the southern-most population was in the derived half of the tree, and toads in the central portion of the state were found throughout the tree.

A Mantel test identified a significant correlation between genetic distance and geographic distance. If toads were to be translocated, moving toads only between the geographically closest populations would maintain a more natural level of genetic divergence across the state.

Two measures of intrapopulation variation were calculated, the number of genotypes per population and the phylogenetic distance within each population. The Chaffee County metapopulation showed a high value for phylogenetic diversity and had numerous genotypes. This metapopulation might be a good source population for translocations in the central portions of the state. The Clear Creek drainage had an unusually low number of genotypes.

Bufo boreas in Colorado

Declines of *Bufo boreas boreas* in Colorado during the 1970's left only three self-sustaining populations and a number of isolated smaller populations distributed across the mountainous central portions of the state (Cary 1993; Corn 1994; Stuart and Painter 1994; Loeffler 1998a and b). Recent declines alone, throughout such a large portion of the toad's range, warranted strong conservation efforts and *Bufo boreas boreas* was listed as endangered by the State of Colorado in 1993. The U. S. Fish and Wildlife Service (USFWS) consistently ruled that federal listing was "warranted but precluded" due to lack of funding. Conservation programs were established by the State of Colorado which included extensive monitoring and research concerning possible causes of declines (Loeffler 1997; Loeffler 1998a and b). Other research concerned methods of reintroduction (Scherff-Norris 1998 and 1999), toxicology (Brinkman 1998), ecology (Jones and Goettl 1998; Livo 1998), and genetics (Goebel 1998).

In the last year two of the three large populations have declined further leaving a single population in Chaffee County that may be self-sustaining. Plans for captive propagation and translocations or reintroductions are being formalized (Boreal Toad Recovery Team Meeting, Denver CO, 8 February 2000). Measures of genetic diversity within and among populations in Colorado are needed to identify the appropriateness of translocations among populations.

Early genetic analyses (Goebel 1996) identified a very high degree of mitochondrial DNA (mtDNA) divergence between toads in the southeastern portion of the toad's distribution (Utah, southeast Idaho, and Colorado) and the rest of the range. Within the *B. boreas* species group a number of small populations were identified with highly divergent mtDNAs, including both species *Bufo nelsoni* and *Bufo exsul*. A single population of boreal toads in Kane County, UT, had a similarly high mtDNA divergence (Goebel 1996) and might be a cryptic species. Like the spotted frog (Green et al. 1996), the boreal toad might have a number of previously unrecognized divergent populations in the regions surrounding the Great Basin. Green et al. (1996) hypothesized that as the Great Basin region became drier frogs moved northward or were isolated, allowing a high degree of genetic divergence along their southern edge. Hovingh (1997) suggested that geological conditions within and around the Great Basin might have allowed a similar divergence in many amphibian species. Thus, DNA from all geographically isolated populations of the boreal toad surrounding the Great Basin should be examined for potential genetic divergence (and speciation). Lack of genetic testing may result in the loss of substantial diversity as has been the case in other species (Avise and Nelson, 1989; Daugherty et al, 1990; Greig 1979).

In most cases, genetic divergence among populations will be much less than that expected among cryptic species, but any genetic divergence suggests an independent evolutionary history and may be relevant for conservation management plans. Previous analyses of mtDNA within the Southern Rocky Mountains (Goebel 1997 and 1998) resulted in two major conclusions. First, all specimens analyzed in Colorado and southeast Wyoming (Albany County) fell into a cluster of closely related mtDNA haplotypes. No additional highly divergent populations (equivalent to *B. nelsoni, B. exsul*, or toads in

Kane Co. UT) were identified. Second, the three largest populations in Colorado (Rocky Mountain National Park, the Clear Creek Drainage, and Chaffee County) all had unique mtDNA haplotypes and significantly different frequencies of shared mtDNA haplotypes. Goebel (1997 and 1998) suggested a hierarchical management structure. First, all toads from the southeast distribution of the toad's range should be managed together. In the second level of management, toads in the Southern Rocky Mountains should be managed separately from boreal toads elsewhere. At the final level, toads in the three large divergent populations in Colorado should be managed as independently evolving units; toads in Colorado should not be treated as a single panmictic group.

But all previous genetic analyses were based on mtDNA only and mtDNA is effectively a single locus. Estimates of diversity based on a single genetic locus could be misleading due to lineage sorting especially among closely related lineages. Analyses of nuclear DNA (nDNA) were needed.

Previous and concurrent genetic analyses

Previous studies described mtDNA divergence of *Bufo boreas* in the southeastern portion of its range (Goebel 1996) and suggested that *B. boreas* in the Southern Rocky Mountains (SRM; central Colorado, the Medicine Bow Mts. of southern Wyoming, and extensions of the San Juan Mts. of northern New Mexico) might be a distinct species. In order to examine the taxonomic and phylogenetic relationships of toads in the SRM region further, two related studies were undertaken. In the first project mtDNA of toads in regions geographically closest to those in the Southern Rocky Mountains, including Utah, southern Idaho, and northwestern Wyoming, was examined. The purpose of this study was to identify the geographic distribution of the mtDNA clade that included all animals from the SRM. The project "Geographical Range Delimitation of the Southern Rocky Mountain Cluster of *Bufo boreas*" (project dates September 1997-December 1999) was funded by USGS (Steve Corn). The second major study was made up of three interrelated and serial analyses funded by the Colorado Division of Wildlife. These were: 1) "Molecular Genetic Determination of Management Units of the Endangered Boreal Toad (*Bufo boreas*) in the Southern Rocky Mountains" (project dates 1995-1997); 2) "Genetic relatedness of individuals within and among populations of *Bufo boreas* in Colorado as identified from nuclear minisatellite DNA" (proposal dates 1 July 1996-31 June, 1997); and 3) "Molecular Genetic Analyses of Populations of *B. boreas* in Colorado" (mtDNA and nDNA analyses; project dates July 1997 - December 1999). The purposes of these studies were to identify mitochondrial DNA and nuclear DNA variation within and among populations in Colorado. Variation of mtDNA within and among populations in the SRMs was previously reported (Goebel 1997). Variation of nuclear DNA is reported here. A fifth project, currently ongoing and not reported here, concerns the mtDNA and nDNA analyses of toads from Utah. This study is critical to identifying the species status of toads in the Southern Rocky Mountains. Because the first four studies were interrelated, this report is a summary of the four analyses (including new data for each analysis). However, this report emphasizes the results of nuclear DNA analyses, which have not been included in previous reports.

Mitochondrial DNA (mtDNA) data

Mitochondrial DNA has been used for systematic and phylogeographic analyses of a variety of species (Avise et al. 1987; Moritz et al. 1987; Harrison 1989; Wilson et al. 1985). MtDNA has a high copy number and is therefore easy to amplify and sequence. It has a variety of genes that evolve at

different rates. Ribosomal genes evolve slowly and can be used to identify relationships among distant lineages. Large portions of the "control region" evolve rapidly and are typically used to analyze variation among close lineages such as cryptic species and populations. MtDNA is inherited from a single parent (maternal inheritance) and does not recombine with each generation. Due to nonrecombination, all DNA within the mtDNA molecule is effectively a single, very large, locus. Thus, divergent maternal lineages can be identified with mtDNA more effectively than either maternal or paternal lineages can be detected with nuclear DNA loci.

However, the use of mtDNA (or any single locus) to identify relationships among closely related taxa was challenged because a single-gene tree may not be congruent with a species tree due to random lineage sorting (Quinn et al. 1991; Neigel and Avise 1986; Avise 1989; Wu 1991; Hudson 1990). Lineage sorting occurs when populations diverge and give rise to new populations. Each new (derived) population will have a subset of mtDNA haplotypes from the old (ancestral) population. For some time after the population split, some individuals in each population will be more closely related to individuals in the other population than in their own, even though the populations have truly diverged. The length of time until each population is monophyletic (all members are more closely related to one another than to members of another population) depends on the size of the population, the rate of gene change, and how strongly selective forces may be acting. In a small population (especially one undergoing a series of gene "bottlenecks") a rapidly evolving gene on which there are strong selective forces may become monophyletic in a few generations. Due to lineage sorting, population and species phylogenies may not be identical to any single gene phylogeny. However, among vertebrates in which females are not strongly philopatric, a mitochondrial gene tree has a much higher probability of tracking a species tree than any single diploid nuclear gene (Moore 1995, 1997). If a lineage from a mitochondrial gene conflicts with lineages from several (theoretically up to 16; Moore 1995) nuclear genes, then the mtDNA lineage is still more likely to correctly track an organismal lineage. If a single or only a few genes are to be analyzed, mtDNA is surely one of the best choices.

One commonly used approach (Avise 1994) and the one I used in this analysis, was to examine several independently inherited genes and use the correspondence among genes to infer organismal lineages (Avise and Ball 1990). In analyses of *Bufo boreas*, mtDNA was examined first. In a second analysis a high number of nuclear loci were examined with Amplified Fragment Length Polymorphism (AFLPs; Vos et al. 1995) markers. A high number of nuclear loci might allow phylogenetic signal to be apparent above the "noise" of lineage sorting from the many independently assorting nuclear loci. If mtDNA and nuclear genes do not conflict, then hypotheses of organismal lineages are strongly supported. Where results conflict, support for phylogenetic hypotheses may be based on the degree of support for nuclear or mtDNA loci. MtDNA and nuclear DNA analyses can also conflict when females (which pass on both mtDNA and nuclear DNA) have different migration patterns than males (which pass on nuclear DNA only). This does not seem to occur in toads, but studies of migration patterns are needed.

Nuclear DNA (nDNA) data

Historically allozyme markers were used to examine nDNA subdivision (e. g. Green at al. 1996). Analyses of allozymes in *B. boreas* in California identified no fixed differences among recognized species (Feder 1973) suggesting that analysis of allozymes would not identify variation at a scale appropriate for the analysis of population diversity. In order to examine nDNA I proposed to examine minisatellite loci (Jeffreys 1987; Longmire et al. 1990; Jeffreys et al. 1990 and 1991; Hanotte et al. 1992; Timms et al. 1993), because they typically showed much more variation than allozymes (Avise 1994). However, analysis of minisatellite DNA required large amounts of high quality DNA in comparison to genetic analyses that use PCR (the polymerase chain reaction) to amplify DNA before analysis. In the last few years a number of PCR-based techniques were developed, because molecular markers are critical to studies of population ecology, conservation genetics, and biodiversity evaluation (Hadrys et al. 1992; Milligan et al. 1994; Karp et al. 1997). The most commonly used PCR-based techniques included random amplified polymorphic DNA (RAPDs) and microsatellite analyses. Analysis of microsatellites was considered, but cost and development-time were prohibitive. RAPDs, although cheap and fast, were unusually sensitive to minor changes in PCR amplification conditions.

After a review of recently developed PCR methods, analyses of Amplified Fragment Length Polymorphisms (AFLPs; Vos et al. 1995) were considered a better choice for nDNA markers than minisatellite analyses. By the time data collection began, published studies using AFLPs showed that they represented reliable PCR-based markers for studies of genetic relationships at a variety of taxonomic levels (Hill et al. 1996). AFLP characters were examined in bacteria (Janssen et al. 1996; Anthrax, Keim et al. 1997; Aeromonas, Huys et al. 1996), animals (cattle, Ajmone-Marsan et al. 1997; humans, Vos et al. 1995 and Falcone et al. 1995) and more commonly in plant species (lettuce, Hill et al. 1996; oaks, De Greef et al. 1998; poplars, Winfield et al. 1998 and Arens et al. 1998; barley, Pakniyat et al. 1997; lymegrass, Anamthawat-Jonsson et al. 1999; cotton, Feng et al. 1997).

AFLP analyses were chosen for analyses of the *B. boreas* group primarily because of the reduced sensitivity to small amounts of degraded DNA. Unlike other PCR-based fingerprinting methods (RAPDs: random amplified polymorphic DNA; DAF: amplification fingerprinting; AP-PCR: arbitrarily primed PCR), AFLP methods were based on ligating known DNA fragments to genomic DNA and using the ligated fragments as primer sites. Thus, primer sites were perfect matches. This reduced sensitivity to reaction conditions, DNA quality, and PCR temperature profiles, all of which limited the utility of other methods (Vos et al. 1995). A reduced sensitivity was critical for this project, because only decayed tissues were available from some localities.

AFLPs provided a number of other advantages over other PCR-marker types. AFLPs provided significantly more amplified fragments than RAPDs (De Greef et al. 1998) and more polymorphic fragments than RFLP or RAPD methods (Feng et al. 1997; Russell et al. 1997; Vogel et al. 1994). AFLPs were variable enough for paternity exclusion tests (Krauss 1999). Analyses of DNA with high levels of variation were important, because mtDNA variation within Colorado was low (Goebel 1997). Unlike other markers (e.g., allozymes and RAPDs), AFLP fragments could be identified by size to within a single base pair (bp). This allowed fragments to be compared across analyses. In contrast, gel migration distance in other markers could be compared only within the same gel and could not be compared across independent analyses easily. After the preliminary screening for primer pairs, AFLP analyses identified relatively large data sets quickly. For example, AFLP analyses identified 12 times the number of polymorphic loci of RAPD analyses in soybean varieties (Vogel et al. 1994). If many loci

were to be examined (critical to phylogenetic analyses), then AFLPs were an excellent choice even though more preparation time for template DNA was needed.

MATERIALS AND METHODS

Specimens Analyzed

Samples were collected from localities throughout Colorado (N=203) as well as the surrounding regions in Wyoming (N=3), Utah (N=25), southeastern Idaho (N=10), and central Idaho (N=35; Table 1, Appendix 1). One to 17 animals were sampled from each collection site. Metapopulations were considered to be clusters of collection sites among which animals could migrate within a single lifetime (i. e., populations within 3-5Km of each other). MtDNA was analyzed in 327 toads (109 from the SRM group); nDNA was analyzed in 109 toads (89 from Colorado). Whenever possible, mtDNA and nDNA were analyzed from the same specimen. In a few samples all DNA was used in mtDNA analyses, which were run first. In these cases nDNA was analyzed from additional samples collected from the same site (where more than 10 samples had been collected per collection site). Thus, one to 17 samples were analyzed from each collection site with both mtDNA and nDNA, but the samples were not always identical. Samples used in each analysis are identified by accession number in Table 1.

Table 1: Samples analyzed in mtDNA and nuclear AFLP analyses.

Locality (Abbreviation)	AFLP restriction site data		MtDNA sequence data	
	Number of samples	Specimen Numbers[1]	Number of samples[2]	Specimen Numbers[1]
Southern Rocky Mountain Group				
Medicine Bow Mts, Albany Co., WY[2] (AL)	0	-	2(1)	331,392
California Park, Routt Co., CO (CAL)	1	398	1(2)	398
Lost Lake, Rocky Mountain NP, Larimer Co., CO (LL)	13	58-9,114,161-4,280-1, 256-7,267-8	5(10)	138,257-8,259,281
Kettle Tarn, Rocky Mountain NP, Larimer Co., CO (KT)	5	154-6,159,93	2(10)	154-5
Indian Peaks, Lost Lake, Boulder Co., CO (IP)	2	100,102	2(4)	99-100
Henderson Mine, Clear Creek Co., CO (HM)	17	314,316-8,589-91,593-5, 597,599-60	4(8)	314,317, 635,637
Herman Gulch, Clear Creek Co., CO (HG)	7	177,179-80,182-4,515		6(6) 690-5
Clear Creek (Power Alley), Clear Creek Co., CO (CC)	3	320-322	3(4)	317,320,322
Bethyl Creek Campground, Clear Creek Co., CO (BCC)	0	-	3(6)	181-3
Upper Bethyl Creek, Clear Creek Co., CO (UBC)	0	-	0(1)	-
Georgetown, Clear Creek Co., CO (GO)	4	309,311-313	1(4)	309
Montezuma, Summit Co., CO (MT)		0 -		2(4) 28,30
Pole Creek Golf Course, Grand Co., CO (PC)	3	393-4,396	0(4)	-
Hartenstein Lake, Chaffee Co., CO (HL)	8	200-4,206,211,215	3(10)	202,209,207
Denny Creek, Chaffee Co., CO (DC)	3	217-219	2(7)	219,224
Collegiate Peaks Campground., Chaffee Co., CO (CPC)	6	87,89-91,189,192	2(10)	188,191
Brown Creek, Chaffee Co., CO (BRO)	1	397	0(1)	-
North Chaffee, Chaffee Co., CO (NC)	3	612-4	4(4)	611-14
West Brush Creek, Gunnison Co., CO (WBC)	1	227	1(2)	227
White Rock Basin, Gunnison Co., CO (WRB)	3	232, 234-5	2(2)	229,233
Trout, Fern Creeks, Mineral Co., CO (TC, FC)	6	419-20,422, 468-9,TC4	2(6)	419,422
Brown Creek, Mineral Co., CO (BC)	3	472-4	0(3)	-
Total Southern Rocky Mountain Group	89		47(109)	

Table 1: Continued

Locality (Abbreviation)	AFLP restriction site data: Number of samples	AFLP restriction site data: Specimen Numbers[1]	MtDNA sequence data: Number of samples[2]	MtDNA sequence data: Specimen Numbers[1]
Idaho				
Tin Cup Creek, Caribou Co., ID (TC)	7	438-9,441-2,444-5,447	9(9)	438-40,442-7
Utah				
Curtis Ridge, Rich Co., UT (CR)	0	-	1(1)	408
East Fork Bear River, Summit Co., UT (SU)	0	-	1(1)	332
Gold Hill, Summit Co., UT (GH)	0	-	1(1)	425
Upper Rocky Pass Spring, Box Elder Co., UT (UPR)	0	-	4(4)	403-4,406-7
Red Butte Canyon, Box Elder Co., UT (RBC)	0	-	2(2)	400-1
Kane Co., UT (KA)	6	295-6,492-3,495,672	10(17)	295-303,490-5,671-2
Total in Utah	**6**		**19(26)**	
Outgroups				
B. exsul	1	325 (MVZ)	5(5)	323-327 (MVZ)
B. nelsoni	1	168	2(2)	167-8
B. canorus	0	-	5(5)	328-330(MVZ),293-4
B. boreas, California	4	135-6,246,605	27(56)	10,12,14-21,23,116-7,135-6,152,246,248-9,251,253,255,290-2,603,605
B. boreas, Oregon	1	252	10(10)	245-9,251-5
B. boreas, Washington	0	-	9(9)	25/112,409-416
B. boreas, British Columbia	0	-	5(6)	2,5,7,355-6
B. boreas, Alaska	0	-	26(26)	544-66,568-71,573-4,618,621,624-5,629-30,633
B. boreas, Idaho	0	-	29(32)	270-2,536-543,526-531,533-35,555-563

Table 1: Continued.

Locality (Abbreviation)	AFLP restriction site data		MtDNA sequence data	
	Number of samples	Specimen Numbers[1]	Number of samples[2]	Specimen Numbers[1]
B. boreas, Montana	0	-	15(21)	33,39,86,261,488-9,496-501,586-8
B. boreas, Northwest Wyoming	0	-	6(8)	103-4,106,109, 111,113
Total, Outgroups	7		139(180)	
Total , All samples	109		224(327)	

[1] Numbers given are AMG numbers. Corresponding collection numbers (i. e., museum numbers) are in Appendix 1.

[2] First number refers to samples for which sequence data are available from direct sequencing methods. Specimens are identified in next column. Number in parentheses refers to the number of samples for which sequence data are inferred from restriction site and sequencing methods combined.

DNA Extraction

DNA was extracted from muscle or liver tissue from animals found dead in the field and from newly metamorphosed toads. Tadpole tails, blood from juveniles or adults, and egg tissue were used also. Eggs contain approximately 100 times the amount of mtDNA of muscle tissue, so only mtDNA was analyzed from eggs. Total DNA was extracted using proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation (Maniatis et al. 1982; Hillis and Davis 1986).

Mitochondrial DNA

DNA amplification, sequencing, and sequence alignment — Four mtDNA data types were combined for analysis: restriction sites of the entire mtDNA molecule (390bp) and sequences of three regions including the control region (911 bp including 48 bases of cytochrome b), Cytochrome oxidase I (COI, 390 bp), and 12S ribosomal DNA (12S rDNA, 858 bp). Restriction site data provided the highest level of variation, but the data were highly homoplastic. Due to the intensive labor of data collection and high homoplasticity, restriction sites were not obtained for samples collected after 1994. Control region sequences provided the most phylogenetic information and were collected for all haplotypes identified with restriction sites as well as all samples collected after 1994. Both 12S rDNA and COI were more conserved and were obtained for a few samples only. The COI and 12S rDNA sequences were used simultaneously in analyses of North American bufonids (Goebel 1996) and in the more extensive analyses of the *B. boreas* group described in this report.

Control region sequences were determined using six primers (CytbA-L, ControlO-H, ControlJ-L, ControlK-H, ControlB-H, ControlP-H; Goebel et al. 1999). In some cases, the primer ControlP2-H (5'-CATAGATTCASTTCCGTCAGATGCC-3") was used for sequencing only. ControlP2-H was a few bases internal to the fragment amplified by CytBA-L and ControlP-H and provided superior sequence data compared to the terminal primer ControlP-H. A repeated fragment that varied in number among specimens was not included in the control region sequences. Cytochrome oxidase I sequences were obtained using two primers (CO1e-H, Palumbi et al. 1991; CO1af-L, Goebel et al. 1999). The 12S rDNA was amplified using four primers (12SA-L, Kocher et al. 1989; Palumbi et al. 1991; tRNAphe-L, 12SF-H, tRNAval-H, Goebel et al. 1999). Sequences of both DNA strands were obtained for all 12S rDNA sequences, at least one accession of all unique COI sequences, and at least one accession of all unique control region sequences, with a single exception. Sequence for one strand at the 3' end only was obtained from two specimens, which had many copies of a repeated fragment. When one-directional sequencing indicated identity with previously obtained sequences, the sequence from the second direction was not determined in some cases. Protein coding sequences were aligned by converting DNA sequences into proteins (MacClade, Version 3.0, Maddison and Maddison 1992) and aligning amino acids. Sequences of 12S rDNA and the control region for the *B. boreas* group were aligned manually. There was ambiguity in homology of sites in only one instance due to adjacent gaps in a single accession (control region sites 226-7) and the two bases of sequence were scored as missing data in that accession (281) only.

Restriction-site polymorphisms were identified using standard techniques (Southern 1975; Maniatis et al. 1982). Genomic DNA was cut with 16 six-base cutting restriction enzymes (ApaI, BamHI, BglI, BglII, ClaI, Csp45I, DraI, EcoRI, EcoRV, KpnI, NheI, PstI, PvuII, SmaI, StuI, and XhoI). After digestion, fragments were separated by size with gel electrophoresis and transferred to

nylon membranes with Southern-blot techniques (Koetsier et al. 1993). Four probes, which comprised the total mtDNA of *B. marinus*, were used to detect mtDNA polymorphisms. The probes were radioactively labeled using random priming and hybridized (at 63°C) to complimentary mtDNA on the nylon membranes. Autoradiography was used to identify the migration pattern of mtDNA restriction fragments. Restriction sites were mapped by standard techniques of double restriction-enzyme digests (Maniatis et al. 1982) and serial probing with four mtDNA probes. Overlap of character data occurred between DNA regions sequenced and eight restriction sites. Only two of the sites were variable and in these the restriction-site data were retained and the sequence characters excluded, because restriction-site data were obtained for the largest number of specimens.

Phylogenetic analyses (parsimony)--Phylogenetic analyses using parsimony were performed using PAUP 4 (Swofford 1999) with the heuristic search option. All characters were weighted equally, because sequence divergences among even the most divergent lineages were small (<3%). Nine single-base gaps in the control region were scored as present or absent. Contiguous gaps due to a missing fragment in accession 109 were scored as missing data. To identify a set of most parsimonious trees, 1000 random addition sequence replicate searches were run, using the options of TBR swapping and steepest descent, and saving five trees per replicate. A strict consensus tree of all trees of minimum length was calculated. Even though the total number of most-parsimonious trees was not known, the strict consensus tree described above was considered to reflect the strict consensus tree of all most-parsimonious trees accurately, because further replicates did not result in the collapse of any additional nodes in the strict consensus tree. Additional support for the strict consensus tree was provided by analyses with reverse constraints (e. g., Baum et al. 1994; Catalan et al. 1997). No additional trees of the same length were found in analyses in which the search was constrained to save only those trees not matching the constraint strict consensus tree (1000 random addition sequence replicates were performed using the options of TBR swapping and steepest descent, saving five trees per replicate). Bootstrap (Felsenstein 1985 and 1988) and decay values (Bremer 1988) were estimated in order to compare degree of support for discovered clades. Final bootstrap values were estimated with 1000 bootstraps, using five replicates per bootstrap, a random tree to start each replicate, and saving only the five most parsimonious trees per replicate. Decay values were estimated using the program Autodecay (Eriksson 1997). Calculations for decay values were replicated 100 times. Only the five most parsimonious trees were saved in each replicate. Bootstrap values >88% in this analysis were considered to support clades strongly (Hillis and Bull 1993; Cummings et al. 1995), but some clades with lower bootstrap values were discussed also.

AFLP nDNA

Detection of polymorphisms—One hundred and two samples from the eastern portion of the toad's range were analyzed along with seven samples from the Northwest and Southwest mtDNA clades (clades described in Goebel 1996). The latter were used as outgroups.

AFLP analyses followed the methods detailed in Vos et al. (1995), with minor modifications from Rosendahl and Taylor (1997), Lin and Kuo (1995), Life Technologies AFLP Instruction Manual (anonymous 1997), and Janssen et al. (1996). Briefly, DNA was digested (cut) with two restriction enzymes, MseI and EcoRI. Short DNA fragments (oligos) were ligated (pasted) to the restriction enzyme cutting sites. These oligos served as exact matches for a first round of PCR amplification (preamplification primers, Table 2). A second round of amplification was performed with radioactively labeled primers (selective primers, Table 2) that matched not only the preamplification primers, but extended a few bases into the amplified fragment. The selective primers amplified only a subset of the fragments amplified with preamplification primers, each subset depending on the additional bases of the selective primers. The amplified fragments were then separated on standard sequencing gels and banding patterns, due to the differential migration of fragments, were identified with autoradiography.

Preliminary analyses were run to choose appropriate selective primers. Sixty-four possible combinations of eight MseI and eight EcoRI primers were used to amplify DNA in five samples from Colorado and three outgroup samples. Primer pairs were chosen based on the number of polymorphic loci identified and the total number of fragments amplified. Some primer pairs amplified too many fragments to score easily; some amplified fewer but provided a lower potential for identifying polymorphic loci. Five primer pairs (Table 2) were chosen because they identified variation in the eight test samples and provided many bands that could be scored reliably. These were used to analyze AFLPs in the remaining toad samples.

Table 2. Oligos used to amplify nDNA with AFLP methods. Adapter oligos were from Janssen et al. (1996); all other primers were from Vos et al. (1995).

Oligos	Sequence
Adapter oligos:	
EcoRI adapter 1	5'- CTC GTA GAC TGC GTA CC - 3'
EcoRI adapter 2	3'- CTG ACG CAT GGT TAA -5'
MseI adapter 1	5'- GAC GAT GAG TCC TGA G -3'
MseI adapter 2	3'- CTA CTC AGG ACT CAT -5'
Pre-amplification primers:	
EcoRI	5'-GAC TGC GTA CCA ATT CA-3'
MseI	5'-GAT GAG TCC TGA GTA AC-3'
Selective amplification primer pairs:	
I.	5'- GAC TGC GTA CCA ATT CAC G -3' (EcoRI-8)[1]
	5'- GAT GAG TCC TGA GTAA CTC -3' (MseI-8)[1]
II.	5'- GAC TGC GTA CCA ATT CAA C -3' (EcoRI-3)[1]
	5'- GAT GAG TCC TGA GTAA CTA -3' (MseI-7)[1]
III.	5'- GAC TGC GTA CCA ATT CAG C -3' (EcoRI-9)[1]
	5'- GAT GAG TCC TGA GTAA CTT -3' (MseI-10)[1]
IV.	5'- GAC TGC GTA CCA ATT CAG G -3' (EcoRI-10)[1]
	5'- GAT GAG TCC TGA GTAA CTG -3' (MseI-9)[1]
V.	5'- GAC TGC GTA CCA ATT CAC A -3' (EcoRI-5)[1]
	5'- GAT GAG TCC TGA GTAA CAT -3' (MseI-6)[1]

[1] Primer name used in Appendix 2

Polymorphisms were scored as the presence or absence of an amplified (restriction) fragment. Each primer pair resulted in the amplification of many fragments. Only bands that were variable and were shared among samples (parsimony informative characters) were scored. Due to the high probability of human error in scoring and recording a large number of bands, all samples were scored twice for all bands. All discrepancies between scorings were re-examined and corrected.

Reliability of scoring was determined in two ways. First, bands were scored as present (1), absent (0) or light (2). Characters that were scored as light in three or more samples were deleted from the data set. Any remaining data scored as light were then treated as missing data. In this manner data from all but the clearly intensely labeled bands were deleted (as in Pakniyat et al. 1997). Second, data from several samples were collected from independent analyses (DNA was taken from the same DNA extraction aliquot for analysis, but preamplifications and selective amplifications were independent).

Loci that varied among independent analyses from the same sample were excluded from the data set. It was not apparent why some loci should vary among independent analyses, or score as light in some samples. Potential sources of error include accidental human error or possible variation in DNA concentration (too much DNA might result in light bands in some samples, while too little DNA might result in lack of amplification in all bands). Variation among duplicate samples was identified in other studies (93-100% similar in poplars, Winfield et al. 1998; 95-98.5% in the bacteria Aeromonas, Huys et al. 1996; 98% in poplars, Arens et al. 1998; 99% in barley, Becker et al. 1995). Reasons for variation among duplicates, other than human error in scoring autoradiographs or somatic mutations, were not determined.

Due to the potentially high DNA degradation of some samples, the highest amount of DNA used in previously published analyses was used for some samples of *B. boreas*. A larger amount of DNA should increase the probability of larger fragments being present, and reduce the possibility of bands being incorrectly scored as missing due to the absence of the appropriate sized template DNA. The highest amount of DNA used in previous studies was1000 ng (Janssen et al. 1996) and the lowest was 1.0-0.1 ng (Rosendahl and Taylor 1997). Five hundred nanograms (Vos et al. 1995) were used initially for all samples in this analysis. When the first round of amplification failed or DNA samples were known to be highly degraded, digestion was repeated with 1000ng. If preamplification failed again the samples were eliminated from the analysis. For some samples selective amplification failed or provided poor results (e. g., bands of only high or low size, smeary bands). If all primer pairs resulted in poor bands preamplifications were repeated. If a single primer pair resulted in scorable banding patterns only the selective amplifications were repeated. In a few samples all or most large fragments were absent or greatly reduced in intensity. The lack of all large fragments could be due to severe degradation of the template DNA or severe overestimation of the DNA concentration. A few of these samples were repeated with both increased and decreased volume of DNA, but most were eliminated from the analysis and await further analysis as funding becomes available. Finally, data were excluded for all samples that had missing data for one or more primer pairs. Although missing data for these samples could be obtained, time and financial constraints limited further data collection at this time.

In this study AFLPs were generated from multiple tissue types (tadpole tails, blood, and muscle tissue). Varying AFLP patterns were found from DNA of different plant organs (Donini et al. 1997), but were not detected from different animal tissue types (Vos, personal communication, 1998). Fragments unique to specific tissue types were not detected in this analysis. Variation in banding patterns due to DNA extraction methods was identified in poplars (Arens et al. 1998), but in *Bufo boreas* all DNA was extracted by the same method and was not expected to result in variation in banding pattern.

Bands of the same size were considered to be genetically identical. This assumption can only be confirmed with hybridization studies and/or further analyses to identify the sequence of each fragment. However, PCR amplified bands of the same size are highly likely to be genetically identical, especially among closely related samples (Rieseberg 1996).

All bands were considered to be single loci (independent characters in phylogenetic analyses). Multiple alleles at some loci have been identified (anthrax, four alleles, Keim et al. 1997; soybean, Maughan et al. 1996; potato, Van Eck et al. 1995), but most fragments appeared to be single loci. Although the assumption that all bands are different loci might be violated for a few fragments, it is highly likely that most fragments were separate loci.

Phylogenetic analyses (parsimony and Neighbor Joining) --Phylogenetic hypotheses were estimated in two ways. Parsimony was used to identify the set of trees that required the minimum number of character changes among all genotypes. A distance method, Neighbor-joining (Saitou and Nei 1987; distances estimated by PAUP 4), was used to estimate relationships based on the similarity of genotypes.

Phylogenetic methods to determine the most parsimonious trees from AFLP data were similar to those for analyzing mtDNA sequence data. Parsimony was performed using the heuristic search option. All characters were weighted equally. A strict consensus tree was calculated from 1000 random addition sequence replicate searches, using the options of TBR swapping and steepest descent, and saving five trees per replicate. Analyses of reverse constraints found no additional trees of the same length. Bootstrap and decay values were estimated in order to compare degree of support for discovered clades in the same manner as for mtDNA. Phylogenetic relationships hypothesized by Neighbor-joining methods used pairwise distances calculated from restriction sites (Nei and Li 1979) and assumed minimum evolution. Negative branch-lengths were set to zero.

Principal Coordinates Analysis (PCO) -- A Principal Coordinates Analysis of pairwise genetic distances was performed using the program NTSYS-pc (Rohlf 1992). A PCO is an ordination analysis and very similar to a principal components analysis (PCA). These analyses construct a set of orthogonal coordinate axes such that the projection of points on to them has maximum variance. Pairwise distances (Nei and Li 1979) were calculated between samples using PAUP 4 (Swofford 1999). The distances were double-centered (program DCENTER in NTSYS-pc) and eigen values were calculated (program EIGEN in NTSYS-pc). The data were graphed by the first three eigen values (program MOD3D in NTSYS-pc). The three-dimensional graph was rotated (127 degrees around the x-axis, and 93 degree about the y-axis) to identify the largest difference among clusters and printed as a two-dimensional scatter plot (the third dimension, height, did not provide any additional visual variation and was excluded).

Mantel Test for isolation by distance -- The relationship between the genetic distance and geographic distance between samples was examined using the Mantel test (Mantel 1967; Hubert 1987). Direct line distances between localities were estimated from maps. Pairwise distances (Nei and Li 1979) were calculated as above (PAUP 4: Swofford 1999). Matrices of pairwise distances (both genetic and geographic distances) were compared using the program MXCOMP (NTSYS-pc; Rohlf 1992), with a Mantel test. The normalized Mantel statistic, Z, was calculated where:

$$Z = \sum_{i<j}^{n} X_{ij} Y_{ij}.$$

If the two matrices show similar relationships (i. e. genetic distance correlates with geographic distance) then Z should be large in comparison to what one would expect by chance. An estimate of the significance can be made by comparing observed Z values with their permutational distribution. However, the product moment correlation coefficient, r, is monotonically related to Z (Smouse et al. 1986) and is usually used to estimate significance (Rohlf 1992).

Two tests for isolation by distance were made using the Mantel test. The first included all samples from the Southern Rocky Mountains as well as the phylogenetically closest samples from southeast Idaho. The second included samples from the Southern Rocky Mountains only. Two hundred and fifty random permutations were run for each test.

Variation of AFLP data within populations

Genetic variability within localities, metapopulations, and regions was estimated in three ways. First the number of unique genotypes in each locality was estimated. This number is difficult to interpret, because AFLP restriction site data identified such a high number of genotypes (almost every sample analyzed was unique). Therefore a second measure, the number of characters that varied within each locality, was calculated. Variation in this second value was compared among metapopulations.

A third measure of variation was based on phylogenetic distance or branchlengths. The phylogenetic distance of a locality, metapopulation, or region was calculated by summing the lengths of all branches that connected all members of a locality, metapopulation or region (minimum spanning distance). This method of estimating phylogenetic diversity has been used largely with higher taxa (e.g., May, 1990; Vane Wright et al., 1991; Nixon and Wheeler, 1992) but can be used with any phylogenetic tree. The phylogenetic distance was divided by the number of samples in each locality, metapopulation or region in order to compare values among groups. Localities with a single member were excluded because the minimum spanning distance was 0.

RESULTS

Mitochondrial DNA -- Phylogenetic Analyses (Parsimony)

Mitochondrial DNA data obtained from direct sequencing combined with restriction site data identified 2213 characters. Two hundred twenty were variable and 150 were parsimony informative. One hundred fifty three unique haplotypes were identified from 327 animals. Of the 153 haplotypes, all had a minimum of 545 bp of control region sequence data, most (118) had 943 bp of control region sequence data, many (N=82) had 34 bp of restriction site data, and a few had an additional 1248 bp of sequence data from the 12S rDNA (N=8) and COI (N=19) genes.

A phylogenetic analysis using parsimony identified 40 most parsimonious trees (length 395). Although many more most-parsimonious trees are likely, further searching found no additional trees length 395 that were not consistent with the strict consensus tree of the 40 trees identified. One of the most parsimonious trees (Figure 1) and a strict consensus tree of all most parsimonious trees (Figure 2) identified four major phylogeographic clades that were previously described (Goebel 1996). The major clades were:

The clade in Kane County, UT. All newly analyzed samples from Kane Co., UT, were found to be identical or very closely related to all previously analyzed samples from this county.

The Northwest mtDNA clade – This clade included all samples from northwest Wyoming, Montana, central and northern Idaho, northern California, Oregon, Washington, coastal British Columbia and Alaska. All newly analyzed samples from central Idaho were found in the Northwest clade.

The Southwest mtDNA clade – This clade included samples from central and southern California and western Nevada. No new samples were analyzed from this region.

The Southeast mtDNA clade – This clade included samples from Colorado and south central Wyoming, northern and southern Utah, and southeast Idaho.

These clades were strongly supported; they had high bootstrap values (83-100%; Figure 2) and the branches leading to them were supported by a relatively large number of characters (9-19; Figure 1). No additional mtDNA major clades were discovered from newly analyzed animals.

The southeastern most distribution of *B. boreas* was the focus of this study because it included the Southern Rocky Mountain group. The new mtDNA data identified the SRM group and identified several new minor phylogeographic groups within the Southeast clade (Figures 1 and 2). The minor clades and groups were:

The group in Box Elder Co., UT. Most mtDNAs from these counties clustered closely together in a highly divergent and strongly supported clade.

The group in Caribou Co., ID. All animals examined had identical or very closely related mtDNAs. While there was no mtDNA character that united all samples (i. e., no character was found in all animals of this group but not found in any animals outside this county), all samples clustered together and were basal to samples in northern Utah and the Southern Rocky Mountains.

Animals from northern Utah (excluding Box Elder Co., UT) and all animals from the Southern Rocky Mountain group together formed a mtDNA phylogeographic clade. All newly analyzed animals from Colorado were within this clade, but mtDNA did not identify the SRM group as a monophyletic phylogeographic clade. Two samples (408 from Rich Co., UT, and 425 from Summit Co, UT) were also found within this clade. All samples from southeastern Wyoming (Albany County) were found to be closely related to toads in Colorado. No samples from New Mexico were analyzed.

AFLP nDNA data and Phylogenetic Analyses (Parsimony and Neighbor Joining)

One hundred fifty eight characters were initially scored in 178 animals, 124 characters in 102 samples were used in the final analyses. Sixty-six samples were removed from the analysis due to missing data and 34 characters were removed from the data due to ambiguities in scoring among samples. Many samples with missing data were removed from the analyses, because missing data skews phylogenetic methods using distance estimates (Neighbor-joining methods). Most of the removed samples had only small amounts of missing data. A relatively small investment in these samples could increase sample size considerably. No samples from southeast Wyoming or northern Utah were included in the final analysis.

One hundred and seven parsimony informative characters were used in the phylogenetic analyses. Samples from the Northwest and Southwest mtDNA major clades were used as outgroups to root the trees. A single most parsimonious tree, length 528 (Figure 3), and a Neighbor-joining tree (Figure 4) identified three clades and/or clusters (groups). These clades/groups were:

A group in Caribou Co., ID. Both analyses identified all genotypes from Caribou Co., ID, as closely related. In both analyses toads in Caribou Co. were basal to toads in the SRMs.

A clade in Kane Co., UT. All samples from Kane Co., UT formed a strongly supported clade (Figure 3) and clustered together in Neighbor-joining analyses (Figure 4). Two samples (295-296) had unusually long branch-lengths in both analyses. These two samples should be reanalyzed, because errors in data collection are possible. However, if these data are correct, then two rather divergent genotypes may coexist in Kane Co., UT.

A clade/group comprising all genotypes from Colorado. Both analyses found all samples from Colorado to be closely related. No samples from northern Utah were analyzed, so monophyly may be due to the more limited sampling in AFLP analyses.

Strong support (identified from long branch-lengths and high bootstrap values) was found for relationships among the outgroups, a clade comprising genotypes from Kane Co., UT. Low statistical support for independent groups in southeast Idaho and the SRMs found with AFLP analyses may be due to high homoplasy inherent in restriction site data. Although only 124 characters were used, the total tree length was 528; many characters changed states multiple times on the tree (characters were homoplastic). Homoplasy is not uncommon with restriction site data, because a restriction site loss may be due to a base change in any of six bases (EcorI) or four bases (MseI). Thus the loss of a band (scored as a single character change) may be due to any of six (or four) possible base changes within the restriction site. Analyses cannot distinguish between losses from different base pair changes (making restriction site changes due to multiple base changes homoplastic characters). Additional parsimony analyses (not shown) were conducted weighting site gains (a statistically rare event) over site losses (a more common event). These analyses did not result in a substantial change in phylogenetic pattern. Stability of relationships with variable weighting strategies suggests that enough loci were examined to identify phylogenetic signal above the "noise" generated by high homoplasy. Difficulties in identifying homoplasy were addressed in Neighbor-joining methods by estimating distance values specifically for restriction site data (Nei and Li 1979).

Relationships within the SRMs varied widely between parsimony and Neighbor-joining analyses. Lack of consensus between methods is not uncommon among closely related individuals and relationships were not strongly supported in either analysis. However, missing data may have skewed the Neighbor-joining analyses, because alleles that were unique to individuals were not scored. Excluding autapomorphic characters does not skew parsimony analyses. Therefore, following discussions of relationships within the SRMs are based on parsimony analyses (not Neighbor-joining analyses).

Even though relationships within the SRMs (Figure 3) are not strongly supported, some patterns are evident. In general, samples from populations in the northern portion of Colorado are found in the basal portion of the clade, samples from the southern-most populations are in the most derived portion

of the tree. For example, all samples from Lost Lake, Kettle Tarn (RMNP), California Park (Routt Co.), Pole Creek Golf Course (Grand Co.), and Indian Peaks (Boulder Co.) were in the northern portion of the state and were found in the top (more basal) portion of the SRM clade (Figure 3). The southern most populations (Fern and Brown Creeks in Mineral Co.) were found in the lower (more derived) portion of the SRM clade (Figure 3). Samples from both Chaffee Co. and the Clear Creek Drainage (localities in the central portions of the state) were found throughout the tree. This pattern could reflect a north/south cline and suggests that toads arrived first in northern Colorado and migrated southward.

Comparison of mtDNA and nDNA phylogenetic analyses

Nuclear data (Figures 3 and 4) were similar to mitochondrial data (Figures 1 and 2) in that they identified similar clades and clusters; the toads in Kane Co., UT, toads in southeast Idaho, and toads in the Southern Rocky Mountains. Analyses of nuclear AFLP data did not include toads from southeast Wyoming or northern Utah, which were included in mtDNA analyses. Therefore, strong conclusions concerning the monophyly of toads in the SRM based on AFLP data are precluded at this time.

Principal Coordinates Analysis (PCO) of AFLP Data

The principal coordinates analysis provided another visual representation of the genetic distinction among samples. The first axis (eigen value) accounted for 61.2% of the variation among samples, the second 13.58% and the third 10.2, and the first three accounted for 85% cumulatively. The scatter plot representation of the PCO (Figure 5) was based on the first two axes only, and identified the same three groups identified by the phylogenetic analyses.

Mantel Test for isolation by distance

In the analysis that included all samples from the Southern Rocky Mountains as well as southeast Idaho r (the normalized Mantel statistic Z) was 0.3001. In the approximate Mantel t-test t was equal to 4.393. The probability that a random Z was less than the observed Z was 1.00. Out of 250 random permutations, the estimated Z was less than a random Z in all cases. The probability that a random Z would be greater than or equal to the observed Z was 0.004. Thus, even though r was relatively small, the probability tests identified a highly significant association between geographic distance and genetic distance.

In the analysis that included samples from the Southern Rocky Mountains only, r (the normalized Mantel statistic Z) was 0.14095. In the approximate Mantel t-test t was equal to 2.2633. The probability that a random Z was less than the observed Z was 0.9958. Out of 250 random permutations, the estimated Z was less than a random Z in all cases. The probability that a random Z would be greater than or equal to the observed Z was 0.100. Thus, even though r was small, the probability tests identified a significant association between geographic distance and genetic distance within Colorado. The level of significance for the SRMs alone (90%) was less than for the analysis of the SRMs and southeast Idaho combined (99.6%). Thus, some (but not all) of the significance was found in the greater genetic and geographic distance between Idaho and the SRMs. This result was expected if toads in

Colorado have been isolated from toads in Idaho longer than they have been isolated from each other, as the current geographic isolation of the two groups suggests.

Variation of AFLP data within populations

The number of characters (AFLP bands) that varied per number of animals sampled (column D in Table 3) was compared across metapopulations. Within the four metapopulations, values varied from 2.9 (San Juan) to 1.48 (Henderson). Three of the four metapopulations and one site with 13 animals (Lost Lake, RMNP) had remarkably similar values (RMNP=2.55; Lost Lake=2.69; Hartenstein Lake=2.52; San Jaun=3.11). In comparison, the Henderson metapopulation had a low value (1.48).

Only metapopulations that contained nine or more animals were described above, because results from populations with fewer animals were difficult to interpret. At least ten samples (and probably many more) need to be sampled from each locality. The number of genotypes per locality (column B) was not considered to be a good comparative value, because the number of genotypes was equal to the number of animals sampled in all but two localities

Table 3: Measures of genetic variation within localities, metapopulations, and clades based on AFLP restriction site data.

	nDNA (AFLP Data)				
Localities	N	A	B	C	D
Southern Rocky Mountain Group[1]	89	87	0.98	68	0.76
California Park	1	1	1.00	NA	NA
Rocky Mountain National Park metapopulation	**18**	18	1.00	46	**2.55**
Lost Lake	**13**	13	1.00	35	**2.69**
Kettle Tarn	5	5	1.00	27	5.40
Pole Creek Golf Course	3	3	1.00	17	5.67
Indian Peaks	2	2	1.00	14	7.00
Henderson metapopulation	**27**	27	1.00	40	**1.48**
Henderson Mine	**17**	17	1.00	30	**1.76**
Herman Gulch	7	7	1.00	27	3.85
Clear Creek (Power Alley)	3	3	1.00	8	2.66
Georgetown	4	4	1.00	14	3.50
Hartenstein Lake metapopulation	**17**	17	1.00	43	**2.52**
Hartenstein Lake	8	8	1.00	32	4.00
Denny Creek	3	3	1.00	17	5.67
Collegiate Peaks Cmpgrd.	6	6	1.00	23	3.83
Brown Creek, Chaffee Co.	1	1	1.00	NA	NA
Gunnison Co., metapopulation	4	4	1.00	13	3.25
West Brush Creek	1	1	1.00	NA	NA
White Rock Basin	3	3	1.00	14	4.67
North Chaffee	3	2	0.67	3	1.50
San Juan Mts. Metapopulation	**9**	8	0.88	28	**3.11**
Fern Creek,	6	5	0.83	15	2.50
Brown Creek, Mineral Co.	3	3	1.00	16	5.33

N = Number of samples at this locality, metapopulation, or group
A = Number of unique genotypes at this locality, metapopulation, or group
B = Number of unique genotypes per number of samples in this locality, metapopulation, or group
C = Number of sites that vary within this locality, metapopulation, or group
D = Number of variable sites/number of animals examined within this locality, metapopulation, or group

[1]The Southern Rocky Mountain Group includes all toads in Colorado.

Measures of variation based on phylogenetic distance (minimum spanning distance divided by the number of samples per group) varied between 1.67 and 13.3 (Table 4). Like the measures of genetic variation above, populations with the smallest sample sizes had widely fluctuating values (e. g., Denny Creek was very large, North Chaffee was very small). Therefore, only populations with larger sample sizes (17 and above) were considered informative. The average phylogenetic distance (PD/N) for the entire SRM group was 4.13 and was very similar to that for four other groups with large sample sizes (Rocky Mountain National Park metapopulation (4.27), Lost Lake within RMNP (4.15), Henderson Mine metapopulation (4.26) and Henderson Mine (4.65). In contrast, the Hartenstein Lake metapopulation had a higher value (6.35).

Table 4: Measures of phylogenetic divergence (PD) within localities, metapopulations, and clades based on branch lengths from parsimony analyses of AFLP restruction site data (Figure 3).

	nDNA (AFLP Data)		
Localities	N	PD	PD/N
Southern Rocky Mountain Group[1]	**89**	**368**	**4.13**
California Park	1	-	-
Rocky Mountain National Park metapopulation	**18**	77	**4.27**
Lost Lake	**13**	54	**4.15**
Kettle Tarn	5	39	7.80
Pole Creek Golf Course	3	34	11.33
Indian Peaks	2	17	8.50
Henderson Mine metapopulation	**27**	**115**	**4.26**
Henderson Mine	**17**	79	**4.65**
Herman Gulch	7	35	5.00
Clear Creek (Power Alley)	3	28	9.33
Georgetown	4	44	11.00
Hartenstein Lake metapopulation	**17**	108	**6.35**
Hartenstein Lake	8	76	9.50
Denny Creek	3	40	13.33
Collegiate Peaks Cmpgrd.	6	55	9.16
Brown Creek, Chaffee Co.	1	-	-
Gunnison Co., metapopulation	4	49	12.25
West Brush Creek	1	-	-
White Rock Basin	3	32	10.67
North Chaffee	3	5	1.67
San Juan Mts. Metapopulation	9	56	6.50
Fern Creek,	6	23	3.83
Brown Creek, Mineral Co.	3	33	11.00

N = Number of samples at this locality, metapopulation, or group.

PD = Phylogenetic distance; the sum of branchlengths between all members of a locality, metapopulation or group.

PD/N = Phylogenetic distance divided by the number of samples analyzed.

[1]The Southern Rocky Mountain Group includes all toads in Colorado.

DISCUSSION

AFLP Markers

AFLP methods produced a large number of markers useful for phylogenies at multiple levels of divergence. Analyses identified variation among recognized species (the outgroups), within strongly divergent mtDNA clades (the southeastern group of *Bufo boreas*) and among individuals within the same population. Each of 109 samples in the final analysis had a unique genotype (based on 158 characters) even though characters that were found in single animals only (autapomorphic characters) were not scored. Markers that identified variation among very closely related animals should be useful in monitoring captive breeding programs and may be useful in paternity testing. Much more data from AFLP methods is possible. Five primer pairs based on two restriction enzymes were examined here. There are 576 possible combinations of primer pairs for each pair of enzymes (I looked at only 64 combinations) and the utility of other restriction enzyme pairs is being explored (MseI/SseI, Anamthawat-Johnsson et al. 1999; MseI/PstI, Pakniyat et al. 1997 and Winfield et al. 1998).

Sometimes potential difficulties of new technologies become evident only after many researchers test them. One criticism of AFLP data is the potential of spurious bands due to star activity (false cutting) from some restriction enzymes, particularly MseI. Star activity can be greatly reduced by careful laboratory technique or by using alternate enzymes. The careful elimination of all sites that varied between duplicate samples (as was done in this analysis) may reduce the likelihood of scoring spurious bands.

Phylogenetic analyses

The identification of similar divergent groups (Kane Co., UT, Caribou Co., ID and the SRMs) with both data types is significant. Even though these groups may have had complex origins, they have diverged through time as identified by both mtDNA and AFLP analyses. This divergence suggests that the genetic analyses presented here have discovered real organismal lineages that are now evolving independently of each other.

Nuclear AFLP data, which is based on a number of genes, identified a north-south cline. However, populations were not found as monophyletic clades. When populations diverge to form new populations (i. e., animals migrate to a new locality) complex organismal histories are likely. For example, new populations may have been colonized multiple times, perhaps even from different source populations. Thus, multiple and different gene phylogenies may accurately reflect complex organismal histories. Over time, loss of gene lineages is random and different genes become fixed in different populations; the divergence of populations becomes evident from their gene histories. In contrast phylogenies of groups that have recently diverged may still contain multiple and different gene phylogenies. This complex history may be evident in the lack of monophyletic populations within the SRMs.

Variation of AFLP data within populations

The low variation within the Henderson metapopulation, as estimated from the number or unique haplotypes per population (Table 3) was unusual in comparison to the other metapopulations (RMNP,

Hartenstein and San Juan). Intrapopulation variation based on phylogenetic branch lengths (Table 4) did not suggest a low value for the Henderson Mine metapopulation. Although fewer unique haplotypes are present, they are as genetically divergent as those found across the state. If the pattern above reflects intrapopulation variation accurately, then one might speculate on its cause and use in management. Low haplotype variation might be due to a population size reduction (perhaps in the 1970s) that was more severe than size reductions in the other three metapopulations. The Henderson population might have been smaller in numbers with a relatively recent expansion to the current size of the other metapopulations. It is possible that there has been greater selection at this site, and therefore a reduction in variability. If this is an accurate comparative measure of population variation, then Henderson might not be the best (in terms of genetic variability) source population for translocations.

In contrast, intrapopulation variation based on number of unique haplotypes (Table 3) was not unusually high at the Hartenstein Lake metapopulation, yet intrapopulation variation based on phylogenetic branch lengths (Table 4) suggested a higher value for the Hartenstein Lake metapopulation than any other group. A good number of unique haplotypes remained and they were more genetically divergent than those found across the state. If the pattern above reflects intrapopulation variation accurately, then one might speculate on its cause. Perhaps Hartenstein Lake metapopulation was derived from a higher number of divergent immigrants than other populations, which resulted in a higher phylogenetic divergence. Perhaps Hartenstein Lake metapopulation did not undergo as severe a bottleneck as the Henderson Mine metapopulation, allowing a higher number of unique haplotypes to remain at this site.

The small numbers of populations with large sample sizes preclude strong conclusions until further data become available. However, there seems to be a general pattern of low variation at Henderson Mine metapopulation and a higher level or variation at Hartenstein Lake metapopulation. In a case where all other criteria for reintroduction are equal, Hartenstein Lake metapopulation would provide a better source population for reintroductions or translocations than the Henderson Mine metapopulation.

Toads in Colorado showed a trend of isolation by distance as estimated by the Mantel test. In other words, the closer two toads are in geographic space, the greater the probability that they are more closely related. *Bufo boreas* in the SRMs is not a panmictic group. This result is somewhat intuitive considering the limited mobility of toads. However, if the state had been recently or randomly populated, then a greater degree of genetic panmixis would be expected. These results suggest that measurable genetic differentiation has occurred across the state. If management plans are to preserve the genetic divergence among populations across the state, then founders for translocations and reintroductions should come from the geographically closest populations. A correlation between geographic proximity and genetic similarity is not unique to toads and has been found in poplars (Winfield et al., 1998) where closely located trees were at least 96% similar and usually identical and among Lactuca spp. (Hill et. al., 1996).

Finally, the conservation programs that treat species as panmictic groups is inadequate (Goebel 1999). The needed conservation of diverse lineages requires conservation of the evolutionary processes that maintain the diversity (e. g., natural selection, mutation, random drift, environmental fluctuations, migration routes, natural cycles of population size fluctuations, etc.). Realistic conservation efforts for populations require an understanding of the critical components of diversity and how they evolve. Some

biologists argue that populations, rather than species, are the units within which evolutionary processes are most critical. If this is true then recognition of diversity within and among populations (rather than species) and conservation of population lineages is most critical to conservation programs.

Further analyses

Due to time and financial constraints, a large number of samples from Colorado (66 of 167 analyzed) still have some missing data and were eliminated from analyses reported here. Most of the removed samples had only small amounts of missing data. A relatively small investment in these samples could increase sample size considerably.

The inclusion of missing data would allow better analyses of variation within and among populations in Colorado. Measures of genetic variation based on AFLP data were estimated (Tables 3 and 4), but results were difficult to interpret due to the small number of animals examined in each population. Recent declines have left only three self-sustaining populations and a number of isolated smaller populations distributed across the mountainous central portions of the state. Estimates of the level of genetic divergence within and among the remaining populations could assist conservation efforts for the remaining toads.

Analyses of animals from the geographically close regions in western Wyoming and Utah are critical. MtDNA analyses identified some samples in northern Utah that were very closely related to toads in Colorado. A large number of samples should be analyzed in order to determine if this is a rare occurrence (and possible due to the retention of "old" haplotypes in Utah from lineage sorting) or whether toads in Utah are within the same management unit as toads in Colorado. Nuclear data is also need from the same samples.

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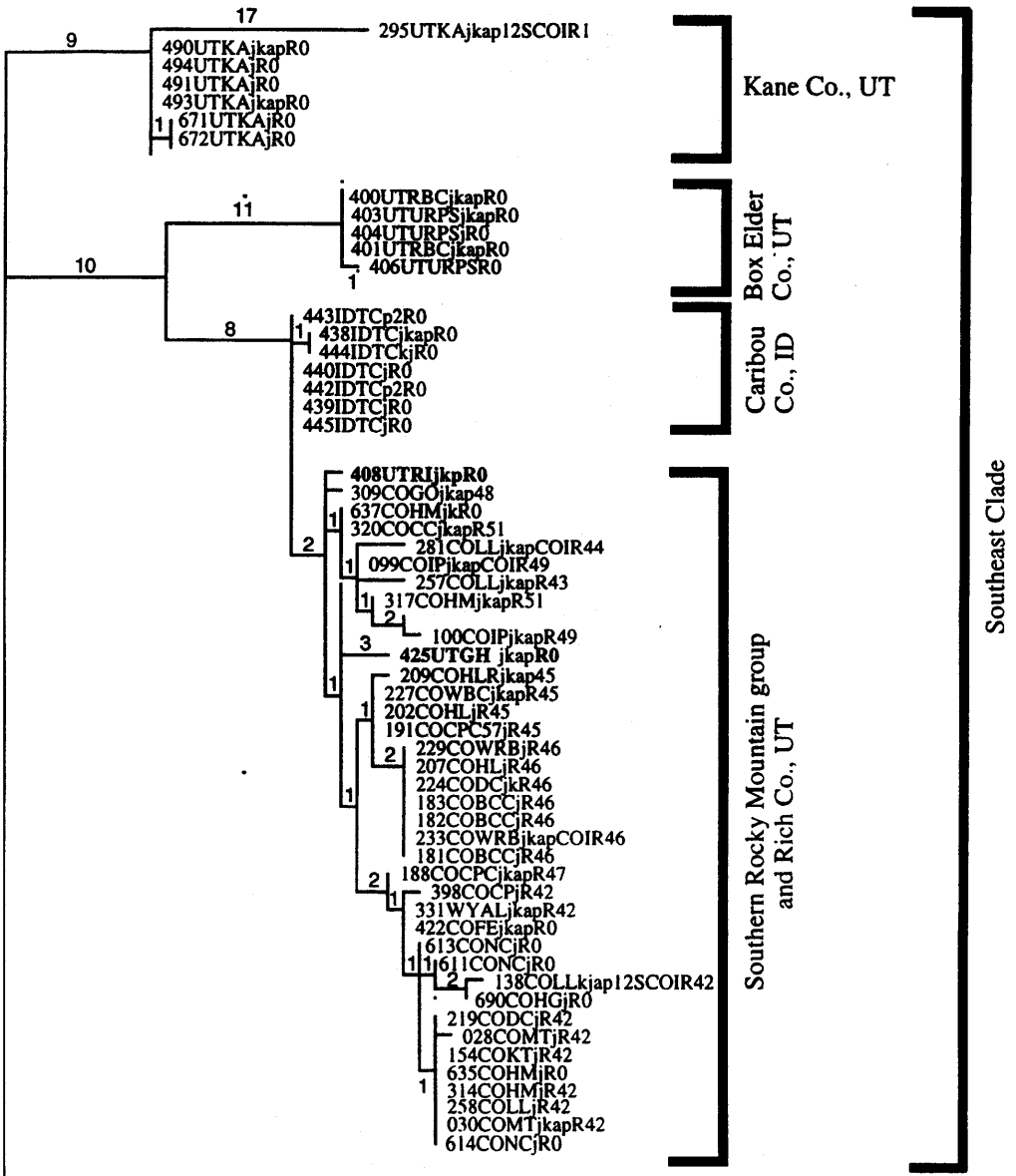
Figure 1. One of the most parsimonious trees (length 395), based on all available mtDNA data. Tree is unrooted. Branches are shown proportional to their length. Numbers above branches are branch lengths. Samples are identified first by their accession number, then by two letter abbreviations representing locality (US State, then locality) and finally by abbreviations representing the data available for each sample. State abbreviations are standard, locality abbreviations are as in Table 1. Data include control region sequence which is identified by four primers j,k,a,p. All four primers indicate complete bidirectional sequence; jk, aj, or pk all indicate complete unidirectional sequence; j, p, or p2 indicate partial unidirectional sequence. The complete12srDNA sequence is indicated by 12S, 390 bp of cytochrome oxidase I by COI, and restriction site data by the letter R followed by a number. R1-R50 represent haplotype number based on restriction sites only, R0 indicates no restriction site data. Note that the presence of varying amounts of data skews some terminal branchlengths. For example, the first sample (295) has a very long branch length due to a large amount of data (12S rDNA and COI sequence data as well as restriction site data) not present in any other sample in this clade. Samples discussed are in bold.



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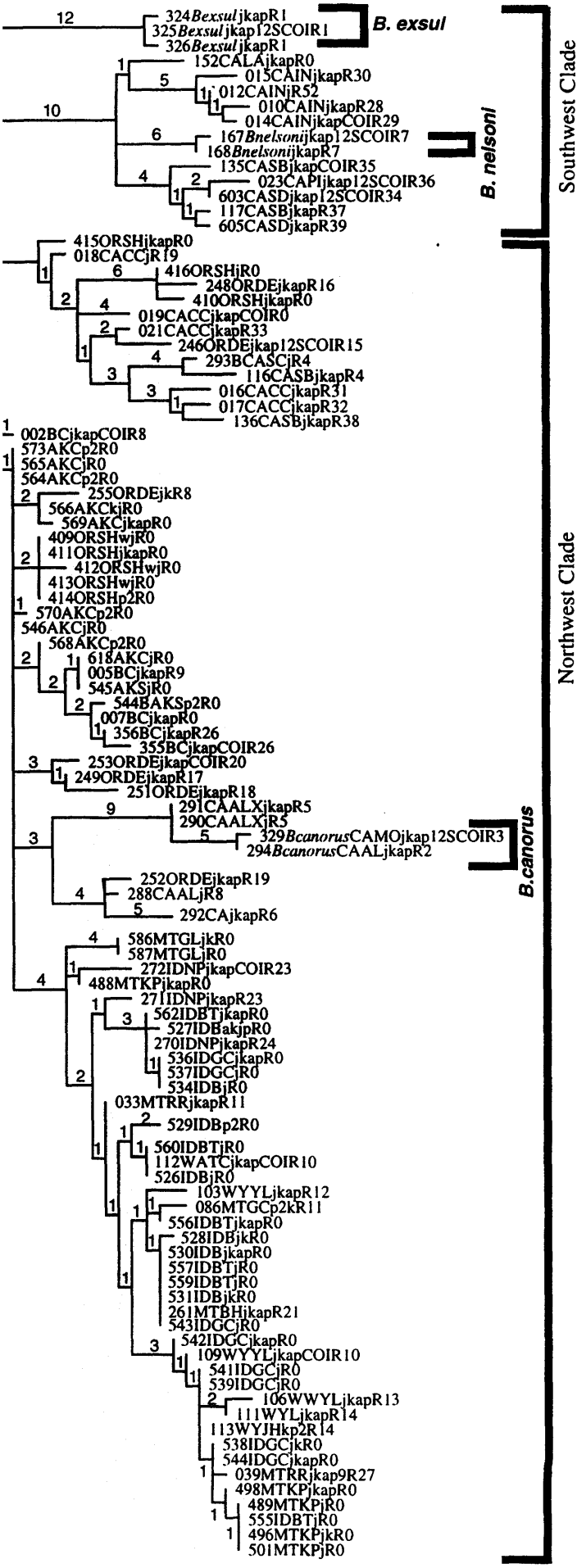
324Bexsuljkap R1
325Bexsuljkap12SCOIR1
326Bexsuljkap R1
B. exsul
152CALAjkapR0
015CAINjkapR30
012CAINjR52
010CAINjkapR28
014CAINjkapCOIR29
167Bnelsonijkap12SCOIR7
168Bnelsonijkap R7
B. nelsoni
135CASBjkapCOIR35
023CAPIjkap12SCOIR36
603CASDjkap12SCOIR34
117CASBjkapR37
605CASDjkapR39
Southwest Clade
415ORSHjkapR0
018CACCjR19
416ORSHjR0
248ORDEjkapR16
410ORSHjkapR0
019CACCjkapCOIR0
021CACCjkapR33
246ORDEjkap12SCOIR15
293BCASCjR4
116CASBjkapR4
016CACCjkapR31
017CACCjkapR32
136CASBjkapR38
002BCjkapCOIR8
573AKCp2R0
565AKCjR0
564AKCp2R0
255ORDEjkR8
566AKCkjR0
569AKCjkapR0
409ORSHwjR0
411ORSHjkapR0
412ORSHwjR0
413ORSHwjR0
414ORSHp2R0
570AKCp2R0
546AKCjR0
568AKCp2R0
618AKCjR0
005BCjkapR9
545AKSjR0
544BAKSp2R0
007BCjkapR0
356BCjkapR26
355BCjkapCOIR26
253ORDEjkapCOIR20
249ORDEjkapR17
251ORDEjkapR18
291CAALXjkapR5
290CAALXjR5
329BcanorusCAMOjkap12SCOIR3
294BcanorusCAALjkapR2
B.canorus
252ORDEjkapR19
288CAALjR8
292CAjkapR6
586MTGLjkR0
587MTGLjR0
272IDNPjkapCOIR23
488MTKPjkapR0
271IDNPjkapR23
562IDBTjkapR0
527IDBakjpR0
270IDNPjkapR24
536IDGCjkapR0
537IDGCjR0
534IDBjR0
033MTRRjkapR11
529IDBp2R0
560IDBTjR0
112WATCjkapCOIR10
526IDBjR0
103WYYLjkapR12
086MTGCp2kR11
556IDBTjkapR0
528IDBjkR0
530IDBjkapR0
557IDBTjR0
559IDBTjR0
531IDBjkR0
261MTBHjkapR21
543IDGCjR0
542IDGCjkapR0
109WYYLjkapCOIR10
541IDGCjR0
539IDGCjR0
106WWYLjkapR13
111WYLjkapR14
113WYJHkp2R14
538IDGCjkR0
544IDGCjkapR0
039MTRRjkap9R27
498MTKPjkapR0
489MTKPjR0
555IDBTjR0
496MTKPjkR0
501MTKPjR0
Northwest Clade

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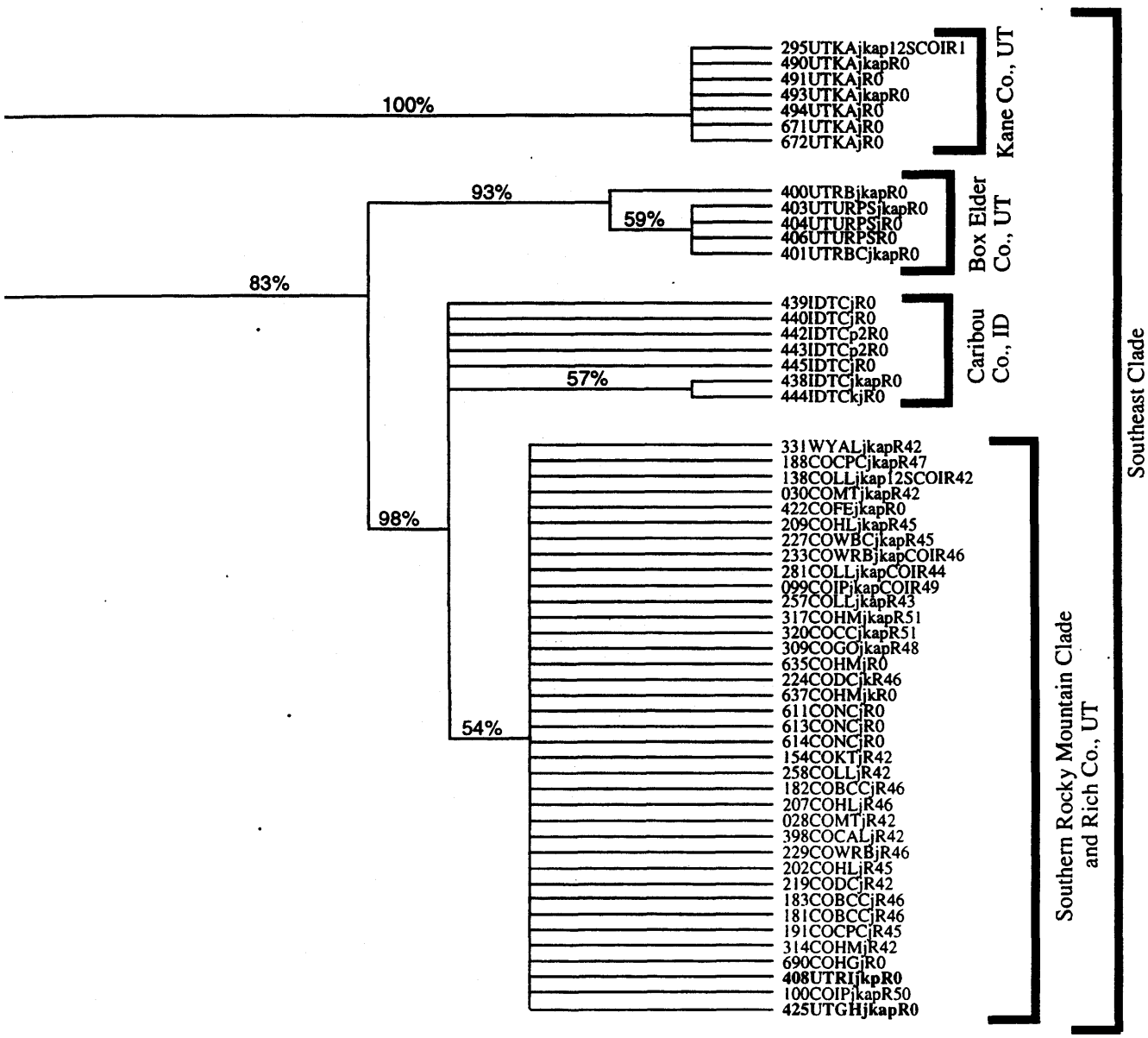
Figure 2. Strict consensus tree of all most parsimonius trees (length 392) based on all available mtDNA data. Tree is unrooted but root probably exists between the four major lineages. Samples and data are identified as in Figure 1. Bootstrap values are below the branches. Values below 50% are omitted, values above 90% generally provide strong support.



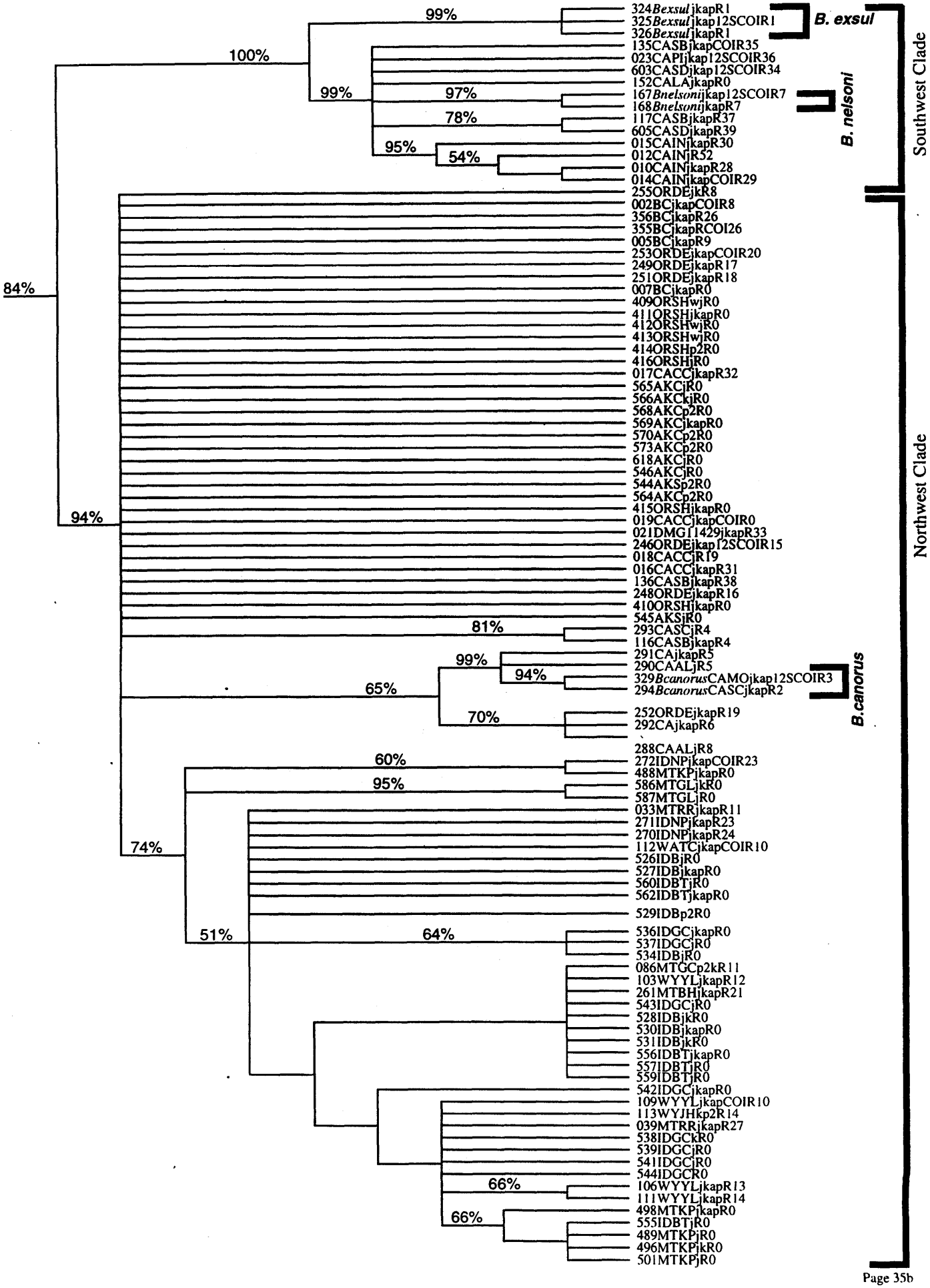

99%
324Bexsuljkap R1
325Bexsuljkap12SCOIR1
326Bexsuljkap R1
B. exsul
100%
135CASBjkapCOIR35
023CAPljkap12SCOIR36
603CASDjkap12SCOIR34
152CALAjkapR0
99%
97%
167Bnelsonijkap12SCOIR7
168Bnelsonijkap R7
B. nelsoni
78%
117CASBjkapR37
605CASDjkapR39
95%
015CAINjkapR30
54%
012CAINjR52
010CAINjkapR28
014CAINjkapCOIR29
Southwest Clade
255ORDEjkR8
002BCjkapCOIR8
356BCjkapR26
355BCjkapRCOI26
005BCjkapR9
253ORDEjkapCOIR20
249ORDEjkapR17
251ORDEjkapR18
007BCjkapR0
84%
409ORSHwjR0
411ORSHjkapR0
412ORSHwjR0
413ORSHwjR0
414ORSHp2R0
416ORSHjR0
017CACCjkapR32
565AKCjR0
566AKCkjR0
568AKCp2R0
569AKCjkapR0
570AKCp2R0
573AKCp2R0
618AKCjR0
546AKCjR0
544AKSp2R0
564AKCp2R0
94%
415ORSHjkapR0
019CACCjkapCOIR0
021DMGl1429jkapR33
246ORDEjkap12SCOIR15
018CACCjR19
016CACCjkapR31
136CASBjkapR38
248ORDEjkapR16
410ORSHjkapR0
545AKSjR0
81%
293CASCjR4
116CASBjkapR4
99%
291CAjkapR5
290CAALjR5
94%
329BcanorusCAMOjkap12SCOIR3
294BcanorusCASCjkapR2
B.canorus
65%
70%
252ORDEjkapR19
292CAjkapR6
Northwest Clade
288CAALjR8
60%
272IDNPjkapCOIR23
488MTKPjkapR0
95%
586MTGLjkR0
587MTGLjR0
033MTRRjkapR11
271IDNPjkapR23
270IDNPjkapR24
74%
112WATCjkapCOIR10
526IDBjR0
527IDBjkapR0
560IDBTjR0
562IDBTjkapR0
529IDBp2R0
51%
64%
536IDGCjkapR0
537IDGCjR0
534IDBjR0
086MTGCp2kR11
103WYYLjkapR12
261MTBHjkapR21
543IDGCjR0
528IDBjkR0
530IDBjkapR0
531IDBjkR0
556IDBTjkapR0
557IDBTjR0
559IDBTjR0
542IDGCjkapR0
109WYYLjkapCOIR10
113WYJHkp2R14
039MTRRjkapR27
538IDGCkR0
539IDGCjR0
541IDGCjR0
544IDGCR0
66%
106WYYLjkapR13
111WYYLjkapR14
66%
498MTKPjkapR0
555IDBTjR0
489MTKPjR0
496MTKPjkR0
501MTKPjR0

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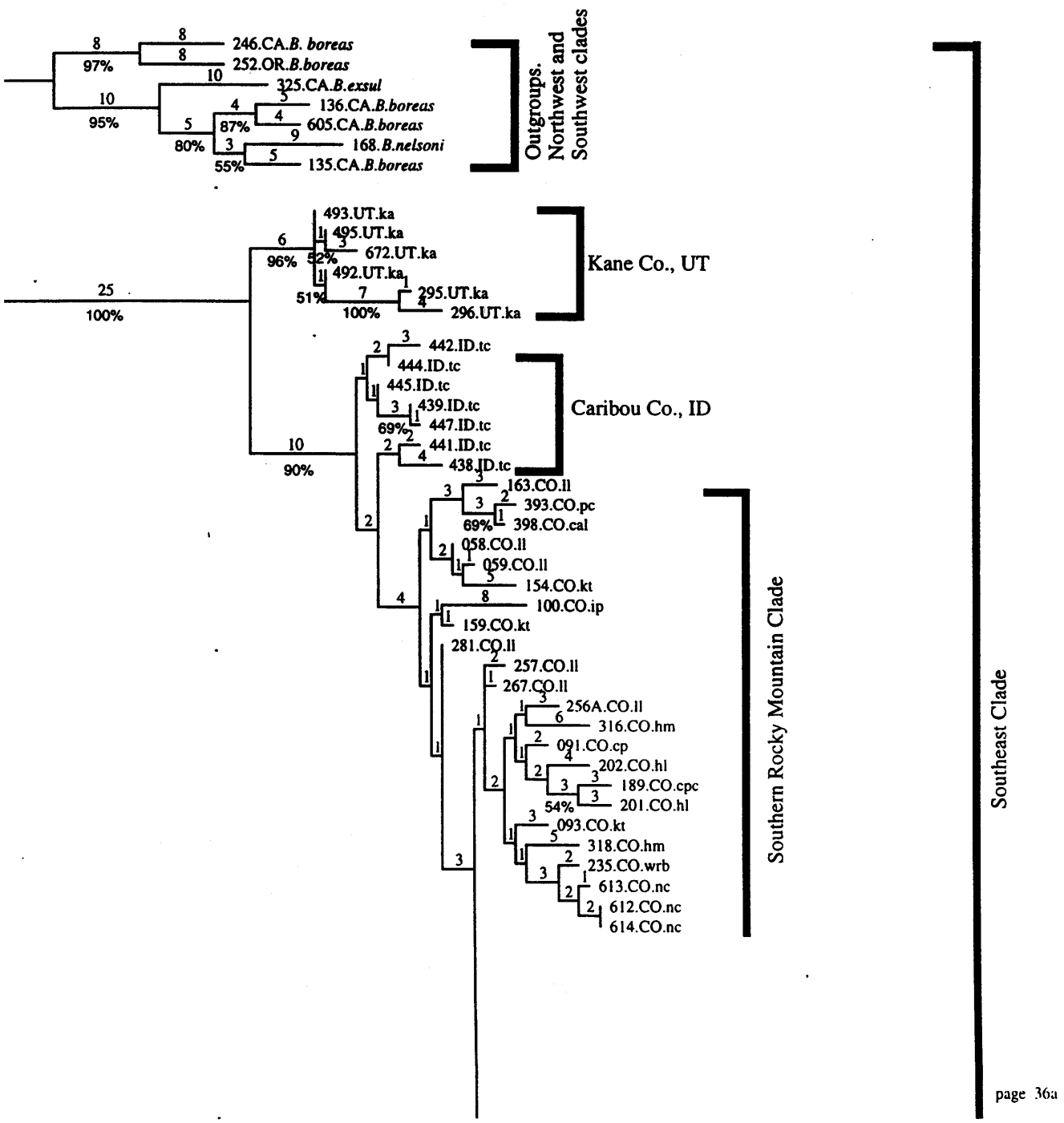
Figure 3. Single most parsimonious tree (length =528) based on nuclear AFLP restriction site data. Branches are proportional to their length. Numbers above the branches are branchlengths. Numbers below branches are bootstrap values (bootstrap values below 50% have been omitted; bootstrap values above 90% are considered to be strongly supported). Samples are identified by accession number, followed by state and county (or locality) abbreviations (Table 1).



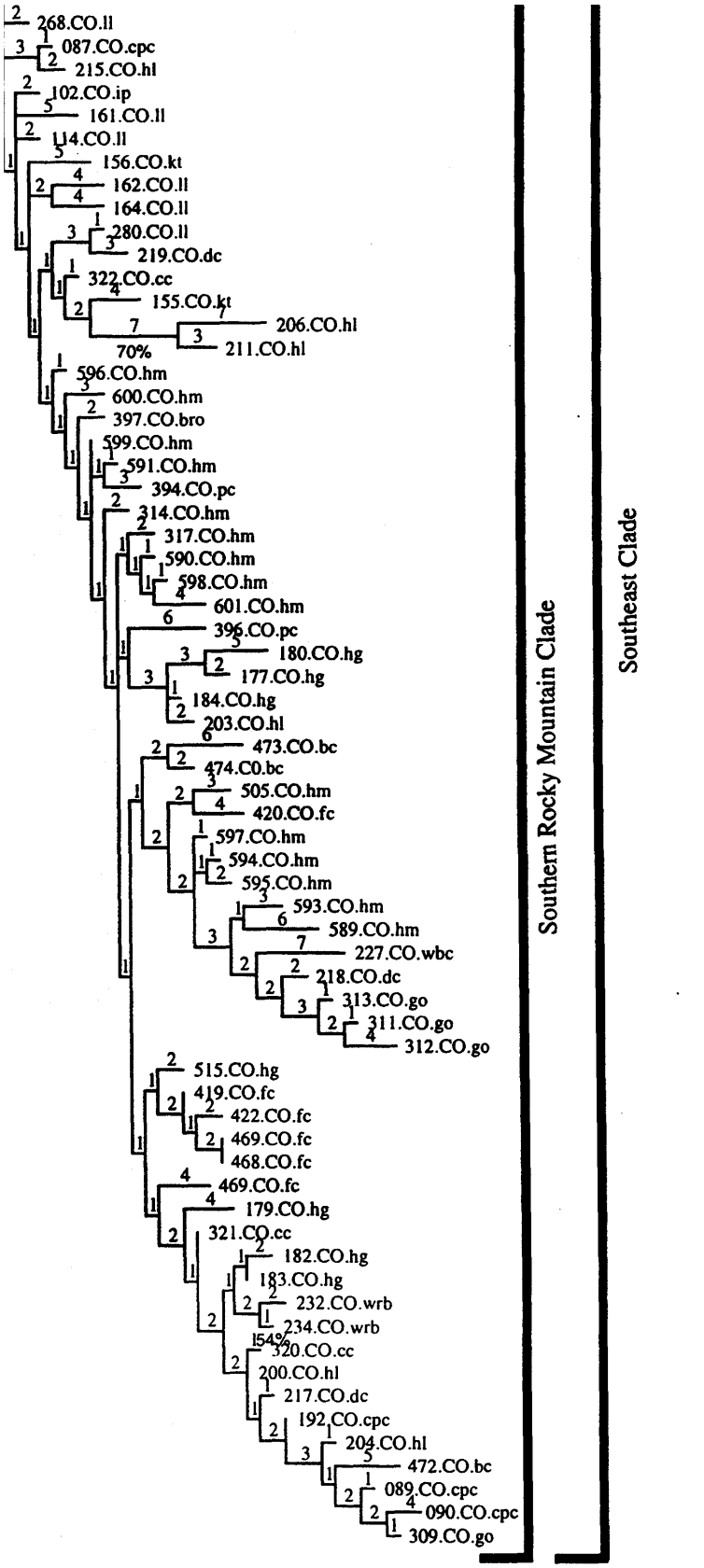
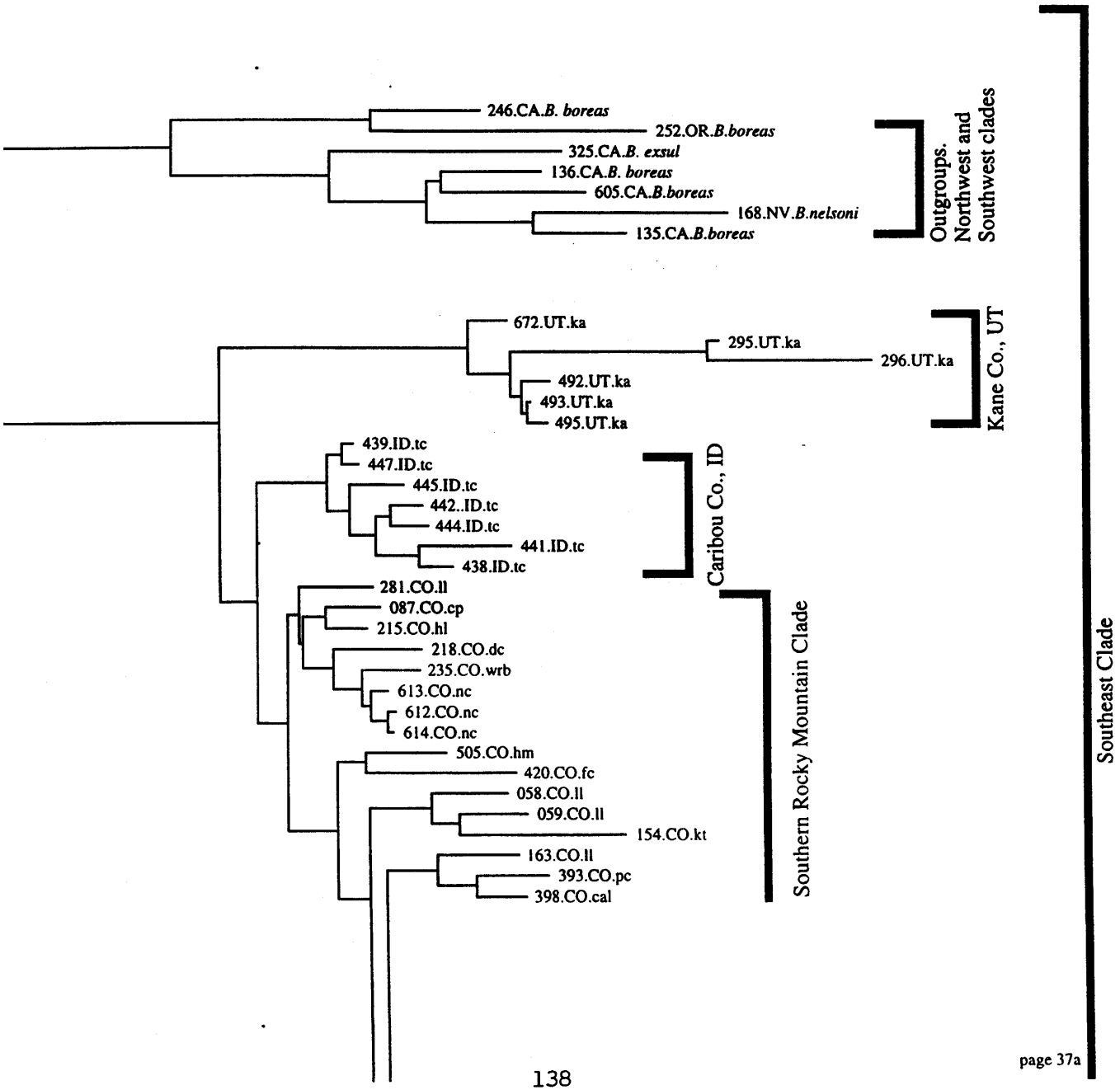
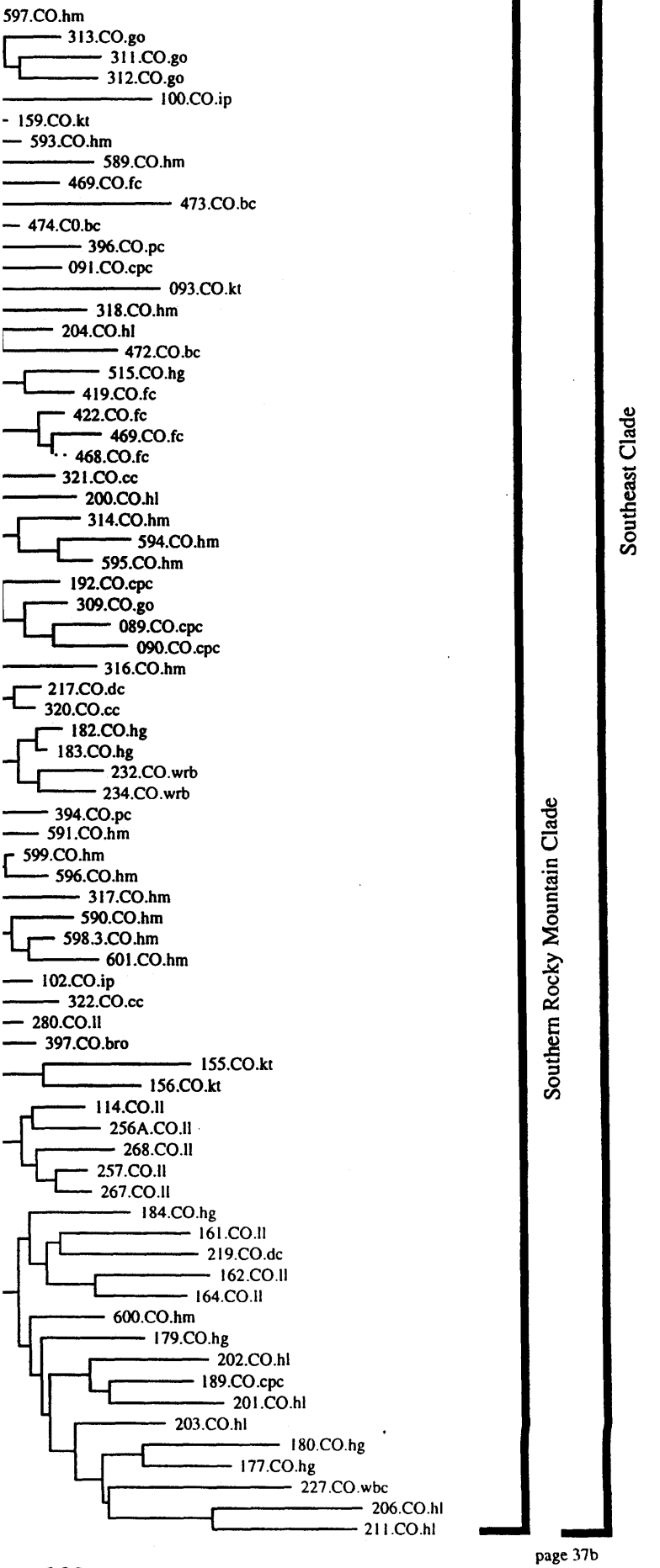

268.CO.ll
087.CO.cpc
215.CO.hl
102.CO.ip
161.CO.ll
114.CO.ll
156.CO.kt
162.CO.ll
164.CO.ll
280.CO.ll
219.CO.dc
322.CO.cc
155.CO.kt
206.CO.hl
70%
211.CO.hl
596.CO.hm
600.CO.hm
397.CO.bro
599.CO.hm
591.CO.hm
394.CO.pc
314.CO.hm
317.CO.hm
590.CO.hm
598.CO.hm
601.CO.hm
396.CO.pc
180.CO.hg
177.CO.hg
184.CO.hg
203.CO.hl
473.CO.bc
474.CO.bc
505.CO.hm
420.CO.fc
597.CO.hm
594.CO.hm
595.CO.hm
593.CO.hm
589.CO.hm
227.CO.wbc
218.CO.dc
313.CO.go
311.CO.go
312.CO.go
515.CO.hg
419.CO.fc
422.CO.fc
469.CO.fc
468.CO.fc
469.CO.fc
179.CO.hg
321.CO.cc
182.CO.hg
183.CO.hg
232.CO.wrb
234.CO.wrb
154%
320.CO.cc
200.CO.hl
217.CO.dc
192.CO.cpc
204.CO.hl
472.CO.bc
089.CO.cpc
090.CO.cpc
309.CO.go
Southern Rocky Mountain Clade
Southeast Clade

Figure 4. Neighbor-Joining tree of nuclear AFLP data from the *B. boreas* species group. Samples are identified by accession number, followed by two two letter abbreviations representing state and county. Clades are identified by bars to the right of the tree. Distances are Nei and Li (1979) distances for restriction site data.




597.CO.hm
313.CO.go
311.CO.go
312.CO.go
100.CO.ip
159.CO.kt
593.CO.hm
589.CO.hm
469.CO.fc
473.CO.bc
474.C0.bc
396.CO.pc
091.CO.cpc
093.CO.kt
318.CO.hm
204.CO.hl
472.CO.bc
515.CO.hg
419.CO.fc
422.CO.fc
469.CO.fc
468.CO.fc
321.CO.cc
200.CO.hl
314.CO.hm
594.CO.hm
595.CO.hm
192.CO.cpc
309.CO.go
089.CO.cpc
090.CO.cpc
316.CO.hm
217.CO.dc
320.CO.cc
182.CO.hg
183.CO.hg
232.CO.wrb
234.CO.wrb
394.CO.pc
591.CO.hm
599.CO.hm
596.CO.hm
317.CO.hm
590.CO.hm
598.3.CO.hm
601.CO.hm
102.CO.ip
322.CO.cc
280.CO.ll
397.CO.bro
155.CO.kt
156.CO.kt
114.CO.ll
256A.CO.ll
268.CO.ll
257.CO.ll
267.CO.ll
184.CO.hg
161.CO.ll
219.CO.dc
162.CO.ll
164.CO.ll
600.CO.hm
179.CO.hg
202.CO.hl
189.CO.cpc
201.CO.hl
203.CO.hl
180.CO.hg
177.CO.hg
227.CO.wbc
206.CO.hl
211.CO.hl
Southern Rocky Mountain Clade
Southeast Clade

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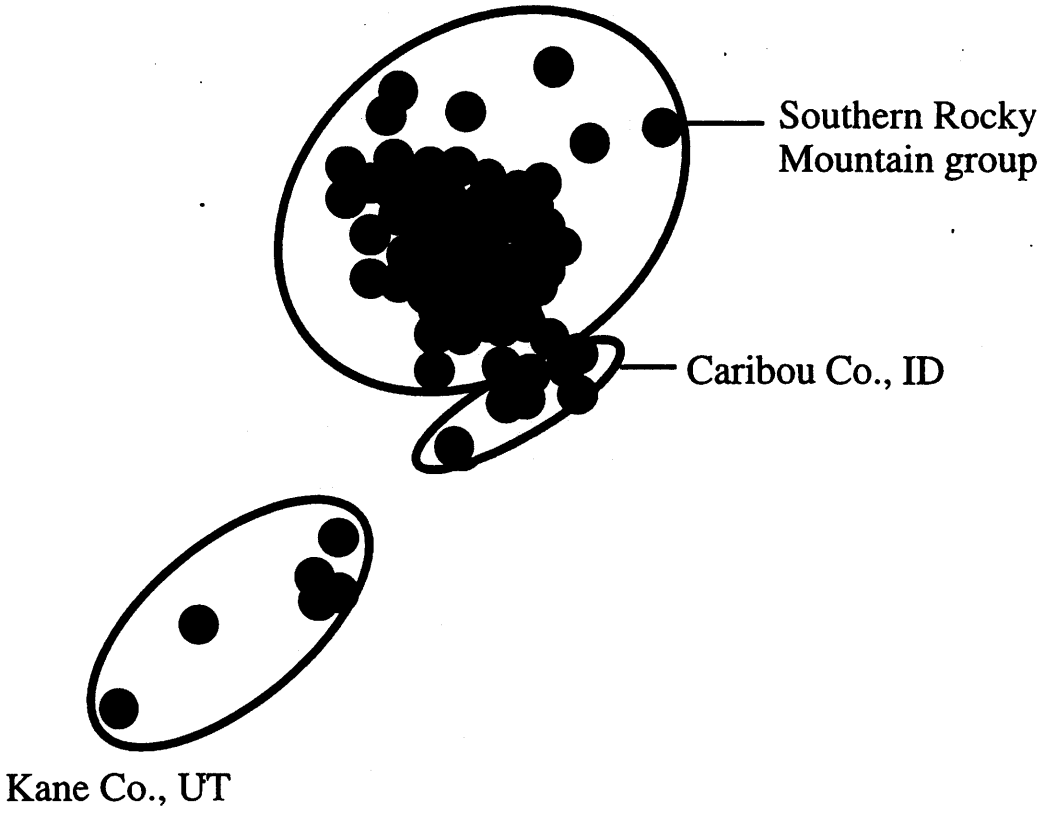

Figure 5. Principal coordinates analysis (PCO) of AFLP restriction site data. The X and Y axis represent the first two principal components which account for 75% of the variation. Circles encompass the three groups identified with both mitochondrial and nuclear DNA phylogenetic analyses.

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APPENDIX 1.

Specimen capture localities for most specimens are in Goebel (1996). Below are localities for additional specimens, or localities that were missing data or found to be in error in Goebel (1996).

AMG293: *B. canorus.* Santa Clara Co., CA. South peculation pond in HWY 17 cahin. 14 June 1993. Collector: D. L. Martin. **AMG294**: *B. canorus.* Alpine Co., CA, Stanislaus N.F., Tryon Mdw. T8N R2OE Sec 32. 25 June 1993. Collector: D. L. Martin. **AMG291:** Alpine Co., CA, Stanislaus N.F., Wheeler K. T8N R18E Sec 27. 7 July 1993. Collector: D. L. Martin. **AMG290:** *B. boreas x B. canorus.* Alpine Co., CA, Stanislaus N.F., Wheeler K. T8N R18E Sec 27. 7 July 1993. Collector: D. L. Martin. AMG25: *B. boreas*, Columbia Co., WA, N. Fork Touchet R., 3.5 mi S. Umatilla N.F. boundary. 18 August 1991. Collector: M. J. Adams. **AMG400**: *B. boreas*, Red Butte Conyon, Grouse Creek Mtns, Box Elder Co., UT, 262503N. 6 June 96, Collector: D. A. Ross. **AMG425**: *B. boreas*, Gold Hill, Military Creek, Bear River Drainage, Summit Co., UT, T1NR9ES23, NE1/4 Lat4517745 LON509403. Metamorph, 7 Sept 96, Collector: D. A. Ross. **AMG438**: *B. boreas*, Tincup creek, about 500M W. of Pine Bar Camp, Caribou Co., ID, T55 R45E SE NW NW20. Metamorph, 1 Sept. 1996, Collector: S. Burton. **AMG422:** *B. boreas*, Fern/Troutcreek Mineral Co., CO. Adult, 6 June 1996, collector: John Goettl. Photo ID, animal left in field. **AMG408**: *B. boreas*, Stock pond, Walton Gulch, Curtis Ridge, Rich Co., UT. 13 June 1996, collector: D. A. Ross. **AMG410:** *B. boreas*, Mt. St. Helens, collector: P. S. Corn. **AMG490-5**: *B. boreas*, Kane Co., UT, 6 August 1997, collector: P. S. Corn. **AMG488-9, 496-501:***B. boreas*, Kramis Pond (next to campground at Como Lake), Ravalli Co., MT, 46 04'03.93"N, 114 14'39.84"W, 18 May 1998, collector: P. S. Corn. **AMG504-508**: egg tissue, Henderson Mine, Hesbo, CO., 23 June 1997, collector: Lauren Livo. **AMG509-518:** egg tissue, Herman Gulch, Bethel Campground, CO., Collected 22-28 May 97, collector: Lauren Livo. **AMG519:** egg tissue, Mt. Bethyl, Clear Creek, Clear Creek Co., CO, 23 June 1997, collector:Lauren Livo. **AMG 521-2** egg tissue, Jumper Creek, 3 June 1997, collector: Craig Fetkavich. **AMG 523-4** tadpoles, Jumper Creek, 3 June 1997, collector: Craig Fetkavich. **AMG525**:juvenile found dead in the field. Brown Creek, Chaffee Co., Co., 22 May, 1997, collector Craig Fetkavich. **AMG526-535**: newly metamorphosed toadlets, Missouri Mine, Boise Co., ID UTM N 4872111.156 E 591873.692, July 1997, collector: Ed Wessman. **AMG536-544:** newly metamorphosed toadlets, Grouse Creek, Washington Co., ID T.12N, R.7W, SW1/4SE1/4 section 8, July 1997, collector: Ed Wessman. **AMG554-563:** newly metamorphosed toadlets, south end of Bull Trout Lake, Boise Co., ID T.11N, R.10E, SW1/4SE1/4 section 10, October 1997, collector: Ed Wessman. **AMG544-544**: adults, Stikine River, Alaska, June 1997, collector: Keith Pahlke. **AMG546-7:** newly metamorphosed toadlets, Chickamon River, Alaska, June 1997, collector: Keith Pahlke. **AMG564-75**: newly metamorphosed toadlets, Chickamon River, Alaska, August 1997, collector: Keith Pahlke. **AMG548-551**: newly metamorphosed toadlets, Rock Springs, Rock Creek, Thousand Springs Creek, Bonneville Basin, Elko Co., NV, T44N,R67E, s. 4 SE1/4 SE1/4, 11 August 1997, collector: Peter Hovingh. **AMG586-588**: Logan Pass, Glacier National Park, MT, UTM zone 12, 300260E 5395277N, 23 July 98, collectors: P. S. Corn and S. K. Meegan. **AMG589-601:** Blood tissue, Henderson Mine, Clear Creek Co., CO, collector: Mark Jones. **AMG 610**: adult found dead in field, Triangle Pass, Gunnison Co., CO, UTM E332700 N 4318180, collector: Andy Holland. **AMG611-614**: blood tissue, North Chaffee Co., CO, 18 July 1998, collectors: Anna Goebel and John Wortman. **AMG635-41**, animals found dead in the field, Henderson Mine, Clear Creek Co., CO, May-July, 1998, collector: Mark Jones.

Modeling Boreal Toad (*Bufo boreas*) Breeding Habitat

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INTRODUCTION

Despite its existence in relatively pristine environments, the distribution and abundance of the southern Rocky Mountain population of boreal toad (Bufo boreas boreas) has declined (Corn et al. 1989, Carey 1993, Loeffler 1998). The fungus *Batrachochytrium dendrobatidis* (Longcore and Pessier 1999) has been identified as a mortality factor for boreal toads in Colorado. These issues have prompted the Boreal Toad Recovery Team to decide to bring individuals from several evolutionarilly significant units into captivity (M. S. Jones, Colo. Div. of Wildl., pers. commun.). The eventual reestablishment of populations and translocation of captively reared toads into new or historic sites requires knowledge of boreal toad breeding habitat relationships. This project was developed to address this issue as outlined in sections 1.1, 3.5, and 5.0 of the Boreal Toad Conservation Plan and Agreement (Loeffler 1998). Quantifying habitat suitability is important because translocation into historic areas is not necessarily the best procedure because breeding habitat changes over time. This practice would also be unwise if *Chytrid* fungus still remains at these sites (M. S. Jones, Colo. Div. of Wildl., pers. commun.). Knowledge of exactly what constitutes suitable habitat is also required when identifying and protecting boreal toad breeding sites from human activity and encroachment.

The availability of suitable breeding sites is a highly influential factor on boreal toad distribution and abundance. There are undoubtedly many areas with suitable terrestrial habitat that do not support populations because they lack a suitable breeding site. The objectives of this study are to evaluate boreal toad breeding habitat requirements and use habitat-based modeling to identify and predict suitable boreal toad breeding habitat. These models will focus on the characteristics that make breeding sites successful at attracting breeding adults and producing metamorphosed individuals.

Breeding takes place in the margins of ponds and lakes. Ideal boreal toad breeding sites have very shallow margins, stable water levels, and warm water temperatures. Females deposit one egg mass averaging 6,661 ± 294 eggs (Carey et al. in press) every 2 to possibly 6 years (M. S. Jones, Colo. Div. of Wildl., pers. commun.). Tadpole development is density dependent (Wilbur 1987, Berven 1990) and directly related to water temperature (Herreid and Kinney 1967). Successful metamorphosis depends on warm water temperatures and the persistence of the breeding site (Wilbur 1987).

The presence and amount of shallows are often used to evaluate amphibian breeding habitats. Loman (1988) found that common frog, *Rana temporaria*, breeding ponds were best predicted by the amount of shallows they contained. Similarly, gradually sloping pond banks are a significant factor in predicting use by naterjack toads, *Bufo calamita* (Banks and Beebee 1987). Shallows that reach warm temperatures are especially important for the boreal toad because of the cold water temperatures and short growing season at high elevation. Boreal toads in Colorado prefer water depths of 5 to 10 cm for egg deposition.

Water level stability during egg development is critical for successful development. Dessication from receding water levels is a common cause of boreal toad egg mortality (Livo 1999). Rising water levels from spring snow melt and American beaver activity (*Castor canadensis*) can also decrease hatching success. Greater depth reduces water temperature (Pearman 1995) which in turn increases egg mortality (Herreid and Kinney 1967).

Since egg and larval development are directly proportional to water temperature (Herreid and Kinney 1967), the temperature regime of breeding ponds is highly influential on the success or failure of the eggs deposited in them. Choice of breeding sites, then, has a strong influence on offspring survival (Barandun and Reyer 1997). Many studies have examined water temperature to explain amphibian breeding site selection. Banks and Beebee (1987) found that ponds used for breeding by the natterjack toad were 5° C warmer than unused ponds. Yellow-bellied toads, *Bombina variegata*, selected the warmest parts of the warmest ponds to deposit their eggs (Barandun and Reyer 1997). Likewise, occupied pool frog breeding sites had higher water temperatures than unoccupied ponds (Sjogren-Gulve 1994).

It is not known how pond size contributes to breeding site suitability for boreal toads. Most boreal toad breeding occurs in active or abandoned American beaver ponds that are small to medium in size. However, even large glacial tarns and high mountain lakes are suitable breeding sites if they have shallow margins that elevate water temperatures. The size and suitability of ovipositioning areas may be more attractive to amphibians than actual pond area (Reading et. al 1991). Pond characteristics affect water temperature regimes. Larger ponds are generally cooler and more stable with respect to water temperature (Caldwell 1985). Various sizes of ponds may ultimately result in similar numbers of degree days, thereby creating similar larval growing seasons. Degree days is a measure of the aquatic growing season calculated by summing daily mean temperatures above 0°C, or above a developmental threshold for a species (Allan 1995). Degree days can be used as a composite variable for water temperature and persistence of a breeding site. Small ponds reach higher maximum daily temperatures, but larger ponds could store enough heat at night to allow continued tadpole growth. Other factors controlling water temperature include: solar radiation received by the pond, mixing by wind, turnover rate, and the temperature of in flowing streams.

Pond size has been well studied for other amphibians as a variable in habitat-based models. Natterjack toad use was correlated with pond surface area at one site and not at another (Banks and Beebee 1987). Seale (1982) found the number of wood frog metamorphs and egg masses increased with pond surface area. Chorus frog survival, mass at metamorphosis, and length of larval period also respond strongly to pond area (Pearman 1995). Larger ponds provide a more stable and persistent environment for larval development.

Surprisingly, pond duration is not always beneficial to amphibians. Most amphibians balance tradeoffs between predation and pond duration when selecting breeding sites. At one extreme, predators are small and scarce, but pond drying is likely to occur before larval development is complete. At the opposite extreme, ponds are more persistent, but predators are more numerous and larger (Skelly 1996). Predaceous insect larvae are a significant mortality factor for boreal toad larvae (Livo 1999). Larger more stable ponds may benefit boreal toad tadpoles in that they are not only more persistent but are more likely to sustain trout populations. Trout do not prey on boreal toad tadpoles but they do consume predaceous insect larvae.

Breeding site choice may not be solely based on physical factors. Boreal toads often select one breeding pond out of several adjacent and physically similar ponds. Only using some of the available ponds seems counter intuitive in light of the fact that larval development is density dependent. This may be explained by the fact that many amphibians exhibit high site fidelity to breeding sites. Toads reproducing for the first time may simply return to their natal pond. Berven and Grudzien (1990) found

adult wood frogs to be 100% faithful to the breeding site in which they first bred and documented that only 18% of juveniles dispersed to breed in ponds other than their natal pond. At two different sites, 96% and 89% of common toad (*Bufo bufo*) males were faithful to a breeding site while 93% and 79% of females exhibited site fidelity (Reading et al. 1991). Eighty-three percent of these males and 100% of females returned to their natal pond to breed. Ninety-four percent of male boreal toads return to the same breeding site every year in Clear Creek County, Colorado (M. S. Jones, Colo. Div. of Wildl., unpubl. data). Possible explanations for the adaption of communal egg deposition are breeding behavior, predator swamping, egg and tadpole modification of water temperatures, and larval densities in the wild that are not severe enough to cause density dependence.

Alternatively, in some areas boreal toads may use many of the available ponds in an area. New ponds are also quickly colonized by adult boreal toads (M. S. Jones, Colo. Div. of Wildl., pers. commun.). By using traditional or natal ponds unless another suitable site is encountered first boreal toads may exhibit more flexibility in their selection of breeding sites than some other amphibians.

Many amphibians show plasticity in the timing of metamorphosis and their size at metamorphosis. Tadpoles respond to decreasing per capita food level by metamorphosing earlier and at a smaller size (Newman 1994). The date and size at metamorphosis can in turn influence the age at maturity (Smith 1987). Berven (1990) found juvenile wood frog survival to be positively correlated with size at metamorphosis. Larger metamorphs were also larger throughout their life. Metamorphic size can be indicative of both larval conditions, related to density, and adult fitness.

Successful metamorphosis and size at metamorphosis are important indicators of the quality of the breeding pond. Even though adults are fairly faithful to a breeding site, dispersal and breeding in other ponds does occur. These "experimental" egg masses may or may not successfully rear metamorphs. Adult red-spotted newts, *Notophthalmus viridescens*, bred in many ponds, but for 3 years only a single pond recruited and was responsible for all adults (Gill 1978).

Several researchers have modeled an amphibian species' presence or absence in relation to environmental variables (Sjogren-Gulve 1994, Corn et al. 1997, Demaynadier and Hunter 1998, Munger et al. 1998, Vos and Chardon 1998). Still others have related density, reproduction, or survival with environmental variables (Wilbur 1987, Berven 1990, Bury et al. 1991, Block and Morrison 1998, Vos and Chardon 1998). Several studies have used logistic regression to examine the effects of habitat variables on the presence of amphibians. Munger et al. (1998) found habitat-based models using logistic regression to be relatively successful at predicting Columbia spotted frog (*Rana luteiventris*) and Pacific treefrog (*Hyla regilla*) presence. In addition to using logistic regression to evaluate presence-absence, Vos and Chardon (1998) conducted linear regression, using the number of egg masses as the response variable.

The objectives of modeling these relationships are to formalize current understanding, learn which environmental factors affect distribution and abundance of a species, generate hypotheses, and ultimately make predictions (Morrison et al. 1992). The purpose of this study is to identify habitat variables that can be used to predict selection of a pond by breeding adults and the estimate the probability of metamorphosis. While many variables are measured, the majority of the *a priori* models consist of parameters for degree days, bank slope, and water level stability. An underlying hypothesis is

that a model based largely on habitat variables can be successful at predicting probability of metamorphosis and ultimately identifying breeding habitat requirements for boreal toads.

METHODS

Work completed

In 1999, 22 breeding sites and 14 nonbreeding sites were studied. Breeding sites were selected to ensure that the entire gradient of number of egg masses and elevations were represented in the samples. Nonbreeding sites were picked by first assigning all suitable nonbreeding sites, within the same breeding location as a breeding site, a number and randomly selecting one pond. In most cases study sites were visited at least monthly.

These study sites were located in Chaffee County, Summit County, Clear Creek County, and Larimer County. The Chaffee County breeding locations evaluated in 1999 were Collegiate Peaks, Denny Creek, Hartenstein Lake, South Cottonwood Creek, South Cottonwood Creek West, Morgan's Gulch, Brown's Creek, Four Mile Creek, and Sayer's Gulch. I included Peru creek, Cucumber Gulch, and Lower North Tenmile Creek in Summit County. In addition to many sites associated with the Henderson Mine, Mt. Bethel and Herman Gulch were sampled in Clear Creek County. Only Lost Lake and Kettle Tarn were visited in Larimer County.

Breeding and nonbreeding ponds were determined by the presence or absence of egg masses. Tadpole development was evaluated by developmental stage and mass at metamorphosis. Independent variables consisted of attributes such as degree days, bank slope, water level consistency, pond area, pond volume, and presence of fish.

Degree days were calculated by placing Onset Computer Corporation Optic stowaway ® temperature loggers in ponds. Egg masses are typically deposited communally in the shallowest area of the breeding pond. Temperature loggers were placed at 30 cm depth, as close to the egg masses as possible. Temperature loggers were placed in similar shallow areas in the nonbreeding ponds. Thirty cm depth was selected to avoid dessication as water levels fluctuate naturally and to attempt to capture the temperature range used by tadpoles. On sunny days tadpoles often aggregate in as little as 2 cm of water and then move to deeper water to feed and at night where deeper waters remain warmer. Loggers were programmed to record the water temperature hourly, 24 hours a day. Depths of temperature loggers were measured throughout the summer and fall to monitor water level stability and allow comparisons between and within ponds. This measurement is necessary because water temperature varies with depth.

Three temperature loggers were placed at 10cm, 30cm, and 60cm in 3 different sizes of ponds to estimate depth's influence on degree days. This is necessary to allow adjustment and comparison of degree days for ponds where the temperature loggers ended up being at different depths.

Water temperatures were recorded at egg masses or tadpole aggregations, 5 cm, 15 cm, 30 cm, 60 cm, and at maximum pond depth using a digital thermometer equipped with 10 ft probe. This will

allow comparison of temperatures recorded from loggers to temperatures experienced by tadpoles. Air temperature, weather, and time of day were also taken in conjunction with the water temperatures for comparative purposes.

A laser rangefinder was used to estimate pond surface area. A maximum length and 3 width measurements were taken. Area was estimated using the ellipse formulas of Millar (1973). Pond volume was estimated by taking 4 depth measurements along 3 transects across the width of the pond.

Bank slope was estimated by taking the depths at .30 m, 1m, and 5 m from shore on the depth transects. One depth transect was positioned to record bank slope at the ovipositioning area.

The number of egg masses and rough estimates of the number (Newman 1994) of tadpoles was also taken. These will be used in conjunction with volume estimates to estimate larval density. This information will be necessary when considering mass at metamorphosis as a variable because of the relationships that exist between larval development and density. Masses of metamorphs were estimated either by weighing 3 batches of 25 metamorphs at each site, soon after tail absorption, or by weighing individual metamorphs. The first individuals encountered at the site, that meet all the requirements, were selected for measurement.

Water samples are routinely taken at breeding sites by the Colorado Division of Wildlife (CDOW) and other agency personnel. Water samples are collected from nonbreeding sites unless they are adjacent to breeding sites and the water flows directly from one to the other.

Spring and Summer 2000

Maximum likelihood estimates of the probability of tadpoles reaching a specific Gosner (1960) stage will be estimated with multinomial likelihood methods. Logistic and multiple linear regression will be use to select best approximating models from the *a priori* model sets. Breeding in a pond and successful metamorphosis will be the dependent variables for the logistic regression models. Number of egg masses deposited in the breeding pond, Gosner (1960) stage, and mass at metamorphosis will be dependent variables for the multiple regression models. Independent variables for these models include all the previously mentioned variables such as degree days, pond area, pond volume, pond bank slope, water level stability.

A correlation analysis will be conducted using PROC CORR in SAS (SAS 1990), to potentially reduce the number of model parameters included in the models. This is especially important because sample sizes (i.e., breeding sites) are limited. The logistic regression models will then be analyzed with PROC LOGISTIC or PROC GENMOD and the linear regression models will be analyzed using PROC REG and PROC GENMOD (SAS 1989). The best approximating models will be selected from the *a priori* models using Aikaike's Information Criteria (AIC) (Burnham and Anderson 1998). Posterior modeling will also be conducted using additional variables and models found to be potentially important during the study.

In 2000, breeding sites will be randomly selected. Pond productivity and the number and proximity of other suitable breeding sites will be estimated as well. This year will provide a valuable test of predictions which will be made from the summer of 1999 data collection.

SUMMARY

Gaining a better understanding of boreal toad breeding habitat requirements will provide information that is necessary for its effective management and preservation. Models resulting from this project will allow current breeding sites, historic unoccupied sites, and new sites to be ranked according to their suitability as breeding sites for boreal toads. These models may also be useful for mitigation by determining habitat suitability of wetlands proposed for development. This research will become even more valuable if additional boreal toad populations are impacted by *Chytrid* fungus and are no longer available for habitat relationships studies in the wild.

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