U.S. FISH AND FISH WILDLIFE SERVICE
<u>SPECIES ASSESSMENT AND LISTING PRIORITY ASSIGNMENT FORM</u>

SCIENTIFIC NAME: *Bufo boreas boreas*

COMMON NAME: Boreal toad, southern Rocky Mountain DPS

LEAD REGION: Region 6

INFORMATION CURRENT AS OF: July 12, 2004

STATUS/ACTION:
___ Initial 12-month Petition Finding: __ not warranted
__ warranted
_X_ warranted but precluded (also complete (c) and (d) in section on petitioned candidate species- why action is precluded)
___ Species assessment - determined species did not meet the definition of endangered or threatened under the Act and, therefore, was not elevated to Candidate status
___ New candidate
_X_ Continuing candidate
___ Non-petitioned
_X_ Petitioned - Date petition received: __________
_X 90-day positive - FR date: 07/22/1994__________
_X 12-month warranted but precluded - FR date: 03/23/1995___________
_ Is the petition requesting a reclassification of a listed species?
___ Listing priority change
Former LP: ___
New LP: ___
Latest Date species became a Candidate:
___ Candidate removal: Former LP: __
___ A - Taxon is more abundant or widespread than previously believed or not subject to the degree of threats sufficient to warrant issuance of a proposed listing or continuance of candidate status.
___ F - Range is no longer a U.S. territory.
___ I - Insufficient information exists on biological vulnerability and threats to support listing.
___ M - Taxon mistakenly included in past notice of review.
___ N - Taxon may not meet the Act's definition of "species."
___ X - Taxon believed to be extinct.

ANIMAL/PLANT GROUP AND FAMILY: Amphibians, Bufonidae.

HISTORICAL STATES/TERRITORIES/COUNTRIES OF OCCURRENCE: Wyoming, Colorado, New Mexico.

CURRENT STATES/ COUNTIES/TERRITORIES/COUNTRIES OF OCCURRENCE: Wyoming (protected species), Colorado (State endangered), New Mexico (State endangered, believed to be extirpated)

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LEAD FIELD OFFICE CONTACT: Terry Ireland, (970) 243-2778

BIOLOGICAL INFORMATION:

Species Description
In the southern Rocky Mountain (SRM) population, female boreal toads may reach a length (snout to vent) of 11 centimeters (cm) (4.3 inches (in)), while males seldom exceed 9.5 cm (3.7 in). Both sexes have warty skin and oval parotoid glands. Although more prominent in females, both sexes often have a distinctive light mid-dorsal stripe. Unlike other *Bufo* species, the male boreal toad has no vocal sac and, therefore, has no mating call. The vocal sac also is absent in the female (Hammerson 1999). However, males are known to emit a chirping sound when distressed, clasped by other males or when gathered in breeding aggregations (Baxter 1952, Campbell 1970a). Females also will emit a chirping sound, but are usually silent. During the breeding season males will have a dark patch on the inner surface of the innermost digit. Tadpoles are dark black or brown with a head-body length of 14-17 millimeters (mm) (0.014-0.017 in) and a tail length of about 16-20 mm (0.016-0.02 in). The eggs are black and approximately 1.5-1.8 mm (0.0015-0.0018 in) in diameter and are deposited in long double-layer jelly strings with 1-3 rows of eggs (Hammerson 1999).

In the mountainous regions of the southern Rocky Mountains adult boreal toads emerge from overwinter refugia when snowmelt has cleared an opening from their burrow and daily temperatures remain above freezing (Campbell 1970a, 1970b). Breeding may begin in the lower altitudes in May and in the higher altitudes in July or early August (Hammerson 1999). Males generally arrive first at breeding sites and position themselves along the shoreline waiting for the arrival of gravid females (Black and Brunson 1971). Breeding assemblages are generally male biased. Black and Brunson (1971) indicated that males greatly outnumbered females in breeding ponds in western Montana. Campbell (1970a) found that in nonbreeding populations the sex ratios were male biased (2.75:1), except in two localities where the sex ratio was equal in one case and female biased in the other. Deviation from the male biased sex ratio may be a function of habitat type or age structure of the population (Campbell 1970a). Olson (1991) suggested that skewed sex ratios were the result of females not breeding every year. In the Oregon Cascade Range females may skip 1 to 3 years between breeding attempts, depending on their physical condition (Olson 1991). At the Native Aquatic Species Restoration Facility (NASRF) in Alamosa, Colorado, captive breeding efforts have resulted in 1:1 sex ratios (L. Livo, pers. comm. 2004). Cyclic breeding by females is believed to protect reproductive populations from year specific catastrophic mortality (Olson 1991).
Breeding is initiated when males vigorously pursue and clasp receptive females. Females deposit up to 16,500 eggs in 2 strings, which are ordinarily laid in shallow (< 15 cm (6 in)) water (Stebbins 1954). Selection of breeding locations is primarily dependent on the availability of shallow water. Campbell (1970a) noted that breeding would occur in very large bodies of water

or very small pools, irrespective of the surrounding terrestrial or aquatic vegetation. Egg and tadpole development is temperature dependant; in high, cold locations, development from hatching to metamorphosis can take 75 days. At lower, warmer locations, tadpole development requires about 45 days (Carey 1987). Persistent, shallow bodies of water are critical for a successful breeding season. If the breeding site dries before metamophosis is complete, desiccation of the tadpoles and/or eggs will occur. Low snow accumulation or early runoff may result in lowered or complete drying of some bodies of water before juvenile toads metamorphose (Campbell 1970a). Successful breeding appears also to be altitude dependant. Campbell (1970a) noted that breeding above 3,353 meters (m) (11,000 feet) (ft) was typically unsuccessful. At 3,353 m (11,000 ft), there is insufficient time for metamorphosis to complete before the onset of winter. Overwinter survival of larvae has not been documented (Fetkavich and Livo 1998). Campbell (1976) and Baxter (1952) observed that at extremely high elevations, reproductive efforts usually failed, because tadpoles did not have sufficient time to metamorphose before the onset of winter.

After hatching and throughout larval development, tadpoles remain in aggregations in shallow water. Brattstrom (1962) indicated that aggregating behavior in association with dark coloration resulted in slight increases in water temperature within the group. Warmer water enhances metabolic activity, thereby shortening larval development time (Burger and Bragg 1947). Therefore, tadpole aggregations may act to reduce vulnerability by shortening the length of time individuals remain in this stage. Others have suggested that aggregations of tadpoles may be a behavioral trait that enhances kin recognition (Waldman and Alder 1979; O'Hara and Blaustein 1982; Blaustein et al. 1990). Hews and Blaustein (1985) suggested that aggregating may be a form of chemical defense against predators. Recently metamorphosed juveniles have been observed to aggregate within a few meters of the water. Black and Black (1969) indicated a thermoregulatory advantage to clustering, while Lillywhite and Wassersug (1974) suggested that aggregations serve primarily as a social response to the presence of other toads. Aggregations of post-metamorphic toads also may serve as an adaptation to reduce predatory risk. An aggregation can reduce the probability of predation for individual group members (Arnold and Wassersug 1978). During this life stage toads are particularly susceptible to dehydration because of their high surface to volume ratio. As a result, recently metamorphosed toads will remain near permanent water sources where they also will feed. Juvenile toads begin to disperse into the surrounding wet meadows, marshes, and forested areas as the summer foraging season shortens.

Survival of embryos from laying to hatching is normally high, but catastrophic mortality has been observed (Blaustein and Olson 1991). Survival of tadpole and juvenile boreal toads is very low. Smallow (1980) estimated that 95 to 99 percent of boreal toads die before reaching their second year of life. Predation and adverse environmental conditions are primarily responsible for mortality at these life stages (Campbell 1970a). In the central Oregon Cascade Range surviving toads may live to at least 11 years and begin breeding at year 4 or 5 (Olson 1991). Skeletochronology studies indicated that the maximum life span of the boreal toad is approximately 12 years (Mark Jones and Craig Fetavich, CDOW, pers. comm., 2000).

After mating, adult males and females leave the water and disperse into nearby wet meadows, marshes or forested areas where they are diurnally active during early and late summer (Mullally 1958; Campbell 1970a; Carey 1978). During warmer summer evenings, toads may be diurnally and nocturnally active (Campbell 1970a; Carey 1978). Boreal toads in the southern Rocky Mountains tend to be more diurnal than boreal toads at lower elevations. This is due to frequent subfreezing night temperatures and the subsequent thermal stress, lack of available prey, and decreased metabolic activity associated with cooler night temperatures of high altitudes (Mullally 1952, 1958; Lillywhite et al. 1973; Carey 1978, Hailman 1984). During active foraging periods adult toads feed primarily on ants, beetles and spiders (Schonberger 1945; Campbell 1970a), although many other invertebrates will be taken opportunistically (Campbell 1970a).

Throughout the summer foraging season adults remain primarily in established territories. Campbell (1970a) indicated that males generally tended to have larger home ranges than females, but that home range size was dependent more on the physical nature of the habitat than sex. Muths (2003) found females had 4 times the home range of males and attributed this to increased energetic demands prior to reproduction. In northwestern Wyoming, Carpenter (1954) found no difference in the home ranges of males and females but concluded, as did Campbell (1970a, 1976), that boreal toads were generally sedentary. Both Carpenter's and Campbell's studies showed toads remained within a broad area in the vicinity of permanent water or moist substrates. Average daily movements of 1.5-4.2 m (5-10 ft) (Campbell 1970a) and 2.0 m (6 ft) (Carpenter 1954) suggest that food, cover, and thermoregulatory refugia can be obtained in very small areas. Campbell (1970a) found that home ranges expanded late in the summer, generally in the direction of former hibernacula. Site tenacity for summer home ranges diminishes in late September and early October when boreal toads may move 900 m (2,953 ft) to winter hibernacula (Campbell 1970b).

Taxonomy

The *Bufo boreas* complex (the western toad), first described by Baird and Girard (1852), contains two subspecies--the boreal toad (*B. b. boreas*) and the California toad (*B. b. halophilus*). Camp (1917) recognized the boreal toad and the California toad as subspecies of the western toad. However, recent DNA (deoxyribonucleic acid) analysis may warrant a taxonomic change to this complex. Goebel (1996) analyzed mitochondrial and nuclear DNA of *B. boreas* populations from the southeastern portion of the range (Utah, Idaho, Wyoming, and Colorado) and found that the SRM population may be distinct enough to warrant recognition as a separate species. Prior to Goebel's work, noted morphological, biochemical, and vocal differences already existed between western toads of the Pacific Northwest and the southern Rocky Mountains (Burger and Bragg 1947, Hubbard 1972).

Habitat

In Colorado, the boreal toad inhabits a variety of wet habitats (i.e., marshes, wet meadows, streams, beaver ponds, glacial kettle ponds, and lakes interspersed in subalpine forest) at altitudes primarily between 2,438-3,505 m (8,000-11,500 ft) with reported occurrences at 3,615 m (11,860 ft) in the San Juan Mountains (Campbell 1976) and 3,640 m (11,940 ft) in Clear Creek County (Livo and Yeakley 1997; Hammerson 1999). Toads emerge from hibernation sites in May and return to them in late August or early September (Hammerson 1999). Activity is primarily during the daytime when ambient temperatures are between 12-20°C (54-68°F). Toads will thermoregulate by basking in the sun or entering into the water if temperatures suddenly plummet (Campbell 1970a). During the summer, toads may become more terrestrial and inhabit drier habitats (Campbell 1976), but typically will stay near moist areas. Summer habitat and post-breeding habitat use is variable. Toads are commonly found where the vegetation can provide cover and allow basking. These areas are typically perimeters of wetlands dominated by willows, sedges, and mossy hummocks (Campbell 1970a). Although less common, toads have been found in dry upland forest-types (Campbell 1970a). Winter habitats (i.e., hibernacula) include--beaver lodges and dams, rodent burrows, and rock-lined chambers and require some type of continuous subsurface water flow (Campbell 1970b; Jones and Goettl 1998; Hammerson 1999). Over-wintering sites are typically not far from summer habitats. In a study along the Front Range of Colorado, Campbell (1970b) documented most toads over-wintered within 900 m (2,953 ft) of their summer habitat.

Movement of boreal toads is variable. Females tend to move further than males (Loeffler 2001). In Idaho, a female boreal toad was documented to have moved 2.5 kilometers (km) (1.5 mi) from a breeding site through dry upland forests to reach summer range (Bartelt 2000). In Chaffee County, Colorado, Lambert (2003) found toads moving up to 8 km (5 mi). By mid-September, toads move towards winter habitats. Winter habitats (hibernacula) include; beaver lodges and dams, rodent burrows, and rock-lined chambers and require some type of continuous subsurface water flow (Campbell 1970b; Jones and Goettl 1998; Hammerson 1999). Over-wintering sites are typically not far from summer habitats. In a study along the Front Range of Colorado, Campbell (1970b) documented most toads over-wintered within 900 m (3,000 ft) of their summer habitat.

Home range of the boreal toad is variable and often dependant on the physical nature of the habitat. Adults remain primarily in established territories throughout the summer foraging season (Campbell 1970a). Although Campbell (1970a) found males to have larger home ranges than females, females typically have larger home ranges. In Rocky Mountain National Park (RMNP), females were shown to have four times the home range of males (Muths 2003). At the Henderson Mine in Climax, Colorado, a radio-telemetry study estimated female home ranges at 16.9 hectares (1 hectare = 2.47 acres) and males at 7.1 hectares (Jones 2000). This study also showed that males tended to stay at the breeding site longer, while females quickly dispersed after mating into the adjacent upland forest.

<u>Historical Range/Distribution</u>
The western toad occurs throughout much of the mountainous areas of the western United States. The current taxonomic arrangement recognizes two subspecies within the *B. boreas* complex--the California toad and the boreal toad. The California toad occurs from northern California to the Baja peninsula of Mexico, and western Nevada. The Pacific Northwest population of the boreal toad ranges from southeastern Alaska south to northern California, British Columbia (Canada), central and northern Idaho, Montana, and northwestern Wyoming. Intergradation occurs in northern California, where the ranges of the California toad and the boreal toad overlap (Stebbins 1985). The SRM Distinct Population Segment (SRM-DPS) of the boreal toad is distributed primarily on public lands in southeastern Wyoming, Colorado, and New Mexico.

<u>Current Range/Distribution</u>
Currently, the SRM-DPS of the boreal toad is known to occur in 14 counties in Colorado and 2 in southern Wyoming (Jungwirth 2004). Although boreal toads have been documented in New Mexico, they are believed to be extirpated from the State (New Mexico Department of Game and

Fish 1988). However, the Fish and Wildlife Service (FWS) continues to recognize New Mexico as being within the range of the SRM-DPS. In Wyoming, the boreal toad was found in the Snowy and Sierra Madre Ranges in southeastern Wyoming (Baxter and Stone 1985). Historical records also exist for the Laramie Mountains in southeastern Wyoming. At the southern periphery of their range in the San Juan Mountains of New Mexico, boreal toads were found at only three localities--Lagunitas, Canjilon, and Trout Lakes (Campbell and Degenhardt 1971; Jones 1978; New Mexico Department of Game and Fish 1988). Surveys conducted in 1993 revealed no populations at the three previously known locations in Rio Arriba County, New Mexico (Stuart and Painter 1994).

Declines in isolated populations were first documented in New Mexico in the mid-1980s (Woodward and Mitchell 1985, Carey 1987) and in Colorado and southern Wyoming from 1986 through 1988 (Corn et al. 1989). Although the boreal toad is believed to be extirpated from New Mexico, there has been an unverified observation (Stuart and Painter 1994). The boreal toad was once common throughout much of the high elevations in Colorado (Burger and Bragg 1947, Stebbins 1954, Smith et al. 1965), but not in the Sangre de Cristo, Wet Mountains, or Pikes Peak regions (Hammerson 1999). Corn et al. (1989) found that boreal toads were absent from 83 percent of locations in Colorado and 94 percent of locations in Wyoming previously known to contain toads. Carey (1993) noted the disappearance of 11 populations in the West Elk Mountains in Colorado between 1974 and 1982. To date, there has been no evidence of recolonization at these sites.

Population Estimates/Status

The SRM-DPS of the boreal toad is divided into 11 geographic areas--Park Range, Elkhead Mountains, Medicine Bow Range, Front Range, Gore Range, Mosquito and Ten-mile Range, Sawatch Range, White River Plateau, Grand Mesa, Elk and West Elk Mountains, and the San Juan Mountains. These 11 geographic areas currently contain 67 known breeding localities comprising 32 populations. However, only the Cottonwood Creek population in Chaffee County is considered viable (Jungwirth 2004). Although many of the 67 known breeding localities have not produced evidence of breeding (e.g., Cucumber Gulch) in the past few years, evidence of breeding only needs to be documented within the past 5 years for a site to be considered a breeding locality.

THREATS:

A. The Present or Threatened Destruction, Modification, or Curtailment of Its Habitat or Range.

The lack of suitable habitat does not appear to be a significant limiting factor for boreal toads in the southern Rocky Mountains (Loeffler 2001b). However, there have been documented instances where certain land management practices had deleterious effects on boreal toads and their habitat. Some activities and their effects on boreal toads are speculative and, therefore, are not discussed (i.e., introduction of tiger salamanders, aerial insect spraying, acid deposition), but do warrant further investigation. This section discusses documented activities known to have had some adverse impact on boreal toads (i.e., water development, domestic livestock grazing, and hardrock mining).

(1) Water Development

Numerous artificial lakes and reservoirs have been developed within the range of the SRM boreal toad population. Large bodies of water (i.e., reservoirs and lakes) typically have well defined shorelines that lack the peripheral shallow-water habitat needed for boreal toad reproduction. With the filling of Left Hand Reservoir in Boulder County, Colorado, in 1967, numerous mountainous wetlands were flooded, which resulted in hundreds of displaced toads aggregating around the shoreline of the newly formed reservoir (Campbell 1970a). However, water developments of this type are rare occurrences and are not considered to be a significant factor in the widespread decline of the boreal toad in the southern Rocky Mountains.

(2) Domestic Livestock Grazing

In Colorado, mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*) are native grazers that have always occurred in boreal toad habitat. However, the tendency for domestic livestock to congregate in riparian ecosystems (Fleischner 1994) has created unnatural impacts to boreal toad habitat. The impacts include the loss of the herbaceous canopy, increased erosion, eutrophication, and the physical alteration of the habitat. When livestock graze in riparian areas, it is inevitable there will be a loss of herbaceous canopy. As toad larvae metamorphose, the newly metamorphosed toadlets rely on the herbaceous canopy for protection.

Domestic livestock grazing (i.e. cattle, sheep, and horses) is nearly ubiquitous in the western United States. From Montana, Wyoming, Colorado, New Mexico and westward, approximately 70 percent of this region is grazed. Although grazing occurs in nearly all habitats, from alpine meadows above the timberline to arid deserts (Fleischner 1994), the altitude at which boreal toads occur is not commonly grazed by cattle. Although sheep grazing in the southern Rocky Mountains is not at the same levels as it was 20 years ago, direct mortality has been attributed to sheep (Bartelt 1998). Bartelt (1998) documented hundreds of newly metamorphosed boreal toads trampled to death by grazing sheep in southeastern Idaho.

From the 1940s through the 1960s, toads were ubiquitous at a time when livestock grazing on National Forests was much higher (Loeffler 1998). Livestock grazing is not considered a major factor in the decline SRM-DPS of the boreal toad. Considering that grazing has been occurring in the southern Rocky Mountains for over a hundred years, this would not explain the sudden decline of the boreal toad in southern Rocky Mountains over the past 30 years. Overall, the high altitude of the relatively small number of sheep grazing operations in the SRM region makes sheep grazing an uncommon threat.

(3) Hardrock Mining

Acid mine drainage produced from hardrock mining has been indicated as a possible factor in the decline of the boreal toad through ground water transport, snow melt, mine tailing contact with rain, and surface flow (Loeffler 2001b). Although boreal toads can occur in habitats known to be contaminated with heavy metals, these sites have artificial high temperatures and high pH, which precipitates some soluble metals from the water (Loeffler 2001b). Acid mine drainage can lower the pH of water, which can result in the decline of the algal community--a primary food source for tadpoles. In addition, a low pH can work synergistically with other variables in creating adverse conditions for amphibians (Long et al 1995).

During the nineteenth century gold, silver, lead and zinc mining was widespread throughout the mountainous region of Colorado. Although many of these mining operations are inactive, the effects on the environment are still evident. Water draining from many of these mines continues to contaminate streams with acids and metallic compounds (Porter and Hakanson 1976). Boreal toads do have the potential to absorb cadmium, chromium, copper, mercury, manganese, nickel, lead, and zinc to concentration levels greater than the surrounding environment (Loeffler 2001b). Acute levels of copper have been found to be lethal to boreal toad tadpoles (Porter and Hakanson 1976; Loeffler 2001b).

Although heavy metal contamination has been shown to be deleterious to boreal toad survival, most boreal toad habitats do not have high enough concentrations of heavy metals to be a significant factor. In isolated instances, hardrock mining may have localized impacts on boreal toads, but is not considered a a major factor in the decline of the boreal toad in the southern Rocky Mountains.

B. <u>Overutilization for Commercial, Recreational, Scientific, or Educational Purposes</u>.

Overutilization for commercial, recreational, or educational purposes has not been identified as a threat to the SRM-DPS of the boreal toad. The boreal toad has no commercial value.

C. <u>Disease or Predation</u>.

(1) Chytridiomycosis (*Batrachochytrium dendrobatidis*)

1There have been recent reports from around the globe of disease and pathogen-related population declines and mass die-offs of amphibians (Blaustein and Wake 1990, Carey 1996). The BD is believed to be the major factor in the decline of the SRM-DPS of the boreal toad. Whether adult anurans (frogs and toads) acquire this fungus from tadpoles or whether the fungus is retained through metamorphosis is unknown. The BD affecting wild anuran populations was not documented until the late 1990s. Since then, it has been reported in numerous species of amphibians worldwide (Berger et al. 1999). Although BD has not been found in non-amphibian hosts, it is possible other hosts may eventually be discovered.

Chytridiomycete fungi, a large and diverse group which includes BD, are found in a wide range of habitats. These fungi serve as important primary decomposers in soil or water where they can be found living on various substrates such as chitin, insect cadavers, plant cellulose, keratin from hair and skin, or pollen (Berger et al. 1999). Chytrid has an evanescent nature that is characterized by a sudden appearance, followed by rapid multiplication and then decline. This cycle is dependent on seasonal temperature changes, water pH, light, nutrition and dissolved oxygen. Chytrids are found primarily in aquatic habitats within a stationary sporangium. The sporangium discharges mobile flagellated zoospores. Once the spores are mobile, they seek an appropriate substrate through the use of chemotaxis (movement of a cell in response to a chemical gradient). Employing the use of an adhesive substance, the zoospores encyst on their host. In Australia, the transport of chytrid fungi has been documented in the movement of fruit boxes and through the pet trade (Berger et al. 1999).

Once a host is infected, clinical signs include lethargy, inappetence, skin discoloration, and excessive amounts of sloughed skin (Berger et al. 1999). Externally, chytrid will inhabit the keratinized layers of the epidermis, primarily in the ventral region and on the limbs and feet. The fungus will produce fungal discharge tubes through the surface of the skin, which results in an internal infection of the host. Once internally infected, it is believed that the fungus releases proteolytic enzymes that digest (break-down) proteins. Histologic examinations show that chytrid is not the ultimate cause of death, but suppresses the immune system to a point that other diseases are considered the cause of death. Concurrent fatal diseases include septicaemis (blood stream infection), microsporidial hepatitis (intestinal infection), and hyphal mycotic dermatitis (skin ulcers). There are three postulates as to why BD has become a major world-wide problem in amphibian populations--1) BD is a relatively recent introduction, 2) for some unknown reason has become pathogenic to the host, and 3) due to changes in the environment, amphibians are less resistant to BD. The first postulate is the most plausible. Based on previously discussed epidemiological factors and that environmental changes have not been detected in many of the infected populations, the chytrid problem is mostly likely a recent introduction (Berger et al. 1999).

In boreal toads, *Aeromonas* or red-leg disease is typically a secondary infection following a BD infection. Carey (1987, 1993) indicated the proximate cause of the widespread decline of boreal toads in northern New Mexico and west-central Colorado was a result of infection by *Aeromonas* bacteria. However, *Aeromonas* is common in the microfauna carried by amphibians and it does not cause infection or death in healthy individuals. As a result, toads in northern New Mexico likely were stressed by other adverse environmental factors and later succumbed to *Aeromonas* infection (Carey 1987). Additional work needs to be done to determine if other environmental or anthropogenic factors may be working synergistically with pathogens to cause death in boreal toads.

In 2003, Livo (in Jungwirth 2004) visited 64 boreal toad localities. At 46 of the localities, 417 boreal toad samples and 189 samples from 7 other amphibian species were collected. Because boreal toads or surrogate amphibians could not be found at 18 localities, samples could not be obtained. Of the 417 boreal toad samples, 33 samples from 8 localities tested positive. Livo also discovered a high rate of infection in adult boreal toads--33 of 43 tested positive. This translates to a 77 percent infection rate (Jungwirth 2004). Livo also found that metamorphs often did not test positive at known BD positive sites. Since BD appears to have a greater potential to infect the terrestrial life stage of boreal toads, Livo theorized that the metamorphs may not have had enough exposure time to the terrestrial habitat. She also did not advise testing for BD in boreal toad tadpoles or metamorphs, since tadpoles and metamorphs often test negative in localities where adults test positive. This is not to say metamorphs will not test positive for BD, but that metamorphs are inconsistent test samples. Berger et al (1998) believes the lack of keratinized body parts in tadpoles may explain why BD is only found on the mouth parts. The Torso Creek breeding site exemplifies this situation. In 2003, 20 metamorphs tested negative, while 5 of 5 adults tested positive.

In summary, 9 of 32 breeding sites tested positive for BD in 2003, which comprises 28 percent of the breeding populations. We recognize that the other 24 sites did not necessarily test negative for BD. Many of these sites were void of any amphibians to sample; and are only considered to be a breeding site based on breeding activity having occurred since 1995. Although it cannot be proven, BD is suspected in the disappearance of boreal toads from these sites (Lauren Livo, pers. comm., 2004).

Testing for BD can be influenced by several factors. The following factors are believed to cause false-negatives when testing for BD (L. Livo, pers. comm., 2004)--1) sample size; 2) possible seasonal fluctuations in BD; 3) the lifestage of the specimen (i.e., tadpole, metamorphs, or adult); 4) the location on the body from which the sample was taken; and/or 5) the use of surrogates for sampling. At Kannah Creek on the Grand Mesa, 39 western chorus frogs (*Pseudacris triseriata*) tested positive for BD. The year before, BD testing had negative results. This problem also has been noted in tiger salamanders (*Ambystoma tigrinum*) (Jungwirth 2004).

Even though Rocky Mountain National Park (RMNP) is one of the most protected environments within Colorado, boreal toad populations have still declined (Corn et al. 1997). Although amphibian populations undergo "boom and bust" cycles, this is not believed to be the case in RMNP, since toad population declines within the park are synchronous with the decline of other SRM populations. Monitoring and research has indicated that BD has caused serious declines in two large breeding populations within RMNP (Livo and Loeffler 2003). Muths et al. (2003) noted significant declines in boreal toad populations at two of four monitored sites (i.e., Kettle Tarn and Lost Lake). From 1996 and 1999 the annual survival rate sharply declined. From 1991 to 1994 the average survival rate was 78 percent. This rate dropped to 45 percent in 1995 and 3 percent between 1998 and 1999. Coinciding with this decline in adults was a decline in the number of egg masses. Muths et al. (2003) also found six adult toads infected with BD and concluded (based on 11 years of monitoring) that the metapopulations of boreal toads within RMNP are in danger of extinction.

In the southern Rocky Mountains, BD appears to be widespread and have impacted at least 50 percent of the known range of the SRM-DPS of the boreal toad. The 2003 BD test results showed the following localities as BD positive--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 North Fork Snakes (Summit County), and Peru Creek (Summit County). In summary, as BD testing continues, more BD-positive sites are discovered. If the Sawatch Geographic Area, which contains the only viable population, was to become infected with BD, the future of the boreal toad in the southern Rocky Mountains could be in critical peril.

(2) Natural Predation

In the SRM-DPS, predators of adult boreal toads include the garter snake (*Thamnophis elegans*), raccoon (*Prycyon lotor*), red fox (*Vulpes vulpes*), common raven (*Corvus corax*) (Olsen 1989), badger (*Taxidea taxus*), and domestic dog (Hammerson 1999; Livo 1999). Predators of larvae metamorphs and newly metamorphosed toads include the mallard (*Anas platyrhychos*), spotted sandpiper (*Actitis macularia*), American robin (*Turdus migratorius*), gray jay (*Perisoreus* *candensis*), Steller's jay (*Cyanocitta stelleri*), western terrestrial garter snake (*Thamnophis elegans*), tiger salamander larvae (*Ambystoma tigrinum*), Dytiscid beetle larvae, and wood frog (*Rana sylvatica*) (Beiswenger 1981, Petranka et al. 1994, Hammerson 1999). In the eastern United States, American toads *(Bufo americanus*) will often avoid breeding in sites occupied by wood frogs (Petranka et al. 1994). In Colorado, Hammerson (1999) noted that boreal toads infrequently breed in areas occupied by wood frogs.

Both garter snakes and sandpipers were often encountered at boreal toad breeding sites (Lambert 2003). At the Brown's Creek site, garter snakes were encountered on nearly every visit and are suspected to be responsible for poor survivorship of boreal toad larvae. The occurrence of garter snakes at the South Cottonwood site also was documented (Lambert 2003). In addition to garter snakes at the South Cottonwood site, a pair of sandpipers nested near the main breeding site. Their tracks were commonly found along the shoreline where boreal toad larvae often congregated. Garter snakes also were frequently encountered at the Rainbow Lake site (Lambert 2003).

(3) Parasites

Internal parasites have been recorded from boreal toads in several locations. Koller and Gaudin (1977) found nematodes, cestodes and trematodes from toads in California. In Colorado, trematodes were found in toads in Garfield County (Tiekotter and Mantor 1977). While Frandsen and Grundmann (1960) reported protozoans, trematodes, and nematodes in Utah boreal toads, Waitz (1961) found boreal toads in Idaho infected with trematodes. However, none of these accounts suggests that helminth parasites affected toad survival.

D. <u>The Inadequacy of Existing Regulatory Mechanisms</u>.

(1) Overview of State, Federal and Private Conservation Efforts

The boreal toad is listed as State endangered in Colorado and New Mexico. In Wyoming, the boreal toad is a regulated nongame species protected by State laws. After the boreal toad became listed as State endangered in 1994, the Colorado Division of Wildlife (CDOW) developed a recovery plan (revised 1997), and formed an interagency team: The Boreal Toad Recovery Team (BTRT). This plan was subsequently revised and combined with the existing draft conservation strategy developed by the Forest Service. In 1998, these 2 plans were consolidated into 1 document: The Boreal Toad Conservation Plan and Agreement for the Southern Rocky Mountains. This plan was revised in 2001. Part of the conservation planning process was the signing of a Conservation Agreement between 8 State and Federal agencies and the Colorado Natural Heritage Program. The intent of the plan is to provide comprehensive guidance for the recovery and management of the boreal toad and its habitat in the southern Rocky Mountains, and to provide a means for all involved and interested parties to make a formal commitment to the implementation of the action recommended in this plan.

To date the BTRT has involved numerous individuals from State, Federal, academic and private backgrounds. BTRT management activities include; 1) conducting surveys of historic and potential suitable habitat, 2) annual monitoring of known breeding populations, 3) research to identify and evaluate biotic and abiotic factors influencing toad survival, 4) research into boreal toad habitat and biology, 5) development and testing of captive breeding and rearing techniques, 6) experimental repatriation efforts, 7) coordination with Federal and private land managers to protect toads and their habitat, and 8) programs to increase public awareness through informational/educational activities and publications. However, the BTRT and the associated Conservation Plan has not been able to curtail the continuing decline of the boreal toad.

During the early 1990s, captive rearing and breeding techniques were developed by the Wyoming Game and Fish and the CDOW. In 1999, the BTRT decided to establish a disease-free breeding stock of boreal toads. This stock was obtained from various locations in SRMP. The housing for this project was established at the CDOW's Native Aquatic Species Restoration Facility (NASRF), near Alamosa, Colorado. To prevent catastrophic loss of captive stock, other stocks were established at several other facilities, including the Saratoga National Fish Hatchery in Wyoming, Cheyenne Mountain Zoo (Colorado Springs, Colorado), the Cincinnati Zoo (Cincinnati, Ohio), Morrison Museum of Natural History (Morrison, Colorado), and Colorado's Ocean Journey (Denver, Colorado).

The Cheyenne Mountain Zoo, Henry Doorly Zoo, and Toledo Zoo have been actively involved with limited success in husbandry, captive propagation and rearing. However, the BTRT plans to continue to work with these institutions. One project planned is to develop a "stud book" database for the purpose of tracking all Southern Rocky Mountain captive boreal toads and their progeny (Jungwirth 2004).

Through captive breeding, the CDOW is trying to reintroduce toads into historic sites. Currently, the only repatriation effort being conducted by the CDOW is located on the Grand Mesa. In May 2003, approximately 20,000 eggs were harvested from a boreal toad breeding site in Chafee County, Colorado. These eggs were then reared to a predetermined lifestage at the Native Aquatic Species Restoration Facility (NASRF). Over 12,000 tadpoles were then translocated and released at three sites on the Grand Mesa. The remaining tadpoles were held at NASRF for 3 three weeks post metamorphosis and released at these same sites. At this time, it is too soon to determine if this repatriation effort has been successful (Livo and Loeffler 2003).

In Mineral County, Colorado, 300 newly metamorphosed toadlets were released at Love Lake in August, 1996. This stock of 300 captive-reared toadlets was taken from nearby Jumper Creek in Mineral County. Subsequent surveys later that summer found some toadlets had survived their first month. Unfortunately, no toads have been found at this site since 1996 (Jungwirth 2004).

In 1995, 800 toadlets, egg masses, and 100 captive-raised toads were released at Glacier Basin at RMNP in Colorado. From 1997 to 2000, the National Park Service and U.S. Geological Survey monitored the results of this effort and only found 3 adult females. In 2000, no toads or evidence of breeding was found (Jungwirth 2004).

In 1995, 1996, and 1997 several thousand toadlets, an unknown number of adult toads, and an unknown number of tadpoles were released at the Lost Lake in Boulder County, Colorado, in an effort to determine the effect of releasing a large number of taodlets. No boreal toads have been observed at Lost Lake since 1999 (Jungwirth 2004).

At Lake Owen in Albany County, Wyoming, 4,000 captive reared tadpoles were released in 1996. In 1997, an additional 1,500 captive-reared tadpoles were released and 3 one-year-olds were observed. Although the 3 one-year-olds indicate some survival, survey efforts in 2000, 2001, and 2002 found no toads or signs of boreal toads (Jungwirth 2004).

Over 90% of known boreal toad breeding sites in the southern Rocky Mountians occur on Federal land (Loeffler 2001b). Region 2 of the U.S. Forest Service (FS) and the Intermountain Region of the National Park Service both address the SRMP of the boreal toad as a sensitive species. Except for the White River National Forest (WRNF), neither agency currently has a management plan specific to boreal toad conservation. The WRNF 2002 Land and Resource Management Plan has set-forth standards and guidelines for the conservation of boreal toads and their habitat. Both agencies typically follow the guidelines and recommendations of the Boreal Toad Conservation Plan and Agreement (revised 2001). Although the FS does have a Watershed Conservation Practices Handbook, this handbook does not protect the upland habitat component (i.e., overwintering areas) critical to boreal toad survival.

Although the CDOW, Wyoming Game and Fish and numerous private interests have made considerable efforts in captive breeding and repatriation, these efforts have had little or no success. In addition, the CDOW and Wyoming Game and Fish lack regulatory authority to protect the species and its habitat from various Federal, State or private land management practices.

(2) Overview of Current Research Efforts

The CDOW is conducting research into various aspects of boreal toad conservation. The Service believes this research is invaluable to the conservation of the species. The following research project descriptions are brief. For more detailed descriptions of these research projects, see the 2003 Report on the Status and Conservation of the Boreal Toad in the Southern Rocky Mountains.

The Colorado Natural Heritage Program (CNHP) is gathering baseline data to evaluate population size and trends, and movement between breeding sites. Since 1998, the CNHP has captured and tagged 826 male and 220 female toads in the area of Cottonwood Creek in Chaffee County, Colorado.

Pisces Molecular LLC is working on developing a real-time polymerase chain reaction (PCR) assay for detecting the presence of BD in environmental samples. This project is focused on 1) developing and testing the parameter of a quantitative, real-time PCR assay, which has been completed; and 2) developing a sample collection and DNA extraction protocols.

Lauren Livo of the University of Colorado at Boulder has and continues to investigate the connection between BD and the decline of the boreal toad and other amphibian species in the southern Rocky Mountains.

(3) Actions Involving Private Land Ownership

The Service and the CDOW are currently working on a Conservation Agreement with Assurances (CCAA) with the Climax Molybdenum Mine in Climax, Colorado. Unfortunately, the toad population on Climax's property has tested positive for BD. In addition to the Climax CCAA, the Service is currently working on a statewide CCAA with the State of Colorado. The Service and the State hope that a statewide CCAA will provide a conduit for private landowners to become more proactive in the conservation of this species. Although the Climax CCAA will provide a location for additional BD research, and the statewide CCAA a conduit for private landowners to be proactive in toad conservation, neither CCAA is a solution to the BD problem, the primary factor for the proposed listing of the SRMP of the boreal toad.

In cooperation with the FS and the CDOW, Vail Associates helped fund survey work in Summit County. Vail Associates has cooperated with both State and Federal agencies in reducing adverse impacts to the Cucumber Gulch wetlands, a known boreal toad breeding site. In addition to Vail Associates, the Pole Creek Golf Course has been working closely with the CDOW in preserving and enhancing 2 known breeding sites located on property owned by Pole Creek Golf Course.

E. Other Natural or Manmade Factors Affecting Its Continued Existence.

(1) Overview

Available data indicate that boreal toad populations have decreased substantially over the past 10 years. Additionally, declines in several other pond breeding amphibians have been noted (Hayes and Jennings 1986; Stolzenburg 1989), and several causes have been proposed, including acid precipitation (Harte and Hoffman 1989), application of fish toxicants (Burress 1982), droughts (Corn and Fogleman 1984), pollution, increased incidence of ultraviolet radiation (UV-B), and natural population fluctuations (Pechmann et al. 1991). However, many of these are only theories and have not been definitively proven. Some may not be a problem individually, but work synergistically to have adverse effects on amphibians (i.e., UV-B).

(2) Drought

The SRM-DPS has been affected by the current drought cycle (Livo and Loeffler 2003). Several SRM breeding sites have either remained dry throughout the breeding season or have dried-up prior to metamorphosis. Although, droughts are a natural factor in the "boom and bust" cycle that many amphibian populations experience. Droughts may be a stressor that exacerbates the BD problem. The magnitude of which drought can or has contributed to the decline of the boreal toad in the southern Rocky Mountains is unknown.

(3) Recreation

The following recreation activities have been identified as posing a significant threat to boreal toads: camping, hiking, biking, skiing, fishing, and off-highway vehicle use (Loeffler 1998). The direct effects from these activities include: trampling, loss of vegetation, reduced water quality, and loss of habitat. In association with human activities around boreal toad breeding sites, the presence of ravens and jays will inevitably increase, which may increase predation on boreal toads. The overall impact of these activities needs further research to determine their significance.

(4) Ultraviolet-B Radiation

Worldwide, declines in amphibian populations have mostly gone unexplained. The effect of increased ultraviolet-B (UV-B) radiation resulting from ozone depletions is speculated to be a contributing factor, particularly on those amphibian species inhabiting mountainous regions. Blaustein et al. (1994) demonstrated a direct correlation between increased levels of UV-B and amphibian mortality in *B. boreas* and the Cascades frog (*Rana cascadae*), but found no effect of ambient UV-B radiation on red-legged frog (*Rana aurora*) hatchling success. However, Corn (1998) found no evidence linking UV-B levels to the decline of the *B. b. boreas*. In addition, Blaustein's study does not explain why the SRM-DPS is in decline and the coastal Pacific populations appear to be stable. In a study of the pH/UV-B synergistic effect on amphibians, Long et al. (1995) found that individually neither pH nor UV-B had any adverse affects on northern leopard frogs (*Rana pipiens*). However, UV-B and pH synergistically did lead to significant decreases in embryonic success. In conclusion, there is no evidence that shows that increases in UV-B exposure have led to the decline of the SRM boreal toad population.

(5) Sportfishing Management

Fish toxicants are used widely in Colorado to manage sport fisheries and reestablish native fish populations. Extremely high concentrations of rotenone (5 μl/l) have been shown to cause mortality in larval northern leopard frogs (*Rana pipiens*) in controlled conditions (Burress 1982). The effects of rotenone, under field conditions and concentrations, on boreal toads are unknown. In 1986, the fish toxin Antimycin was used in Lost Lake, RMNP to eradicate the fish population. Although information on the presence of toad larvae and the effects of Antimycin treatment were not gathered, subsequent amphibian surveys in 1990 and 1991 indicated a large adult boreal toad population. Antimycin also was used in 1990 to eradicate fish in Spruce Lake, RMNP. Park biologists found late stage larval boreal toads dead, but the overall effect on the 1990 year class was unknown (David Stevens, RMNP, pers. comm.).

The introduction of nonnative fish, and their subsequent predation on amphibian larvae, also has been widely reported to affect amphibian populations (Voris and Bacon 1966, Cochran 1983, Hayes and Jennings 1986, Bradford 1989). However, toad eggs and tadpoles reduce predation by producing chemical toxins that make them unpalatable (Licht 1968, Formanowicz and Brodie 1982, Hews 1988). Licht (1968, 1969) showed that *Bufo* eggs were unpalatable to two species of sunfish (*Lepomis* spp), catfish (*Ictalurus melas*), stickleback (*Gasterosteus aculeatus*) and cutthroat trout (*Salmo clarkii*). Similarly, Voris and Bacon (1966) found that larval forms of *Bufo* were eaten less frequently by *Lepomis* when offered with tadpoles of other genera. Carey (1987) is the only author identifying nonnative fish as a possible reason for declining boreal toad numbers. She observed fathead minnows (*Pimephales promelas*) foraging in shallow waters where boreal toad tadpoles were and proposed that minnow activity resulted in energetic stress for the larvae. However, because fathead minnows are not present in all locations where boreal toad declines have been documented, it is unlikely they are responsible for rangewide toad declines. Similarly, unpalatability of eggs and larvae and declining toad numbers in trout-free waters suggests that salmonid introductions in the montane regions of the southern Rocky

Mountains are not responsible for declining toad numbers. Stomach analysis of brook trout (*Salvelinus fontinalis*) at a site occupied by boreal toads did not produce evidence of predation on tadpoles (Jones et al 1999).

Several of the previously mentioned factors may not be relevant to the decline of boreal toad populations. For instance, drought has been implicated in localized amphibian reproductive failures (Pechmann et al. 1991) and extinctions (Corn and Fogleman 1984) but it is unlikely that it can be a primary factor responsible for the decline of this wide-ranging, long-lived species. Corn and Vertucci (1992) examined inputs of acid-producing anions to the mountains of Colorado and Wyoming and concluded that acid precipitation was unlikely to have caused or contributed to the decline of boreal toads in the southern Rocky Mountains.

Until the 1970s, the boreal toad was considered abundant throughout the southern Rocky Mountains. Over the past 30 years, dramatic declines and localized disappearances of numerous populations have been well documented (Corn et al. 1989, Carey 1993). Boreal toads have disappeared from 83 percent of their historic locations in Colorado and 94 percent in Wyoming (Corn et al. 1989) and are believed to be extirpated in New Mexico (New Mexico Department of Game and Fish 1988). Habitat quality and quantity does not appear to be a significant factor in the decline of this species, because many historic sites appear to be in good condition. Many of the aforementioned factors (drought, hardrock mining, grazing etc.) are not believed to be major factors in the decline of the boreal toad in the southern Rocky Mountains. As researchers investigate the decline of the SRMP of the boreal toad, the chytrid fungus Batrachochytrium dendrobatidis continues to be implicated as the primary threat.

SUMMARY OF REASONS FOR ADDITION, REMOVAL OR LISTING PRIORITY CHANGE:

____Is the removal based on a Policy for Evaluation of Conservation Efforts When Making Listing Decisions (PECE) finding? If "Yes", summarize the specific PECE evaluation criteria that were met in determining that the conservation effort is sufficiently certain to be implemented and effective so as to have contributed to the elimination or adequate reduction of one or more threats to the species identified through the section 4(a)(1) analysis.

FOR PETITIONED CANDIDATE SPECIES:

a. Is listing warranted? **Yes** ___
b. To date, has publication of a proposal to list been precluded by other higher priority listing actions? **Yes** ___
c. Is a proposal to list the species as threatened or endangered in preparation? **Yes** ___
d. If the answer to c. above is no, provide an explanation of why the action is precluded.

LAND OWNERSHIP: Approximately 90 percent of the known breeding localities are on Federal land (i.e., USFS), the remaining 10 percent is on private land.

PRELISTING: 1The CDOW has led an interagency Recovery Team for the boreal toad following advice of the FWS to include the toad's range in Wyoming and New Mexico into the recovery planning process. The Recovery Team is currently made up of personnel from the BLM, USGS-BRD, CDOW, Environmental Protection Agency, NPS, New Mexico Department of Game and Fish, FWS, USFS, and Wyoming Game and Fish Department. There also was a Technical Advisory Group created from former members of the Recovery Team. These include people from Waldorf College, University of Wyoming, University of Colorado, FWS, CDOW, Denver Water Department, USGS-BRD, and Colorado Natural Heritage Program. Members of the Recovery Team and Technical Advisory Group developed a Conservation Plan to address management and research needs in 1998 (Loeffler 1998) which was revised in 2001 (Loeffler 2001). The following paragraphs describe actions carried out under guidance of the Boreal Toad Recovery Team and the Conservation Plan.

Many of the involved entities have already expended large amounts of staff time and money for recovery efforts. In 2002, the CDOW continued a mark-recapture study using Passive Integrated Transponder (PIT) tag identification at Climax Molybdenum Mining Company's Henderson Mine in Clear Creek County to determine population size, movements, habitat use, and hibernacula locations. This population is known to be declining due to BD, but information gathered has been useful. The USGS continued a mark-recapture study in RMNP that has been ongoing since 1991 and also has provided information on population estimates and decline due to BD, as well as habitat use information. Few toads remain in this population. Another mark-recapture study was conducted by the Colorado Natural Heritage Program in the Chaffee County toad population which has not been infected with BD. Twenty-two out of 174 females PIT-tagged since 1998 have been recaptured in different years at breeding sites revealing that 55 percent of the females returned for breeding annually, 40 percent returned every other year, and 5 percent returned after a 2-year absence. This shows a varied breeding cycle but assumes that females captured at breeding sites were actually attempting to breed (CDOW, unpubl. lit. 2003).

Several BD and other disease studies were conducted by involved organizations in 2001 and 2002 to determine extent of area of BD infection, detection ability of the fungus by various methods and means, identification of other possible diseases contributing to declines, environmental factors contributing to BD infection, exposure levels contributing to mortality, life-stage acquisition and detection of BD, physiological changes in amphibians to BD exposure, and transmission of BD and another disease between amphibian species (CDOW, unpubl. lit. 2003). An international BD working group has been formed to address the fungus problem since declines have been noted in central America and Australia as well as the United States. Members of the international working group and the Boreal Toad Recovery Team and Technical Advisory Group have been working on determining the life history of the fungus and infection pathways. Specifically, work is proposed to determine if temperature, heavy metals, UV-B radiation, pH, and perhaps pesticides are impacting anti-microbial peptides on toads skin which, if so, would allow the BD to invade and cause death in boreal toads (Cindy Carey, University of Colorado, pers. comm. 2000). A Polymerase Chain Reaction (PCR) protocol and primer sequences were developed in 2001 and 2002 by Dr. Seanna Annis (University of Maine) to detect the BD on the toads. It is hoped, through ongoing refinements in the PCR test methodology, that the test will be able to be used for detection of the fungus in soil and/or water in the near future (CDOW, unpubl. lit. 2003).

A BD study was conducted at the Henderson Mine, the Henderson Mill site in Grand County, Lost Lake in Boulder County, and the Grand Mesa in Delta County (site of planned reintroduction) in 2002. The BD study's purpose was to identify detection methods of BD using sentinel tadpoles. Samples of chorus frogs and tiger salamanders also were taken from the Grand Mesa to determine if the planned reintroduction site has BD, but none was detected on the specimens. A CDOW employee conducted another BD study in 2002 and collected 213 boreal toad samples from 34 sites in north-central Colorado. Eight of the boreal toad samples were chytrid positive. Dr. John Wood from Pisces Molecular has conducted much of the PCR testing to date and conducted methodology studies to determine sample collection procedures and sensitivity of detection of the fungus. Dead boreal toads and other amphibians also were sent to Dr. Allan Pessier at the University of Illinois for histologic and pathologic study. Dr. Pessier also initiated a study on efficacy of treatments for BD infections but this has not yet been completed. Of the 13 boreal toads submitted to Dr. Pessier in 2001, 1 toad from the Henderson Mine was infected with BD. In 2002, five boreal toads were submitted but none showed evidence of infection. In 2001 and 2002, Dr. Cindy Carey, from the University of Colorado and Lauren Livo from the CDOW, conducted environmental interaction experiments with boreal toads and BD. Isolates of differing strains of BD also were tested to determine mortality rates. Tadpoles also were experimentally exposed to BD in two experiments. Furthermore, a study to determine if BD grown in a broth creates toxins was conducted with northern leopard frogs.

Lastly, Dr. Carey and Ms. Livo exposed tiger salamanders, boreal toads, and northern leopard frogs to *Ambystoma tigrinum* virus (ATV) to determine if this virus caused illness or mortality to other amphibian species. Final analysis of all these studies is pending (CDOW, unpubl. lit. 2003).

Additional actions carried out by CDOW, and other organizations are described below. A graduate student, funded by the CDOW, developed a computer model to identify suitable boreal toad habitat (Holland 2002). An amphibian field guide was developed through funding by CDOW to help people identify the boreal toad and other amphibian species. Several of the entities have been involved over the last several years, and especially the last 5 years, conducting inventory and breeding area monitoring actions (Boreal Toad Recovery Team 1998b, 1999, 2000; CDOW, unpubl. lit. 2001, 2003). The monitoring includes counting adults, egg masses, and tadpoles as well as recording breeding, non-breeding, and wintering habitat and recording water quality at breeding sites. The CDOW maintains a population database and GIS maps of the historic and current distribution of the boreal toad. Extensive genetic work has been accomplished by Dr. Anna Goebel, during and after her PhD internment at the University of Colorado, to determine relatedness of boreal toads within and outside of the SRM population range (Goebel 1996; Goebel 2000). Furthermore, the USGS-BRD funded a graduate student to examine theoretic causes of decline in the Lost Lake and Kettle Tarn breeding localities. The USGS-BRD and the University of Colorado have conducted some studies on ultraviolet radiation. Ultraviolet radiation (UV-B) research conducted by Dr. Cindy Carey (University of Colorado, pers. comm. 2000) found that boreal toads are not affected by current levels of UV-B in the environment but additional studies are proposed to determine if UV-B allows for BD invasion. Contaminant studies on heavy metals and transition elements also have been conducted by and funded through the University of Colorado, FWS, and CDOW.

Additionally, salmonid and predacious diving beetle, tiger salamander, and wandering garter snake predation have been investigated and other predators have been recorded. Boreal toad tadpole palatability tests with native and nonnative salmonids have been conducted in hatchery raceways by CDOW. The trout appeared to react negatively to the taste of tadpoles and largely ignored the tadpoles. Wild brook trout (*Salvelinus fontinalis*) inhabiting a pond with boreal toad tadpoles also were examined by CDOW, but no tadpoles were found in the trout stomachs (John Goettle, CDOW, pers. comm. 1996). Livo (1999) investigated predacious diving beetle larvae (*Dytiscus* sp.), tiger salamander larvae (*Ambystoma tigrinum*), and western terrestrial garter snake (*Thamnophis elegans*) predation and influences on boreal toad tadpoles. It was discovered that the beetle larvae not only eat the tadpoles but also can affect their foraging behavior, timing of metamorphosis, and size of young toads. Color variation in one group of tadpoles was thought to be a cryptic response to garter snake predation. Boreal toads appeared to occur more frequently at ponds without tiger salamanders and predacious diving beetles.

Propagation and rearing of boreal toads also has been conducted under the direction of the Boreal Toad Recovery Team and by CDOW to maintain genetic stocks in case of BD or other related die-offs. A "Hatchery Manual for Rearing and Propagation of Captive Boreal Toads" was produced by CDOW in 1997. Facilities currently housing toads for propagation and possible future reintroductions include the Native Aquatic Species Restoration Facility operated by CDOW (Alamosa, Colorado), Saratoga National Fish Hatchery operated by FWS (west of Laramie, Wyoming), Cheyenne Mountain Zoo (Colorado Springs, Colorado), Henry Doorly Zoo (Omaha, Nebraska), and the Toledo Zoo (Dayton, Ohio). There also are toads being reared primarily for education, display, and research at Ocean Journey Museum (Denver, Colorado); the CDOW offices in Ft. Collins and Durango, Colorado; the Morrison Museum of Natural History (Morrison, Colorado); and, the Cincinnati Zoo (Cincinnati, Ohio (CDOW, unpubl. lit. 2003)). Additionally, some toads will be given to members of the Integrated Research Challenges in Environmental Biology-National Science Foundation (IRCEB) group for BD research. A revised husbandry manual was produced in 2002 for NASRF called the "Native Aquatic Species Research Facility Boreal Toad Husbandry Manual." Experimental reintroductions have occurred in four sites in Colorado and one in Wyoming, but with limited success or no evidence of success (CDOW, unpubl. lit. 2003). A reintroduction is planned on the Grand Mesa for 2003. Short distance translocation and alteration of habitat to help survival of eggs and tadpoles also has occurred with limited success. There also have been a number of newspaper articles and televised reports about the boreal toad in order to inform the public about the boreal toad's status and what agencies are doing to recover the toad.

Conservation agreements, signed by participating entities, that describe actions to be carried out by the entities, have been included in the Conservation Plan. One parcel of private land within the range of a small breeding site was placed in an Open Space designation. A mining company west of Denver has initiated preparation of a Candidate Conservation Agreement with Assurances (CCAA). A golf course containing another breeding locality near Winter Park also is interested in entering into a CCAA, and Breckenridge Open Space near the Town of Breckenridge recently expressed interest in entering a CCAA. Impacts to several occupied or potentially occupied breeding sites have been avoided through informal consultation with the

FWS by the USFS or U.S. Army Corps of Engineers.

Despite numerous conservation actions funded and implemented to date, additional populations or breeding localities of the toad being found in the last several years, and protection of the toad afforded by State and Federal laws, the FWS should retain the toad at a listing priority of 3. This is due to the high magnitude (extent) of the BD infection; high likelihood of imminence of extirpation in boreal toad populations infected with the BD, high likelihood of imminence for potential endangerment of the boreal toad should the BD spread to the Chaffee County population. Additionally, existing State and Federal measures are not preventing loss of habitat (and resultant population loss) on private, State, and Federal land.

DESCRIPTION OF MONITORING: The CDOW and the Colorado Natural Heritage Program conduct and supervise all monitoring and survey efforts, and on a yearly basis the CDOW publishes a Monitoring and Survey report. The FWS believes this has been a sufficient and successful method of monitoring and keeping informed the FWS on the status of this species.

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LISTING PRIORITY

THREAT			
Magnitude	Immediacy	Taxonomy	Priority
High	**Imminent**	Monotypic genus	1
		Species	2
		Subspecies/population	3*
	Non-imminent	Monotypic genus	4
		Species	5
		Subspecies/population	6
Moderate to Low	Imminent	Monotypic genus	7
		Species	8
		Subspecies/population	9
	Non-imminent	Monotypic genus	10
		Species	11
		Subspecies/population	12

_X_ Have you promptly reviewed all of the information received regarding the species for the purpose of determining whether emergency listing is needed? **Yes**

Magnitude
In 2003, 28 percent of the known breeding localities tested positive for BD. This is a conservative number, considering the problems with false-negatives as described under Factor C of Threats. In addition, there is a high rate of infection (77 percent) at BD-positive sites. It is believed that BD is widespread and there is a high likelihood of uninfected sites becoming infected. We base this presumption on the lack of knowledge of why BD has become a problem and how it moves through the environment. Once sites become infected, there is no known treatment to prevent toad mortality.

Imminence:
Disease (i.e., BD) has been identified as the primary threat to this species. The rapid decline in the SRM-DPS of the boreal toad and the inability to stop or prevent the spread of BD poses an imminent threat to the continued existence of this species.

Is Emergency Listing Warranted?

The disease problem within the SRM-DPS of the boreal toad is not so imminent a threat that emergency listing is warranted.

APPROVAL/CONCURRENCE:

Approve:____Ralph Morgenweck______________________ ______August 30, 2004
Regional Director, Fish and Wildlife Service Date

Concur:______Matt Hogan, Acting__________________________ ______5/2/05
Director, Fish and Wildlife Service Date

Do not concur:____ _____________
______
Director, Fish and Wildlife Service Date

Director's Remarks:____
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Date of annual review:_____________

Conducted by:_________________________________

Comments:____
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