# REPORT ON THE STATUS AND CONSERVATION OF THE BOREAL TOAD

Bufo boreas boreas

IN

### THE SOUTHERN ROCKY MOUNTAINS

2003



Prepared By The Boreal Toad Recovery Team Tina Jungwirth, Coordinator/Editor



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March, 2004

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### INTRODUCTION AND SUMMARY

This is the sixth in a series of reports providing a summary of boreal toad conservation work in the Southern Rocky Mountains, and a current progress report on recovery efforts for this species.

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

Research in the mid-1990s regarding the genetics of the boreal toad in the Southern Rocky Mountains has revealed that this population is a genetically unique lineage, and may warrant classification as a separate subspecies, or even a separate species, within the genus *Bufo*. Hammerson (1999) recognizes this information and suggests that *Bufo boreas* in the Southern Rocky Mountains be considered a separate species. Such recognition may lead to giving this species a higher priority in consideration for listing under the Endangered Species Act. For the purpose of this report, the names *Bufo boreas boreas*, and 'boreal toad' will continue to be used.

The boreal toad is presently listed as an endangered species by both Colorado and New Mexico, and is a protected species in Wyoming. The U.S. Fish and Wildlife Service has classified the Southern Rocky Mountain population of the boreal toad as a candidate species which is "warranted but precluded" for federal listing - meaning there is adequate justification and information to warrant federal listing as threatened or endangered, but listing has been postponed, as there are presently other species in greater need of listing, and the US Fish & Wildlife Service has limited resources to prepare and process listing packages. Pursuant to the listing of the boreal toad as endangered in Colorado, a recovery plan for the boreal toad was developed by the Colorado Division of Wildlife in 1994 (revised Jan. 1997), and an interagency recovery team was formed that same year. In 1998, the existing Recovery Plan was updated and combined with an existing draft Conservation Strategy to create a comprehensive Boreal Toad Conservation Plan for the Southern Rocky Mountains. As part of the conservation planning process, Conservation Agreements have been signed by eight involved state and federal agencies, and by the Colorado Natural Heritage Program, outlining and confirming respective roles in implementing the Conservation Plan. No new agreements were appended to the plan in 2000. A revised and updated version of the Boreal Toad Conservation Plan was completed in 2001.

For the past six years, the recovery team has worked on plans and actions to implement recovery and conservation efforts for the boreal toad. Work to date has involved several state and federal resource management agencies, personnel from universities, the Colorado Natural Heritage Program, and various other interested parties, including local land use planners and private land owners. Management activities to date have included (1) the conducting of surveys of historic and potential suitable habitats for new toad populations, (2) the annual monitoring of known breeding populations, (3) research work to identify and evaluate biotic and abiotic limiting factors to toad survival, (4) research to better define good boreal toad habitat and boreal toad biology/ecology, (5) development and testing of techniques and protocols for captive breeding and rearing of boreal toads, (6) experimental reintroductions of toads to vacant historic habitat, (7) protection of boreal toads and their habitats via coordination with land management agencies - in particular with the US Forest Service, (8) work with local land use planners and developers aimed at avoiding or minimizing potential impacts of private land development on boreal toads and their habitat, and (9) efforts to increase public awareness of this species and its plight via informational/educational activities and public involvement in searches for new populations of boreal toads.

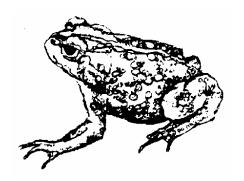
As of February 2004, the boreal toad (SRMP) is known to occur in fourteen counties (Chaffee, Clear Creek, Eagle, Grand, Gunnison, Hinsdale, Jackson, Larimer, Mesa, Mineral, Park, Pitkin, Routt, Summit) in Colorado and two counties (Albany and Carbon) in southern Wyoming. Indications are that boreal toads may also still occur in Boulder, Garfield, Gilpin, Lake, Rio Blanco, Saguache, and Conejos counties in Colorado. No confirmed reports of boreal toads have been obtained during the past two decades from Rio Arriba County, New Mexico, and it may be extirpated from that state. This is based on surveys, monitoring of breeding sites, and on confirmed or reliable observations of individual boreal toads during recent years. Breeding populations have been documented during the past six years in 13 counties in Colorado, and at three locations in Wyoming. There are presently 63 known breeding localities - some having more than one breeding site - located in nine of the eleven geographic areas, or "mountain ranges of historic occurrence". The White River Plateau, an historic area of occurrence, has no recent confirmed records of occurrence of boreal toads, although unconfirmed sightings exist. A second historic area of occurrence, the Grand Mesa, had confirmed sightings of adult boreal toads in 2002 and 2003, but breeding localities were not located. Based on the definition of "Breeding Population" (Loeffler 1998), the 63 breeding localities comprise 32 separate populations, of which only one (1) presently meets the criteria to be considered "viable". (See summary in Table 1). The decline in the number of "viable" populations from 1999 to 2003 is due to recent revision of the viability criteria, and the discovery of die-offs caused by the chytrid fungus in at least two of the populations which were formerly considered to be viable.

The criteria for recovery of the boreal toad in the Southern Rocky Mountains were reviewed and edited in 1998 to make them more objective and measurable, and again revised at the end of 2000 to reflect improved knowledge of boreal toad population dynamics. Due to the changes in the criteria, direct comparisons of the level of achievement of recovery goals from 1997 to subsequent years may not accurately reflect actual progress towards recovery (See "Recovery Objectives and Status", page 7). Significant progress has been made with the boreal toad recovery and conservation effort in the past six years, and it is anticipated that much can be accomplished towards recovering this species in the next five years, provided adequate funding and personnel time is available. The recovery team

recognizes that time and funding are in short supply, and will pursue innovative approaches to accomplish needed work, including partnerships, and other cooperative efforts. However, *without* a significant, continued commitment of funds and time from all the involved agencies, recovery will be difficult, if not impossible, to achieve in the foreseeable future.

### **ACKNOWLEDGMENTS**

The Boreal Toad Recovery Team appreciates the substantial contributions made towards the implementation of the Boreal Toad Recovery Plan by various agencies - particularly the Colorado Division of Wildlife, the USGS/Biological Resources Division, and the US Forest Service. Also appreciated is the funding which has been provided by *Great Outdoors Colorado* (GOCO) in support of the boreal toad conservation and recovery effort during the past several years, and technical assistance and advice provided by scientists from the Integrated Research Challenges in Environmental Biology - National Science Foundation (IRCEB) group in regard to evaluation and management of the chytrid fungus problem.



### MANAGEMENT STATUS & ADMINISTRATION

### **Legal Status of the Boreal Toad**

The boreal toad has been state listed as an endangered species in New Mexico since 1976 and in Colorado since November, 1993. It is a protected species in Wyoming and federally classified as a candidate species ("warranted but precluded" - meaning there is adequate data to warrant federal listing as threatened or endangered, but listing has been postponed as there are presently other species in greater need of listing, and the US Fish & Wildlife Service has limited resources to prepare and process listing packages).

### The Recovery Team and Technical Advisory Group

### **Boreal Toad Recovery Team**

The Recovery Team for the Southern Rocky Mountain Population of the Boreal Toad was formed in late 1994. Initially a loosely organized group of people from various agencies, it had been working on boreal toad issues for two to three years prior to that time. Since 1994, it has evolved in to a multi-agency team, consisting of a core recovery team and a technical advisory group.

This group has primary responsibility for the development and implementation of a recovery/conservation plan, and represents all agencies which have legal responsibility and authority to implement management actions. Members of this group have the "voting" authority to make decisions and recommendations for, and to, their agencies regarding management actions. It is composed of one representative from each such agency, or in the case of the US Forest Service, one representative from each involved region. As of March 2004, the team consists of the following personnel:

Colorado Division of Wildlife
New Mexico Game & Fish Department
Wyoming Game & Fish Department
US Fish & Wildlife Service
USGS/Biological Resources Division
US Forest Service (Region 2)
US Forest Service (Region 3)
NPS/Rocky Mountain National Park
Bureau of Land Management
Environmental Protection Agency

Tina Jungwirth, Denver, CO Charles Painter, Santa Fe, NM Bill Turner, Laramie, WY Terry Ireland, Grand Jct., CO Erin Muths, Ft. Collins, CO Doreen Sumerlin, Granby, CO Donna Storch, Taos, NM Therese Johnson, Estes Park, CO Jay Thompson, Lakewood, CO Ed Stearns, Denver, CO

The Recovery Team meets at least twice each year, once in the spring and once in the fall, to review and plan needed field work and other management actions. A mailing list of numerous interested parties is used to disseminate information on Recovery Team actions and boreal toad conservation efforts. Minutes of Recovery Team meetings are available upon request from the team coordinator

(see below).

The Colorado Division of Wildlife (CDOW) has assumed the responsibility for leadership and coordination of the Boreal Toad Recovery Team. Contact with the Recovery Team may be made via Tina Jungwirth as follows:

By Mail: Tina Jungwirth, Species Conservation Section, Colorado Division of Wildlife, 4255

Sinton Road, Colorado Springs, CO 80907

*By Phone:* 719-227-5237

By E-Mail: Tina.Jungwirth@state.co.us

### Boreal Toad Technical Advisory Group

This group is composed of persons who have specialized or technical expertise and knowledge regarding the species, habitat, and/or other specific areas of knowledge which are vital to the implementation of recovery and conservation efforts. In the process of plan development, formulation of guidelines and protocols for implementation, and weighing of alternatives in decision making, this group is relied upon to help guide and advise the recovery team. As a general rule, technical/biological recommendations which represent a majority consensus of this group will be accepted and followed by the Recovery Team, unless there are overriding socio-economic and/or political factors which dictate other courses of action.

The Technical Advisory Group may meet outside of the twice yearly Recovery Team meetings as necessary to handle relevant issues.

The present recognized composition of this group is as follows, and is open to other qualified and interested participants:

Paul Bartelt Waldorf College, Forest City, IA
Ron Beiswenger University of Wyoming, Laramie, WY
Cynthia Carey University of Colorado, Boulder, CO

Steve Corn USGS/Biological Resources Division, Missoula, MT

Craig Fetkavich Colorado Division of Wildlife, Alamosa, CO

Anna Goebel University of Colorado, Boulder, CO

Mary Jennings US Fish & Wildlife Service, Cheyenne, WY

Kevin Rogers Colorado Division of Wildlife, Steamboat Springs, CO

Don Kennedy Denver Water Board, Denver, CO

Brad Lambert Colorado Natural Heritage Program, Ft. Collins, CO

Lauren Livo University of Colorado, Boulder, CO

Michelle Geraud US Fish & Wildlife Service, Cheyenne, WY

### **Recovery and Conservation Plans**

Boreal toad recovery work from 1994 through 1998 was based primarily on the Boreal Toad

Recovery Plan, which was prepared by and for the State of Colorado pursuant to the listing of the boreal toad as a state endangered species in 1994 (Revised in 1997). The Recovery Team, with primary direction from the US Fish & Wildlife Service and the US Forest Service, also developed a draft Conservation Strategy, which focused on actions needed to protect and conserve boreal toad habitats on public lands - primarily US Forest Service lands.

In 1998, the Recovery Team agreed that it would be in the best interests of the recovery effort to revise and combine the State Recovery Plan and the draft Conservation Strategy into a single, comprehensive document. Therefore, in October, 1998, the existing documents were combined in the new *Boreal Toad Conservation Plan and Agreement*. This document was revised and updated in early 2001, and provides guidance to all participating agencies in regard to management and conservation of the boreal toad and its habitat, and provides the opportunity for each agency to sign a Conservation Agreement to define and confirm its commitment to the boreal toad conservation effort. As of February, 2001, eight state and federal agencies and the Colorado Natural Heritage Program, based at Colorado State University, have signed such agreements, which are appended to the Conservation Plan. Copies of this plan are available upon request from the Recovery Team coordinator (see previous page for contact information). The plan may also be accessed via the Internet at the following address: <a href="http://wildlife.state.co.us/aquatic/boreal/index.asp">http://wildlife.state.co.us/aquatic/boreal/index.asp</a>

### **Recovery Objectives and Status**

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

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

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

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

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

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

1. There must be documented breeding activity *and* recruitment to the population in at least four (4) out of the past ten (10) years. However, if breeding activity has not been documented in the past four (4) years, there must be reliable observations of toads, including at least one sub-adult age class, in the area during at least two (2) of those four years.

### OR

2. There has been an average observed total of at least twenty (20) breeding adults in the population, producing an average of at least four (4) viable egg masses per year, and the number of breeding adults observed in the population has remained stable or increased over a period of at least ten (10) years.

### AND

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

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

### Breeding population:

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

### Breeding Locality:

A geographic area containing one or more breeding sites which are separated by a distance of no more than  $\frac{1}{2}$  mile (approx. 0.8 km).

### **Breeding Site:**

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

### Recruitment:

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

\* \* \*

### MONITORING & STATUS OF BREEDING POPULATIONS

Based on various historic reports and observations since the early part of the 20th century, boreal toads were considered to be fairly common in much of the Southern Rocky Mountains, from southern Wyoming to northern New Mexico. One of the earliest published reports of boreal toads in Colorado is from the Buena Vista area, in Chaffee County, where numerous toads were seen under street lights and along irrigation ditches (Ellis and Henderson, 1915). Records of boreal toad observations over the years are somewhat sparse and scattered. Most are associated with a few specific studies, such as James Campbell's work in the late 1960's and early 1970's (Campbell, 1970; Campbell, 1972).

By the early 1980s, the boreal toad was still considered fairly common throughout its known range in Colorado (Hammerson and Langlois 1981), but evidence of dramatic declines had already been noted. Carey (1993) observed the disappearance of 11 populations of boreal toads between 1974 and 1982 in the West Elk Mountains. Subsequent surveys have shown no re-colonization of these former breeding sites. Surveys of 38 historic breeding locations in eight national forests in Colorado covering Boulder, Chaffee, Delta, Gunnison, Jackson, Larimer, Mesa, and Summit counties from 1982 to 1992 revealed only one occupied site in Chaffee County (Lauren Livo, pers. obs.). In 1989, Hammerson (1989) surveyed 143 sites in the Arapaho Lakes, Big Creek Lakes, and Lone Pine Creek areas of Jackson County; 31 sites in the White River plateau within Garfield and Rio Blanco counties; five sites in the Elkhead Mountains in Moffat and Routt counties; 49 sites on the Grand Mesa including Delta and Mesa counties; and 22 sites in Chaffee, Clear Creek, Gilpin, Gunnison, and Park counties. Boreal toads were found in only two of these 250 sites, in Chaffee and Garfield counties. In 1991 Hammerson (1992) surveyed 377 sites in the following Colorado locations or river basins: Upper Alamosa, Upper Arkansas, Conejos, Upper Eagle, Grand County, Grand Mesa, Upper Gunnison, Upper Rio Grande, San Juan, San Luis Valley, Upper San Miguel, and Upper South Platte, and observed only a single population of boreal toads which was subsequently confirmed in 1992 by Livo. Corn et al. (1989) found that toads were absent from 83 percent of historic locations in Colorado and 94 percent of the historic sites in Wyoming. This represented a decline from 59 to 10 known localities from 105 sites surveyed in 1986-1988 in Boulder and Larimer Counties, Rocky Mountain National Park, and in the Park Range in Colorado, and in Albany and Carbon Counties in Wyoming. Boreal toads were thought to be extirpated from the southern periphery of their range in the San Juan Mountains in New Mexico (Stuart and Painter 1994; New Mexico Department of Game and Fish 1988), but an unconfirmed report of a sighting of one adult boreal toad and one boreal toad tadpole in September 1996 gives hope that a breeding population may still exist in New Mexico (C. Painter, unpubl. 1996).

Since the listing of the boreal toad as a state endangered species in Colorado in 1993, efforts to survey known historic and potential toad habitats, and to monitor known existing breeding populations, have been intensified. The following is a summary of what is known about boreal toad occurrence, distribution and status as of late 2003.

### **Breeding Populations by Geographic Area**

The objectives for recovery of the boreal toad in the Southern Rocky Mountains, as outlined in the Boreal Toad Conservation Plan (1998, revised 2001), are based on the documentation and/or establishment of a certain number of secure populations within each of the "mountain ranges of its historic distribution." These are presently recognized to include the Park Range, Elkhead Mountains, Medicine Bow Range, Front Range, Gore Range, Mosquito & Ten-Mile Range, Sawatch Range, White River Plateau, Grand Mesa, Elk & West Elk Mountains, and the San Juan Mountains. The "mountain ranges of historic occurrence" are presented in this report in roughly geographic order from north to south. See Figure 1 for a map of general locations.

The borders or limits of these mountain ranges are often difficult to define precisely. For the purpose of boreal toad recovery, and for clarification, the descriptions in the following pages will serve to define these areas, and provide a brief summary of boreal toad status in each. In cases where toad populations may be found which do not fit neatly in to one of these areas, the Boreal Toad Recovery Team will make a determination as to which "mountain range of historic distribution" the population is most closely linked.

Based on the definition of "Breeding Population" (Loeffler 1998), there are presently 63 breeding localities comprising 32 separate populations, of which only one (1) presently meets the criteria to be considered "viable" (See Summary in Table 1). This population is the Cottonwood Creek population in Chaffee County. The decline in the number of "viable" populations from 1999 to 2003 is due to revision of the viability criteria, and the discovery of die-offs caused by the chytrid fungus in at least two of the populations which were formerly considered to be viable. In most cases, breeding populations are defined such that there is normally no migration of toads between populations. However, due to the continuity of habitat, and the fact that breeding populations can occur in separate drainages which are in close proximity at their headwaters, some populations may be closer to each other than the minimum 5-mile separation, and some toads may occasionally migrate from one to the other by crossing high mountain passes. A case in point would be the Conundrum Creek population in Pitkin County and the Triangle Pass population in Gunnison County. In a straight line they are within 5 miles of each other, but they are located in different primary drainages, separated by a 12,500'+ mountain pass. Whereas these localities are in different major drainages, they are considered parts of different populations.

Monitoring in 2003 of 63 known breeding localities, indicates that 38 of the sites had breeding activity, 14 sites apparently were inactive, and 11 sites are of unknown status due to lack of adequate monitoring. Breeding activity was documented in at least 22 of the 32 known populations in 2003.

Overall, boreal toad populations showed fair to good reproduction. However, in 2003, several breeding localities remained dry or dried prior to metamorphosis due to the effects of drought.

Additional testing of breeding sites for the presence of chytrid occurred in 2003. McQueary Lake

and Buzzard Creek were found to be chytrid positive, bringing the total number of positive breeding sites to nine. The following populations contain one or more chytrid-positive breeding localities: California Park (Routt County); Clear Creek West Fork (Clear Creek County); Conundrum Creek (Pitkin County); North Fork of the Big Thompson (Rocky Mountain National Park, Larimer County); Pole Creek (Grand County); Snake River (Summit County); South Cache le Poudre (Larimer County); and Upper Williams Fork (Grand County).

The 2003 survey efforts located three previously undocumented breeding localities within known populations. The new breeding localities are in the Snake River (Summit County), Clear Creek South (Clear Creek County), and Holy Cross City (Eagle County) populations. In addition, adult boreal toads were again found in the Buzzard Creek drainage of eastern Mesa County, indicating the almost certain presence of a breeding population on Grand Mesa. Figure 2 illustrates current known boreal toad breeding localities as well as miscellaneous recent (1992-2003) boreal toad observations thought to be reliable.

### **Interpretation of Breeding Locality Tables**

<u>Locality Numbers</u>: These are assigned chronologically to localities on a county-by-county basis. The two-letter designation indicates the county, and the number is the chronological number of the locality for that county, based on when the locality was originally found. All breeding localities within a specific county may not fall within the same geographic area or mountain range

<u>Locality and Population Names</u>: After the locality number will be the name of the locality, followed by the name of the population of which it is considered a part. The population name is in parentheses, and in some cases may be the same as the locality name.

In this version of the Status Report, chytrid status is reported to the right of the locality and population name. Chytrid status may be *negative* (at least one individual tested and no chytrid-positive results obtained), *positive* (at least one individual tested positive for chytrid), or *not tested* (no toads from that locality sampled). For positive sites, the year chytrid fungus was first detected at the site is reported in parentheses. For negative sites, the year of last testing at the site is reported in parentheses.

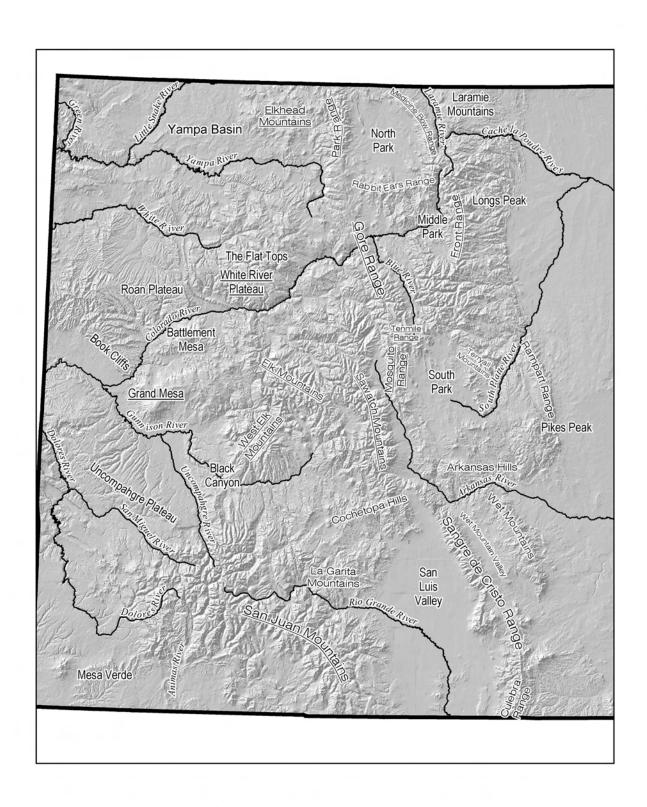
M/F/Egg Masses: This column shows the *minimum* number of breeding-age males (M), females (F), and number of viable egg masses at the locality in each year. These numbers may represent actual counts, or they may be presumed, based on other evidence. For instance, if tadpoles are observed at a locality, it is assumed that there had to be at least one adult male and one adult female present. If three separate egg masses are observed, but no adults are seen, the table will still show 3/3/3, as it is assumed that one pair of breeding toads was present to produce each of the egg masses. A question mark "?" in this column indicates that data are lacking or ambiguous. It should be noted that more intensive studies, using PIT tagging, in Rocky Mountain National Park, the Urad/Henderson Mine area, and the Cottonwood Creek drainage in Chaffee County demonstrate that standard monitoring reveals only a small proportion of adult toads actually present at a site or in a population.

Recruitment: A "Yes" entry means that one-year-old toadlets were observed at the site in the Spring

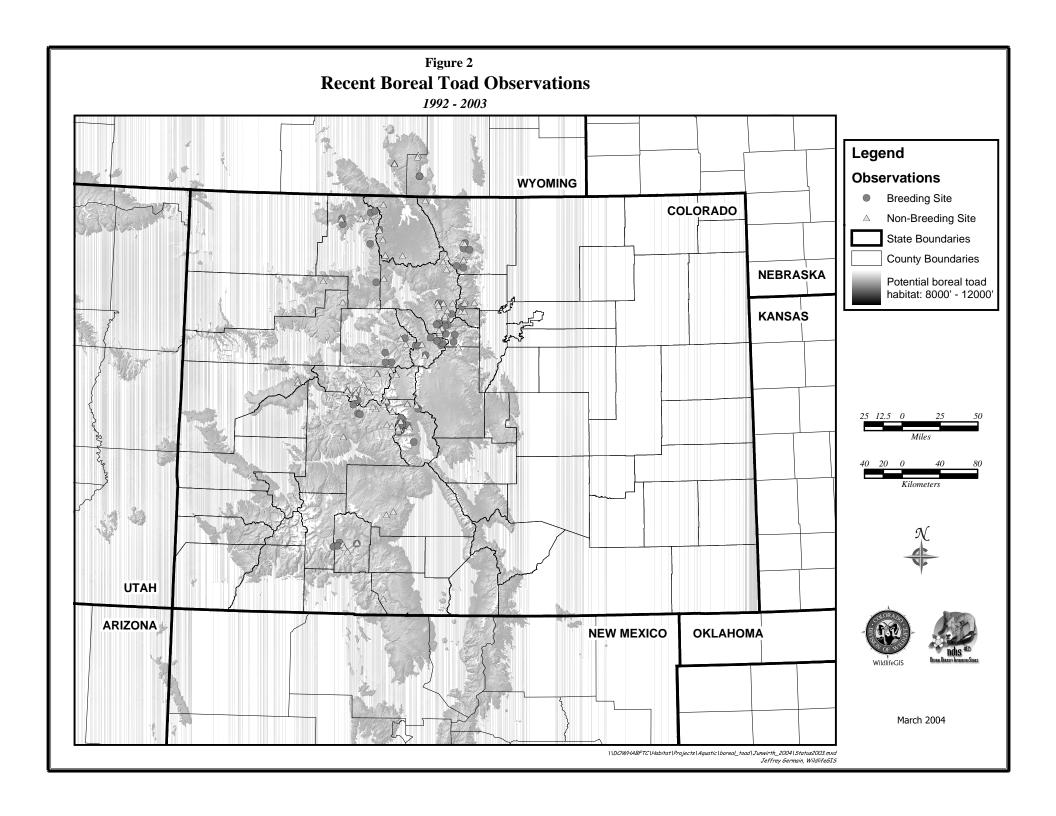
of the *following* year, or two-year-old toads were seen the second year. For example; one year old toadlets in June, 1997, would indicate successful recruitment from the 1996 breeding season, and would be noted by a "Yes" entry in 1996. Therefore, all sites will, at this time, show either a "Unk" (unknown) entry or a "No" entry for 2002, as success can not be determined until the Spring or Summer of 2003, or it is known that there were no metamorphosed toadlets produced at the site in 2002.

<u>Age Classes</u>: The first number in the entry indicates the minimum number of age classes observed/reported at a specific site. Numbers within parentheses indicate which age classes were observed: M = Metamorphs (young of the year), 1 = one year olds (new "recruits"), S = Subadults (generally two to three year old toads), 2 or 3 = Subadults which were specifically identified as either two or three year old toads, A = Adult toads (generally 4 years old and older).

\* \* \*



# MOUNTAIN RANGES IN WESTERN COLORADO (Mtn. ranges of historic occurrence of boreal toads shown underlined)



Five year summary of boreal toad breeding populations in the Southern Rocky Mountains

Mar. 2004

Geographic area	Number of	Populations w/ breeding/recruitment				Populations w/ 20+ breeders & 4+				"Viable"		
(Mtn. range of historic	populations						egg m	asses				populations
occurrence)		1999	2000	2001	2002	2003	1999	2000	2001	2002	2003	
Park Range	3	1/1	1/1+	2/1	2/1	1/?	0	0	0	0	0	0
Elkhead Mountains	1	0/?	1/1	1/1	1/1	1/?	0	0	0	0	0	0
Medicine Bow Range	1	0/0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
Front Range	12	6/5	6/4	8/5	8/5	8/?	2	3	3	3	2	0
Gore Range	3	3/2	3/1	3/2	4/2	3/?	0	1	0	1	0	0
Mosquito & Ten-mile Range	2	2/0	0/0	1/1	1/0	1/?	1	1	0	0	0	0
Sawatch Range	6	5/2	3/1	4/3	5/2	5/?	0	0	1	2	1	1
White River Plateau	0	0/0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
Grand Mesa	0	0/0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
Elk & West Elk Mountains	2	2/1	2/2	2/2	2/1	2/?	1	1	1	1	1	0
San Juan Mountains	2	1/0	2/1	1/1	1/1	1/?	0	0	0	0	0	0
TOTALS	32	18/11	18/11	22/16	24/13	22/?	4	6	5	7	4	1

Number of populations: Number of toad populations, based on the definition of "population" in the Boreal Toad Conservation Plan.

Populations w/Breeding/Recruitment: Populations where any type of breeding activity was documented and/or recruitment of toadlets from that year was observed in the following year: # Before / = Breeding; # After / = Recruitment. NOTE: Recruitment from 2003 production can not be determined until 2004 surveys are done.

Populations w/ 20+ Breeders & 4+ Egg masses: Indicates number of populations where 20 or more breeding adults were observed and 4 or more viable egg masses were produced.

"Viable" Populations: Represents the number of populations in the historic area of occurrence which meet the criteria for "viable populations" as presented in the Boreal Toad Conservation Plan, and can be counted towards delisting goals.

### Park Range

This area extends from south-central Carbon County, WY, through western Jackson County and eastern Routt County, CO, along the Continental Divide to approximately Rabbit Ears Pass. It is located primarily within the Routt and Medicine Bow National Forests.

There are presently four known boreal toad breeding localities in this area. The Soda Creek population has only one known breeding locality, and the North Fork of the Elk River population has two localities as of 2002. In 2001, a breeding locality in Jackson County (deemed the Red Canyon population) was discovered.

### **ROUTT COUNTY**

Locality RO02 - Soda Creek (	(Soda Creek)	Chytrid Status: Not sampled
------------------------------	--------------	-----------------------------

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	1/1/1	Unk	3 (M,2,A)	Nine metamorphs seen
1997	1/1/1	Yes	2 (M,A)	Numerous metamorphs
1998	0/0/0	No	1(1)	Inadequate monitoring
1999	1/1/0	Yes	1(A)	One female toad seen.
2000	0/0/0	Unk	1(1)	One yearling toad seen
2001	0/0/0	Unk	None seen	Inadequate monitoring
2002	0/0/0	Unk	None seen	Inadequate monitoring
2003	0/0/0	Unk	None seen	Site visited 3 times

Locality RO03 - Diamond Park (N. Fork of Elk River) Chytrid Status: Not sampled

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	1/1/1	Yes	2 (M,A)	20 metamorphs seen
1997	1/1/1	Yes	3(M,1,A)	Few metamorphs seen
1998	0/1/0	No	1 (1,A)	Inadequate monitoring
1999	0/2/0	No	1(A)	Only two toads seen
2000	0/0/0	Unk	None seen	Site visited three times
2001	0/0/0	Unk	None seen	Inadequate monitoring
2002	0/0/0	Unk	None seen	One site visit
2003	0/0/0	Unk	None seen	Site visited twice

Locality RO06 - Upper Buck Mountain (N. Fork of Elk River) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2000	9/4/4	Yes	3 (M,S,A)	Est. <50 metamorphs
2001	6/2/2	Yes	4(M,1,S,A)	Est. 100-500 metamorphs
2002	5/2/2	Yes	3(1,S,A)	Metamorphs not observed
2003	6/6/6	Unk	3(M,1,A)	Est. 50-100 Metamporphs

### JACKSON COUNTY

Locality JA01 – Spike Lake (Red Canyon) Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2001	1/1/1	Unk	1(M)	Two visits after discovery
2002	1/1/1?	Unk	?	Site info not provided*
2003	0/0/0	Unk	none seen	_

This breeding locality was discovered in 2001; tadpoles and metamorphs, but no adult toads, were observed.

\* \* \*

### **Elkhead Mountains**

This mountain area is in western Routt County and eastern Moffat County, CO, northeast of Craig. It is located primarily within the Routt National Forest. The only known boreal toad breeding population in this area is in California Park. There are two known breeding localities at this time (First Creek and Torso Creek). Although evidence of reproduction has been observed in several locations, a specific breeding site was not found until 2000 near Torso Creek.

### **ROUTT COUNTY**

Locality RO01 - First Creek (California Park)

Chytrid Status: Not tested

Vear M/F/Egg Masses Recruitment Age Classes Comments

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	0/0/0	Yes	2(2,3)	Numerous sub-adults
1996	1/1/1	Unk	2(S,A)	Larvae seen
1997	1/0/0	Unk	2(S,A)	Toads along Elkhead Cr.
1998	0/0/0	No	1(S)	<b>Inadequate Monitoring</b>
1999	0/0/0	No	None seen	Monitoring adequate
2000	0/0/0	No	None seen	Monitoring adequate
2001	0/0/0	No	None seen	Monitoring inadequate
2002				Not monitored
2003	0/0/0	Unk	None seen	Site visited once

<sup>\*</sup>Tadpoles taken from this site to NASRF in 2002, but monitoring information not submitted.

Locality RO04 - Torso Creek (California Park) Chytrid Status: Positive (2001)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	0/1/0	Unk	3(1,S,A)	Numerous 1-yr. olds.
2000	2/2/2	Unk	3(M,2,A)	Approx. 400 metamorphs
2001	2/1/1	Yes	4(M,1,S,A)	>50 metamorphs
2002	1/1/1	Yes	3(1,S,A)	Site dried by August visit
2003	3/2/1	Unk	2(M,A)	<50 metamorphs

An enclosure was constructed around the breeding area to exclude sheep.

\* \* \*

### **Medicine Bow Range**

This is an area extending from southeastern Carbon County and western Albany County, WY, south through eastern Jackson County and western Larimer County, CO, to approx. Cameron Pass. It is situated primarily within the Routt and Roosevelt National Forests and on the Colorado State Forest.

At this time, there is only one known breeding site, Bird Creek, located in Albany County, Wyoming. Based on historic and recent observations of toads in Carbon and Albany counties, it is likely that other breeding populations will be found in the Medicine Bow Range, given adequate survey effort. A confirmed sighting of an adult boreal toad was made in the upper Laramie River drainage, in Larimer County, CO in 1998, but surveys in 1999 and 2000 failed to find a breeding site or toads.

### **ALBANY COUNTY, WY**

Locality WY01 - Bird Creek (Albany) Chytrid Status: Not tested

		· <i>j</i> /		
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1993	1/1/1	Yes	1(A)?	No counts of adults/eggs
1994	4/1/1	Yes	3(1,S,A)	
1995	4/1/1	Yes	3(1,S,A)	
1996	2/1/1	Yes	4(M,1,S,A)	17 toadlets collected
1997	3/3/3	Yes	4(M,1,S,A)	Some eggs collected
1998	0/0/0	No	2(1,S)	No reproduction seen
1999	0/0/0	No	None seen	Surveys adequate
2000	0/3/0	No	1(A)	Three &toads seen*
2001	0/1/0	No	1(A)	One female toad seen*
2002	0/1/0	Unk	1(A)	One female toad seen*
2003	1/0/0	Unk	1(A)	One male toad seen

This site is the source for stock used for reintroductions at Lake Owen

\* \* \*

<sup>\*</sup>Two of the three female toads found in 2000 were placed in captivity at the Sybille Wildlife Research Station; the female toads seen in 2001 and 2002 were not taken into captivity.

### **Front Range**

This is an extensive area in northern Colorado, which includes southwestern Larimer County, eastern and southern Grand County, the western portions of Boulder, Gilpin, and Clear Creek counties, and eastern Summit County. It extends from the Mummy Range, in the north, south through Rocky Mountain National Park, to Loveland Pass and the Mt. Evans Wilderness Area. Much of the area is situated within the Arapahoe/Roosevelt National Forest.

There are twenty-one (21) known breeding localities, comprising twelve (12) populations, within the Front Range area as of 2002. Two sites, comprising the Upper Williams Fork population, were discovered in 2001. These breeding populations and localities are located in five counties, as follows:

### LARIMER COUNTY

Locality LR01 - Lost Lake (North Fork, Big Thompson, RMNP) Chytrid Status: Positive (2000)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1990	?/?/22	Unk	1(A)	Incomplete data
1991	206/28/15	Unk	1(A)	No data on sub-adults
1992	143/23/23	Unk	1(A)	No data on sub-adults
1993	77/10/?	Unk	1(A)	Incomplete data
1994	110/35/35	Unk	1(A)	No data on sub-adults
1995	122/32/32	Yes*	1(A)	No data on sub-adults
1996	43/15/15	No	1(A)	No data on sub-adults
1997	112/15/15+	No	3(M,2*,A)	15 to 20 egg masses
1998	106/12/12	Unk	2(M,A)	150+ Metamorphs seen
1999	10/10/10	Unk	1(A)	Metamorphs possible
2000	3/3/3	Unk	1(A)	Positive for chytrid
2001	0/3/0	Unk	1(A)	Only females observed
2002	0/1/0	Unk	1(A)	One female observed
2003	0/0/0	Unk	None seen	Surveys adequate

<sup>\*</sup> Recruitment in 1995 based on observation of 2-yr. old toads in 1997.

Locality LR02 - Kettle Tarn (North Fork, Big Thompson, RMNP) Chytrid Status: Positive (2001)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1990	?/?/13	Unk	1(A)	Incomplete data
1991	21+/23/23	Unk	1(A)	No data on sub-adults
1992	63/18/18	Unk	1(A)	No data on sub-adults
1993	54/25/25	Unk	2(M,A)	
1994	120/21/21	Unk	2(M,A)	
1995	210/24/24	Unk	2(M,A)	
1996	29/13/8	Unk	3(M,2,A)	
1997	15/11/0	No	1(A)	
1998	18/13/10	Unk	1(A)	
1999	15/8/2	Yes*	1(A)	No metamorphs seen
2000	13/5/3	Unk	2(1,A)	One 1-yr. old seen.*
2001	2/4/3	Yes	3(M,S,A)	Metamorphs observed*
2002	2/2/2	Yes	3(M,1,A)	See note**
2003	3/3/3	Unk	3(M,1,A)	500+ metamorphs

<sup>\*</sup> Metamorphs observed, but number not estimated in monitoring form.

Locality LR03 - Spruce Lake (Big Thompson River, RMNP) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	Unk	Yes	Unk	Reproduction presumed
1997	3/1/?	Unk	3(1,S,A)	Limited monitoring
1998	9/3/1	Unk	1(A)	Inadequate monitoring
1999	9/3/1	Yes	2(S,A)	Inadequate monitoring
2000	10/4/2	Unk	3(M,1,A)	Three 1-yr. olds seen.
2001	10/2/2	Unk	2(S,A)	Larvae observed*
2002	15/3/3	Unk	1(A)	No metamorphs observed
2003	12/1/1	Unk	1(A)	No larvae observed

<sup>\*</sup>Last site visit June 20, prior to time of metamorphosis.

Locality LR04 - Glacier Basin (Big Thompson River, RMNP) Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	1/1/0	Unk	1(A)	
1996	1/1/1	Yes	1(A)	Transplant site
1997	0/1/0	No	2(1,A)	
1998	3/0/0	Unk	1(A)	No breeding activity seen
1999	3/0/0	Unk	1(A)	No night survey done
2000	0/0/0	Unk	None seen	Monitoring adequate
2001				Not monitored

This site will no longer be regularly monitored after 2000. Translocation appears unsuccessful (Muths et al. 2001).

<sup>\*\*</sup> Tadpoles from NASRF released at site; it is unknown whether metamorphs observed in 2002 derived from naturally produced clutchs or from these released tadpoles.

Locality LR05 - Twin Lake (South Cache la Poudre)	Chytrid Status: Positive (2001)
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Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1998	1/1/1	Unk	1(A)	Tadpoles observed
1999	0/0/0	Unk	None seen	Site disturbed*
2000	0/0/0	Yes	None seen	Low water
2001	3/2/2	Yes	3(1,S,A)	No metamorphs seen
2002	1/1/1	Unk	2(S,A)	No metamorphs seen
2003	0/0/0	Unk	0	Site disturbed

<sup>\*</sup> In 1999, there was temporary disturbance at this site due to testing of reconstructed dam.

### **BOULDER COUNTY**

Locality BO01 - Lost Lake (Middle Boulder Creek) Chytrid Status: Not tested\*

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	0/1/0	No	2(M,A)	Toadlets introduced
1997	0/1/1	No	3(M,1,A)	Toadlets introduced**
1998	0/2/0	No	3(1,2,A)	No breeding observed
1999	0/0/0	No	None seen	Minimal surveys done
2000	0/0/0	No	None seen	Monitoring adequate
2001	0/0/0	No	None seen	Monitoring adequate
2002	0/0/0	Unk	None seen	Monitoring adequate
2003	0/0/0	Unk	None seen	Site visited 3 times

This is an experimental reintroduction site. Monitoring continued through 2002.

### **GRAND COUNTY**

Locality GR01 - Jim Creek (Winter Park)

Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	5/1/?	Unk	3+(S,A)	Substantial population
1996	?/?/0	Unk	3+(S,A)	Substantial population
1997	0/0/0	Unk	None observed	Monitoring inadequate
1998	0/0/0	Unk	None observed	Monitoring inadequate
1999	0/0/0	Unk	None observed	No night survey done
2000	0/0/0	Unk	None observed	Monitoring adequate
2001	0/0/0	Unk	None observed	No night survey done
2002				Not monitored
2003	0/0/0	Unk	None observed	Site visited 7 times

Population indicates breeding pre-1996, but no actual breeding site found.

<sup>\*</sup>PCR test results were chytrid negative for samples from 5 groups of sentinel tadpoles placed at Lost Lake in 2001.

<sup>\*\*</sup>Tadpoles observed, possibly from mating of a resident female and a translocated male toad.

Locality GR02 - Pole Creek (Pole Creek)			Chytrid Status: Positive(2002)	
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	5/3/3	Unk	2(M,A)	Numerous metamorphs
1996	3/3/3	Yes	2(M,A)	Few metamorphs
1997	10/4/2	No	2(1,A)	Few, if any, metamorphs
1998	5/2/2	Yes*	2(M,A)	Monitoring marginal
1999	5/5/5	Unk	2(M,A)	Metamorphs at #4
2000	6/2/2	Yes	3(M,S,A)	One clutch desiccated
2001	9/7/7	Unk	4(M,1,S,A)	>500 metamorphs
2002	14/6/6	Yes	4(M,1,S,A)	Metamorphs present**
2003	7/2/2	Unk	4(M,1,S,A)	>500 metamorphs

This locality is on Pole Creek Golf Course, near holes #4 and #15.

Locality GR03 - Vasquez Creek (Vasquez Creek) Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	1/1/1	Yes*	1(A)	Found late in season
2000	0/0/0	Unk	None seen	Monitoring adequate
2001	0/0/0	Unk	1(S)	One subadult seen*
2002	0/0/0	Unk	None seen	One site visit
2003				Site not monitored

<sup>\* 16</sup> toadlets from 1999 clutch were captive reared and released in Vasquez Creek drainage in 2000; the subadult observed in 2001 was observed at the release site. No toads were observed at the 1999 breeding site.

Locality GR04 – McQue	ry Lake (Upper Williams Fork	) Chytrid Status: Positive (2	2003)	
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Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2001	2/3/3	Yes	2(1,A)	No metamorphs observed
2002	8/6/6	Unk	2(M,A)	<50 metamorphs seen
2003	2/2/2	Unk	2(S,A)	Desiccation & predation

This site was discovered in 2001.

Locality GR05 – Upper Williams Fork (Upper Williams Fork) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2001	2/2/2	Yes	3(M,1,A)	Metamorphs observed
2002	1/1/1	Yes	3(1,S,A)	No metamorphs seen
2003	1/2/1	Unk	4(M,1,S,A)	< 50 metamorphs

This site was first visited in July 2001.

<sup>\*</sup> Recruitment from 1998 production based on observation of subadult toads in 2000.

<sup>\*\*</sup>Metamorphs sampled on 9/23/02 were chytrid-positive.

### **SUMMIT COUNTY**

Locality SU02 - Montezuma (Snake River)

Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	7/1/1	No	2(S,A)	Breeding unsuccessful
1996	9/?/0	No	1(A)	No breeding observed.
1997	1/1/1	Unk	1(A)	New site, vs. '95 & '96
1998	0/0/0	Unk	None seen	Monitoring inadequate
1999	3/1/1	Unk	1(A)	Tadpoles observed
2000	0/0/0	Unk	None seen	No access to property*
2001				Not monitored
2002	0/0/0	Unk	None seen	2 site visits
2003				Not monitored

<sup>\*</sup>This site is on private property, and permission for ongoing access needs to be obtained.

Locality SU03 - Peru Creek (Snake River)

Chytrid Status: Positive (2001)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	1/1/1	Yes	3(M,S,A)	May be $> 3$ age classes
1997	6/2/2	Unk	4(M,1,S,A)	Good metamorphosis
1998	3/1/1	Unk	2(M,A)	Monitoring inadequate
1999	14/1/1	Unk	1(A)	Monitoring minimal
2000	19/1/1	Yes	1(A)	Tadpoles seen
2001	29/1/1	Unk	2(1,A)	Inadequate monitoring
2002	2/1/1	Unk	2(M,A)	>500 metamorphs
2003				Not monitored

Disturbance from construction was observed in the wetland area, but not the breeding pond itself, on 6/15/01. Monitoring in 2001 did not occur around the time that metamorphosis would be expected.

Locality SU06 - Upper North Fork of Snake River (Snake River) Chytrid Status: Positive (2001)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1998	1/2/1	Unk	3(M,S,A)	1st survey mid-July
1999	1/1/1	Unk	2(S,A)	Some tadpoles seen
2000	1/1/1	Unk	2(M,A)	10-20 metamorphs seen
2001	1/1/1	Yes	2(1,A)	Inadequate monitoring
2002	1/2/1	Unk	2(1,A)	Inadequate monitoring
2003				Not monitored

One male, one female, and 13 additional toads observed 5/24/01; About 100 tadpoles and 23 yearlings observed 7/20/01.

Locality SU07 - Lower North Fork of Snake River (Snake River)			Chytrid Status: Not tested	
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1998	1/2/1	Unk	3(M,S,A)	1st survey mid-July
1999	1/2/0	Unk	1(A)	No breeding observed
2000	1/1/0	Unk	1(A)	No breeding observed
2001	1/0/0	Unk	1(A)	Inadequate monitoring
2002	0/0/0	Unk	None seen	Three site visits
2003				Not monitored

Locality SU08 – Straight Creek (Snake River)			Chytrid Status: Negative (2003)	
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2003	1/1/1	Unk	3(M,S,A)	Site discovered 5/29/03

### **CLEAR CREEK COUNTY**

Locality C	C01 - Vintage (Clear Cr	Chytrid Status: Not tested		
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1994	?/?/?	Unk	Multiple	Little data available
1995	3/2/2	Unk	2(M,A)	Prob. few metamorphs
1996	1/1/1	No	1(A)	No production
1997	1/1/1	No	1(A)	Eggs froze
1998	3/0/0	No	1(A)	No breeding observed
1999	3/0/0	No	1(A)	No breeding observed
2000	0/0/0	No	None seen	Minimal monitoring
2001	0/0/0	Unk	None seen	Minimal monitoring
2002				Not monitored
2003	0/0/0	Unk	None seen	No evidence of breeding

<sup>\*</sup>All site visits in 2001, including night surveys, conducted in May.

Locality CC0	2 - Urad/Henderson (C	Chytrid Status: Positive (2001)		
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	131/19/19	Yes	4(M,1,S,A)	
1996	142/18/18	Yes	4(M,1,S,A)	Few metamorphs
1997	167/33/23	Yes	4+(M,1,S,A)	
1998	203/107/55	Yes	4(M,1,S,A)	Many metamorphs
1999	141/60/60	Unk	4(M,1,S,A)	Chytrid fungus mortality
2000	34/34/34	Unk	2(M,A)	
2001	14/14/14	Unk	3(M,1,A)	Some egg mortality*
2002	25/22/22	Unk	2(M,A)	Several sites dry**
2003	15/15/15	Unk	1(A)	

<sup>\*</sup>Egg mass mortality due to a water fungus observed at the Hesbo site; other sites had good egg mass survival.

Locality CC03 - Herman Gulch (Clear Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1993	?/?/?	Unk	2(M,A)	Breeding observed
1994	11/11/11	Unk	2(M,A)	
1995	52/12/12	Unk	3(M,S,A)	Good production
1996	20/12/12	No	1(A)	Poor larvae survival
1997	19/10/10	Unk	3(M,S,A)	Many metamorphs
1998	10/10/10	Unk	2(M,A)	Few metamorphs seen
1999	11/11/11	Yes	1(A)	High egg mortality
2000	9/5/5	Unk	3(1,S,A)	No metamorphs seen
2001	2/2/4	Unk	3(M,S,A)	<50 metamorphs
2002	0/1/0	Unk	1(A)	No evidence of breeding
2003	1/1/1	Unk	1(M)	<50 metamorphs

This site typically has poor egg survival, probably due to water quality problems in run-off from I-70.

Locality CC04 - Mount Bethel (Clear Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1993	Yes	Unk	2(M,A)	Many metamorphs
1994	Yes	Unk	2(M,A)	
1995	4/1/1	No	2(S,A)	Few, if any, metamorphs
1996	3/3/3	Unk	2(M,A)	Few metamorphs
1997	9/1/1	Unk	2(M,A)	
1998	11/3/3	Unk	2(M,A)	36+ metamorphs seen
1999	23/1/1	Yes	2(M,A)	500+ metamorphs seen
2000	29/3/3	Yes	4(M,1,S,A)	Many metamorphs seen
2001	28/6/5	Yes	4(M,1,S,A)	500+ metamorphs seen
2002	16/4/4	Yes	3(M,1,A)	Metamorphosis early
2003	7/7/7	Unk	3(M,1,A)	<50 metamorphs

Locality CC05 - Bakerville (Clear Creek) Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1994	1/1/1	Unk	2(M,A)	Limited data
1995	Unk	Unk	Unk	Site not monitored
1996	0/0/0	No	None seen	
1997	Unk	Unk	Unk	Site not monitored
1998	0/0/0	Unk	None seen	Inadequate monitoring
1999	0/1/0	Unk	1(A)	Inadequate monitoring
2000	0/0/0	Unk	None seen	Monitoring adequate
2001	3/0/0	Unk	1(A)	Inadequate monitoring
2002				Site not monitored
2003	1/1/1	Unk	1(A)	Few tadpoles found

Locality CC06 - Silverdale (Clear Creek South) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1993	?/?/0	Unk	Multiple	First survey of site
1994	?/?/0	Unk	Multiple	No metamorphs
1995	2/0/0	Unk	2(S,A)	No breeding observed
1996	5/0/0	No	1(A)	No breeding observed
1997	0/0/0	No	None observed	Inadequate monitoring
1998	1/1/0	Unk	2(S,A)	Monitoring marginal
1999	0/0/0	Yes	1(S)	41 sub-adults seen
2000	0/0/0	Unk	2(1,S)	Many sub-adults seen
2001	0/0/0	Unk	2(S,A)	65 subadults, 7 adults*
2002				Site not monitored
2003				Site not monitored

<sup>\*</sup> Breeding site used in 1990s apparently not being used at present, and location of current breeding site unknown.

Locality	CC07 - Otter Mountain (C	Clear Creek Sou	th)	Chytrid Status: Negative (2003)
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2003	1/1/1	Unk		200 tadpoles seen

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### **Gore Range**

This is a geographic area extending from west-central Routt County and northwestern Grand County south to western Summit County, including the Eagle's Nest Wilderness Area. Much of this area is located within the White River and Arapahoe National Forests. Prior to 1999, there were only two known breeding localities in the Gore Range, both in east-central Summit County, and each with two or more breeding sites. Surveys in 1999 located two new breeding populations in the Gore Range. One is at east Vail, in Eagle County, and the other on the North Fork of Morrison Creek, in southeastern Routt County. No new populations or breeding sites were located in 2001 or 2002.

### **ROUTT COUNTY**

Locality RO05 - North Fork Morrison Creek (Morrison Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	10/2/2	Yes	4(M,1,S,A)	Site found late July.
2000	7/3/3	Yes	4(M,1,S,A)	<50 metamorphs seen.
2001	29/10/1	Unk	4(M,1,S,A)	Three site visits
2002	15/1/1	Unk	2(S,A)	Three site visits
2003	13/1/0	Unk	1(A)	Two site visits

### **EAGLE COUNTY**

Locality EA03 - East Vail (Vail) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	3/1/1	Yes	3(M,S,A)	Site found late July.
2000	8/2/1	Unk	3(M,1,A)	Many metamorphs.
2001	32/4/3	Yes	3(M,S,A)	15 metamorphs seen
2002	7/1/1	Yes	4(M,1,S,A)	Hundreds of subadults
2003	4/1/1	Unk	4(M,1,S,A)	50-100 metamorphs seen

This site is near a bike path and surrounded by development.

### **SUMMIT COUNTY**

Locality SU04 - Upper North Tenmile (North Tenmile Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	6/6/6	Unk	2(S,A)	Few, if any, metamorphs
1996	17/6/6	Unk	3(M,S,A)	Good production
1997	13/3/3	Unk	2(M,A)	Limited metamorphosis
1998	18/3/1	Yes	2(S,A)	Inadequate monitoring
1999	2/3/3	Unk	4(M,1,S,A)	Inadequate monitoring
2000	7/4/4	Unk	2(S,A)	Metamorphs likely
2001	8/2/2	Yes	1(A)	Larvae disappeared
2002	8/8/8	Unk	4(M,1,S,A)	No night survey
2003	1/1/1	Unk	1(A)	No larvae/metamorphosis

Locality SU05 - Lower North Tenmile (North Tenmile Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	4/2/2	Yes	2(M,A)	Few metamorphs
1997	1/2/1	Unk	2(1,A)	Little or no reproduction
1998	5/5/5	Unk	3(M,S,A)	Inadequate monitoring
1999	3/2/1	Unk	1(A)	Inadequate monitoring
2000	5/3/2	Unk	2(M,A)	Monitoring adequate
2001	3/4/3	Yes	2(M,A)	100 metamorphs seen
2002	2/2/2	Yes	3(M,1,A)	No night survey
2003	2/2/2	Unk	2(1,A)	Likely many metamorphs

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### **Mosquito and Ten-Mile Range**

This is an area extending from southern Summit County south to the Buffalo Peaks Wilderness Area in western Park County and northeast Chaffee County. Much of it is situated within the Arapahoe and Pike/San Isabel National Forests.

As of 2003 there are only two known boreal toad breeding localities in this geographic area, as follows:

### **SUMMIT COUNTY**

Locality SU01 - Cucumber Gulch (Breckenridge) Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	1/1/1	No	3+(M,S,A)	Mult. age classes seen
1996	?/?/0	No	2(S,A)	No breeding observed
1997	2/1/1	No	1(A)	Recruitment doubtful
1998	1/0/0	Unk	1(A)	Monitoring minimal
1999	1/1/1	Unk	1(A)	No metamorphs seen
2000	0/1/0	Unk	1(A)	Monitoring adequate
2001	0/0/0	Unk	None seen	Monitoring adequate
2002	0/0/0	Unk	None seen	5 site visits by CNHP
2003	0/0/0	Unk	None seen	4 site visites

Development has occurred around this site.

### **CHAFFEE COUNTY**

Locality CF07 - Fourmile Creek (Buffalo Peaks)				Chytrid Status: Negative (2003)
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	3/1/0	No	1(A)	No breeding observed
1996	2/2/2	Yes	2(M,A)	Numerous metamorphs
1997	3/3/3	Yes	4(M,1,2,A)	Good production
1998	1/1/1	Unk	4(M,1,S,A)	Late egg clutch
1999	6/3/2	Unk	2(S,A)	Eggs lost to desiccation
2000	1/0/0	Unk	1(A)	Monitoring adequate
2001	10/4/4	Yes	2(M,A)	Ca. 100 metamorphs
2002	1/2/1	Unk	2(1,A)	Tadpoles disappeared
2003	10/3/3	Unk	3(M,S,A)	Likely many metamorphs

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### **Sawatch Range**

This geographic area includes western Lake and Chaffee counties and eastern Pitkin and Gunnison counties, and extends from the Holy Cross Wilderness Area south to Monarch Pass. It includes the upper Fryingpan drainage and eastern Taylor Park, and is situated primarily within the White River, San Isabel and Gunnison national forests.

There are seventeen (17) known breeding localities within this area. Fourteen (14) of these are located in the Collegiate Peaks area of Chaffee County, two (2) in southern Eagle County, and one (1) in eastern Gunnison County. The twelve sites in the Cottonwood Creek drainage of Chaffee County, including a breeding locality discovered in 2002, comprise the most substantial remaining metapopulation of boreal toads in the Southern Rocky Mountains, and presently is the only population which meets the viability criteria in the Conservation Plan.

### **CHAFFEE COUNTY**

Locality CF01 - Collegiate Peaks Campground (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1993	1/1/1	Yes	1(A)	Reproduction presumed
1994	1/1/1	Unk	4(1,2,3,A)	Larvae observed
1995	11/5/5	Unk	3+(M,S,A)	Subadults not aged.
1996	13/5/5	Unk	3(M,S,A)	Few metamorphs.
1997	10/8/6	Unk	2(M,A)	Numerous metamorphs
1998	38/7/7	Yes	2(M,A)	1st year of PIT tagging
1999	24/3/3	Yes	4(M,1,S,A)	4 one-year olds seen
2000	6/6/3	Unk	3(M,1,A)	1 one-year old seen
2001	12/6/6	Yes	3(M,S,A)	Numerous metamorphs
2002	21/4/3	Yes	4(M,1,S,A)	About 200 metamorphs
2003	23/5/5	Unk	4(M,1,S,A)	~3000 eggs removed

Locality CF02 - Denny Creek (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1994	5/5/5	Unk	2(S,A)	Probably metamorphs
1995	16/10/3	Unk	3(M,S,A)	Sub-adults not aged
1996	4/4/4	Yes	3(M,S,A)	Metamorphs present
1997	10/4/4	Yes	3(1,2,A)	Few, if any, metamorphs
1998	55/22/22	Yes	4(M,1,S,A)	1st year of PIT tagging
1999	63/18/16	Yes	4(M,1,S,A)	Good production
2000	58/23/23	Yes	4(M,1,S,A)	Good production
2001	52/22/22	Yes	4(M,1,S,A)	Numerous metamorphs
2002	27/13/13	Unk	4(M,1,S,A)	Only 1 metamorph seen
2003	33/22/14	Unk	3(M,S,A)	Slow to develop

Locality CF03 - Hartenstein Lake (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1994	5/?/?	Unk	1(A)	Limited data
1995	29/6/6	Unk	1(M,A)	Few metamorphs seen
1996	10/2/2	Yes	2(M,A)	Metamorphs presumed
1997	12/5/5	Unk	2(M,1,A)	Many metamorphs
1998	31/7/5	Yes	3+(M,S,A)	1st year of PIT tagging
1999	64/10/9	Unk	2(1,A)	Predation by mallards
2000	57/14/14	Yes	2(M,A)	Few metamorphs
2001	69/5/5	Yes	3(1,S,A)	Four yearlings seen
2002	21/4/4	Unk	4(M,1,S,A)	Metamorphosis early
2003	11/7/7	Unk	2(S,A)	No metamorphs seen

Locality CF04 - South Cottonwood Creek (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	24/3/3	Unk	3(M,S,A)	Numerous metamorphs
1996	12/4/4	Yes	2(M,A)	Good production
1997	26/3/3	Yes	4(M,1,2,A)	Numerous metamorphs
1998	35/7/7	Yes	4(M,1,S,A)	1st year of PIT tagging
1999	45/11/11	Yes	3(M,1,A)	Numerous metamorphs
2000	54/10/10	Yes	4(M,1,S,A)	Numerous metamorphs
2001	51/5/5	Yes	4(M,1,S,A)	Numerous metamorphs
2002	26/5/5	Yes	4(M,1,S,A)	Low water levels*
2003	62/4/4	Unk	4(M,1,S,A)	>500 metamorphs

<sup>\*</sup>In 2002, in addition to adults caught and gender determined, approximately 15 additional adults seen but not captured; few metamorphs observed.

Locality CF05 - Brown's Creek (Brown's Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	2/3/1	Yes	2(S,A)	Metamorphs unlikely
1996	4/4/4	Unk	3(M,S,A)	Few metamorphs
1997	2/2/2	Unk	3(M,2,A)	Fair metamorphosis
1998	0/1/0	Unk	1(A)	No breeding observed
1999	3/2/2	Unk	2(M,A)	Snake predation
2000	0/0/0	Unk	None seen	Monitoring adequate
2001	1/2/1	Unk	2(M,A)	5 metamorphs seen
2002	2/3/1	Unk	1(A)	Tadpoles disappeared
2003	1/1/0	Unk	1(A)	No evidence of breeding

Locality CF06 - Kroenke L	ake (Cottonwood Creek)	Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	3/2/2	Unk	1(A)	Metamorphs unlikely
1996	2/2/2	Unk	2(M,A)	Fair metamorphosis
1997	9/2/2	Unk	1(A)	Metamorphs unlikely
1998	3/3/3	Unk	1(A)	Metamorphs unlikely
1999	6/3/3	Unk	1(A)	No night surveys
2000	3/2/2	Unk	2(S,A)	One subadult seen
2001	9/1/1	Unk	3(M,S,A)	4 metamorphs, 1 subadult
2002	2/2/2	Yes	2(M,A)	15 metamorphs seen
2003	16/3/3	Unk	3(M,1,A)	Likely many metamorphs

Locality C	F08 - Morgan's Gı	ch (Cottonwood Creel	(i) Chyti	rid Status: Negative (2	(003)
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Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1997	19/6/6	Yes	2(M,A)	Many metamorphs
1998	24/1/1	Yes	4(M,1,S,A)	Eggs late season
1999	40/3/3	Unk	4(M,1,S,A)	One egg mass not viable
2000	17/5/5	Unk	2(S,A)	Few or no metamorphs
2001	12/5/5	Yes	3(M,S,A)	30 metamorphs seen
2002	10/0/0	Unk	2(S,A)	No breeding observed*
2003	21/7/7	Unk	2(S,A)	Likely desiccation loss

<sup>\*</sup>Pond dried by mid-June in 2002.

Locality CF09 - Sayre's Gulch (South Fork Lake Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1997	9/1/1	Unk	1(A)	Site found late in season
1998	34/2/2	Unk	2(S,A)	Metamorphs few, if any
1999	4/4/2	Unk	2(S,A)	Larvae lost to mallards*
2000	8/5/5	Unk	2(S,A)	No early-season survey*
2001	13/5/5	Yes	2(S,A)	Larvae apparently lost**
2002	21/6/6	Yes	4(M,1,S,A)	
2003	9/4/4	Unk	4(M,1,S,A)	Likely many metamorphs

<sup>\*</sup> Most larvae apparently lost to mallard and/or dytiscid predation in 1999 and 2000; the same may have occurred in 2001.

Locality CF10 - South Cottonwood Cr. West (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1998	2/2/2	Yes	2(M,A)	Excellent production
1999	9/9/9	Yes	3(M,1,A)	Good production
2000	19/9/9	Yes	3(M,1,A)	Good production
2001	26/7/7	Yes	4(M,1,S,A)	Numerous metamorphs
2002	14/5/5	Yes	4(M,1,S,A)	Numerous metamorphs
2003	6/6/6	Unk	4(M,1,S,A)	Numerous metamorphs

<sup>\*\*</sup>Observation of one one-year-old toadlet in 2002 indicates at least some survival of tadpoles from 2001.

Locality CF11 - Rainbow Lake (Cottonwood Creek)

Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	4/3/3	Unk	1(A)	Larvae lost to mallards
2000	1/1/1	Unk	2(S,A)	One sub-adult seen
2001	2/1/1	Yes	1(A)	Tadpoles disappeared*
2002	3/2/2	Unk	2(1,A)	Tadpoles disappeared
2003	1/1/1	Unk	1(A)	Few tadpoles found

This site is on private land, and subject to considerable human use.

Locality CF12 - Middle Cottonwood (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	13/1/1	Unk	4(M,1,S,A)	8 one-year olds seen
2000	9/1/1	Unk	3(M,S,A)	Few metamorphs seen
2001	11/4/4	Yes	3(M,S,A)	100 metamorphs seen
2002	14/3/3	Yes	4(M,1,S,A)	15 metamorphs seen
2003	53/5/3	Unk	3(1,S,A)	Likely many metamorphs

Locality CF13 - Denny Creek West (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	5/2/2	Unk	1(M,1,A)	5 metamorphs seen
2000	1/0/0	Unk	1(A)	Minimal monitoring
2001	3/0/0	No	1(A)	Adequate monitoring
2002	3/3/3	Unk	3(1,S,A)	Metamorphosis possible*
2003	2/2/2	Unk	2(M,A)	Adequate monitoring

<sup>\*</sup>Five one-year-olds were observed in 2002 despite no breeding observed at this site in 2001; successful breeding in 2001 may have been overlooked or it is possible that the toadlets were from the Hartenstein or Denny Creek sites. No metamorphs were observed in 2002, but it is possible some were produced.

Locality CF14 - Denny Creek South (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	1/1/1	Unk	3(M,S,A)	4 sub-adults seen
2000	1/0/0	Unk	1(A)	Dried up mid-summer
2001	2/2/2	No	1(A)	Egg masses desiccated
2002	0/0/0	No	None seen	Site dry
2003	0/1/0	Unk	1(A)	Site dry

Marginal site, subject to desiccation.

<sup>\*</sup>Larvae may have been preyed on by mallards and gartersnakes, but at least one from 2001 survived as a one-year-old toadlet in 2002.

Locality CF15 – Holywater Beaver Ponds (Cottonwood Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2002	3/3/3	Yes	1(M)	About 50 metamorphs
2003	5/1/1	Unk	2(1,A)	Some apparent egg loss

<sup>\*</sup>Site discovered on July 3, 2002. No adults or subadults observed, and egg count estimated.

# **EAGLE COUNTY**

Locality EA01 - Holy Cross City (Holy Cross City) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	1/1/1	Unk	1(A)	Predation & late season
1997	1/1/1	Unk	1(A)	Recruitment unlikely
1998	2/2/2	Unk	1(A)	Inadequate monitoring
1999	2/0/0	Unk	1(A)	Inadequate monitoring
2000	1/0/0	Unk	1(A)	Inadequate monitoring
2001	1/1/1	Unk	None seen	5 visits to site*
2002	2/1/1	Unk	1(A)	Breeding pond dried**
2003	2/1/1	Unk	1(A)	5 visits to site

<sup>\*</sup>Report of boreal toad tadpoles at this site in July 2001 by Bill Andree.

Locality EA02 - East Lake Creek (East Lake Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	1/1/1	Unk	3(M,S,A)	Site found 8/13/96
1997	Unk	Yes	Unk	Site not monitored
1998	3/0/0	Yes	2(1,A)	Inadequate monitoring
1999	4/4/4	Yes	3(M,1,A)	No night survey done
2000	2/2/2	Unk	3(1,S,A)	Minimal monitoring
2001	1/0/0	Yes	1(A)	Only one adult male
seen*				
2002	2/2/2	Unk	3(1,S,A)	14 adults seen (not sexed)
2003	2/2/2	Unk	3(M,S,A)	Likely many metamorphs

Two closely associated breeding sites at this locality.

Locality EA04 – Strawberry Lakes (Holy Cross City) Chytrid Status: Not tested

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2003	1/1/1	Unk	1(A)	100-500 tadpoles

<sup>\*\*</sup>In 2002, the breeding pond dried, probably before tadpoles could metamorphose.

<sup>\*</sup>Successful breeding in 2001 assumed due to 2 one-year-olds observed in 2002.

#### **GUNNISON COUNTY**

Locality GU03 - Magdalene Gulch (Texas Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	1/1/1	Unk	2(M,A)	Site found late in season
2000	2/1/0	Unk	1(A)	Adequate monitoring
2001	0/0/0	Unk	None seen	Inadequate monitoring
2002	0/0/0	Unk	None seen	One site visit
2003	0/0/0	Unk	None seen	Inadequate monitoring

\* \* \*

# **White River Plateau**

This geographic area includes southwestern Routt County, eastern Rio Blanco County, northeastern Garfield, and northwestern Eagle County. It includes the Flat Tops Wilderness and is situated primarily on the White River National Forest.

There are presently no known breeding sites in this area, although there have been reports of toad observations in recent years, primarily from the Trapper's Lake area. It is likely that one or more breeding sites may be located in this area, given adequate survey effort.

\* \* \*

## **Grand Mesa**

This area incorporates western Gunnison County, northern Delta County, and eastern Mesa County, and is located primarily on the Grand Mesa and Gunnison national forests.

Historically, boreal toads were abundant on the Grand Mesa. Extensive surveys have been conducted on Grand Mesa, but despite this effort, no confirmed observations of boreal toads were made for approximately 25 years. In 2002, two field crews working in the Buzzard Creek drainage of Mesa County observed a total of three adult boreal toads. Photographs were taken of two of the toads, confirming the identification. In addition, tadpoles were observed along the same reach of stream as two of the toads. However, the identification of the tadpoles as boreal toad tadpoles was not confirmed. Adult boreal toads were again seen in the Buzzard Creek drainage in 2003. Testing on these adults showed them to be chytrid positive. As of 2003, a breeding site has not been located in the Buzzard Creek drainage.

An experimental translocation of boreal toads to the Kannah Creek area in Mesa County was begun in 2003. Over 13,000 tadpoles and 816 toadlets were released at the site between June 25<sup>th</sup> and August 29<sup>th</sup>, 2003. Chytrid testing of resident chorus frogs revealed the site to be chytrid positive. This area is approximately 24 miles southwest of the toad locations along Buzzard Creek. In addition to the straight-line distance, several drainages occur between these two sites that would

impede movement of boreal toads and prevent contact between natural and translocated populations.

\* \* \*

# **Elk and West Elk Mountains**

This area consists of parts of western and northern Gunnison County west of Taylor Park, and southwest Pitkin County. It includes the Maroon Bells/Snowmass and West Elk wilderness areas.

Prior to 2000 there were three known boreal toad breeding sites in this area, one in southern Pitkin County, and the other two in northern Gunnison County. In 2000, new breeding sites were found on Brush Creek in Gunnison County, and on East Maroon Creek in Pitkin County. There have also been recent, reliable reports of toads from other localities within this area, such as Mt. Crested Butte, the Snowmass Lake area, near the town of Aspen, and in the Roaring Fork Drainage. With additional survey effort it is likely that more breeding populations will be located - especially in the Elk Mountains. However, no additional breeding localities were found during the 2003 field seasons.

#### PITKIN COUNTY

Locality PI01 - Conundrum Creek (Conundrum Creek) Chytrid Status: Positive (2001)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1995	3/1/1	Yes	2+(S,A)	Minimal monitoring
1996	1/1/1	Unk	2+(S,A)	Many metamorphs
1997	2/2/2	Unk	2(2,A)	Poor production
1998	2/2/0	Unk	1(A)	Inadequate monitoring
1999	0/0/0	Unk	Unk	Site not monitored
2000	2/2/2	Unk	2(M,A)	Adequate monitoring
2001	3/9/3	Yes	2(M,A)	100 metamorphs seen
2002	1/1/1	Unk	2(M,1)	Many metamorphs*
2003	0/0/0	Unk	None seen	-

<sup>\*</sup>No adults seen during many site visits, but at least one egg mass produced, resulting in hundreds of metamorphs.

Locality PI02 - East Maroon Creek (Conundrum Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2000	3/3/3	Yes	4(M,1,S,A)	Several ponds at site
2001	3/3/3	Yes	3(1,S,M)	Adults not observed
2002	3/3/3	Unk	4(1,M,S,A)	Breeding in 2 ponds
2003	3/3/3	Unk	3(M,S,A)	Numerous metamorphs

In 2001, about 3 egg masses deposited although adults were not observed; 16 subadults and about 50 metamorphs seen.

#### **GUNNISON COUNTY**

Locality GU01 - Triangle Pass (White Rock Mountain) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1993	3/3/3	Unk	1(A)	Metamorphs unlikely
1994	Unk	Unk	Unk	No data
1995	1/1/1	Unk	2(S,A)	Metamorphs unlikely
1996	Unk	Yes	Unk	No monitoring
1997	2/2/2	Yes	4(M,1,S,A)	Many metamorphs
1998	17/5/5+	Unk	4(M,1,2,A)	Many metamorphs
1999	19/5/4	Unk	2(M,A)	No night survey done
2000	13/13/13	Unk	3(M,S,A)	One subadult seen.
2001	18/14/11	Yes	2(M,A)	No night survey done
2002	16/17/16	Yes	3(1,S,A)	No visits after 7/25/02
2003	32/14/14	Unk	4(M,1,S,A)	Numerous metamorphs

This locality has also been referred to as "White Rock Basin".

Locality GU02 - West	Brush Creek	(White Rock Mountain)	Chytrid	d Status: Not tested
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Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1999	1/1/1	Unk	2(M,A)	<50 metamorphs seen
2000	0/0/0	Unk	None seen	Inadequate monitoring
2001	0/1/0	Unk	1(A)	Inadequate monitoring
2002	0/0/0	Unk	None seen	One site visit
2003	1/1/0	Unk	1(A)	One site visit

Locality GU04 - Brush Creek (White Rock Mountain) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2000	3/3/3	Yes	4(1,2,S,A)	Minimal monitoring
2001	6/1/1	Unk	3(1,S,A)	Minimal monitoring
2002	23/5/1	Yes	2(S,A)	Minimal monitoring
2003	7/2/1	Unk	1(A)	Minimal monitoring

\* \* \*

# **San Juan Mountains**

This is a large area in southern Colorado and northern New Mexico, which includes portions of Hinsdale, Archuleta, Mineral, Saguache, western Rio Grande, and Conejos counties in Colorado, and Rio Ariba County in New Mexico. It extends along the Continental Divide from Poncha Pass into northern New Mexico. Most of the boreal toad habitat in this area is located within the Gunnison, Rio Grande, San Juan, and Carson national forests.

Prior to 2000, there were only two known breeding sites in this area, and one of those two sites (Trout Creek) was questionable, as the tadpoles observed there in 1996 may have been the result of an unauthorized translocation from the Jumper Creek site, rather than natural breeding at that location. However, breeding at the West Trout Creek site (in Hinsdale County) supports the legitimacy of the Trout Creek observations.

There have been several good reports of observations of boreal toads from other localities in the San Juan Mountains, most notably from the Elk Creek drainage in Conejos County, Miner's Creek in Saguache County, and from near Chama, New Mexico. Survey efforts in these areas should continue.

# **MINERAL COUNTY**

Locality MI01 - Jumper Creek (Trout Creek)				Chytrid Status: Negative (2003)
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1994	3/0/?	Unk	1(A)	1st toad observation
1995	Unk	Unk	Unk	Breeding likely
1996	4/2/1+	Yes	2(M,A)	Breeding observed
1997	8/3/3	Yes	3(M,1,A)	Many metamorphs
1998	7/1/2	Unk	4(M,1,S,A)	
1999	3/2/2	Unk	3(M,S,A)	<50 metamorphs seen
2000	4/2/2	Yes	1(A)	Site dessicated
2001	4/1/1	Yes	3(M,1,A)	<50 metamorphs seen
2002	0/0/0	Yes	1(1)	Site dry; 3 1-yr-olds seen
2003	1/1/1	Unk	2(1,A)	dry before metamorphosis

Locality MI0	2 - Trout Creek (Trou	Chytrid Status: Not tested		
Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
1996	1/1/1(See note)	No	None seen	Tadpoles observed
1997	0/0/0	No	None seen	
1998	0/0/0	No	None seen	
1999	0/0/0	No	None seen	Only one site visit
2000	0/0/0	Unk	None seen	Minimal monitoring
2001	0/0/0	Unk	None seen	Minimal monitoring
2002	0/0/0	Unk	None seen	Minimal monitoring
2003	0/0/0	Unk	None seen	

NOTE: This site is questionable. 1996 observations may have been result of unauthorized transplant from Jumper Creek. No eggs, tadpoles, or toads have been observed during minimal monitoring efforts associated with site visits to West Trout Creek.

Locality MI03 – Roaring Fork Pond (Goose Creek) Chytrid Status: Negative (2003)

Year	M/F/Egg Masses	Recruitment	Age Classes	Comments
2000	1/1/1	Unk	2(M,A)	Site found late season
2001	3/0/0	Unk	1(A)	Minimal monitoring
2002	1/1/1	Unk	None seen	One egg mass; 2 visits
2003	3/0/0	Unk	1(A)	No breeding activity

Previously listed as Boots Pond; renamed here to conform to a CDOW database of pond names and NASRF records.

# **HINSDALE COUNTY**

Locality HI01 - West Trout Creek (Trout Creek) Chytrid Status: Negative (2003) Age Classes Year M/F/Egg Masses Recruitment Comments 2000 Site found mid-season 2/2/2 Unk 2(M,A)2001 4/4/4 Yes 4(M,1,S,A)Minimal monitoring 2002 Yes 2(1,A)1 visit, 6 1-yr-olds seen 1/1/1 100-200 metamorphs 2003 5/5/5 Unk 3(1,M,A)

\* \* \*

#### **BOREAL TOAD SURVEYS**

In addition to annual monitoring of known breeding sites, surveys of historic and other suitable boreal toad habitats are conducted each year. The amount of survey work has been constrained by the availability of qualified personnel to conduct and supervise the work and by limited funding. Areas where surveys have concentrated over the past five years include the Park Range, Front Range, Gore Range, Sawatch Mountains, Elk Mountains, and the San Juan Mountains in Colorado, Albany County, Wyoming, and Rio Arriba County, New Mexico. In 1999 a cooperative effort was initiated between the Colorado Division of Wildlife, Region 2 of the US Forest Service, and the Colorado Natural Heritage Program to conduct statewide surveys and a considerable portion of the breeding site monitoring work. Surveys since 1999 have resulted in the location of seven previously unknown breeding populations located in Routt, Eagle, Jackson, Grand, Gunnison, and Mineral counties, and fifteen new breeding localities within known populations in Routt, Chaffee, Grand, Gunnison, Summit, Clear Creek, Eagle, Jackson, Mineral, and Hinsdale counties.

In 2003, surveys for SRMP boreal toads in Wyoming resulted in observations at Bird Creek, Little Snake, North Fork Little Laramie, Ryan Park, Silver Run Lake, Sourdough Creek, and White Rock. Extensive surveys also were conducted in western Wyoming in 2003, yielding numerous toad and breeding site observations. Samples were collected for analysis to determine the relationships of these toads with those in the Southern Rocky Mountain population and to document chytrid fungus distribution in Wyoming.

In 2003, personnel from the Carson National Forest surveyed the Trout Lakes and Lagunitas areas of New Mexico. No boreal toads were observed

In 2003, CNHP crews surveyed 56 sites in 7 Colorado counties (Chaffee, Eagle, Gunnison, Lake, Larimer, Park, and Pitkin counties). Five of the surveys resulted in observations of one or more boreal toads.

Data regarding areas surveyed, where no toads were found, is in the process of being gathered from various sources and compiled, and will be used to help plan future survey efforts. Ongoing survey efforts will continue, with a focus on locations from which reliable reports of boreal toad observations have been received in the past two years. Sampling of populations for presence/absence of chytrid fungus will continue.

#### **PUBLIC INFORMATION & INVOLVEMENT**

Ongoing efforts to involve the general public in the search for boreal toad populations include the distribution of picture post cards, which provide basic information about the toad, and directions on how, and where, to report toad observations. In addition, toad "wanted" posters continue to be distributed to inform the public, and personnel in various resource management agencies, about the boreal toad, and to provide information on how and where to report toad observations. Reports of boreal toad observations resulting from the cards and posters have increased somewhat from previous years, indicating that the information is reaching more people.

In the vicinity of known boreal toad breeding populations, information is posted at camp grounds, trailheads, and near breeding sites on National Forest lands to inform recreationists about the presence of the toads, in an effort to prevent inadvertent or intentional damage to the toads and their habitat.

Several news releases and public information videos have been produced to help inform the public about the boreal toad and about ongoing conservation efforts. These have been well received by most news media, and widely distributed. In addition, a 30-minute slide presentation on the boreal toad and its management was produced, and continues to be presented to various groups.

#### CAPTIVE PROPAGATION & TRANSLOCATIONS

Reintroduction or translocation of animals are tools which may be used in the recovery of threatened or endangered species. These actions may involve captive propagation and/or rearing. Preliminary work with experimental translocations and captive rearing of boreal toads has been done in the Southern Rocky Mountains. However, it has been decided by the Boreal Toad Recovery Team that this approach will be used only in cases where no other viable alternatives exist to re-establish boreal toads in areas where they are known to be extirpated, and for experimental/research purposes. The following are the guidelines, as established by the Boreal Toad Recovery Team in 1997, to determine if/when translocations/reintroductions should be done:

- 1. Boreal toads are determined to be extirpated from a historically occupied mountain range, based on thorough surveys\*, and suitable habitat for toads still exists in that area. (\* Methodology outlined in the Boreal Toad Conservation Plan, 1998)
- 2. The chance of natural recolonization of the unoccupied area is minimal.
- 3. There is no known, significant and imminent environmental threat in the area which would preclude successful reintroduction and survival of boreal toads.
- 4. Available source stock of toads for transplants is sufficient to provide the numbers needed without doing harm to the source population(s).
- 5. There is a firm commitment from involved agencies to make the reintroduction effort a top priority for long-term funding, and to do long-term monitoring and evaluation. Ideally, such

commitment should be stated in the form of a Cooperative Agreement or Memorandum of Understanding.

In light of the recent discovery of the presence of the chytrid fungus in Colorado, and ongoing research, these guidelines were reviewed by the Boreal Toad Recovery Team with minor revisions.

# **Captive Propagation and Rearing**

During the early 1990's, techniques and procedures for captive rearing and breeding of boreal toads were developed by the Wyoming Game & Fish Department and the Colorado Division of Wildlife. At the Sybille Wildlife Research Center, in Wyoming, boreal toads were reared in conjunction with efforts to raise captive Wyoming toads, and captive reared boreal toads were subsequently released at the Lake Owen site (see 'Experimental Translocations,' below). In Colorado, a small number of tadpoles were reared to toadlet stage at the University of Colorado in 1993 and 1994, for a subsequent experimental release in Boulder County (see page 43), and numerous toads were reared in captivity by the Colorado Division of Wildlife, at its Fish Research Hatchery in Bellvue, CO, from 1995 through 1997. The Division of Wildlife effort resulted in development of standard practices for rearing of boreal toads, and the "Hatchery Manual for the Rearing and Propagation of Captive Boreal Toads" was produced in March 1997. Captive propagation and rearing of toads in Colorado was discontinued in late 1997, with the intent of reinstating it only if it is needed for a future reintroduction.

After the recent discovery of chytrid fungus in Colorado, and the associated die-off of boreal toads in Clear Creek County in 1999, the Recovery Team decided it would be prudent to establish diseasefree captive stocks of boreal toads from several key populations in the Southern Rocky Mountains. The primary location for housing of this captive stock presently is the Colorado Division of Wildlife's Native Aquatic Species Restoration Facility (NASRF), near Alamosa, CO. In order to minimize risk of losing all captive stock to an unforeseen die-off or accident, and to promote more effort towards development and testing of captive propagation and rearing techniques, selected stocks of toads are also housed at several other facilities, including the Saratoga National Fish in Wyoming, and at various AZA certified zoos, including, as of January 2003, the Henry Doorly Zoo (Omaha, NE), the Cheyenne Mountain Zoo (Colorado Springs, CO), the Cincinnati Zoo (Cincinnati, OH), Morrison Museum of Natural History (Morrison, CO), Ocean Journey (Denver, CO), and the Toledo Zoo (Dayton, Ohio). The primary purpose of establishment of captive stocks is to preserve genetic diversity in the event of catastrophic die-offs. Secondarily, captive stocks will be used to develop and test propagation and rearing techniques, and to provide source stock for possible future reintroductions to areas where the species has been extirpated. In December 2002, revised husbandry methods for NASRF were summarized in the "Native Aquatic Species Restoration Facility Boreal Toad Husbandry Manual."

## Colorado Native Aquatic Species Restoration Facility (NASRF)

Currently 552 toads are at NASRF, of which 492 are being hibernated during the winter of 2003-2004. NASRF houses representatives from 18 different boreal toad breeding localities throughout the state. In the spring of 2003, NASRF personnel were unable to obtain successful reproduction from captive stock.

## Saratoga National Fish Hatchery

On December 18, 2000, Saratoga National Fish Hatchery (SNFH) received official notification of approval from the Director, U.S. Fish and Wildlife Service, to house in refugia and breed Boreal toads (*Bufo boreas boreas*). Due to the increased loss of boreal toads housed at Sybille Wildlife Research Center (Sybille), the Wyoming Game and Fish Department, along with the Boreal Toad Recovery Team, made a decision to move all remaining captive populations from Wyoming of the Southern population Boreal toads to SNFH. The Hatchery received 1 male and 3 female Bird Creek boreal toads on December 12, 2001.

On July 28, 2001, at the direction of the Wyoming Game and Fish Department, a private landowner from Ryan Park delivered a female boreal toad to SNFH. In July 2002, U.S. Forest Service employees delivered 3 juvenile boreal toads to SNFH. One of these toads died in March of 2003 with a ruptured stomach, leaving one female and one male from this group.

Four more juvenile boreal toads were delivered by the U.S. Forest Service during the summer of 2003 from Ryan Park. There is one female and 3 males in this group.

In July 2003, the U.S. Forest Service delivered 5 juvenile boreal toads from Sourdough Creek. Three of these toads died between August 12<sup>th</sup> and August 15<sup>th</sup>. The necropsy report from Dr. Allen Pessier states that they died from chytridimycosis. While the possibility that infections were acquired in captivity at Saratoga N.F.H. can not be completely excluded, strong consideration should be given to the possibility of chytridimycosis in wild populations of boreal toads in Wyoming.

Boreal toads at Saratoga National Fish Hatchery (as of March 2004)

	Male	Female	Unknown
Bird Creek boreal toads	1	3	0
Ryan Park boreal toads	4	3	0
Sourdough Creek boreal toads	1	1	0

# Cheyenne Mountain Zoo

In 1993, personnel from the Cheyenne Mountain Zoo, in Colorado Springs, collected three yearling toadlets and 17 tadpoles from the Denny Creek breeding site, in Chaffee County, Colorado. These tadpoles were reared to metamorphs at the zoo, and some were over-wintered in a Percival Environmental Chamber. As of late 1997, all boreal toads at the Cheyenne Mountain Zoo had died due to unknown causes.

In 2000, the Cheyenne Mountain Zoo, in cooperation with the Colorado Division of Wildlife, has revived its effort to captive rear boreal toads. Twenty toads (10 from each of two different lots of

eggs collected at Hartenstein Lake, and reared at the CDOW's Native Aquatic Species Restoration Facility) have been provided to the Cheyenne Mountain Zoo for captive rearing and propagation work. As of September, 2002, all these toads remained alive.

### Henry Doorly Zoo

Due to the limited number of known breeding boreal toads remaining in the San Juan Mountain area as of the mid 1990s, it was thought advisable to attempt to establish a captive brood stock of boreal toads from that geographic area. In 1996, the Henry Doorly Zoo, in Omaha, Nebraska, obtained boreal toads from Colorado for experimental propagation projects. Forty toadlets, originating from Mineral County, Colorado, were sent to the zoo. Most of these died within the first two to three months due to unknown causes. As of late 1997, three boreal toads (one male and two females) remained in captivity at Henry Doorly Zoo. Unfortunately, these three toads died of unknown causes in 1998. The CDOW provided 10 metamorph toadlets, taken from the Jumper Creek site in Mineral County, to Henry Doorly Zoo in August, 1998, to be used for further captive rearing and breeding work. Ten additional toadlets from 2000 egg masses were sent to Henry Doorly Zoo. As of March 2004, a total of nine toads were still alive.

#### Toledo Zoo

In October, 2000, one lot of 10 toadlets from the North Fork of Morrison Creek breeding locality, and one lot of 12 toadlets from the West Trout Creek breeding locality were sent to the Toledo Zoo, in Ohio. As of September 2002, thirteen toads were alive and in good condition.

In addition to the toads at the locations mentioned above, there are boreal toads at several other sites, primarily being used for educational, display, and research purposes. These include (1) Colorado's Ocean Journey, in Denver, (2) Colorado Division of Wildlife, in Ft. Collins, (3) the Morrison Natural History Museum, in Morrison, and (4) the Cincinnati Zoo, in Cincinnati, Ohio. Some toads will also be provided to specific members of the IRCEB (Integrated Research Challenges in Environmental Biology - National Science Foundation) group, for essential research on the chytrid fungus.

The Boreal Toad Recovery Team plans to work in cooperation with the AZA and various accredited zoos to initiate a "stud book" database for the purpose of tracking all captive Southern Rocky Mountain boreal toads and their progeny.

#### **Experimental Translocations**

Prior to the development of specific guidelines for translocations and reintroductions of boreal toads, in 1999, some translocations did take place. Although these were, in general, done according to acceptable standards, they did not follow strict and consistent protocols, which should be adhered to for any future translocations.

In August of 1993 and 1994, 44 and 200 boreal toadlets, respectively, were released near **Caribou**, in western Boulder County, CO, to determine if such releases could ultimately result in creation of a new breeding population at a site at which toads historically existed, but at which no toads had been

seen in 20 years. The source of the tadpoles was a breeding site along Interstate 70, west of Denver, in Clear Creek County. The toadlets were released about a month after metamorphosis. They were fed as much as possible during the entire time they were being raised in order to maximize their growth and their chances of surviving the first winter. One-day surveys in 1995 and 1997 indicated that sub-breeding sized individuals were still present in the area. In 1998, males from the first cohort should have been of breeding size. No surveys were conducted in the area in 1998, and brief surveys in 1999 and 2000 failed to find any toads at the site.

Glacier Basin, in Rocky Mountain National Park, was the site of an experimental translocation of boreal toads, which began in 1995. It is a cooperative effort between Rocky Mountain National Park and the USGS/Biological Resources Division. Toadlets (n=800) were released in 1995, and egg masses and 100 captive-reared toads were translocated in 1996. The stock for this transplant came from the Lost Lake breeding site, in Rocky Mountain National Park (See Muths et al., 2001).

From 1997 through 2000, NPS and USGS/BRD staff continued to monitor the Glacier Basin site. No egg masses or tadpoles have been found to date. Although three adult female toads were observed in 1999, no male toads or breeding activity were seen. Surveys were conducted in the Glacier Basin area in 2000, but no toads or breeding activity were observed.

In 1995, 1996, and 1997, several thousand boreal toad toadlets, and several adult toads, and some tadpoles were released at **Lost Lake, Boulder County**, to determine if translocation of large numbers of young toads is an effective reintroduction method, to monitor the dispersal behavior and habitat use by the reintroduced toadlets, and to assess the survival rates of various age classes of toads. The transplanted animals originated from eggs taken from the Henderson Mine site, in Clear Creek County, and reared at the CDOW's Research Hatchery, in Bellvue, CO (see Loeffler, ed. 1999 for a complete report). This locality will continue to be monitored for several years to determine the result of the translocation. No toads have been observed at Lost Lake since 1999, although some monitoring has continued through 2003.

In Wyoming, an experimental reintroduction at the **Lake Owen** site, in Albany County, was initiated. In 1996, 4000 captive reared tadpoles, which originated from eggs taken at the Bird Creek breeding site, were released at Lake Owen. In 1997, an additional 1500 captive-reared tadpoles were released, and three one-year-old toads were observed, indicating that there was some survival of toadlets from the 1996 release. No additional toads have been released since 1997, but plans are to monitor the site for the next few years to determine the success of the reintroduction effort. Surveys at the site in 2000-2002 found no toads or sign of breeding activity.

Love Lake, in Mineral County, CO, was the site of a release of approximately 300 newly metamorphosed toadlets in early August, 1996. These were captive reared toadlets from tadpoles collected at the nearby Jumper Creek site in Mineral County. Subsequent searches during late summer of 1996 found some live and some dead toadlets at the site. No toadlets were seen during surveys at the site since 1996. Monitoring at this location should continue, however, due to its relative proximity to the Trout Creek population.

Grand Mesa, in western Colorado, was intensively surveyed from 1997 to 1999, and is a high priority site for an experimental reintroduction of boreal toads. In addition to intensive aquatic habitat mapping, approximately 780 hours of inventory effort was expended in historically occupied habitats on Grand Mesa in 1998. No toads, eggs, or larvae were found. Six potential reintroduction sites were selected from 80 possible sites, using standardized criteria. Administrative groundwork for initiation of an experimental translocation was started in early 1999, but the project was put on hold due to the finding of chytrid fungus in Clear Creek County, and evidence of the presence of chytrid fungus in at least two other populations. Some initial testing of resident amphibians has been conducted at the Kanah Creek drainage (Mesa County), with no chytrid positive specimens of tiger salamanders or chorus frogs in 2002. During a January, 2003 meeting of a subgroup of the Boreal Toad Recovery Team and Technical Advisory Group, it was determined that an effort would be made to experimentally translocate eggs and/or tadpoles derived from Hartenstein Lake (Chaffee County) in 2003. Between June 25<sup>th</sup> and August 29<sup>th</sup>, 2003, over 13,000 tadpoles and 816 toadlets were released at the Kannah Creek site as part of a CDOW research project led by Kevin Rogers. Continued testing of resident chorus frogs revealed the site to be chytrid positive. Research at the site will continue in 2004.

\* \* \*

#### RESEARCH

# **Chafee County Mark-Recapture Study**

Brad Lambert, Colorado Natural History Program (CNHP), Ft. Collins, CO

In 2003, CNHP continued a mark-recapture study in the Cottonwood Creek drainage in Chaffee County. The following breeding sites were monitored with multiple visits to collect data on the adult populations for the study: Collegiate Peaks Campground, Denny Creek, South Cottonwood, South Cottonwood West, Morgan's Gulch, Rainbow Lake, Hartenstein Lake, Holywater beaver ponds and Middle Cottonwood Creek. The purpose of this study is to collect baseline data for evaluating population size and trends, and to detect toad movement between breeding sites.

Since 1998, 826 adult males and 220 adult females have been tagged in the Middle Cottonwood Creek and South Cottonwood Creek drainages. The adult captures continued to be high at the Denny Creek/Hartenstein Lake sites and at the South Cottonwood Creek sites. The Middle Cottonwood Creek site has had a dramatic increase in high counts of adult toads over the last two years. There has been no apparent decline in the Cottonwood Creek metapopulation over the last five years, although breeding success and adult high counts have fluctuated from year to year at several breeding sites. In 2003, successful reproduction was reported at a majority of the breeding sites and chytrid fungus testing by CDOW came up negative for the breeding sites in Chaffee County

Preliminary analysis of the data collected suggests that boreal toads from the Chaffee County sites show a high degree of breeding site fidelity. Despite most of the breeding sites in the Cottonwood Creek drainage being closely situated, only 15 recaptured toads out of the 1,734 total recaptures have been captured at different breeding sites. The data reveals that, although rare, there is movement by toads along the Middle Cottonwood Creek sites and between the South Cottonwood Creek and South Cottonwood Creek West sites. One notable adult male was tagged at Collegiate Peaks Campground in 1999 and was recaptured in 2002 at the South Cottonwood Creek site approximately 8 km away. This is the first time there has been evidence of movement between the population in the Middle Cottonwood Creek drainage and the population in the South Cottonwood Creek drainage.

As a result of the mark-recapture study, breeding cycles in females have also been examined. Data from Chaffee County 1998-2003 shows evidence that females are likely to skip a year or more in between breeding; assuming that a breeding site visit by an adult female in the spring equals a breeding attempt. There have been 29 recaptures of adult females in separate years: 35% were captured in consecutive years, 59% were captured in alternate years, and 6% were captured after an absence of two or more years from the breeding site. The majority of females captured in consecutive years were at the Denny Creek site, which also appears to have a larger female resident population then other sites in Chaffee County.

Currently, the data from 1998-2003 seasons are being pooled to see if survival estimates can be obtained through an open population model, such as Cormack's Jolly Seber model. Some modeling exercises are also being conducted to see if the 2001-2003 data can be fit into a robust design closed population model for survival rates, and population estimates. Air temperature and water temperature at the time of sampling will be looked at as variables that may impact the capture-recapture probabilities.

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# Boreal Toad Research & Monitoring in Rocky Mountain National Park - 2003

Erin Muths, USGS/BRD, Ft. Collins, CO; submitted March 2004

Boreal toads have been monitored in the North Fork Drainage of the Big Thompson River since the early 1990s (Corn et al. 1997). The populations in this drainage (Kettle Tarn and Lost Lake, possibly one metapopulation) crashed between 1995 and 1998 (Corn et al. 1997, Muths et. al. 2003). We have continued to monitor these populations and began intensive monitoring at Spruce Lake, in 2000. In 2001 we detected boreal toads (1 individual near Ypsilong Lake. Two of the four known boreal toad populations are in severe decline (Muths et al. 2003), one of these is likely extirpated; one population is very small and there is very little know about the fourth. Efforts are directed at site protection, monitoring and the completion of the amphibian health evaluation project.

Lost Lake was visited 4 times and no boreal toads were detected. Lake Husted and surrounds were visited once and no toads were found. Kettle Tarn was visited 12 times; boreal toads were detected on 10 of 12 visits. Five individuals were found at Kettle Tarn (Table 1). Two egg masses were observed at Kettle Tarn and at least one hatched, producing thousands of tadpoles and eventually metamorphic boreal toads that were observed on 7 August by personnel from the USFS. Kettle Tarn did not return to its pre-drought level this year, but water remained at the site longer than in 2002. Kettle Tarn was reported dry on 24 August except for 2 damp puddles and metamorphs were present (L. Livo, pers. com.). We visited Kettle Tarn on 16 September. The tarn was completely dry and we found no metamorphs or adults. The campsite at Kettle Tarn was closed early in the spring/summer season and we recommend that the closure continues next year.

Spruce Lake was visited 10 times and boreal toads were detected on 9 visits. Over the course of 3 years we have captured 6 individual female toads and 23 individual male toads. Based on observations and the number of toads we have recaptured, the population at Spruce Lake appears to be relatively small. To date, there is no evidence of chytrid fungus at Spruce Lake (D.E. Green, DVM, pers. comm.). We recommend maintaining the early season (15 May – 15 July), partial closure of Spruce to protect nursery habitat and to continue to encourage anglers to bleach their waders and other equipment before visiting Spruce Lake. We are currently working on our analyses of capture – recapture data for Spruce Lake, although data are sparse and care must be taken in interpretation. The master's degree project, An analysis of the possible causes of decline in a

metapopulation of boreal toads in Rocky Mountain National Park: An information-theoretic approach and population viability analysis, by Rick Scherer, is completed pending his oral defense in April 2004. Two publications derived from this work are currently in preparation.

We found boreal toads in small, unnamed ponds northeast of Ypsilon Lake and northeast of Fay Lakes (Roaring Creek Drainage, RMNP). One adult female toad was observed at this site in 2002. This year we found 1 female, 2 males and > 200 tadpoles. One specimen (tadpole) was collected and preserved to confirm the identification of *Bufo boreas*. Three non-lethal skin scrapings were taken from 3 individual boreal toads (1 female and 2 males) at this site. Samples were submitted to Pisces Molecular (Boulder, CO) for PCR analysis to determine the presence of chytrid fungus. All three samples were negative for chytrid fungus infection.

#### Relevant Publications in 2003:

- Rittmann, S. E. Muths, and D.E. Green. 2003 *PSEUDACRIS TRISERIATA* (Western Chorus Frog) and *RANA SYLVATICA* (Wood frog). CHYTRIDIOMYCOSIS *Herpetological Review* 34(1): 53.
- Muths, E., P.S. Corn, A.P. Pessier and D.E. 2003. Green Evidence for disease related amphibian decline in Colorado *Biological Conservation* 110 (2003): 357-365.
- Muths, E. 2003. Homerange and movements of boreal toads in undisturbed habitats Copeia 2003 (1): 161-165.

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# Repatriation of boreal toads Bufo boreas on the Grand Mesa, Colorado

Kevin Rogers and Tanya Banulis, CDOW

This study explores the efficacy of introducing various boreal toad *Bufo boreas* life stages for establishing a new population in what was thought to be a chytrid negative site on the Grand Mesa, Colorado. While initial testing of resident amphibians (chorus frogs *Pseudacris triseriata* and tiger salamanders *Ambystoma tigrinum*) for chytrid by the PCR test revealed no infection in 2002, subsequent tests on older chorus frogs did come back positive in 2003. The study site lies in the Kannah Creek drainage on the southern end of the Grand Mesa in Mesa County, Colorado. The site is comprised of half a dozen small ponds in close succession that provide a number of potentially suitable breeding areas with excellent breeding shallows. Willow (*Salix* sp.) and large boulders surround the immediate area.

Approximately 20,000 eggs from 14 clutches were harvested from the Chaffee County boreal toad metapopulation in late May of 2003. These eggs were brought to the Native Aquatic Species Restoration Facility (NASRF) in Alamosa, CO and reared to a Gosner stage 25. Genetic material from each clutch was preserved for future analysis if necessary. Over 12,000 tadpoles were released unmarked around the margins of three ponds at the study site on June 25th. An

additional 1200 were divided among six pens (two pens per pond), raised to metamorphosis, toe clipped for future identification, and released. Remaining tadpoles were kept at NASRF until approximately 3 weeks post metamorphosis. Eight hundred sixteen toadlets were then given a different toe clip and divided among the same three ponds on August 29th. Production problems prevented the release of a group raised to metamorphosis in the hatchery as planned.

We had excellent success raising tadpoles to metamorphosis in the pens, with all but one pen having >80% surviving to metamorphosis. Of the mortalities, most were directly attributable to desiccation rather than inadequate husbandry practices. Metamorphosis was initiated the last week in July, and was completed by mid-August. Average water temperature in the pens over that time (release date to metamorphosis) was 17.4 C

# Monitoring:

The reintroduction site was monitored weekly with a modified line-transect survey protocol. Randomly placed transects radiated perpendicularly from the perimeter of each pond and extended for 100 m. When toadlets were observed, the location (distance from line), substrate, length (snout-vent), mass, and presence and condition of toe clips was evaluated. Although the origin of any young boreal toads found this coming field season will be used to evaluate which life stage is best for repatriation efforts, we did notice that pen raised toadlets captured in late August were only half as heavy as those tadpoles released directly into the wild. The ability to behaviorally thermoregulate apparently was critical for maximizing growth, as their counterparts in the pens were fed *ad libitum*.

Unfortunately, the line-transect methodology did not appear to be a viable approach for quantitative estimation of population size or emigration, as we were not able to identify all toadlets on the survey line. This combined with the very low number of individuals spotted on each transect, and the risk of squashing those individuals on the line that were not detected, caused us to suspend this monitoring approach midway through the study. It should be noted that the habitat surrounding the ponds was not complex. Future repatriation efforts in more complex habitats will likely have even more difficulty implementing a line-transect monitoring scheme.

# Chytrid testing:

All amphibians encountered during monitoring were tested for infection by chytrid fungus with the following test: the ventral surface of each individual was scraped with a wooden applicator which was then preserved in 70% ethanol (as per Livo et al. in this document). Each skin scrape sample was mixed then spun at approximately 16,000 G for 3 minutes. The supernatant was drawn off and discarded, while any pellet was resuspended with the addition of tissue lysis buffer and vortexing. Total DNA was extracted from all samples using a spin-column DNA purification procedure. All sample DNA preparations were assayed for the presence of the *Batrachochytrium dendrobatidis* ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle single-round PCR amplification with appropriate controls. A third of the 39 chorus frogs collected from the Kannah Creek site in June tested positive for chytrid fungus. These results were very disappointing, especially after samples collected in 2002 showed no sign of chytrid infection. By the end of the summer it became apparent that tiger salamanders and juvenile chorus frogs are not

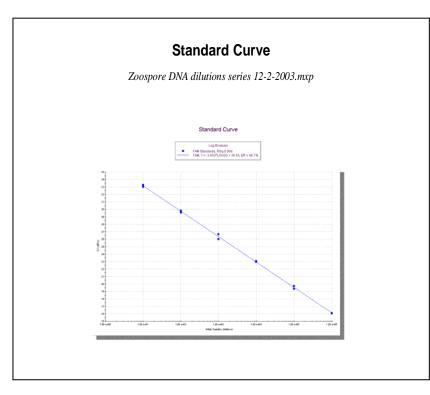
good sentinel animals for evaluating chytrid presence (L. J. Livo, personal communication). As such, our subsequent testing focused on adult chorus frogs well as released boreal toads. It appeared that infection of adult chorus frogs did diminish over the course of the summer, giving renewed optimism toward the persistence of the newly introduced population. In addition, over 100 skin scrapes from introduced boreal toads acquired over the course of the summer also tested negative for the disease. Infection levels in the resident chorus frog population will be monitored closely in subsequent years to examine if the infusion of large numbers of susceptible boreal toads can aggravate infection in the chorus frog population.

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# Developing qPCR Based Environmental Testing Procedures for *Batrachochytrium dendrobaditis*

John Wood, Janet Epp, Patrick Power, Pisces Molecular LLC

Our work during the past twelve months has focused on developing and testing a real-time PCR assay for measuring the presence of *Batrachochytrium dendrobaditis* in environmental samples. This work has been divided into two parts: The first part has been to develop and test the parameter of a quantitative, real-time PCR assay; these experiments are complete. The second part of our work has been to devise sample collection and DNA extraction protocols for a variety of different types of samples from the environment; this work is ongoing.



We examined the DNA sequence of the Batrachochytrium dendrobaditis ("B.d.") ribosomal RNA gene cluster Intervening Transcribed Sequence ("ITS") region determined by Seanna Annis and chose a small portion of the DNA sequence as a target for the fluorescently labeled probe molecule used in quantitative PCR ("qPCR") procedures. Because of their demonstrated sensitivity and specificity in our regular, nonquantitative chytrid PCR assay, we decided to use the same primer sequences for the qPCR assay and design a probe within the region between these two primers. The chosen probe sequence ("ITS-1) was has been tested with purified

B.d. (type strain JEL270 from Joyce Longcore) DNA and functions as expected.

After optimization of the reaction parameters and using serial dilutions of laboratory grown and purified *B.d.* zoospores, we were able to demonstrate that the reaction is close to theoretical maximum efficiency (95+% out of 100%), and log linear across five orders of magnitude (figure left). The minimum sensitivity of the assay is equal to or better than 10 *B.d.* zoospores in a 5 ml sample. We compared our qPCR assay with the assay developed by Alex Hyatt's group in Australia during the IRCEB meeting in Tempe November 19-22, and found that the assays are very similar. Hyatt's group had not used their qPCR assay on non-amphibian, environmental samples yet.

The second part of our work has been to develop sample collection and DNA extraction protocols for environmental samples. Although many amphibian researchers feel that the rapid spread of chytrid infections among amphibian populations worldwide suggests that an alternative host or vector for transmission of *B.d.* must exist, to date there is no concrete demonstration of the presence of *B.d.* in the environment except in/on amphibians - which makes our experiments more difficult since there are no clues about where to look, or what level of sensitivity we need to detect. So far we have tried three different kinds of samples – water filtrates, mud, and insects – from known (amphibian) chytrid positive areas with negative results.

During earlier experiments with Lauren Livo, we had shown that it was possible to trap laboratory grown B.d. zoospores added to pond water on Millipore or glass fiber filters, extract and purify DNA from the filters, and get chytrid positive PCR results. Although these experiments suggested that it might be difficult to filter a sufficient volume of water for good sensitivity before the filter clogged with particulates, the positive PCR results encouraged us when we got twelve filter paper fragments from Karen Lips and Matt Whiles at Southern Illinois University. These filters were used to filter stream water to measure sediment load and composition at different known chytrid positive locations in Costa Rica. The DNA extraction procedure we used for these samples was based on the water filtrate sample procedure we use for trout Whirling Disease (WD) filtered water samples. Because we added "carrier" DNA to these samples (salmon sperm DNA, to prevent losses of sample DNA by sticking to the plastics used in the procedure), we could not directly check the quantity of sample DNA we extracted from these samples, but our procedure has worked very well for thousands of WD filtered water samples, so we are confident worked for these samples as well. We have found that DNA extracts from filtered water samples frequently contain humic acid related potent PCR inhibitors, so we checked one of these filter paper DNA extracts for PCR inhibition by spiking. The spiked sample was negative – indicating that the DNA sample was PCR inhibited - so we treated all twelve of the DNA samples with an additional step to remove PCR inhibitors. Subsequent to this treatment, we re-spiked three of the treated DNA samples and tested them in another PCR run – all three were now chytrid PCR positive. However, again unfortunately, all twelve of the treated (but unspiked) DNA samples were chytrid PCR negative.

We have also developed a preliminary protocol for extracting DNA from mud samples. The principal problem with mud samples has always been volume: DNA extraction spin columns are limited to approximately 0.5 ml in sample volume, which is too small of a volume to get any

useful level of sensitivity with field collected mud samples. The procedure that we have come up with relies on zoospores having a different, and slower, settling rate than sediment particles. By uniformly mixing mud in a larger volume of water (or 70% ethanol which we have recommended as a sample preservative) then letting the largest sediment particles settle relatively rapidly, the upper volume of liquid can be removed and centrifuged without getting a large volume pellet. If zoospores settle more slowly than sediment, they will still be suspended and removed with the upper liquid, and can be concentrated by centrifugation.

As a test of this procedure, we prepped and tested 6 mud samples from Lauren Livo. The samples were 10 to 15 mls each of mud/sediment preserved in a larger volume of 70% ethanol. The samples were mixed by shaking, allowed to settle for three minutes, then the supernatant liquid removed to clean 50 ml centrifuge tubes. The centrifuge tubes were spun at 1000 x G for three minutes, then all of the supernatants except the last 0.5 ml removed with a pipette and discarded. The remaining liquid and any pellets were mixed, then transferred to clean microcentrifuge tubes, and total DNA extracted with our standard spin-column DNA purification procedure.

Although these sample volumes were still small -10 to 15 mls of mud and 10 to 20 mls of 70% ethanol, these quantities can be scaled up considerably with our existing centrifuges - up to 250 to 500 mls of mud and 250 mls of 70% ethanol.

With these preliminary procedural details worked out, we can now test the assumption that zoospores will settle more slowly than sediment particles. By mixing lab grown, and counted, zoospores into 70% ethanol, or mud plus 70% ethanol, letting the mixture settle for different lengths of time, then removing aliquots and testing these with our chytrid qPCR procedure, we can determine the rate at which zoospores settle. (According to Joyce Longcore, little information is currently available about *B.d.* zoospore buoyant density).

Finally, although Lauren's mud samples were from known chytrid positive ponds, all of our PCR results were negative. We checked to make sure that the PCR results were true negatives, and not simply negative due to PCR inhibition, by spiking known positive chytrid DNA into aliquots of the mud sample DNAs – and all of the spiked samples were chytrid PCR positive, indicating no PCR inhibition

In collaboration with Kevin Rogers and Suzanne Rittmann, we have also chytrid PCR tested a variety of insects from known chytrid positive areas. The details of these experiments are reported in Kevin Rogers' report. Unfortunately, these insect samples also tested uniformly negative for the presence of *B.d.* DNA.

Our plans for continuing work will focus on refining our current protocols, and testing a broader number of samples, towards the end of either finding chytrid positive environmental samples, or proving a sufficient level of sensitivity in our protocols and total number of samples tested to convince ourselves and others that *B.d.* does not have a non-amphibian host or vector. Specifically, with Chauncey Anderson, a USGS hydrologist in Portland Oregon, we are exploring alternative water filtration techniques, such as tangential flow filtration, that can process larger

quantities of water without clogging. Alternatively, we are considering the possibility of developing an *in situ* cell lysis and DNA extraction protocol that could avoid the clogging problem by rendering DNA immediately soluble in a sample, then extracting and purifying the DNA from this larger sample volume. We also hope to explore the possibility of using algae, or other macroscopic "biomass" as fortuitous "filters" for *B.d.* zoospores, based on the chance observation by Rick Spears in Australia that zoospores in lab culture appeared to stick to algae.

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# Surveys of *Bufo boreas* and other Colorado amphibians for *Batrachochytrium dendrobaditis*, 2003

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A chytrid fungus, *Batrachochytrium dendrobatidis* (referred to here as BD),has been associated with amphibian population declines and extinctions at many locations throughout the world. In Colorado, populations of the boreal toad (*Bufo boreas*) have been found to be susceptible to infection by this pathogen. In an effort to determine geographic distribution and prevalence of this fungus within Colorado, sampling of Colorado amphibian populations for BD continued during the 2003 field season, with efforts concentrated on boreal toad populations.

Sixty-four boreal toad localities were visited in 2003, and samples obtained from a total of 417 boreal toads at 46 of these sites. In addition, 189 samples were obtained from 7 additional amphibian species (see Table 1).

Thirty-three boreal toads from 8 localities tested positive via PCR for BD in 2003. Positive boreal toad breeding sites (based on all results since 2000) are:

Urad Valley sites (Clear Creek County)
Pole Creek (Grand County)
McQueary Lake (Grand County)
Kettle Tarn (Larimer County)
Twin Lake (Larimer County)
Buzzard Creek (Mesa County)
Conundrum (Pitkin County)
Torso Creek (Routt County)
Upper N. Fork Snake (Summit County)

Peru Creek (Summit County)

Note that all currently known breeding sites were visited at least once in 2003, although toads were not found at all sites.

At BD-positive sites, adults have high rates of infection: at sites with one or more animals testing positive in 2003, 33 of 43 adult or juvenile toads were positive for this pathogen, yielding a prevalence rate of 77%. One important finding is that boreal toad metamorphs, even at known BD-

positive sites, often are BD negative around the time of metamorphosis. For example, in 2003, 20 metamorphs at Torso Creek were BD negative, despite 5 of 5 boreal toad adults having been positive earlier in the season at the same site. Metamorphs at Kettle Tarn (n=20) and Pole Creek (n=15) were also BD negative this season. On the other hand, it is possible to encounter BD positive metamorphs: 8 of 11 metamorphs at Pole Creek were BD positive in 2002, perhaps after being able to move around in the terrestrial habitat for a longer time prior to sampling.

Previous PCR testing of sentinel tadpoles had very low rates of positive tests, even with tadpoles placed at known BD-positive sites. Consequently, neither boreal toad tadpoles, nor boreal toad metamorphs are particularly good individuals to sample when trying to determine the BD status of a site.

Table 1. Samples obtained from amphibians during 2003.

Species	Number of samples	Positive samples	Negative samples	Overall prevalence
Bufo boreas	417	33	384	8%
Bufo cognatus	10	0	10	0%
Bufo woodhousii	10	0	10	0%
Pseudacris triseriata	127	27	100	21%
Rana pipiens	9	3	6	33%
Rana sylvatica	3	0	3	0%
Spea bombifrons	5	0	5	0%
Ambystoma tigrinum	25	0	25	0%
TOTAL	607			

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## A look at aquatic macroinvertebrates as reservoirs of chytrid infection

Kevin Rogers and Suzanne Rittman, CDOW John Wood, Pisces Molecular LLC

Since chytrid fungus *Batrachochytrium dendrobatidis* can persist at a location even in the absence of amphibian species, we suspect that amphibians are not the primary host, and that infection can be maintained through other organisms. As aquatic insects contain a large amount of chitin that the chytrid fungus degrades, it is not unreasonable to assume that they may also harbor chytrid. A preliminary look at aquatic macroinvertebrates as reservoirs of chytrid infection was initiated by collecting insect samples from two known chytrid positive sites. Samples were collected from Hesbo Pond on the Henderson Mine and Ranch Pond #4 on the Pole Creek Golf Course, CO in early August of 2003. These samples included a variety of families such as *Notonectidae*, (backswimmers), *Coenagrionidae* (damselflies), *Dytiscidae* (diving beetles), and *Hydrophilidae* (water scavenger beetles).

The standard sample digestion and DNA extraction procedure was modified slightly to accommodate these sometimes relatively large, and hard chitinous shelled insects. We found that the detergent and proteinase based tissue lysis buffer did not break down the chitin exoskeleton, even after an extended incubation time. However, physically breaking apart or puncturing the insect exoskeleton did allow the lysis buffer to digest the softer internal tissues and release the cellular contents, and would have presumably released chytrid DNA on the surface of the exoskeleton as well. The remainder of the procedure followed the standard spin column DNA extraction protocol. We checked the quantity and quality of DNA extracted from all the different types of insects, and all yielded desirable high molecular weight DNA. All sample DNA preparations were assayed for the presence of the B. dendrobatidis ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle singleround PCR amplification using the assay developed by Seanna Annis and modified for greater specificity and sensitivity at Pisces. No evidence of chytrid was detected in these DNA samples. An aliquot of DNA from one sample of each of the insect types was spiked with known chytrid positive DNA (JEL270), and tested by PCR to make certain that the extracted insect DNA samples did not contain PCR inhibitors, which could cause false negatives. All spiked samples were chytrid PCR positive, indicating that the original unspiked DNA samples were true negatives.

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#### HABITAT MANAGEMENT

#### Overview

Boreal toad habitat consists of areas with suitable breeding habitat in lodgepole pine, spruce-fir forests, and alpine meadows. Breeding habitat consists of shallow, quiet water in lakes, marshes, bogs, ponds, and wet meadows, often with egg placement optimizing thermal effects of the summer sun. Young toads are restricted in distribution and movement by available moist habitat, while adults can move several miles and reside in marshes, wet meadows, or upland forested areas. Although availability of adequate suitable habitat does not appear to be a significant factor in the decline of boreal toad populations, protection of such habitats and the preservation of reliable and stable water levels in breeding habitat, are essential to the long-term viability of toad populations.

# Public Lands

The large majority of known existing and potential boreal toad populations and habitats in the Southern Rocky Mountains are located on US Forest Service lands and in Rocky Mountain National Park (see summary by geographic areas, earlier in this publication). Therefore, efforts to protect and enhance habitat for boreal toads are focused mainly on these lands.

At this time, protection and consideration of boreal toad habitats on US Forest Service lands is achieved via management guidance provided in various USFS documents, such as the Watershed Conservation Practices Handbook and the Region 2 Sensitive Species List. A significant number of known breeding populations are located within USFS Wilderness Areas and within Rocky Mountain National Park, which provides additional protection of habitats from potential disturbance by disruptive land uses. In addition, cooperative efforts with individual forests are pursued in localities where boreal toad breeding populations exist. These efforts are focused at informing recreationists about boreal toads & habitats, making land managers aware of the toads' habitat needs, and incorporating considerations for boreal toad habitat protection in land use decisions on forests. It is anticipated that specific direction for boreal toad habitat conservation measures will be incorporated in individual forest management plans after review under the National Environmental Policy Act (NEPA).

#### Private Lands

There are a few boreal toad populations and habitats located on private lands. In Colorado, the Colorado Division of Wildlife has worked with private land owners and developers, mainly in Summit, Clear Creek, and Grand counties, on cooperative efforts to protect existing toad populations and habitats. At the Cucumber Gulch site, in Summit County, cooperative work with the town of Breckenridge and a local land developer has resulted in the adoption of a number of conditions and criteria which will help to minimize any potential impacts on boreal toads at that site. This effort will help to set a precedent for consideration of boreal toad habitats in other pending land developments in

Summit County. In 1998, Vail Associates helped fund boreal toad survey work in Summit County in cooperation with the USFS and CDOW, and is working closely with several local, state, and federal agencies to minimize potential negative impacts of planned development at the Breckenridge Ski Resort on the Cucumber Gulch wetlands, and boreal toads.

In Grand County, cooperative efforts with managers of the Pole Creek Golf Course have helped to gain consideration for boreal toads on that property, and managers of the golf course have agreed to pursue cooperative work to preserve and enhance the habitat at the two known breeding sites.

In Clear Creek County, the Climax Molybdenum Company has worked in cooperation with the Colorado Division of Wildlife at the Henderson/Urad Mine, since 1995, to help facilitate research work on boreal toads and to protect and enhance toad breeding habitat on their property. However, a Candidate Conservation Agreement with the US Fish & Wildlife Service is still pending for this property as of March 2004.

Although the boreal toad populations on private lands represent a relatively small portion of the total toad population and habitat, efforts will continue to protect such sites and to minimize and mitigate impacts of land development and land use changes.

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