Native Aquatic Species Restoration Facility

Boreal Toad Husbandry Manual

by
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Acknowledgements

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Chapter 1 Introduction

The boreal toad, *Bufo boreas boreas*, once a common species in the mountains of Colorado, southern Wyoming, and northern New Mexico, experienced dramatic population declines beginning in the mid- to late-1970s. In 1994, a State Recovery Plan was written for the boreal toad, and an interagency Recovery Team was formed. As of December 2002, the boreal toad is listed as "endangered" by the Colorado Division of Wildlife and as "warranted but precluded" for Federal listing by the US Fish & Wildlife Service.

Rearing and propagating boreal toads in captivity were identified as research needs to assist with recovery of the boreal toad in the Boreal Toad Conservation Plan and Agreement (Loeffler 2001). The John W. Mumma Native Aquatic Species Restoration Facility (NASRF) in Alamosa, Colorado, began operation in 2000. This facility was funded in part by the Colorado Division of Wildlife (CDOW), Great Outdoors Colorado, and the Colorado Water Conservation Board. At the NASRF, the CDOW maintains a captive population of boreal toads that represent a genetically diverse and geographically extensive cross-section of existing populations. In addition, several zoos and other institutions now maintain boreal toads.

When the captive rearing program was started at the NASRF, the specific purpose was to house a backup reserve of toads in captivity due to concerns about the status of wild populations. Two of the three main groups in the wild were declining dramatically due to infection from a pathogenic fungus. Although at least some populations appear to be stable, the boreal toad has a vastly reduced distribution and many of the remaining populations are extremely small compared to pre-decline levels. Further, because it is unlikely that toads will recolonize vacant historical areas naturally, captive-reared boreal toads may be required for translocation efforts, making successful captive propagation extremely important.

In addition to use in reintroductions, a limited number of surplus captive boreal toads may be used for research purposes. There is strong evidence that chytrid fungus (*Batrachochytrium dendrobatidis*) is a major threat to boreal toads. Research toads may be used to learn more about the toad as well as the interaction of this chytrid fungus with toads.

This manual is focused on boreal toads in Colorado and is designed to be updated as new husbandry techniques are developed, and/or current practices are revised.

Chapter 2 Bringing toads into captivity

To ensure that captive stocks of toads adequately represent the genetic variation present in populations of wild boreal toads, collection of wild eggs, tadpoles, toadlets, or toads may be necessary. The Boreal Toad Recovery Team will determine which wild populations should act as donor populations in a given year, considering available space at the NASRF.

Considerations used to identify donor populations

Collection of small numbers of individuals representing early life history stages (especially eggs or tadpoles, but also metamorphs) is not considered to have a significant impact on native populations (for example, see Johnson 1994). However, if later life history stages (juveniles or adults) are to be taken, source populations should be of adequate size so that collection of these individuals will not impact the population's viability and continued persistence.

Considerable genetic variation has been observed in boreal toads in the Southern Rocky Mountain populations (see Chapter 3). When selecting wild populations to serve as donors for the captive rearing efforts, the Boreal Toad Recovery Team must consider the following:

- Specific goals of the captive rearing efforts (such as preservation of genetic diversity versus production of animals for release to translocation sites)
- Capacity of the NASRF to allocate resources for both existing and new animals

In addition, considerations from the table below (Table 1) can be used to guide decisions regarding donor site selection:

Table 1. Considerations in donor site selection

Assessment of risk	Chytrid status	Negative population trend	Unique genetics secured at the NASRF?
High	Positive	Yes (>75%)	No
Medium	Positive	Yes (25 to 75%)	No
Low	Negative	None observed	No
Not a crisis	Negative	None observed	Yes

When a donor site has been selected, one objective for the upcoming field season is to collect sufficient numbers of eggs or tadpoles from that site to ensure that approximately 15 individuals survive to breeding age. With a lot size of 15 individuals, there likely will be at least a few individuals of each sex (rather than all individuals of one sex). This would provide any captive breeding efforts greater flexibility when selecting individuals for a breeding program, and therefore a greater chance of success. Practical experience since 2000 indicates that obtaining 50 eggs or 25 tadpoles usually results in at least 15 individuals from a lot surviving through metamorphosis and over their first winter.

Collection and transfer protocol

The Boreal Toad Recovery Team prioritizes sites and determines how many populations, and from which specific geographic areas, to target for collection efforts. The Team usually makes this determination before the field season begins and gives specific individuals responsibility for egg and tadpole collection from the selected breeding populations. Individuals making egg or tadpole collections must possess a valid Scientific Collection permit from the State of Colorado.

Biologists believe that eggs are less likely to be infected with chytrid fungus than tadpoles, and that tadpoles are less likely to be infected than toadlets or toads. In addition, because a single boreal toad egg mass contains thousands of eggs, removal of a small number of eggs or tadpoles for captive propagation has minimal effect on the donor population (c.f., Johnson 1994). Consequently, eggs are the preferred life history stage for collection, followed by tadpoles. Do not collect toadlets or toads from a site unless you have specific instructions to do so.

Before collecting eggs or tadpoles, coordinate with hatchery personnel to ensure that rearing facilities are available and that someone will be at the hatchery to receive the eggs or tadpoles.

Collection checklist

- Waterproof containers with secure lids
- Cooler with ice or snow
- Net (for tadpoles)
- Battery-operated aerator with air stone

Collecting eggs

To collect boreal toad eggs, gently lower the container into the water near an egg strand; this results in a portion of the egg strand entering the container. Pinch the jelly surrounding the eggs so that about 50 eggs (usually less than 3 inches of egg strand) remain in the container. Fill the container with water from near the egg mass. Because decomposing matter may deplete oxygen levels, especially in a closed container, minimize the amount of dirt, debris, and vegetation included with the eggs.

Collecting tadpoles

Only collect tadpoles if eggs are unavailable. Use a small net (such as used for tropical fish) to collect about 25 tadpoles. Fill the container with water from the pond. Because decomposing matter may deplete oxygen levels, especially in a closed container, minimize the amount of dirt, debris, and vegetation included with the tadpoles. Ensure that the tadpoles are from separate clutches, or, if this is not possible, on the container's label note the possibility that the tadpoles are from multiple clutches.

Collecting toadlets or toads

Only collect toadlets or toads from a boreal toad site if you have explicit instructions to do so. These instructions should also include the number and gender of toads to collect. The container used should have air holes in the lid or sides and a moist paper towel in the bottom. If multiple toads are transported, ensure that the container is large enough to avoid crowding.

Transporting eggs or tadpoles
Label each container with:

- Site name
- Collection date
- Stage (egg or tadpole) of sample

Keep individual egg or tadpole samples in separate containers. Keep the containers cool (for example, in a cooler) during transport. If snow is available at the boreal toad breeding site, you can place some around the containers to prevent overheating (See Figure 1).

Figure 1 Snow being placed in cooler with tadpoles in labeled containers in preparation for the hike out from the breeding site

Eggs in particular may be fragile; avoid unnecessary jostling during transportation. Do not use an All Terrain Vehicle (ATV) to transport eggs. (Tadpoles have survived transport by ATV.) Check the eggs or tadpoles every hour or two to assess their condition. If you have a prolonged stopover during transport, remove the lid from the container, but be sure to replace it before resuming your trip. You also may use an aerator, such as used in fish tanks if it provides gentle water movement. For example, the NASRF has a tadpole transport cooler with a hole drilled in it for the aerator tubing. Do not use a forceful aerator that causes the eggs or tadpoles to swirl around in the water.

Call personnel at the NASRF with your estimated time of arrival. Ensure that the eggs or tadpoles arrive at the hatchery within 24 hours of collection.

Transporting toadlets or toads

Place toadlets or toads in a container with airholes in the lid or sides and a moist paper towel in the bottom. Label the container with the site name and collection date. Prevent overheating during transport, for example by placing the container in a cooler.

Chapter 3 Ensuring genetic tracking

Boreal toad genetics

Two genetic data sets are being collected on wild animals, as well as those in captivity, to assist captive breeding programs. First, mitochondrial DNA (mtDNA) control region sequence data are being collected. Mitochondrial DNA is effectively one locus with many characters and recombination is extremely rare, if present at all. These two features make it the most reliable single locus for identifying lineages. Second, nuclear amplified fragment-length polymorphism (AFLP) data are being collected for 124 loci. AFLP data are useful because they identify variation at many levels (between siblings as well as between species in the *Bufo boreas* group) and the data are made up of many loci, overcoming potentially misleading data when a single locus only is analyzed. Both kinds of data have been and continue to be collected on both wild animals and toads in the captive-breeding program.

Previous studies on animals in the wild indicated two complimentary genetic patterns, "isolation by distance," and a differential north/south distribution, which may be a cline. A significant correlation between genetic distance and geographic distance was identified. This means that the more distant two populations were, the more isolated their gene pools were found to be with AFLP data. This same pattern is seen in the north/south distribution of mtDNA haplotypes and nuclear alleles. The most northern populations share the fewest characters with the most southern populations. Central populations have both unique alleles and share a few alleles with both northern and southern populations. It is not clear whether this is due to a cline from a single invasion to Colorado, or is due to two separate invasions, one from the north and one from south (as is suggested by the mtDNA data). One single data set, mtDNA haplotypes from restriction site data of the whole mtDNA, identified the formerly large population in Rocky Mountain National Park as being considerably different from the two other largest populations in the Clear Creek Drainage and Chaffee County.

Both data sets are used to measure and identify patterns of diversity. Diversity within and between populations was estimated in wild populations. AFLP data are being analyzed within three sibling sets from the captive-breeding program. These data will be used to identify appropriate mating pairs both to avoiding inbreeding and to match patterns of diversity found in wild populations. Specific breeding strategies can be tailored to the specific goals of the captive-breeding program. For example, if the goal is to increase animal numbers at current wild populations, then mating pairs can be chosen such that mates are as divergent as the maximum divergence found in the wild population they represent. This would avoid inbreeding, maximize diversity, and mimic wild patterns of diversity. Other strategies can be designed for different goals.

Critical genetic data are still needed. The current sibling sets examined are from egg masses brought in from the wild. Therefore they may not be full siblings (there may be multiple fathers) and the parents were not examined along with the offspring. Parents and their sibling sets are needed to identify patterns of inheritance for the AFLP alleles. Data should be collected on all individuals that will be bred in captivity and from some parent and sibling sets to make a database for identifying patterns of diversity in closely related animals.

Naming conventions for individuals/cohorts

When hundreds or thousands of toads are kept in captivity, it is imperative that a naming convention be used to identify groups of toads. The current naming convention is described below.

Breeding site
Each breeding site has been assigned a unique four-character code (see Table 3 and Figure 17 in Chapter 14). The first two characters are alphabetic and indicate the county in which the site is located. For example, "JA" represents Jackson County and "CF" represents Chaffee County. The second two characters are numeric and describe the breeding site number within the county. For example, "JA01" indicates the first site (Spike Lake) in Jackson County and "CF07" is site number 7 (Fourmile) within Chaffee County. Each boreal toad breeding locality has one of these unique site codes assigned to it.

Year brought to facility
The next two digits on the code indicate the year the clutch was brought to the facility. With the exception of a few metamorphosed toads brought in prior to 2000, this code also indicates the year that the toads hatched. For example, the code "CF07-01" represents a lot that was brought in from Fourmile in Chaffee County in 2001.

Clutch number
The last two digits of the code indicate the clutch number brought into the NASRF that particular year from the site. For example, the code "CF07-01-02" represents the second clutch brought into the NASRF in 2001 from the Fourmile site in Chaffee County.

Progeny arising in captivity
Progeny born in captivity from toads captured as adults in the wild are given the same lot codes as though they were from a clutch produced at the site (same breeding site, year, and clutch number information). Progeny arising in captivity from toads taken as eggs or tadpoles from the wild will be assigned a ten-character code with the first two characters NA (indicating the NASRF). For example, toads with the code "NA-CF07-03-01" would be the first lot in the hatchery in 2003 from the Fourmile site in Chaffee County, and would be the product of adult toads collected as eggs or tadpoles from the Fourmile site in Chaffee County.

Age when taken into captivity
If juvenile or adult toads are brought into captivity, the last two digits of the code are "AD." For example, the code "CF07-00-AD" would be used for one or more juvenile or adult toads brought into the NASRF in 2000 from the Fourmile site in Chaffee County. As almost all animals are brought in as eggs, tadpoles, or metamorphs, the –AD suffix is rarely used.

Studbook

A studbook is a true record of the history of a captive population. It includes pedigrees of animals and lists the various locations in which animals have been held. Not only does a

studbook contain the history of an individual in a population, it also contains information on general biology and ecology of the species, the status and distribution of wild populations, as well as a bibliography of relevant publications.

Studbooks are primarily used for monitoring and managing populations in captivity, whether in a zoo, aquarium, or breeding facility. They provide an accurate database for a particular species, which allows for detailed genetic and demographic analyses. The most common use of studbook data is for making breeding decisions so that genetic variation can be retained and close inbreeding avoided. The data also provide information regarding the stability of a population, and whether numbers are declining or increasing.

The American Zoo and Aquarium Association's Wildlife Conservation and Management Committee (WCMC) and Taxon Advisory Group (TAG) have approved over 350 studbooks for species managed in North American collections. By establishing a studbook for the boreal toad, there will be a database that contains important information regarding individual pedigrees and demographic data. This information will assist in breeding the toads in captivity without compromising the genetic integrity of a specific population.

Once the studbook is established (mid-2003), the information contained therein will be kept in several accessible locations, such as the NASRF, the Cheyenne Mountain Zoo, and with the Aquatic Research Section at the Colorado Division of Wildlife. The keeper of the studbook will be Mark Kombert, DVM, at the Cheyenne Mountain Zoo.

Chapter 4 Housing

Successfully rearing boreal toads in captivity depends on a thorough understanding of the toad's natural history. Therefore, the culturist should be knowledgeable about the existing and developing research on boreal toads and related species. Cooperation with the Colorado Division of Wildlife's Aquatic Research group and members of the Boreal Toad Recovery Team will be helpful in this endeavor.

Because boreal toads are ectothermic, they depend on their environment to maintain their body temperature, so there should be a range of temperatures available in toad enclosures. This will allow each individual to use different areas, including water, basking areas, and hiding areas to thermoregulate. Since boreal toads do not drink, but depend on open water or moist substrates to stay hydrated, a range of moisture levels and humidities will help toads stay in osmotic balance. Hiding places provide areas for toads to escape and feel secure. These hiding places should be provided throughout the temperature and humidity range in an enclosure, so that toads "are not forced to choose between meeting physiological needs and security" (Barnett et al. 2001). Hiding places can be made from plastic reptile huts, overturned clay pots with entrance holes, or pieces of PVC piping (see Figure 2). Plastic aquarium plants can also provide hiding spots throughout the enclosure. Toad enclosures should be away from areas of high activity and loud noises, as this can be stressful to the animals (Barnett et al. 2001).

Figure 2 Toad hiding places

Toad housing should be easy to maintain and disinfect, while also allowing for as many natural behaviors (such as hiding, swimming, and hunting) as possible so that the captive-reared animal has a higher likelihood of survival following release to the wild. Soil should be avoided for husbandry tanks, but it is used in display tanks. Electrical "hot rocks" should not be used in

amphibian husbandry because of their very high temperature (S. Taylor, pers. comm.). If gravel is used in boreal toad enclosures, it should either be too big for the toads to ingest or small enough to pass through the toad's digestive tract easily. If it is necessary to rear boreal toads in tap water, the water should be run through an activated charcoal filter or allowed to sit in an open container for at least 24 hours to allow chlorine to dissipate; adding an airstone to the water will reduce this time (Barnett et al. 2001).

Details on housing specific to each life stage of boreal toads are given on the following pages.

Egg, tadpole, and metamorph housing

Enclosure
When brought into captivity, eggs and tadpoles are placed in tanks in an isolation room. Individual clutches are maintained in separate tanks. Tanks are made of 0.25-inch non-tempered glass, and are 40" long x 15" wide x 15" deep. The tank has a drain with a standpipe to maintain appropriate water levels, and a secondary drain that serves as a backup in case the primary drain fails. Screening is placed over drains to prevent tadpole loss. Aquarium sealer used in construction was allowed to cure for seven days before the tank was used for eggs or tadpoles. See Figure 3 for photo of tank used for tadpoles and eggs.

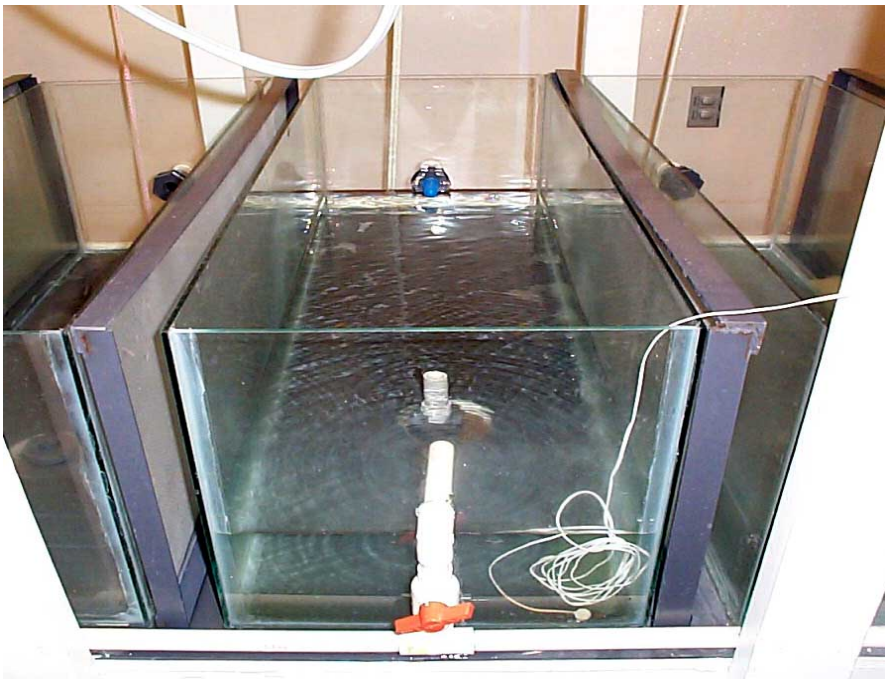

Figure 3 Tank used for eggs and tadpoles

Tadpole survival is density-dependent. Recommended tadpole density for breeding stock is approximately 25 tadpoles per tank, which results in 90 percent survival to metamorphosis. Toadlets are transferred to conventional tanks approximately 3 to 5 days after metamorphosis (see *Toadlet and toad housing* section).

For convenience, tadpoles identified for research use can be maintained in 16’ fiberglass raceways, and can be reared at much higher densities, with up to 2000 tadpoles per raceway. However, due to these high densities, as well as insufficient metamorphic area at the peak of metamorphosis, the post-metamorphic mortality of these tadpoles at the NASRF has been as high as 85 percent.

Lighting
A Reptisun 5.0 UVB fluorescent bulb (48-inch, 290-315 nm spectrum) is suspended over the length of the tank so that the light is approximately 15” above the floor of the tank, as is a Zoomed incandescent daylight blue reptile bulb (UV-A). Lights are replaced every 6-12 months to ensure effectiveness. Because ultraviolet light will not pass through glass or plastic, full-spectrum lights should be placed over an open tank or outside only a screened lid (Barnett et al. 2001). Lights are on timers with a 12L:12D photoperiod.

Water depth
During the egg and most of the tadpole stage, water is maintained at a depth of 8-9”. As tadpoles approach metamorphosis, the water level is lowered to 5”. A sheet of perforated plastic the width of the tank is folded to an oblique angle and placed in the tank so that metamorphosing tadpoles have access to a dry area (see Figure 4).

Figure 4 Ramp provided for metamorphosis

Water quality & temperature
Water at the NASRF comes from artesian wells that have a pH of 8.8 to 8.9; it is stripped of nitrogen, oxygenated, and cooled to 68-70ºF. It is then used without further treatment for boreal toad eggs, tadpoles, and metamorphs. The water quality at facilities housing boreal toads other than the NASRF should meet values found at boreal toad sites, or at least general water quality

recommendations for amphibians. Facilities without flow-through systems should routinely conduct water quality testing. Water quality values observed at boreal toad sites, as well as general water quality recommendations for amphibians, are listed in Chapter 15.

Air temperature and humidity
All rooms are kept at 68ºF or above. As there is no air conditioning, summer temperatures can approach 80ºF. No effort is made to control humidity, which averages 60 percent.

Labeling
Each tank is labeled with the inhabitants' unique identifying code (see Chapter 3). Clipboards with monthly datasheets (see Chapter 17) are attached to each enclosure.

Cleaning
Tadpole tanks are cleaned with a siphon hose as needed, when debris accumulates in the tank.

Toadlet and toad housing

Enclosure
All toadlets and toads are maintained in Gemini fiberglass tanks with interior dimensions of 60" long x 24" wide x 12" deep. Each tank is tilted a few degrees by cutting approximately 6" off of the 12" legs at the drain end. Water flows into the standing water end of the tank and exits via a screened drain. The "land end" of the tank is provided with half of a 6" diameter PVC pipe (forming a "hut") and a sheet of perforated plastic bent into the shape of an inverted V (forming a "ramp") where toads often bask under the light (see Figure 5). Typical dimensions for the ramp are 7" to 10" wide x 17" long, with the fold at 11", resulting in a height of 3" to 5". The plastic is purchased through United States Plastics Corporation, and can be cut using a table saw or hand saw. Use an EMX strip heater to bend the sheets. To prevent adult toads from climbing out, the tank corners above the dry end of the tank are covered with triangles of plastic. To prevent toadlets from escaping, an overhang made with plastic sheeting cut into strips is installed along the top edge of tank (see Figure 6). See Figures 7 & 8 for photos of the NASRF rearing facilities.

Figure 5 Toads basking on plastic ramp

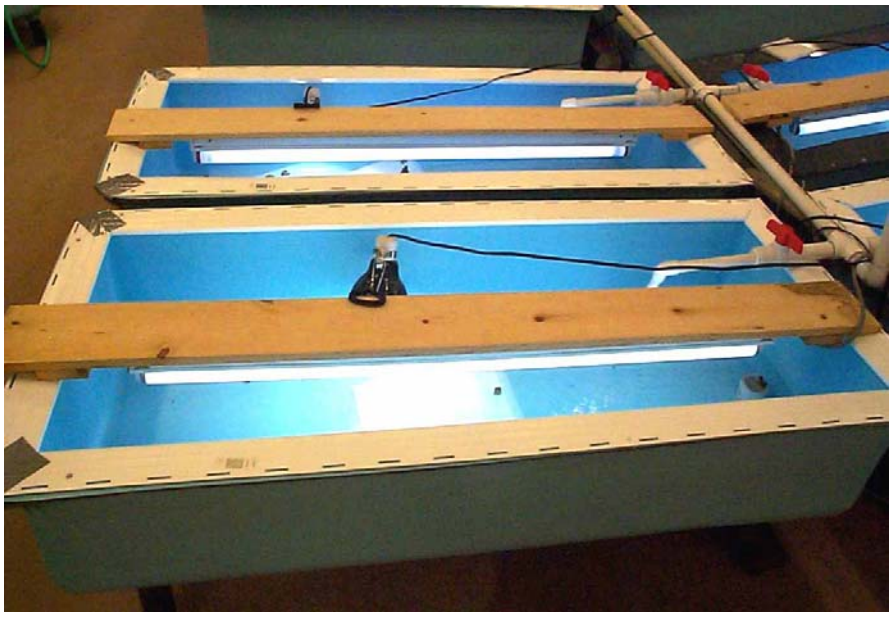

Figure 6 Plastic material placed along top edge of tanks to prevent toadlets from escaping

Figure 7 Toad tanks on the production floor at the NASRF

Figure 8 Tadpole tanks inside trailer at the NASRF

Lighting

The ceiling lights for the facility are fluorescent tubes that illuminate the room whenever hatchery personnel are working. Lights at one end of the building remain on 24-hours a day during the summer, but are turned off at night during the winter. Each fiberglass tank has a

Reptisun 5.0 UVB fluorescent bulb (48-inch, 290-315 nm spectrum) suspended over the length of the tank so that the light is approximately 12" above the floor of the tank; these lights lose their effectiveness when suspended at distances of more than 15". Lights are replaced every 6-12 months to ensure effectiveness. Because ultraviolet light will not pass through glass or plastic, full-spectrum lights should be placed over an open tank or outside only a screened lid (Barnett et al. 2001). Each tank also has a 100-watt incandescent daylight blue reptile bulb (UV-A) suspended above the dry end of the tank. This light provides heat and become extremely hot. Toads must be kept at least 6 inches from the bulb. (**NOTE**: Do not place objects such as the PVC pipe "huts" or perforated plastic ramps directly under the lamps, as toads will stack on top of one another and may receive severe burns or die). Tank lights are on timers with a 12h:12h photoperiod. See Figure 9 for photo of toad tank with lighting.

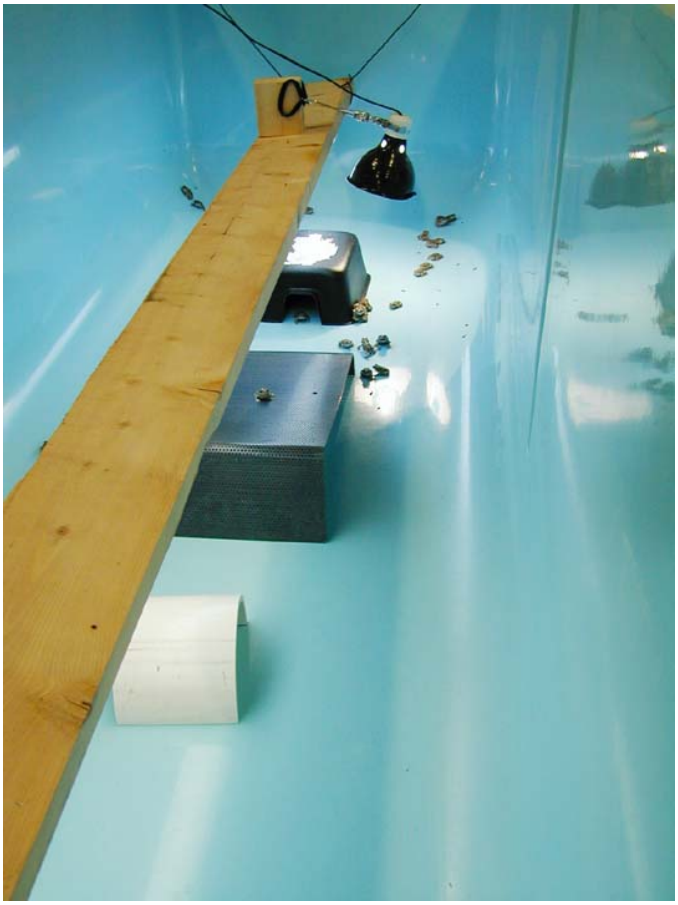

Figure 9 Toad tank with lighting

Water depth
Approximately 50 percent of the tank surface is covered with water. Maximum depth is 2.5".

Water quality & temperature
Water at the NASRF comes from artesian wells that have a pH of 8.8 to 8.9; it is stripped of nitrogen, oxygenated, and cooled to 68-70ºF. It is then used without further treatment for boreal toad toadlets and toads. The water quality at facilities housing borealss other than the NASRF should meet values found at boreal toad sites, or at least general water quality recommendations for amphibians. Facilities without flow-through systems should routinely conduct water quality testing. Water quality values observed at boreal toad breeding sites, as well as general water quality recommendations for amphibians, are listed in Chapter 15.

Air temperature and humidity
All rooms are kept at 68ºF or above. As there is no air conditioning, summer temperatures can approach 80ºF. No effort is made to control humidity, which averages 60 percent.

Labeling
Each tank is labeled with the inhabitants' unique identifying code (see Chapter 3). Clipboards with monthly datasheets (see Chapter 17) are attached to each enclosure (see Figure 10).

Cleaning
Toadlet and toad tanks are cleaned once per week.

Figure 10 Toad tank with datasheets attached

Chapter 5 Prevention of disease transmission

Amphibians, including boreal toads, are vulnerable to a number of bacterial, fungal, and viral pathogens. Follow the procedures described in this section to minimize the opportunity for these microorganisms to become established in captive populations. These procedures will also help prevent transmission of pathogens from one lot of toads to another or introduction of a pathogen from NASRF animals to a translocation site.

Prevention of disease transmission has five basic steps:

- Minimize the likelihood of bringing pathogens into captivity
- Prevent contact among individuals from different lots of toads
- Use equipment for only one group of toads prior to disinfection or disposal
- Disinfect equipment after use
- Appropriately quarantine new or ill individuals

Minimize likelihood of bringing pathogens into captivity
In natural populations, there is an increasing chance for contact with pathogens as an individual progresses through its life cycle. In recognition of this, the order of preference for bringing individuals into captivity from a wild population is: eggs > tadpoles > metamorphs > older toadlets/toads.

Individuals of later life stages, such as metamorphs, may be brought into captivity, but only under special circumstances. One such circumstance would be if the metamorphs represent a breeding locality discovered late in the season and there is no opportunity to acquire earlier life stages that year. Other situations, such as bringing in adult toads, require prior approval from the Boreal Toad Recovery Team.

Prevent contact among individuals from different lots of toads
Once in captivity, toads derived from different clutches or localities must be kept in separate tanks. When moving animals, for example when placing individuals into hibernation chambers, check labels carefully to ensure that toads are not inadvertently placed in the wrong container.

Captive breeding efforts are the exception to contact among animals from different lots. In this situation, all animals used in captive breeding should appear healthy, including normal behavior and good body condition. These toads can be returned to their lots at the end of breeding efforts.

Use equipment for only one group of toads prior to disinfection or disposal
Change disposable gloves before moving from one lot of toads to the next. All equipment (nets, containers, etc.) must be disinfected before the equipment is used on a new group of toads. When a particular item is used frequently, such as a net for tadpoles in a tank, individual nets can be assigned to specific tanks, allowing for a longer interval between disinfections.

Disinfect equipment after use
Wright and Whitaker (2001a) provide the following important distinction between the terms "cleaning," "disinfecting," and "sterilizing":

> Cleaning: action of physically removing organic and inorganic debris from an item.
>
> Disinfecting: reduces the load of contaminating organisms to a large extent, but not completely.
>
> Sterilizing: renders…[an] item devoid of all life.

While cleaning is necessary prior to disinfection or sterilization, cleaning alone is not sufficient to maintain a healthy habitat for captive toads.

Disinfect equipment by placing it in a 10% bleach solution (one part commercial bleach containing 6% sodium hypochlorite to nine parts water) and allow it to soak for 10 minutes. A circular tank located in the southwest corner of the hatchery floor at the NASRF is used as a disinfection tank; the bleach solution is replaced weekly prior to the scheduled cleaning. After removing equipment from the bleach solution, rinse the equipment thoroughly. If toads are present in the room where equipment is disinfected, adequate ventilation is vital, as chlorine gas has been associated with amphibian disease outbreaks (B. Spencer, pers. obs.). Also, be careful to remove all traces of bleach, as residual bleach can be fatal, especially for tadpoles.

If a disease outbreak occurs despite normal disinfection procedures, sterilization of tanks and other equipment may be required.

> Guidelines for sterilization (Wright and Whitaker 2001a):
- Many pathogens can be killed with bleach and ammonia.
- *Mycobacterium* spp. can be killed with prolonged exposure to steam (usually not practical) or formalized saline (>500ppm formalin) (adequate ventilation and respiratory protection are necessary with this option).
- Contact time with disinfectant should be 30+ minutes for sterilization.

Appropriately quarantine new or ill individuals
When boreal toads of any life stage are brought into a facility from the wild, they should be quarantined from resident toads. The recommended quarantine period for wild-caught amphibians is 90+ days (Wright and Whitaker 2001a). Keeping these toads away from the established population at a facility will reduce the likelihood of disease or parasite transmission to the resident population (Wright and Whitaker 2001a). Eggs and tadpoles brought into the NASRF are reared in an isolation room. They are placed in tanks on the main floor after metamorphosis. Individual lots of toads remain in separate tanks throughout their lives, with the exception of healthy animals selected for captive breeding efforts.

Quarantine housing can be in plastic boxes or glass aquariums that are easily disinfected. These tanks should be in a separate room from where the established population of boreal toads is to prevent airborne pathogens from traveling between the tanks (Wright and Whitaker 2001a).

Quarantine may also be necessary if there is a sudden catastrophic disease outbreak. If a disease outbreak occurred at the NASRF, toads would be isolated by placing them in an isolation room or isolation trailer.

On occasion, toadlets or toads escape from their tanks. When an escaped toad is caught, maintain it in an individual container for 90 days. If, during this quarantine period, it shows no evidence of disease and no disease outbreak is occurring with other captive toads, the escapee can be returned to the tank with others from its lot.

Chapter 6 Feeding

There has been little research on nutritional needs of amphibians or the nutrient content of potential prey species, and no known research on these issues relative to boreal toads. Relevant developments in this field should be included in future versions of this manual. In the interim, some guidelines based on past experiences and current practices are given in the following pages. In general, toads of all life stages should be offered a variety of food items to provide a diversity of nutrition. Recording the food given to individuals may be valuable if an animal becomes sick or dies.

Tadpole feeding

Tadpoles are fed the food items listed below, daily. Excess food is siphoned out of the tanks at the end of each day.

- Prepared Tadpole Gel Strips
  To make tadpole gel strips: Use approximately a 50:50 ratio by weight of Mazuri dry gel to hot (>200ºF) water (e.g., 250 g of feed and 250 ml water). Blend with spoon and pour to a depth of one inch into a flat pan. Chill overnight in a refrigerator. Store unused gel covered in a refrigerator for no more than two weeks; discard if mold is present.

  To make tadpole feed coating: Grind Hikari algae wafers in the coffee grinder used for producing the tadpole food. Mash Spirulina Aquarium Flake Food and shrimp flakes with a mortar and pestle to reduce flake size. In approximately equal amounts by volume, combine the ground algae wafers, Spirulina flakes, and shrimp flakes. Store in closed container at room temperature for up to one year.

  Feeding to tadpoles: To prepare, cut gel into 1” x 1” x 0.25” strips. Coat each strip of gel with the tadpole coating. Place 7 to 8 of the coated strips in a 25-tadpole tank.

- Romaine lettuce
  Small pieces (3”) of frozen romaine lettuce can be given to tadpoles. Lettuce is placed on the water’s surface. **Note**: Do not substitute spinach or other greens, as side effects such as oxalate kidney stones have been observed in other amphibian species.

- Spirulina Aquarium Flake Food and shrimp flakes
  Approximately 10 g of Spirulina Aquarium Flake Food and shrimp flakes are sprinkled into tank.

Toadlet feeding

Newly metamorphosed toadlets are “catch fed” wingless fruit flies (*Drosophila melanogaster*) twice a day for about a week. To do this, a few fruit flies are shaken gently from the culture jars into a small plastic container. The container is then placed in a freezer for approximately 45 seconds so the fruit flies stop moving but are not frozen. The flies are then powdered with a mixture of Flukers vitamin and mineral supplement. Toadlets are then placed in a small, lidded

plastic container with at least 2-3 fruit flies per toadlet. The toadlets are allowed to free feed for 10 minutes. **NOTE**: Toadlets must not remain in these containers for a prolonged time (>20 minutes), as they will become desiccated. As toadlets grow, they are graduated to a diet of pinhead crickets twice a day for approximately a week.

When toadlets are large enough to eat 1-week-old crickets, feedings decrease to once per day. Two-week-old crickets and three-week-old crickets are provided once per day as the toadlets grow to a sufficient size to feed on them. It is important not to feed food items that are too large, as the risk of prolapsing increases (see *Illness and treatments* section).

Toad feeding

Adult toads are fed 3-week old crickets, powdered with Repta-calcium and Repta-vitamin mix just prior to feeding. Adult toads are fed daily.

Culturing toad food items

Most food items given to boreal toads will require vitamin and mineral supplementation. Wright and Whitaker (2001b) describe two techniques to accomplish this:

1. Dusting: use calcium carbonate or other non-phosphorus forms of calcium. Recommended for food items that will be killed from gut loading or for whom gut loading is impractical (fruit flies, pinhead crickets).
2. Gut loading: recommended for larger crickets and waxworms. Feed no more than 48 hours before prey item will be eaten by amphibian. Gut loaded crickets should be given access to water or cut fruit (without moisture, crickets will die quickly when eating a gut loading diet). May be less effective for older crickets that eat less near the end of their lives.

With both techniques, prey items should be eaten within a few hours of gut loading or dusting to maximize calcium intake by the amphibian.

Fruit flies

Wingless fruit flies (available from Carolina Biological) are cultured as follows:

1. Combine ½ cup of Instant Potatoes and ½ cup of water with mold inhibitor (1 g mold inhibitor per 3 cups water) in mason jar. (The mold inhibitor and water must be boiled together prior to use.)
2. Add 60-90 grains of yeast.
3. Let stand 1 minute.
4. Add fruit flies to jar (preferably from a 2-3 week old culture that has not had flies removed for other purposes), cover with fabric, and seal with rubber band or metal ring.
5. Put new cultures to the right of other cultures and label with today's date and six weeks from today's date (discard date).

Crickets

Crickets (available from Bassets) are kept in Rubbermaid bins (approximately 40" long x 20" wide x 16" deep). Small bedding pots are placed in the Rubbermaid containers to provide cover for crickets. These pots are also used to dispense crickets into plastic deli containers for feeding to toads. Crickets are fed commercial rabbit pellets. Hydrosource polymer crystals are used as a

water source for crickets. To prepare, put 1 cup Hydrosource into a 5 gallon bucket and add water (2 to 3 gallons water) until Hydrosource is saturated; cover bucket and keep at room temperature. To hydrate crickets, place a quarter cup of Hydrosource onto a shallow, roughened dish and place it in the cricket bin; replace weekly. The advantages of Hydrosource are that crickets do not drown in it and it is inexpensive.

Waxworms

Waxworms (available from Bassets) can be stored in the refrigerator with minimal care. If waxworms are stored at warm temperatures, they will pupate.

A daily checklist for culturing food items at the NASRF is in Chapter 17.

Chapter 7 Hibernation protocol

Hibernating captive toads may serve an important role in preparing toads for reproduction. Hibernation may also increase the lifespan of toads, as it provides a "resting period" of several months from the demands of active living. Finally, hibernating toads reduces over-wintering feeding and maintenance costs for the facility housing the toads.

Determining which toads should be hibernated

Space limitation
As of the winter of 2002-2003, there are two environmental chambers at the NASRF. Each chamber can hold 12 to 16 individual hibernacula. Because hibernation space is limited, not all groups of toads may have hibernating individuals.

Adult toads
Because each representative group of toads must be kept separate from other groups, and 15 toads per group are retained permanently in captivity, in the past only 5 from each group of adults were hibernated while the remaining 10 remained active through the winter in their normal enclosures. In general, 3 females and 2 males were selected for hibernation; these were randomly selected from the group. For adults, PIT tag numbers are recorded for all toads as they are assigned to either the hibernating group or the non-hibernating group. At present, as many toads as possible will be hibernated; however, metamorphs will stay on the hatchery floor to continue feeding throughout the winter.

Juvenile toads
Because gender cannot be determined for young-of-year (YOY) and juvenile toads, 5 individuals per group are randomly selected for hibernation. YOY toads may be hibernated if they have reached a mass of at least 2.5g.

Population priority
Because hibernation space is limited, priority is given to toads from sites considered more valuable or more at risk than other sites. For example, sites from the northern subpopulation or chytrid-positive sites may be given preference for hibernation over thriving populations from sites in the southern subpopulation.

Health of individuals
If any individual toad appears to be unhealthy (dark skin, emaciated, bloated, or behaving abnormally), it will not be considered for hibernation.

Environmental chamber

Disinfection
Each environmental chamber is disinfected by spraying the interior with a 10% bleach solution and allowing the solution to remain on the interior of the chamber for at least 15 minutes (see Chapter 5 for explanation). The interior is then rinsed with water and allowed to air dry. If this

procedure is completed after toads are taken out of hibernation in the spring, it need not be repeated in the fall.

Temperature
The environmental chamber temperature is set at 41ºF for the entire hibernation period. There are three safety settings plumbed into the hatchery dialer: loss of power, a high temperature alarm set at 58ºF, and a low temperature alarm set at 35ºF. When notified by a pager, the panel in the Hatchery Manager's office displays whether one of the environmental chambers is malfunctioning. If the chamber malfunctions, remove the toads until a repair can be effected or toads can be transferred into another chamber.

Humidity
Because of the high level of humidity on the production floor of the NASRF, the humidity within the environmental chamber has remained at ≥75 percent without any adjustment of the chamber.

Lighting
There are no lights in the environmental chamber; hibernating toads are kept completely in the dark except for the brief examination periods every two weeks.

Monitoring conditions
The environmental chamber should be adjusted to hibernation settings approximately one month prior to the hibernation period (adjusted on September 1 for a hibernation period beginning October 1) to insure that it is operating correctly. Thermometers and hygrometers should be placed inside the environmental chambers to verify that temperature and humidity are being maintained.

Individual hibernaculum

Container
For adult toads, the individual hibernaculum is a plastic box (13" long x 9" wide x 7" tall) with a tight-fitting plastic lid. A smaller box may be used for toadlets, but details have not yet been determined. Drill approximately 25 quarter-inch holes in bottom of the box for drainage and approximately 20 in the lid for air. Clean, disinfect, and rinse boxes before use (See Chapter 5).

Contents of hibernacula
On bottom of plastic box, place 1" of disinfected pea gravel. Above this, place 1" of carbon or charcoal (available in 2 pound bag from Home Depot or similar store), followed by 2-3" of coarse sand (mortar-mixing sand or winter traction sand are recommended; playbox sand is too fine, and will not allow water flow through it). Cover half of the hibernaculum with a loose layer of sphagnum (green) moss. Pour water over hibernaculum until thoroughly wet. See Figure 11 for photo of individual hibernaculum.

Figure 11 Individual hibernaculum for overwintering toads. Left to right: plastic container; pea gravel; charcoal; sand and moss.

Putting toads into hibernation

Timing
Hibernation usually takes place from October 1 through May 1.

Preparing toads
Any PIT tagging or batch marking must be completed at least 1 week prior to the toads entering hibernation. No adjustments in lighting or ambient temperatures are made prior to hibernation. However, other toad husbandrists have found that toads should be fasted for 1 week prior to hibernation, and that toads should be gradually acclimated to hibernation by reducing lighting and ambient temperature in the week preceding hibernation (B. Spencer, pers. obs., M. VanVleet, pers. comm.). Boreal toads should be carefully monitored to determine if the latter practices are advantageous prior to hibernation. Each toad should have its PIT tag number, mass, and length recorded prior to going into hibernation. Toadlets without PIT tags can be weighed as a group, to determine a mean mass.

Putting toads into environmental chambers
Place a maximum of 5 adults or 15 toadlets (SVL <30 mm) into each hibernaculum and put all hibernacula into environmental chambers.

Water for toads
Place two 1-gallon jugs of water in each environmental chamber. Storing the water in the environmental chamber helps prevent temperature shock to the toads when water is added to their individual hibernaculum.

Maintaining toads in hibernation

Toad examination and hibernacula watering dates are scheduled every two weeks on a Microsoft Outlook calendar that is posted on the outside of the environmental chambers.

Examination of toads
On scheduled inspection dates, briefly remove each individual hibernaculum from the environmental chamber, then examine and count the toads within (minimizing disturbance). Remove any dead toads. Note any mortality or comments on the Microsoft Outlook calendar on the outside of environmental chamber. These mortalities are recorded electronically, and hard copies of mortality records are kept in a notebook. Remove and replace moss if there is any mold.

Watering hibernacula
Remove lid from hibernaculum. Place a US Plastic drain pan over the chamber; pour approximately 2 cups of water from the jugs into the drain pan to evenly moisten the hibernaculum. Replace lid and return the hibernaculum to the environmental chamber. Pour out water from jugs in environmental chamber, replace with fresh water, and return jugs to chamber.

Removing toads from hibernation

When toads are brought out of hibernation, the individual hibernacula are removed from the environmental chamber during the day and allowed to come to room temperature for 2 to 3 hours before toads are returned to husbandry tanks. However, other toad husbandrists have found that toads should be gradually acclimated to ambient temperatures following hibernation by allowing toads to remain in hibernacula chambers at least overnight before returning them to husbandry tanks (M. VanVleet, pers. comm.). Boreal toads should be carefully monitored to determine if the latter practices are advantageous following hibernation.

Examination of toads
Each toad should have its PIT tag number, mass, and length recorded after coming out of hibernation. Toadlets without PIT tags can be weighed as a group, for a mean mass. General body condition should also be noted. Any toad that appears unhealthy should be held in quarantine (see Chapter 5) until it recovers.

Return to husbandry tanks
Adult toads, regardless of whether they will be used for breeding attempts, are removed from the hibernaculum and returned to the tank with any non-hibernating individuals from their group. Toadlets are maintained in separate tanks from their non-hibernated cohorts. They are not placed with others from their same clutch until they have reached the approximate size of non-hibernated individuals from their group (in order to avoid cannibalism of smaller, hibernated toadlets).

Feeding
Toads coming out of hibernation typically require one or two days before they begin eating, but should be offered a small amount of food immediately.

Chapter 8 Breeding protocol

Much research and experimentation remains to be conducted before breeding boreal toads in captivity will be highly successful. The five egg clutches produced at the NASRF in 2002 had relatively low survival rates, with approximately 50 percent survival to hatching. Conversely, survival of eggs brought in from the wild was high, approaching 95 percent survival to hatching. Prior attempts to breed boreal toads have produced viable eggs, but resulted in high (>80 percent) mortality of breeding adults. Following are guidelines that may be updated as more is learned about the physiology of boreal toad reproduction in captivity. Determinations of which toads should be bred will be made based upon genetic information in the boreal toad studbook (see Chapter 3).

Feeding following hibernation

Two different approaches for feeding toads following hibernation have been used. Recent practices at the NASRF have been to delay feeding toads until after breeding attempts. Previous practices were to feed toads for approximately one week prior to beginning breeding attempts because, based on previous breeding attempts with boreal toads, this feeding period prior to breeding appeared to provide time for toads to come into breeding condition following hibernation (S. Taylor, pers. comm.). Both methods will be tried until it is determined which is more successful.

Breeding environment

Tank characteristics
For toad pairing, tanks the same size as normal husbandry tanks should be filled with 1.5” of water. Water temperature and quality should be the same as for the husbandry tanks (see Chapter 4). Water flow into tanks is turned off to prevent the eggs from going down the drain (which occurs with even a small amount of flow).

Lighting
Lighting should mimic the natural photoperiod during the breeding season of the boreal toads, as this may serve as an environmental cue for toad breeding.

Breeding attempt

As male boreal toads come into reproductive condition, they develop dark brown “nuptial pads.” These hard, dark callouses serve to provide easier grasping of the female for breeding. In the wild, boreal toads reach breeding age at approximately 4 years old for females and 3 years old for males. However, in captivity, boreal toads have been bred at 1 year old with limited success.

Males (3 or 4) should first be put into breeding tanks. When they exhibit breeding behavior (grasping each other), or after 24 hours, females should be added to the tanks (2 or 3 females). Alternatively, a combination of females and males can be put into breeding tanks initially. Natural amplexus is the ideal outcome. However, hormone injections may be necessary to induce breeding in some or all toads.

Hormonal stimulation
Following is the existing protocol for hormonal stimulation of boreal toads. However, based upon current research, intraperitoneal, dermal, or oral application of hormone may replace this protocol.

The hormone used for stimulating spermatogenesis or recruitment of new oocytes is LHRH (des-Gly10(D-ALA6) Leutenizing Hormone Releasing Hormone Ethylamide). To prepare, the lot (1 mg) should be dissolved with 10 cc of sterile water. A 1cc (100 µg) syringe with a 27 gauge needle should be used for injecting subcutaneously into the groin area. Dosage is 1 µg LHRH solution/10 g of female toad and 0.5 µg LHRH solution/10 g of male toad. For example, a 70 gram female toad receives a 7 µg injection, while a 40 gram male toad receives a 2 µg injection.

If male toads are displaying breeding behavior (grasping others), but no pairs are in amplexus within 36 hours of being placed in the breeding tank, inject a female toad as described above. The female can be injected again 4 hours later if no pairings are made. If, at the end of that day, there are still no pairings, inject a single or multiple male(s). This can be repeated daily until eggs are produced or breeding attempts are stopped (4-5 days maximum). Once breeding attempts are stopped, remove toads from the breeding tank and return them to husbandry tanks. Prolonged clutching by males may result in open wounds on the chests of grasped females. After toads have been allowed to rest and eat for at least a week, breeding may be attempted again. Repeated pairings of animals, as well as administration of hormones should be done cautiously, as numerous deaths of both successful and unsuccessful breeding boreal toads have been observed after many days of breeding attempts.

Amplexus

After a pair of toads is in amplexus for 3-4 hours and no eggs are laid, inject the female with the above dosage of LHRH hormone. If no eggs have been laid 8 hours later, inject both the female and male as outlined above. The pair can be injected daily until eggs are produced or breeding attempts are stopped (2-3 days maximum after pairing). The PIT tag numbers of all pairs should be recorded for future breeding information.

Egg laying

Once a pair of toads begins to lay eggs, transfer other non-amplexed toads to another breeding tank. Toads moved while in amplexus may take longer to lay eggs than those that are undisturbed (L. Livo, pers. obs.).

After an egg mass is completely deposited, remove the pair from the breeding tank and increase water depth to 8” to provide a water quality buffer for the eggs. Water temperatures and quality should be equivalent to those in adult toad tanks (see Chapter 4). Do not disturb eggs during development, except to siphon out infertile eggs, the decomposition of which will degrade water quality.

Chapter 9 Toad health

Routine health checks

Routine health checks of groups or individuals allow evaluation of housing conditions, feeding regimes, and other husbandry practices. Scheduled examination and weighing of toads is a minimally invasive method to track the health and mass of captive animals.

Eggs and Tadpoles

- Record the number of days that each clutch takes to reach metamorphosis, so that differences in environmental conditions that yield faster metamorphosis can be documented. On average, it takes 75-90 days to proceed from egg to metamorphosis at the NASRF. Time to metamorphosis varies by tadpole density and water temperature.
- Note abnormalities for groups of tadpoles.
- Record survival/mortality rates to metamorphosis. This can help determine proper densities and feeding practices for tadpoles.

Toadlets and Toads

- Note physical condition (including specific checks for abnormalities in limbs and digits, scoliosis, skin color, bloating, etc.).
- Behavioral changes should be documented, such as decreased interest in feeding, hiding, etc. as they may indicate an underlying environmental problem or illness.
- Individuals should be counted daily (see tank datasheet in Chapter 17). Boreal toad toadlets have been observed cannibalizing each other; counting individuals will help to assess this occurrence, and if so, make feeding or housing adjustments.

Manual restraint

If it is necessary to hold or restrain a boreal toad, the following guidelines should be followed.

Tadpoles

Be certain tadpole's skin stays moist during handling, and do not hold tightly, as tadpoles are easily injured. A small plastic spoon is often useful for handling individual tadpoles. If an aquarium net is used to capture a tadpole, the net can be used to provide moisture as you handle the tadpole with disposable gloves from below the net.

Toadlets and toads

Wear disposable gloves to avoid damaging toad's skin. To ensure that the toad does not escape while being held, grasp it "immediately anterior to the hindlimbs, and a second grip secured around the forelegs" (Wright 2001a).

Chemical restraint

Tricaine methanesulfonate (MS-222 or FINQUEL®, Argent Chemical Laboratories, Redmond, WA) is the preferred agent for chemical restraint. In order to maintain a physiologically neutral solution, prepare MS-222 for use as follows (Wright 2001a):

Stock solution
Mix 2 g MS-222 with 2 liters of well oxygenated distilled water to which has been added 34-50 ml of 0.5 M Na_2HPO_4. This yields a solution of 1 g/L. Use this concentration for adult amphibians, and a concentration of 0.2 g/L for tadpoles. Sodium bicarbonate (baking soda) should be used to buffer MS-222; use enough sodium bicarbonate to bring the pH of the solution to 7.0 (Fellers et al. 1994).

To anesthetize a toad, place it in a small, covered container (such as a jar) and add about 5 to 10 ml of the MS-222 solution. It usually takes less than 30 minutes soaking in this solution to achieve anesthesia. Once the animal is anesthetized, it should be put in a bath of clean water, observing to be sure animal does not drown.

Euthanasia

Soaking the amphibian in an MS-222 solution as described above for an extended period is the preferred method of euthanasia. This technique is minimally stressful and does not create artifacts that can interfere with pathological examination (Wright 2001a). Once the animal is no longer responsive, its head can be removed to ensure death. If MS-222 is unavailable, barbiturate overdose is an alternative; however, administration into the heart or body cavity can produce tissue artifacts.

Mortalities

Tanks are checked daily for dead animals (see datasheet in Chapter 17). See Chapter 10 for the required samples to prepare when a dead toad is found. Logs of mortalities are kept electronically and hard copies of mortality records are kept in a notebook. Unexplained deaths or the deaths of multiple animals should be investigated by a veterinarian or veterinary pathologist.

Illness and treatments

The following is a brief outline of selected amphibian diseases with an emphasis on those commonly observed in captive toads. These include nutritional diseases, bacterial, fungal and parasitic infections, and miscellaneous conditions. Wright and Whitaker's Amphibian Medicine and Captive Husbandry is a recently published text which can provide additional information on amphibian illnesses and treatments. **NOTE**: Any toad that exhibits signs of an infectious disease should be isolated immediately. Contact a cooperating amphibian veterinarian or veterinary pathologist for assistance with the diagnosis and treatment of sick toads.

Nutritional deficiencies

- Metabolic Bone Disease
  Metabolic bone disease (MBD) is usually caused by an imbalance in dietary calcium, phosphorus, and vitamin D_3. Other factors that may be involved in some cases of amphibian MBD include excess dietary vitamin A (usually in animals on rodent-based diets) or low water calcium hardness (Wright 2001a, 2001b). Signs suggestive of MBD include bone deformities – especially of the mandible and spine – long bone fractures, splayed limbs, reluctance to move, tetany, bloating, subcutaneous and body cavitary fluid retention (edema), and prolapse of the stomach or cloaca. Tetany refers to muscular spasms that can resemble seizures and that are often induced by excitement or handling. Slight drooping of the mandible not related to metabolic bone disease has been observed in some boreal toads and may be a developmental defect. Diagnosis of MBD is by characteristic clinical signs (bone deformity, tetany, etc.), demonstration of reduced bone density by radiography, or by pathologic examination of sentinel animals. Treatment of individuals with MBD includes supplementation with oral calcium and vitamin D_3 and exposure to ultraviolet radiation. More important than treatment is prevention of MBD. Invertebrate prey items should be gut-loaded or dusted, as discussed in Chapter 6 of this manual, to help prevent calcium/phosphorus/vitamin D imbalances.
- Short Tongue Syndrome/ Vitamin A Deficiency
  This is a condition frequently observed in captive Wyoming toads (*Bufo baxteri*), but has recently been observed in a small number of long-term captive boreal toads (Pessier et al. 2002). Affected animals gradually lose the ability to catch prey despite vigorous efforts. Microscopic examination of the tongue from affected animals shows replacement of the sticky mucus-producing epithelium of the tongue (mucus helps to apprehend prey) with squamous epithelium. Preliminary data in Wyoming toads suggest the cause may be vitamin A deficiency; however, additional study is required. Animals suspected to have short tongue syndrome should be reported and submitted for pathologic examination. Treatment with vitamin A without veterinary supervision is discouraged because of risks associated with supplementation of this vitamin.
- Obesity
  Overeating combined with lack of physical activity can lead to obesity in captive toads. Treatment may include decreased feeding, increased exercise, and increased ambient temperature (to increase metabolism)
- Inanition (Starvation)
  Inanition is frequently observed when toads are housed in large groups and compete for food. Other possible causes include inappropriate prey items (too large or small), and systemic illness and environmental conditions that interfere with feeding. Affected animals are thin with atrophied muscles and prominent bones and are often dramatically smaller than similarly aged tankmates. Treatment consists of movement into a less competitive environment or correction of other predisposing husbandry conditions.
- Gastric overload and impaction
  Gastric overload is caused by overeating or consumption of a food item that is too large. Impaction can occur when a non-food item, such as gravel, is ingested and becomes lodged in the stomach or intestine. Animals may be lethargic and display abdominal bloating. Veterinary assistance is suggested to relieve gastric overload or impaction. Prevention of

gastric overload and impaction can be accomplished by proper feeding practices and selection of appropriate substrates.

- Scoliosis
  Scoliosis can be detected in tadpoles by the presence of a crooked tail. If left untreated, the metamorphosing toadlets will have visibly crooked spines. In a non-flow system, Wright and Whitaker (2001b) used 1 ml of vitamin B complex per gallon of water. They observed that either the vitamin B or the associated algae growth reduced scoliosis in giant monkey frog (*Phyllomedusa tarsius*) tadpoles. Because all tanks at the NASRF are flow-through, addition of vitamin B to water is impractical. At the NASRF, algae placed into tadpole tanks may have reduced scoliosis in boreal toad tadpoles. Any algae used for this purpose must be chytrid free. For example, at the NASRF, algae is produced in outdoor fiberglass tanks set above ground level to preclude access by resident amphibians.

Bacterial infections

- Localized infections
  These often occur following skin injury or other trauma such as implantation of identification microchips. Signs might include discoloration and swelling at the site or region of injury (cellulitis). Abscesses are swellings with accumulations of pus-like material (exudate). Treatment should ideally be conducted with veterinary advice and includes cleaning and flushing the affected area and draining any exudate. Microscopic examination (Gram's stain) and bacterial culture of exudate can help to determine the causative agent. Local or systemic treatment with antibiotics may be helpful in some cases. Superficial skin infections can be treated topically with silver sulfadiazine cream. Abscesses can also be caused by mycobacteria (see Mycobacteriosis, below) and microscopic examination of exudate by methods for acid-fast bacteria may be required for diagnosis (Wright 2001a, Pessier 2002)
- Systemic infections (Red Leg Syndrome)
  Systemic (involving multiple tissues) bacterial infections are most often observed in amphibians under stress or in poor husbandry conditions. Examples include recent shipment, overcrowding, poor water quality, hibernation, other diseases (chytridiomycosis or iridovirus infection), and high environmental organic and bacterial loads. Signs include reddening of the skin, particularly the ventral thighs, abdomen, and toes (red leg), skin ulcers, and accumulation of fluid under the skin (see Edema Syndrome, below). Other signs might include cloudy eyes, seizures, or head tilt. In a group situation, mortalities may be very high. Reddening of the skin is **NOT** a specific sign of bacterial infection and can also be seen with handling or heated substrates (usually reddening is mild in these cases), fungal infections such as chytridiomycosis, iridovirus infection, and chlamydiosis. Veterinary assistance in diagnosis should be sought if animals are noted with signs of "red leg." Antibiotic treatment of individual animals can be with enrofloxacin at 5-10 mg/kg once daily orally or by injection. For large groups of animals, ciprofloxacin baths at 500-700 mg/75 L for 6-8 hours daily can be attempted. Any identified lapses in husbandry (infrequent cleaning, water quality, etc.) should be **IMMEDIATELY** corrected (Wright 2001a, Pessier 2002).
- Mycobacteriosis
  Mycobacterium spp. are a unique group of bacteria common in aquatic environments that infect amphibians opportunistically. Infection often occurs following skin wounds with local infection and often dissemination to internal organs. Visible signs of infection can include discrete skin nodules, skin ulcers, or localized swelling of a limb or foot (cellulitis).

Diagnosis is by demonstration of "acid-fast" bacteria within samples of the lesion. Mycobacteriosis in the skin can closely resemble other conditions including bacterial abscesses or cellulitis (see Localized infections, above), fungal infections such as chromomycosis, and neoplasms (tumors) (Pessier 2002). There is no effective treatment for infected amphibians. Control within a colony situation could include depopulation of infected animals, disinfection of enclosures, and identification of predisposing factors such as rough environmental substrates or opportunities for tankmate-induced skin trauma. Humans working with infected animals should take precautions such as wearing disposable gloves, as transmission to humans can occur under some circumstances.

Fungal infections

- Chytridiomycosis

  First described in arroyo toads, chytridiomycosis is caused by a true fungus in the phylum Chytridiomycota named *Batrachochytrium dendrobatidis.* Infection with *B. dendrobatidis* has been recognized worldwide in a variety of captive and wild amphibians and has been implicated as a cause of some population declines. Chytridiomycosis has been recognized in wild boreal toads as early as 1995 and may play a role in the decline of this species as well. Lethal fungal infections in Wyoming toads and boreal toads previously attributed to another fungus, *Basidiobolus ranarum*, are now known to be infections with *B. dendrobatidis* (Pessier 2002).

  Signs of chytridiomycosis can range from death without evidence of illness to animals with obvious skin disease. Skin signs include excessive shedding (sloughing) and discoloration of skin usually of the ventral body and feet. The discoloration is usually brown, but can be red, particularly if secondary bacterial infections are present, and can mimic red leg syndrome. Shedding of the skin in chytridiomycosis should not be confused with normal skin shedding which usually occurs in large pieces. Other potential signs of chytridiomycosis include changes in posture in which animals may try to avoid contact of the ventral abdomen with substrate, avoidance or increased preference for water, decreased or absent appetite, and lethargy.

  Diagnosis of chytridiomycosis is by demonstrating of characteristic fungal thalli in skin scrapings or on histologic examination of a skin sample. In addition, a Polymerase Chain Reaction (PCR) test specific to *Batrachochytrium dendrobatidis* is available from Pisces Molecular (5311 Western Avenue, Suite E, Boulder, CO 80301; phone (303) 546-9300; email: jwood@pisces-molecular.com). Samples should be submitted for testing for any toad suspected of being infected with this fungus.

  Successful treatment of chytridiomycosis with itraconazole, an antifungal drug, has been described (Nichols and Lamirande 2001). For treatment, animals are placed in daily 5-minute baths of an 0.01% solution of itraconazole for 10 days. The solution is prepared by diluting the commercially available 10 mg/ml solution (Sporanox Oral Solution, Ortho Biotech Inc., Raritan NJ, USA) in amphibian Ringer's solution (10 ml commercial itraconazole solution to 990 ml of amphibian Ringer's). Baths should be shallow enough that that the toad can stand to avoid drowning; however, attempts should be made to ensure that itraconazole solution is

applied to all areas of the skin. Tadpoles or young toadlets **SHOULD NOT** be treated with itraconzole baths as described because deaths have been observed.

Saprolegniasis

This condition can be caused by numerous species of Oomycete watermolds that are common in aquatic environments. Infections are usually opportunistic and often associated with skin injuries as might occur with rough substrates or improper animal handling. Other factors that can be contributory to lesion development are low environmental temperature, high environmental organic loads, and poor water quality. Tadpoles may be frequently affected on the skin or oral disc. Saprolegniasis has a characteristic appearance which consists of mats of white cottony material at the site of infection. Described treatments include sea salt baths (10-25 g/L for 5-30 minutes or benzalkonium chloride soaks at 1:4,000,000 continuously with water changes three times weekly or 2 mg/L as a daily 60 minute bath). Elevation of environmental temperatures above 20°C may be helpful as an adjunct to therapy (Wright 2001a). Any lapses in husbandry that may have contributed to infection should be corrected. For tadpoles, evaluation of water quality parameters such as pH, ammonia, and temperature are especially important.

- Other Fungal Infections

  These include chromomycosis and zygomycosis. Common signs of infection are skin nodules and ulcers that must be differentiated from lesions of mycobacteriosis or neoplasia. Non-specific signs might include weight loss and decreased appetite. Diagnosis is by veterinary examination of aspirates or histologic sections of the lesions. Successful treatment has not been described. Chromomycosis in particular can be associated with skin trauma (rough substrates, injury from tankmates) and is acquired from soil. Correction of predisposing factors and disinfection of captive environments is required for control of infection in groups of animals. Gloves should be worn while cleaning contaminated substrates as chromomycosis can infect humans via skin wounds.

Parasitic infections

Amphibians can serve as hosts for many types of protozoan and metazoan parasites. Parasitism has not been a major problem in captive boreal toads to date. Most of these organisms can be identified by a pathologist or veterinarian from a skin scrape, fresh fecal sample, urine sample, blood sample, or visual examination, depending on the specific organism. In colony situations, parasites such as the lungworm *Rhabdias* are most likely to be of concern and may reflect lapses in husbandry (hygiene) that allow for clinically significant superinfections.

Miscellaneous conditions

- Skin Injuries

  Abrasions and other skin injuries are common in amphibians. Predisposing factors can include rough handling (dry, ungloved hands), rough tank furniture or substrate, use of coarse nets, and tankmate aggression. Abrasions on the nose are often seen post-shipment or in animals housed in clear containers. Small, superficial wounds may not require any treatment if the inciting cause is eliminated. Larger abrasions or wounds may require topical cleaning and treatment with antibacterial/antifungal medications such as silver sulfadiazine cream or gentamicin-based ophthalmic solutions (Wright 2001a). Secondary bacterial infections may require systemic antibiotic therapy or more extensive wound care under direction of a veterinarian.

- Edema Syndrome
  Accumulation of fluid both under the skin (edema of subcutaneous lymph sacs) and within the body cavity (hydrocoelom) is extremely common in captive toads. Affected toads are usually bloated or have distinct abdominal enlargement. Abdominal enlargement due to fluid accumulation should not be confused with that seen in obese animals or gravid females. There are many causes of fluid accumulation including septicemia (red leg), heart failure, lymph heart failure, kidney disease, liver disease, hypocalcemia (metabolic bone disease), and osmotic imbalances secondary to housing in water with low dissolved solutes (Wright 2001a). In a small number of boreal toads, edema and hydrocoelom have recently been associated with heart failure resulting from chronic inflammation of the tissues around the heart (pericarditis). The cause is under investigation. In captive Wyoming toads, the most common cause is end-stage kidney disease. Diagnosis of fluid accumulations can be difficult and often will require veterinary assistance. Soaking of affected animals in amphibian Ringer's solution may be helpful in some cases; however, this does not always address the underlying cause. Evaluation of husbandry for possible contributory factors (water quality and source, and dietary factors that could lead to hypocalcemia/metabolic bone disease) should be conducted. Necropsy of affected animals can be helpful in reaching a definitive diagnosis. In tadpoles with generalized edema, evaluation of water quality for parameters such as ammonia and hardness should be an initial consideration. Submission of affected tadpoles for pathologic examination is helpful to rule out conditions such as iridovirus infection.
- Cloacal and rectal prolapse
  A prolapse occurs when cloacal tissue protrudes from the vent. This can be caused by intestinal inflammation (enteritis), intestinal parasitism, hypocalcemia (metabolic bone disease), gastric overload, or impaction of fecal matter. Treatment of the prolapse is by application of a hyperosmotic saline or sugar solution to reduce swelling followed by gentle replacement of the prolapsed tissue. A pursestring suture may be required in some circumstances. At the NASRF, treatment has been to use an eyedropper to put several drops of a 7.2% hypertonic saline solution on the prolapse. Attempts to determine and correct the underlying cause of the prolapse should be made.
- Hyperthermia
  Hyperthermia is caused when an amphibian is exposed to inappropriately high temperatures. Signs might include agitation, excitability, seizure-like activity, and changes in skin color. If circumstances and signs suggest hyperthermia, immediately place the affected animals in cool water. Veterinary assistance should be sought for severely affected toads.
- Hypothermia
  Hypothermia is caused when an amphibian is exposed to inappropriately low temperatures, usually as the result of improper hibernation techniques. Animals in hibernation can be "freezer burned" due to cold temperatures and desiccation. Therefore, it is imperative that hibernating amphibians be kept damp at all times.
- Dehydration and desiccation
  Amphibians are quite susceptible to dehydration and subsequent desiccation. Low humidity associated with heating or air conditioning systems may be contributory in some cases. More often, dehydration occurs secondary to loss of access to water as may occur with malfunctioning water delivery devices, neglect, or escape of the animal from its enclosure into the facility. Dehydrated toads are lethargic with tacky, dry skin that is often wrinkled.

Initial treatment is to place the animal in a shallow bath of cool water. For severe cases, intracoelomic injection of fluids, such as two parts non-lactated electrolyte solution (or normal saline) to one part 5% dextrose may be helpful. Intramuscular injection of 1-2 mg/kg dexamethasone can be used supportively (Wright 2001a). The latter should be conducted with veterinary advice.

- Spindly leg

  Spindly leg is a poorly defined syndrome in metamorphosing frogs. Characteristic features include markedly thin limbs (usually forelimbs) which may have some degree of abnormal rotation or position. The cause is poorly understood, but nutritional, genetic, environmental, and traumatic factors have been proposed. Supplementation of B vitamins, as well as a varied diet is recommended for tadpoles and parents. Pairing for breeding should be done with the recommendations of a geneticist. Finally, tadpoles' environment should be monitored to be sure that dissolved oxygen levels are appropriate, and that no toxins are present in the water. Lighting should be installed as described in Chapter 4 of this manual. Tadpoles and toadlets should not be crowded, as this may cause spindly leg syndrome, small size at metamorphosis, and/or cannibalism.

Chapter 10 Preservation protocols

Institutions holding boreal toads should submit dead animals for complete necropsy examination (including histopathology). Submitting animals for necropsy also ensures that screening for other diseases that may affect the captive population (edema syndromes, short tongue, etc.) is conducted. Results of the necropsy and histopathology report should be sent to Allan Pessier and Kevin Rogers to enhance tracking of disease trends and mortality within the captive population.

Contact information:

> Dr. Allan Pessier, DVM: Loyola University Medical Center, Room 0745, Building 101, 2160 South First Street, Maywood, IL 60153, telephone: (708) 216-1185, email: apessie@lumc.edu
>
> Dr. Kevin Rogers: Colorado Division of Wildlife, Steamboat Springs Service Center, P.O. Box 775777, 925 Weiss Drive, Steamboat Springs, CO 80477, telephone: (970) 870-2866, email: Kevin.Rogers@state.co.us

A PCR test specific to *Batrachochytrium dendrobatidis* is available from Pisces Molecular (5311 Western Avenue, Suite E, Boulder, CO 80301; phone (303) 546-9300; email: jwood@pisces-molecular.com). A sample preserved in 70% ethanol should be submitted for testing for any toad suspected of being infected with this fungus.

When multiple animals die, often a single factor is suspected. For example, tadpoles may die if their water is contaminated by bleach, metamorphs may desiccate if they inadvertently are kept in a feeding container for an extended time, and toads may die in an environmental chamber that overheats. In circumstances such as these, with an apparently obvious cause of mortality, samples should be collected and the carcasses preserved. However, actual processing of samples, such as PCR samples to detect chytrid fungus, may be postponed indefinitely. For example, such samples may be tested at a later date to determine the onset of a disease outbreak.

Sample labels and datasheets

Assign a unique record number to each specimen and use this number to label all samples and correspondence associated with that specimen. A facility abbreviation followed by a date code (year-month-day) provides a useful base for a series of record numbers. For example, a record number of NASRF021106-02 would represent the second toad that died at the NASRF on November 6, 2002.

Specimen datasheets and labels
Mortality datasheets should be maintained in a notebook and include the following information:

- Record number
- Lot identifier
- Date of death
- Comments (such as suspected cause of death or where specimens were sent and a summary of any pathology results)

Carcasses should be labeled with, at a minimum, record number. Tags are available from biological supply houses, or can be made from 110 weight 100% cotton or linen fiber paper. Use pencil or an ink that will not run in water or the fixative solution. For containers with multiple specimens, tie the tag to individual animals. The standard site for attaching a tag on a toad is to tie it on the knee of the rear left leg. The tag should be inserted into the jar with tadpoles.

Each carcass or tadpole lot label should contain at a minimum, the following information:

- Record number
- Type of preservative solution
- Date
- Lot identifier

Handling specimens

When a small number (1 or 2) of toads is found dead, collect a sample for PCR testing and then preserve the carcass. To obtain a sample for PCR, snip off a small portion of the pelvic patch and/or rear toe and preserve it in 70% ethanol in a small (2 ml) plastic tube with screw cap. Place the record number on the outside of the tube.

After the PCR sample has been collected, institutions that have veterinary support should perform a complete necropsy as suggested by the protocol at the end of this section. If veterinary support is not available, fix the carcass in 10% neutral buffered formalin by incising the carcass along the ventral midline, opening the coelomic cavity, and immersing the carcass in 10% neutral buffered formalin. When large numbers of toads die, obtain PCR samples as described above. Fix half of the carcasses in formalin. Place the other half of the carcasses in a freezer (see Freezing, below).

Ethanol

If a stock solution of 70% ethanol is not available, this concentration can be obtained by diluting absolute ethanol. **Note**: Shortly after a bottle of absolute (100%) ethanol is opened, it becomes 95% ethanol; the following dilution calculation is based on 95% ethanol.

To obtain 1000 ml of 70% ethanol, add 737 ml of 95% ethanol to 263 ml of distilled water.

Neutral buffered formalin

To make a 10% solution of neutral buffered formalin from a 37% solution of formaldehyde, add 100 ml formaldehyde to 900 ml water (to which has been added 6.5 grams of sodium phosphate dibasic and 4.0 grams sodium phosphate monobasic). Formaldehyde or formalin solutions with a white precipitate in the bottom are old; avoid using them.

Freezing

Specimens to be frozen should be placed in a plastic bag with a label, as described above. Avoid using a “frost-free” freezer if possible, as the defrost cycle in these units results in large temperature fluctuations and damage to specimens. Freezing at -70ºC preserves specimens best.

Boreal toad necropsy protocol
Summary and Tissue Checklist

Tissues for Histopathology (Samples can all be placed in a single container of 10% buffered formalin)

____ skin of ventral pelvic region ("drink or pelvic patch")
____ skin of ventral hindlimb
____ skin of dorsal body
____ cross section of foot at the level of the metatarsals

____ heart	____ esophagus
____ larynx/trachea	____ stomach
____ lung	____ small intestine (at least 2 sections)
____ liver and gallbladder	____ large intestine
____ spleen	____ pancreas
____ kidney	____ bone/bone marrow
____ ovary	____ brain
____ testicle and Bidder's organ	____ eye
____ urinary bladder	____ skeletal muscle
____ tongue	____ peripheral nerve

____ any other organs/tissues with significant abnormalities

Chytrid PCR:

____ rear toe or skin of ventral pelvic region in 70% ethanol for chytrid PCR

Microbiology:

____ routine aerobic bacterial culture (heart blood or liver, see written protocol item # 3)
____ other (coelomic fluid or other grossly identified lesions)

Tadpoles (see written protocol specific to tadpoles):

____ whole (opened) formalin-fixed carcasses
____ whole frozen (-70ºC) carcasses

Necropsy Protocol for the Boreal Toad (*Bufo boreas boreas*)

General guidelines for amphibian necropsy have recently been published and may be useful (Nichols 2001).

Postmetamorphic Animals:

1. Deaths of all advanced subadult (yearlings) and adult animals should be investigated by gross necropsy with submission of samples for histopathology and ancillary testing as detailed below. Exceptions might include animals in advanced stages of autolysis; however, limited histopathology (such as examination of the skin for chytridiomycosis) could still provide valuable information about group/colony health.

2. While necropsy and histopathology of all small, recently metamorphosed animals may not be feasible, examination of a subset of these animals from each institution is strongly recommended.

3. For freshly dead to mildly autolyzed animals, routine aerobic bacterial culture is recommended. Suggested samples include aseptically collected heart blood from adult animals or alternatively, liver from subadult to adult animals. Coelomic fluid or other grossly visible lesions, if present, are also a desirable sample.

4. Freezing at –70ºC or –80ºC portions of gross lesions (examples include visceral nodules, cutaneous or mucosal ulcers, organs with petechiae, among many others) is highly recommended. If grossly observed lesions are too small to accurately section and divide for multiple purposes (histopathology, microbiology, freezing, etc.), samples for histopathology should be given preference.

5. All major organs, including the brain, should be sampled for histopathology. A tissue checklist is provided with this protocol. Complete dissection of individual organs and submission/fixation of representative samples is encouraged, particularly for adult animals. **Important tissues that are frequently overlooked in toad necropsies include tongue, urinary bladder, and multiple skin sections** (as indicated on tissue checklist). Following standard pathology practice, all samples should be fixed in 10 times their volume of 10% neutral buffered formalin.

6. Small animals (less than 10-15 grams) can be submitted whole after opening the coelomic cavity to allow penetration of the fixative. Large full-thickness incisions into the coelomic cavity work best; separation of the head from the body allows more optimal fixation of the brain. Under some circumstances, larger animals (adults) can be submitted whole as for smaller animals; however, this results in varying degrees of sample degradation and is suboptimal.

Tadpoles:

1. In tadpole mortality events, euthanized sick animals or animals known to be very recently dead are the preferred samples. Whole representative animals should be selected for histopathology and others frozen whole at –70ºC or –80ºC.

2. Samples for aerobic bacterial culture can be collected from animals designated for histopathology. The coelomic cavity is opened as aseptically as possible and sterile swabs of coelomic contents/fluid are obtained. Alternatively, a small section of liver (usually very large in tadpoles) can be aseptically collected for culture. The remainder of the opened carcass can be immersed in 10% neutral buffered formalin as described below.

3. In animals for histopathology, the coelomic cavity should be opened as described for small toads and the carcass immersed in 10% neutral buffered formalin.

Chapter 11 Marking methods

To differentiate toads from one other, they must be marked by some method. Marking is useful to distinguish toads of different ages, husbandry history, and genetics. Which of the following marking methods is used to mark a specific toad depends on the purpose of the marking and the size of the toad.

PIT tagging

Using a passive integrated transponder (PIT) tag allows for individual identification of toads. PIT tagging is a type of microchip marking system, and each tag has a unique 10 space alphanumeric code that is read using an electromagnetic scanning device that activates the transponder (see Figure 12). PIT tagging is only recommended for toads weighing 10 grams or more. The brand of PIT tags used is AVID.

Figure 12 PIT tags

To insert a PIT tag:

1. Scan the tag to verify that it is functioning properly, and can be read.
2. Wear disposable gloves.
3. Hold toad with back towards you, and hind legs pointing away from you.
4. Swab a small amount of Bactine on the incision site (about 2/3 of the way up the back from the cloaca, on either side of the backbone) to disinfect and anesthetize the site.
5. Pinch a small amount of skin and gently pull it away from the toad's body to ensure that no muscle is cut (see Figure 13).
6. Using disinfected, fine, sharp scissors (such as surgical or small sewing scissors), snip a very small (1/8") hole through all layers of skin, perpendicular to the backbone.

7. Manually slide the PIT tag through the hole, being sure it is fully inserted.
8. Seal the wound using Nexaban or Vet Seal.

Figure 13 Pinching dorsal skin of a boreal toad to prepare it for clipping a small opening with scissors

PIT tags often move within the toad's body once inserted, and can be difficult, if not impossible, to read at times. Attempt scanning the toad weekly for several weeks before assuming that the PIT tag has been lost, or is no longer functioning and needs to be replaced.

Visible implant fluorescent elastomer

Visible Implant Fluorescent Elastomer (VIE) provides a batch marking system that can be used on animals as small as boreal toad metamorphs that are too small for PIT tags. VIE is available in four colors (red, orange, yellow, and green) that fluoresce under ultraviolet light (see Figure 14). In addition, there are three non-fluorescent colors (blue, black, and purple) available. Limited experience with VIE batch marking to date indicates no mortality due to this method.

VIE is injected under the skin (see Figure 15). In order to remain visible (either under normal lighting conditions or under an ultra-violet light source), VIE must be injected under relatively thin and unpigmented skin, such as that found on the ventral surface of boreal toads. Rear foot webbing may also be an acceptable site for injection for larger individuals. Although specific sites on the ventral surface can be used as injection sites (such as the pelvic patch area or the area under the arm), post-injection movement of VIE has been observed, so separation by injection site is not a reliable form of batch mark (e.g., a green VIE mark placed in the pelvic area could migrate to the arm or chest area, and vice versa). Movement from the ventral surface into the limbs has also been observed.

Figure 14 VIE being mixed for injection

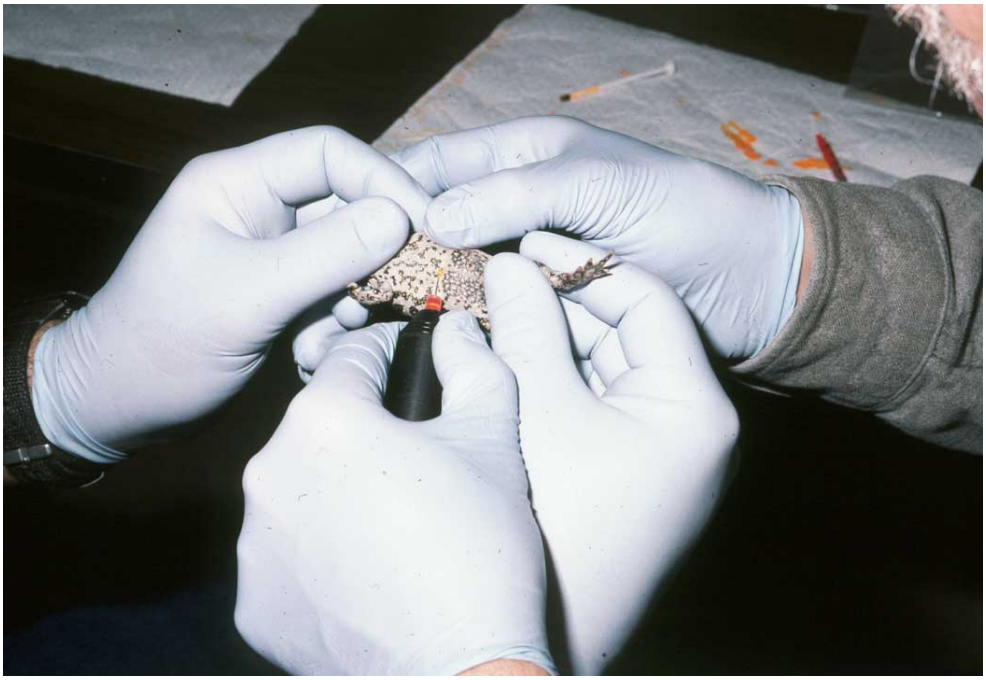

Figure 15 Injecting VIE into a toadlet

Multiple colors can be injected to expand the number of batch marks available. However, we recommend that no more than three colors be used for a single group of toads.

VIE is available in sample kits that each contain the elastomer and other equipment (curing agent, syringe, etc.). Because of the small size of metamorphs, use of hand injector (purchased separately) is recommended (see Figure 16).

Figure 16 VIE injector (with pencil for size comparison)

Toe clipping

Toe clipping has been used in the past to "batch mark" boreal toads, by clipping a specific toe on each toad to identify it as a member of a certain group. Toe clipping has also been used to individually mark amphibians, but this requires clipping multiple toes on each toad, and is not recommended. Toes clipped on boreal toad toadlets have regenerated, although the regenerated toe appears slightly different to the trained observer.

If toe clipping is deemed appropriate, the procedure is as follows:

1. Wear disposable gloves.
2. Pull the skin on the toad's toe back, towards the toad's body. (Pulling the skin prior to clipping allows more skin to cover the wound after clipping.)
3. Clip the toe at the base of the toe with disinfected, fine, sharp scissors (such as surgical or small sewing scissors).
4. Swab the wound with Bactine to disinfect and "deaden" the wound.
5. Seal the wound using Nexaban or Vet Seal.

Chapter 12 Facilities rearing boreal toads

Facilities currently rearing boreal toads

The NASRF in Alamosa, Colorado, holds the majority of captive Colorado boreal toads. However, there are other facilities participating in raising and propagating boreal toads across the country (Table 2). These facilities housing toads from various locations around Colorado play an important role in protecting boreal toads, as they house genetic diversity of toads at locations away from the NASRF. If there is a catastrophic loss of toads at the NASRF, the boreal toad's genetic diversity is still represented at the various participating facilities.

Table 2. Facilities holding Colorado *Bufo boreas*
(as of September 2002)

Facility	**Number of toads**	**Source Locality and lot code (number of toads)**	**Contact information**
Cheyenne Mountain Zoo	20	Hartenstein CF03-00-01 (10) CF03-00-02 (10)	Mark Kombert, Associate Veterinarian Cheyenne Mountain Zoo 4250 Cheyenne Mountain Zoo Road Colorado Springs, CO 80906 Email: mkombert@cmzoo.org Phone: (719) 633-9925 Ext.128
Cincinnati Zoo	77	Jumper Creek MI01-00-01 (36) MI01-00-02 (41)	Terri Roth, Director of Animal Sciences Center For Research of Endangered Wildlife Cincinnati Zoo 3400 Vine Street Cincinnati, OH 45220 Email: Terri.roth@cincyzoo.org Phone: (513) 961-2739
Colorado Division of Wildlife, Durango Office	2	Unknown	Mike Japhet, Aquatic Biologist Colorado Division of Wildlife 151 E. Street Durango CO 81301 Email: Mike.Japhet@state.co.us Phone: (970) 247-0855
Colorado Division of Wildlife, Fort Collins Office	4	Henderson Mine CC02-00-05 (4)	Mark Jones, Aquatic Research Leader Colorado Division of Wildlife 317 W. Prospect Road Fort Collins, CO 80526 Email: Mark.Jones@state.co.us Phone: (970) 472-4361

Table 2. Facilities holding Colorado *Bufo boreas* continued (as of September 2002)			
Facility	**Number of toads**	**Source Locality and lot code (number of toads)**	**Contact information**
Henry Doorly Zoo	11	Jumper Creek MI01-98-01 (3) Jumper Creek MI01-00-01 (4) Jumper Creek MI01-00-02 (4)	Cathy Meier Omaha's Henry Doorly Zoo 3701 South 10th Street Omaha, NE 68107 Email: cathym@omahazoo.com Phone: (402) 733-8401
Morrison Museum of Natural History	6	Jumper Creek MI01-00-?? (6)	Matt Mossbrucker Morrison Museum of Natural History 501 Colorado Highway 8 P.O. Box 564 Morrision, CO 80465 Email: museum@town.morrison.co.us Phone: (303) 697-1873
Ocean Journey	3	Jumper Creek MI01–00-02 (3)	Richard A. Lerner, Curator of Fishes Ocean Journey US WEST Park 700 Water Street Denver, CO 80211 Email: rlerner@oceanjourney.org Phone: (303) 561-4424
Toledo Zoo	13	Morrison Creek RO04-00-01 (2) West Trout Creek HI01-00-01 (11)	Andrew Odum, Curator of Herpetology Toledo Zoo P.O. Box 140130 Toledo, OH 43614-0801 Email: Raodum@aol.com Phone: (419) 385-5721
Saratoga National Fish Hatchery	7	Toads from Wyoming; not Colorado toads	Deedee Roberts Saratoga National Fish Hatchery PO Box 665 Saratoga WY 82331 Email: Dee_Dee_Roberts@fws.gov Phone: (307) 326-5662

Potential facilities to rear boreal toads

In addition to the facilities listed in Table 2, new facilities with adequate accommodations and knowledgeable staff should be sought to house and rear boreal toads. Increasing the number of facilities holding boreal toads will expand the genetic holding potential and help protect against catastrophic loss at any one facility. If possible, a facility that is willing to participate in raising boreal toads should have demonstrated the ability to rear amphibians, preferably temperate toad species.

The NASRF will serve as a clearinghouse for transfers of Colorado boreal toads to other facilities, and hatchery staff will keep detailed logs of location and status of boreal toads at the various facilities. It would be helpful to establish a routine conference call among participating husbandrists to periodically discuss toad husbandry techniques, similar to that used by participants in the Wyoming Toad Species Survival Plan.

Guidelines for inter-facility transfer of boreal toads

Facilities receiving and possessing boreal toads from Colorado must have a letter from the Colorado Division of Wildlife giving permission and outlining conditions for possession.

Tadpoles can be shipped in plastic fish transport bags, commonly used in fish hatcheries. Put water and tadpoles into a plastic bag, and pump air (preferably pure O_2) into the bag to provide adequate oxygen while the tadpoles are in transit. Tie the bag shut (hog ties have been successfully used) and place it inside a second bag. Fold and tie off corners of the bags to prevent larvae from being crushed in the corners. Next, place bags in a shipping container surrounded with shredded newspaper or other material to prevent the bags from rolling during transport.

Toadlets can be shipped in shallow plastic containers (such as a Rubbermaid #5 container with dimensions of 9.25” long x 5” wide x 1.5” deep) with holes in the lid and damp sheet moss, moistened paper towels, or a damp cotton washcloth in the bottom to prevent desiccation. Adult toads can be shipped in plastic containers with damp paper towels or damp sheet moss in the bottom. Pack these individual containers inside the shipping container with shredded paper to prevent injury to the toadlets/toads. During hot weather, use Styrofoam containers with cold packs inside as shipping containers.

Packaging for all animal shipments must conform to the International Air Transport Association (IATA) regulations (web site: www1.iata.org).

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Chapter 14 Breeding site codes

Table 3. Site origin, site code, county, and number of lots at the NASRF as of October 2002

Locality	Site code	County	Number of lots at the NASRF
Lost Lake (Boulder)	BO01	Boulder	
Collegiate Peaks Campground	CF01	Chaffee	
Denny Creek	CF02	Chaffee	
Hartenstein	CF03	Chaffee	3
South Cottonwood Creek	CF04	Chaffee	
Brown's Creek	CF05	Chaffee	2
Kroenke	CF06	Chaffee	
Fourmile	CF07	Chaffee	2
Morgan's Gulch	CF08	Chaffee	
Sayre's Gulch	CF09	Chaffee	
South Cottonwood Creek West	CF10	Chaffee	
Rainbow Lake	CF11	Chaffee	
Middle Cottonwood	CF12	Chaffee	
Denny Creek West	CF13	Chaffee	
Denny Creek South	CF14	Chaffee	
Holywater Beaver Ponds	CF15	Chaffee	
Vintage	CC01	Clear Creek	
Urad/Henderson	CC02	Clear Creek	5
Herman Gulch	CC03	Clear Creek	
Mount Bethel	CC04	Clear Creek	
Bakerville	CC05	Clear Creek	
Silverdale	CC06	Clear Creek	
Holy Cross City	EA01	Eagle	
East Lake Creek	EA02	Eagle	
East Vail	EA03	Eagle	3
Jim Creek (Winter Park)	GR01	Grand	
Pole Creek	GR02	Grand	2
Vasquez Creek	GR03	Grand	
Triangle Pass	GU01	Gunnison	2
West Brush Creek	GU02	Gunnison	
Magdalene Gulch/Texas Creek	GU03	Gunnison	
Brush Creek	GU04	Gunnison	
West Trout Creek	HI01	Hinsdale	3
Spike Lake	JA01	Jackson	2
Lost Lake (RMNP)	LR01	Larimer	
Kettle Tarn (RMNP)	LR02	Larimer	4
Spruce Lake (RMNP)	LR03	Larimer	1
Glacier Basin	LR04	Larimer	
Twin Lake	LR05	Larimer	
Jumper Creek	MI01	Mineral	3
Trout Creek	MI02	Mineral	
Roaring Fork/Boots Pond	MI03	Mineral	2
Conundrum	PI01	Pitkin	
East Maroon Creek	PI02	Pitkin	2
First Creek (California Park)	RO01	Routt	
Soda Creek	RO02	Routt	

Table 3. Site origin, site code, county, and number of lots at the NASRF as of October 2002 continued			
Locality	**Site code**	**County**	**Number of lots at the NASRF**
Diamond Park	RO03	Routt	
Torso Creek (California Park)	RO04	Routt	2
Morrison Creek	RO05	Routt	3
Buck Mountain	RO06	Routt	2
Cucumber Gulch	SU01	Summit	
Montezuma (Snake River)	SU02	Summit	
Peru Creek	SU03	Summit	
Upper North Tenmile	SU04	Summit	
Lower North Tenmile	SU05	Summit	
Upper North Fork Snake River	SU06	Summit	
Lower North Fork Snake River	SU07	Summit	

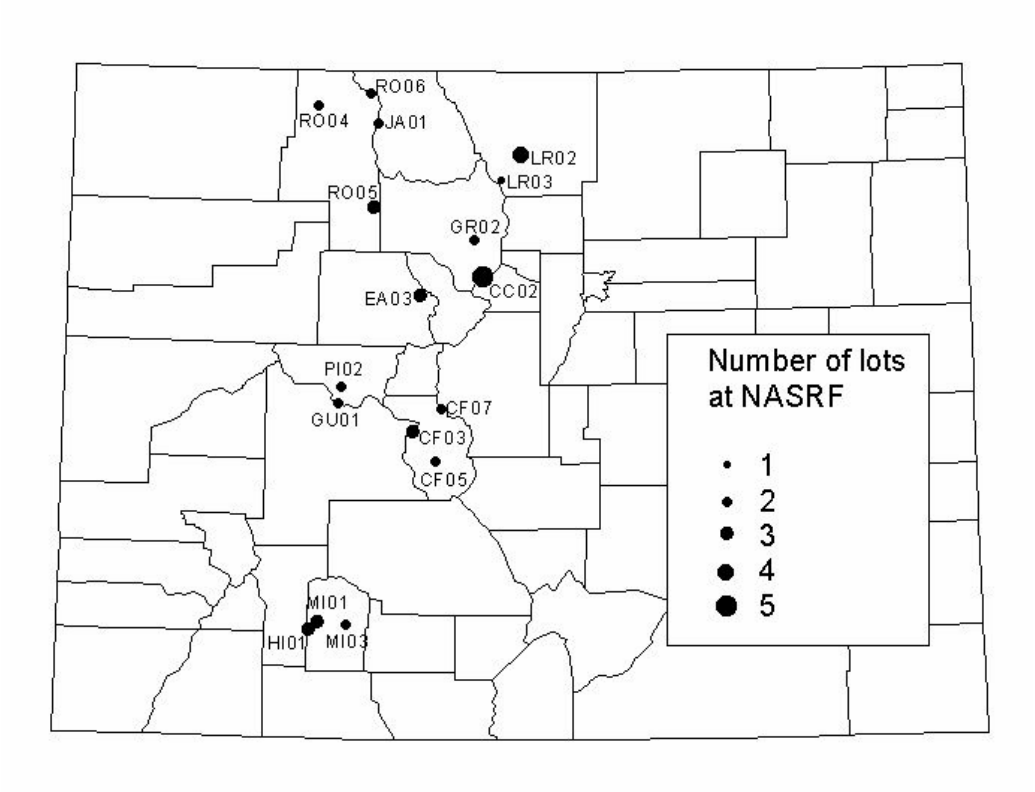

Figure 17. Geographic distribution of boreal toad populations represented at the NASRF

Chapter 15 Water quality data

Table 4. Suggested water quality parameters for most adult and larval amphibians (adapted from Table 12.1 in Whitaker 2001)

Parameter	**Measure**	**Comments**
Temperature (highland anurans)	18-23ºC	In most cases water temperature and air temperature are similar; requirements are highly species-specific and may vary with seasonal change in temperate species; increased growth rate in many larval forms occurs at slightly higher temperatures; water temperatures lower than what the animal is adapted to may negatively affect digestion and suppress immunological function
PH	6.5-8.5*	
Salinity	0-5 ppt	Most species do not tolerate elevated salinity for long periods of time
Hardness	75-150 mg/L	Measures mineral ions needed by animals; requirements dependent on origin of animals
Alkalinity	15-50 mg/L	Measures buffering capacity of water; requirements vary greatly with species of animal
Dissolved oxygen	>80% saturation	Low oxygen levels are tolerated well by many amphibians
Carbon dioxide	<5 mg/L*	Agitation and aeration minimized carbon dioxide accumulation; elevated carbon dioxide associated with decreasing pH
Un-ionized-ammonia	<0.02 mg/L	Undetectable levels accomplished with proper filtration and established biofilter; the indiscriminate use of chemicals in the water may result in a sudden increase in ammonia
Nitrite	<1 mg/L	Very low levels maintained with an active biological filter
Nitrate	<50 mg/L	Control with water changes and removal of organic debris; see nitrite
Chlorine	undetectable	Oxidizing compound easily removed using sodium thiosulfate or agitation of water
*Subcommittee on Amphibian Standards. 1974. Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals. National Academy of Sciences, Washington, DC, 153 pp.		

Table 5. Water quality data from boreal toad sites

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
ABOVE TRICKLE PARK RES 215GM1	08/03/98	12.3	50.5	7.82	28.0	132	<10	<0.20	4.2	1710	26.2	<5.0	<5.0	<5.0
ANN'S POND	05/16/00		114	6.36	4.8	1576	<15	3.93	10.7	148	4764	7.6	<2.0	1064
BEAVER POND 215 GM2	08/03/98	12.4	127.5	8.04	80.0	223	<10	<0.20	4.5	1096	287.0	<5.0	<5.0	5.2
BROWN'S CREEK	08/22/97	10.1	41	7.45	21.0	32	<10	<0.20	2.3	448	56.7	<5.0	<5.0	<5.0
BROWN'S CREEK	06/29/99		044	7.69	20.6	365	<15	<0.15	<1.0	227	<10	<2.0	<2.0	<10
BROWN'S CREEK	08/29/99					34	<15	<0.15	<1.0	54	<10	<2.0	<2.0	<10
BROWN'S CREEK	05/11/00		59	7.28	23.2	229	<15	<0.15	<1.0	133	<10	<2.0	<2.0	<10
BRUSH CREEK	06/14/00		203	7.56	132.0	15	<15	<0.15	<1.0	132	14	<2.0	<2.0	<10
BUCK MTN POND #2	06/19/00		26	6.52	12.6	117	<15	<0.15	2.2	1128	21	<2.0	<2.0	<10
CALIFORNIA PARK	08/05/00		176	7.26	101.0	645	<15	0.23	15.8	1475	389	<2.0	<2.0	99
CALIFORNIA PARK ELKHEAD CREEK	06/15/00		207	7.56	86.6	1202	<15	<0.15	4.3	2168	159	<2.0	<2.0	<10
CALIFORNIA PARK ROUTT N. FOREST	05/26/99		065	5.5	41.2	943	<15	<0.15	1.4	2330	96	<2.0	<2.0	<10
COLL. PEAKS CAMPGROUND	07/28/99		091	7.50	58.0	67	<15	<0.15	1.0	929	17	<2.0	<2.0	<10
COLLEGIATE PEAKS	08/23/97	9.2	67	7.63	37.8	80	<10	<0.20	1.4	369	17.8	<5.0	<5.0	5.6
COLLEGIATE PEAKS	05/22/99					1300	<15	<0.15	18.7	23474	93	2.4	<2.0	11
COLLEGIATE PEAKS CHAFFEE CO	08/28/99		097	7.55	60.8	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS EAST	05/10/00		69	7.23	33.0	32	<15	<0.15	<1.0	213	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS EAST	06/21/00		76	7.15	36.8	31	<15	<0.15	1.5	287	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS EAST	07/19/00		79	7.07	38.0	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
COLLEGIATE PEAKS LOWER POND	05/28/98		83.8		46.0	137	<10	<0.20	1.1	760	21.5	<5.0	<5.0	<5.0
COLLEGIATE PEAKS MIDDLE POND	05/28/98		71.6		34.8	66	<10	0.21	1.5	395	17.7	<5.0	<5.0	<5.0
COLLEGIATE PEAKS WEST	05/10/00		83	7.02	48.6	201	<15	<0.15	1.5	876	14	<2.0	<2.0	<10
COLLEGIATE PEAKS WEST	06/22/00		105	7.20	59.6	112	<15	<0.15	1.7	1372	30	<2.0	<2.0	<10
COLLEGIATE PEAKS WEST	07/19/00		108	6.92	59.2	41	<15	<0.15	1.2	1258	24	<2.0	<2.0	<10
CONUNDRUM	05/28/00		62	6.76	8.0	83	<15	<0.15	<1.0	593	24	<2.0	<2.0	<10

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
CONUNDRUM SILVER DOLLAR SITE	07/23/00		242	8.59	43.8	40	<15	<0.15	4.1	1830	26	<2.0	<2.0	<10
CRAIG'S POND FETKAVICH	06/08/99					54	<15	<0.15	1.1	196	<10	<2.0	<2.0	<10
CUCUMBER GULCH	06/18/97	21.8	44	7.08	14.2	83	<10	<0.20	1.4	368	17.7	<5.0	<5.0	5.5
CUCUMBER GULCH	06/24/98					59	<10	<0.20	1.6	192	12.5	<5.0	<5.0	<5.0
CUCUMBER GULCH	05/25/00		174	7.52	35.2	81	<15	<0.15	3.5	2395	22	<2.0	<2.0	<10
DENNY #1	06/23/00		42	7.17	19.0	32	<15	<0.15	1.0	165	11	<2.0	<2.0	<10
DENNY CREEK	08/23/97	8.8	38	7.51	23.0	224	<10	0.33	8.4	2280	45.1	5.7	<5.0	26.7
DENNY CREEK	05/28/98					104	<10	<0.20	<1.0	262	13.1	<5.0	<5.0	<5.0
DENNY CREEK	05/27/99					236	<15	<0.15	<1.0	604	28	<2.0	<2.0	<10
DENNY CREEK	09/04/99		045	7.72	29.0	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
DENNY CREEK	09/04/99		034	7.71	18.8	55	<15	<0.15	<1.0	316	14	3.0	<2.0	<10
DENNY CREEK POND 2	05/16/00		37	7.28	16.8	125	<15	<0.15	<1.0	380	17	<2.0	<2.0	<10
DENNY CREEK POND 4	05/16/00		38	7.24	17.6	142	<15	<0.15	<1.0	582	36	<2.0	<2.0	<10
DENNY CREEK WEST	06/23/00		42	6.93	17.8	44	<15	<0.15	1.5	2172	127	<2.0	<2.0	<10
DENNY CREEK WEST	07/01/00		62	7.23	31.6	90	<15	<0.15	2.9	3433	140	<2.0	<2.0	<10
DENNY EAST	07/06/00		44	6.85	21.8	114	<15	<0.15	1.7	224	19	<2.0	<2.0	<10
DIAMOND PARK	06/27/97	24.0	73	7.53	30.6	184	<10	<0.20	3.3	886	82.4	<5.0	<5.0	8.0
DIAMOND PARK	06/02/99		057		34.8	95	<15	<0.15	2.3	309	10	<2.0	<2.0	<10
DIAMOND PARK	07/07/99					126	<15	<0.15	3.6	267	<10	<2.0	<2.0	<10
E. LAKE CREEK LOWER	06/30/00		20	6.54	6.8	53	<15	<0.15	49.4	164	<10	<2.0	<2.0	<10
E. LAKE CREEK UPPER	05/30/00		53	5.77	2.0	103	<15	<0.15	1.4	61	15	<2.0	<2.0	14
E. MAROON CREEK	05/29/00		1144	7.12	90.4	363	<15	1.48	20.4	1384	37	47.6	<2.0	151
E. MAROON CREEK	06/08/00		1112	7.79	52.2	<15	<15	<0.15	2.7	113	17	<2.0	<2.0	<10
EAST LAKE CR	07/12/00		66	6.30	1.8	112	<15	<0.15	<1.0	30	24	<2.0	<2.0	25
EAST MAROON UPPER BREEDING	07/24/00		1360	7.44	98.6	259	<15	1.50	7.2	1474	98	29.4	<2.0	51
FOUR MILE CREEK CHAFFEE, CO	08/19/97	9.6	47	8.12	21.5	442	<10	<0.20	1.6	1558	106.0	<5.0	<5.0	<5.0
FOUR MILE CREEK CHAFFEE, CO	08/04/98		53.2		22.6	145	<10	<0.20	83.3	1302	110.6	<5.0	<5.0	10.1
FOUR MILE CREEK CHAFFEE, CO	09/19/98		63.8		27.6	254	<10	<0.20	1.3	2188	122.3	<5.0	<5.0	<5.0
FOUR MILE CREEK CHAFFEE, CO	06/09/99					381	<15	<0.15	<1.0	754	64	<2.0	<2.0	<10
FOURMILE	05/16/00		40	7.27	14.4	252	<15	<0.15	<1.0	658	41	<2.0	<2.0	<10

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
GORE CREEK SOUTH SIDE	07/22/99		166	6.65	91.2	52	<15	<0.15	<1.0	239	20	<2.0	<2.0	<10
GUNNISON TRIANGLE PASS	06/19/98	14.4	11.9	6.5	8.6	176	<10	<0.20	1.2	406	15.9	<5.0	<5.0	5.4
HARTENSTEIN LAKE	08/30/97	10.1	18	6.93	10.6	72	<10	<0.20	<1.0	162	3.7	<5.0	<5.0	<5.0
HARTENSTEIN LAKE	09/07/98		27.4		13.8	74	<10	<0.20	1.8	470	16.5	<5.0	<5.0	6.2
HARTENSTEIN LAKE	06/24/99					56	<15	<0.15	<1.0	87	<10	<2.0	<2.0	<10
HARTENSTEIN LAKE	07/13/99		15.1		8.2	66	<10	<0.20	1.2	95	3.4	<5.0	<5.0	<5.0
HARTENSTEIN LAKE	06/01/00		25	6.46	2.8	302	<15	<0.15	2.8	971	14	<2.0	<2.0	<10
HARTENSTEIN LAKE OUTLET POND	07/06/00		20	6.84	10.8	33	<15	<0.15	<1.0	142	26	<2.0	<2.0	<10
HARTENSTEIN LAKE PROPER	07/06/00		17	7.02	7.6	34	<15	<0.15	<1.0	78	<10	<2.0	<2.0	<10
HATRENSTEIN LAKE NON-BREEDING	07/06/00		26	6.74	12.2	63	<15	<0.15	1.6	1754	201	<2.0	<2.0	<10
HERMAN GULCH	05/28/97					2471	<10	<0.20	7.1	1441	38.2	<5.0	<5.0	21.2
HERMAN GULCH	06/24/97		92	7.21	19.8	181	<10	0.26	3.1	665	264.5	<5.0	<5.0	<5.0
HERMAN GULCH	05/20/98	12.1	100.0	7.23	34.2	159	<10	0.26	1.9	1047	286.4	<5.0	<5.0	<5.0
HERMAN GULCH	05/20/98	12.1	100.0	7.23	34.2	179	<10	0.30	2.4	1415	621.8	<5.0	<5.0	<5.0
HERMAN GULCH	07/23/98	12.5	100.0	8.10	48.6	175	<10	<0.20	2.2	102	4.7	<5.0	<5.0	5.6
HERMAN GULCH	08/22/98		589		59.4	103	<10	<0.20	<1.0	265	13.2	<5.0	<5.0	<5.0
HERMAN GULCH	07/11/00		725	7.36	47.6	25	<15	<0.15	1.3	777	78	<2.0	<2.0	<10
HERMAN GULCH (RUT)	06/24/97		711	7.30	61.6	419	<10	0.33	4.3	1400	225.4	<5.0	<5.0	7.2
HOLY CROSS S UPPER POOL EAGLE	07/31/98		16.1		8.2	127	<10	<0.20	1.1	26	2.1	<5.0	<5.0	6.4
JL2 SUMMIT CO	06/29/98					36	<10	<0.20	2.4	33	5.8	<5.0	<5.0	<5.0
JUMPER CREEK	05/21/98	12.5	51.6	6.75	29.8	611	<10	<0.20	1.5	323	8.2	<5.0	<5.0	<5.0
JUMPER CREEK	08/18/98		81.2		42.6	21	<10	<0.20	1.0	121	2.4	<5.0	<5.0	<5.0
JUMPER CREEK	05/31/99					491	<15	<0.15	<1.0	259	<10	<2.0	3.7	<10
JUMPER CREEK	07/05/99					527	<15	<0.15	3.0	440	45	<2.0	<2.0	<10
KETTLE TARN II	05/01/00		20	7.03	6.4	66	<15	<0.15	2.1	1323	39	<2.0	<2.0	<10
KETTLE TARN RMNP	05/05/00		23	6.52	10.0	112	<15	<0.15	1.7	1372	30	<2.0	<2.0	<10
KETTLE TARN RMNP	07/12/00		34	6.04	9.0	57	<15	<0.15	1.2	230	10	<2.0	<2.0	18
KROENKE LAKE	08/29/97	10.5	22	6.93	10.4	150	<10	0.22	4.0	861	14.3	<5.0	<5.0	8.2

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
KROENKE LAKE	06/30/98					63	<10	<0.20	1.6	142	10.1	<5.0	<5.0	<5.0
KROENKE LAKE	06/30/99					48	<15	<0.15	1.5	561	18	<2.0	<2.0	<10
LAKE OWEN BEAVER POND	08/20/99					70	<15	<0.15	2.5	837	68	<2.0	<2.0	12
LILY PAD LAKES TRAIL	07/01/98					87	<10	<0.20	1.7	1355	94.4	<5.0	<5.0	7.3
LOST LAKE	06/05/00		44	6.98	16.4									
LOST LAKE BOULDER, CO	08/22/97	20.8	46	6.98	17.4	290	<10	<0.20	3.6	437	17.9	<5.0	<5.0	11.0
LOST LAKE BOULDER, CO	08/18/98		48.3		24.2	38	<10	<0.20	1.2	253	12.2	<5.0	<5.0	6.9
LOST LAKE RMNP	05/16/00		18	6.61	6.0	41	<15	<0.15	2.0	778	83	<2.0	<2.0	<10
LOST LAKE RMNP	08/08/00		15	6.13	5.6	20	<15	<0.15	1.2	243	12	<2.0	<2.0	<10
LOWER N. FORK SNAKE	05/23/00		154	7.02	54.6	562	<15	<0.15	4.7	579	16	<2.0	<2.0	<10
LOWER N. TENMILE	05/23/00		61	6.74	33.0	39	<15	<0.15	1.8	77	<10	<2.0	<2.0	<10
LOWER N. TENMILE	07/11/00		77	7.15	37.8	26	<15	<0.15	<1.0	161	<10	<2.0	<2.0	<10
MAGDALENE GULCH	09/01/99		038	7.67	22.4	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
MIDDLE COTTONWOOD	05/10/00		87	7.24	44.0	<15	<15	<0.15	1.4	1129	16	<2.0	<2.0	<10
MIDDLE COTTONWOOD CHAFFEE	07/28/99		042	7.60	25.8	19	<15	<0.15	<1.0	66	<10	<2.0	<2.0	<10
MIDDLE COTTONWOOD CHAFFEE CO	09/11/99		049	7.90	28.4	<15	<15	<0.15	<1.0	77	<10	<2.0	<2.0	<10
MORGAN'S GULCH	05/31/00		40	7.08	20.4	74	<15	<0.15	<1.0	510	<10	<2.0	<2.0	<10
MORGAN'S GULCH CHAFFEE CO	07/12/99		045	7.39	26.0	104	<15	<0.15	1.3	1215	<10	<2.0	<2.0	<10
MORGAN'S GULCH CHAFFEE CO	09/11/99		040	7.62	21.2	208	<15	<0.15	3.2	2058	14	<2.0	<2.0	<10
MORGANS GULCH CHAFFEE, CO	09/06/97	12.1	52	6.98	28.2	52	<10	<0.20	2.7	2442	243.7	<5.0	<5.0	6.0
MORGANS GULCH CHAFFEE, CO	06/15/98		32.9		17.4	73	<10	<0.20	1.0	204	2.4	<5.0	<5.0	<5.0
MOUNT BETHEL	06/02/97					343	<10	<0.20	2.8	208	6.3	<5.0	<5.0	<5.0
MOUNT BETHEL	06/16/97					204	<10	<0.20	7.2	131	10.0	17.5	<5.0	5.0
MOUNT BETHEL	05/20/98	11.8	39.7	7.51	22.8	200	<10	<0.20	1.5	151	3.8	<5.0	<5.0	<5.0
MOUNT BETHEL	07/23/98	14.4	67.4	8.66	38.4	20	<10	<0.20	<1.0	37	4.1	<5.0	<5.0	<5.0
MOUNT BETHEL	08/22/98		83.5		44.8	24	<10	<0.20	<1.0	67	6.4	<5.0	<5.0	<5.0
MOUNT BETHEL	06/09/00		49	6.93	25.4	89	<15	<0.15	1.9	103	<10	<2.0	<2.0	<10
MOUNT BETHEL	07/13/00		82	7.87	39.8	77	<15	<0.15	<1.0	138	11	<2.0	<2.0	<10
MOUNT BETHEL	07/31/00		95	7.75	46.6	39	<15	<0.15	1.0	112	15	<2.0	<2.0	<10

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
N. FORK ELK RIVER UPPER SITE	06/08/00		32	6.87	16.4	37	<15	<0.15	3.1	225	<10	<2.0	<2.0	<10
N. TEN MILE CREEK NT6	06/22/98					27	<10	<0.20	2.7	115	13.3	<5.0	<5.0	<5.0
N. TEN MILE HIGHEST	06/23/97	22.0	70	7.55	35.0	28	<10	<0.20	<1.0	103	7.1	<5.0	<5.0	<5.0
N. TEN MILE RELOCATION POND	06/23/97	22.6	56	7.22	19.8	79	<10	<0.20	2.1	227	13.3	<5.0	<5.0	8.2
N. TEN MILE UPPER	06/23/97	21.9	63	7.42	30.4	132	<10	<0.20	1.5	583	48.9	<5.0	<5.0	16.2
N. TEN MILE UPPER LITTLE POND	06/23/97	21.8	105	7.5	58.2	80	<10	<0.20	<1.0	1356	122.2	<5.0	<5.0	42.8
N. TENMILE CREEK NT4,5,&6	06/22/98					34	<10	<0.20	1.6	120	9.2	<5.0	<5.0	<5.0
N. TENMILE CREEK NT5	06/22/98					18	<10	<0.20	<1.0	151	30.2	<5.0	<5.0	<5.0
N. TENMILE CR-LOWER BREEDING	07/20/00		72	7.15	36.0	38	<15	<0.15	<1.0	166	<10	<2.0	<2.0	<10
N. TENMILE CR-UPPER BREEDING	07/20/00		78	7.12	42.2	17	<15	<0.15	<1.0	150	<10	<2.0	<2.0	<10
N. TENMILE UPPER	06/07/00		59	7.55	35.4	34	<15	<0.15	<1.0	67	<10	<2.0	<2.0	<10
NFS3	07/16/99		6.95	109	41.8	37	<15	<0.15	2.5	880	37	<2.0	<2.0	<10
NORTH WILLOW CREEK	07/09/98	16.0	25.1	6.5	13.0	152	<10	<0.20	1.9	299	15.3	<5.0	<5.0	<5.0
NT2	07/02/99		74	7.12	40.4	109	<15	<0.15	3.3	2907	213	2.4	2.8	<10
NT4	05/28/99		53	6.91	26.2	143	<15	<0.15	5.6	341	19	2.0	<2.0	<10
NT4	06/11/99		59	6.97	29.2	43	<15	<0.15	2.2	55	<10	<2.0	<2.0	<10
NT4	07/02/99		59	7.08	36.6	132	<15	<0.15	1.5	179	13	<2.0	<2.0	<10
PC1	06/09/99		63	6.78	15.8	68	<15	0.66	7.4	696	95	<2.0	<2.0	424
PERU CREEK	07/15/97	22.3	80	7.12	18.2	58	<10	0.41	10.3	410	61.0	5.3	<5.0	242.5
PERU CREEK	06/25/98					30	<10	0.41	6.8	500	42.0	<5.0	<5.0	343.5
PERU CREEK	05/23/00		57	6.98	11.8	26	<15	0.24	4.7	519	64	<2.0	<2.0	388
PINGREE PARK TWIN LAKES U POND	07/24/98	13.0	23.6	7.81	15.4	1704	<10	<0.20	3.6	1212	19.3	<5.0	<5.0	10.9
POLE CREEK	06/02/00		71	7.35	34.6	418	<15	<0.15	2.6	985	231	<2.0	<2.0	<10
POLE CREEK	08/12/00		187	7.70	105.8	238	<15	<0.15	2.1	1009	123	<2.0	<2.0	<10
POLE CREEK #15	06/15/97					225	<10	<0.20	1.5	286	24.5	<5.0	<5.0	<5.0
POLE CREEK #4	06/09/97	21.2	61	7.10	28.6	306	<10	<0.20	1.1	301	15.0	<5.0	<5.0	<5.0
POLE CREEK HOLE 15	06/02/00		54	7.31	28.0	121	<15	<0.15	<1.0	212	19	<2.0	<2.0	<10
POND AT EGGELSTON	08/03/98	12.5	74.2	8.27	42.4	117	<10	<0.20	1.0	100	10.8	<5.0	<5.0	<5.0
POND S OF MESA/DELTA LINE E65	08/04/98	12.5	17.1	7.87	8.8	67	<10	<0.20	2.0	122	11.4	<5.0	<5.0	6.6

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
RAINBOW LAKE	05/22/99					<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
RAINBOW LAKE	05/14/00		79	7.44	42.8	89	<15	<0.15	1.6	2047	76	<2.0	<2.0	<10
ROCK CREEK PARK	08/05/99					18807	28	0.79	19.8	28041	540	14.6	4.6	57
S. COTTONWOOD	07/19/00		95	7.12	44.8	96	<15	<0.15	<1.0	414	58	<2.0	<2.0	<10
S. COTTONWOOD BREEDING	05/16/00		92	7.48	49.2	226	<15	<0.15	1.9	467	<10	<2.0	<2.0	<10
S. COTTONWOOD NEW POND	07/21/00		103	7.10	50.0	109	<15	<0.15	2.1	1919	272	<2.0	<2.0	<10
S. COTTONWOOD NON -BREEDING	06/23/00		108	7.13	53.4	20	<15	<0.15	1.2	243	12	<2.0	<2.0	<10
S. COTTONWOOD NON-BREEDING	05/16/00		93	7.27	48.2	33	<15	<0.15	1.1	294	<10	<2.0	<2.0	<10
S. COTTONWOOD WEST	05/16/00		110	7.37	48.0	55	<15	<0.15	1.6	1193	53	<2.0	<2.0	<10
S. COTTONWOOD WEST NEW POND	06/22/00		99	7.25	40.4	41	<15	<0.15	2.0	778	83	<2.0	<2.0	<10
S. COTTONWOOD WEST OLD POND	06/22/00		148	7.27	56.8	66	<15	<0.15	2.1	1323	39	<2.0	<2.0	<10
SAYRES GULCH CHAFFEE CO	09/06/97	10.5	92	7.1	57.4	68	<10	<0.20	<1.0	45	1.7	<5.0	<5.0	<5.0
SAYRES GULCH CHAFFEE CO	09/13/98		112.1		59.2	56	<10	<0.20	1.8	203	19.2	<5.0	<5.0	<5.0
SAYRES GULCH CHAFFEE CO	06/17/99					30	<15	<0.15	4.1	507	26	<2.0	<2.0	<10
SECOND POND ABOVE TRICK215GMI2	08/03/98	13.2	38.5	7.66	17.6	40	<10	<0.20	1.3	208	21.1	<5.0	<5.0	<5.0
SNAKE RIVER SR2	06/25/98					13	<10	<0.20	1.5	75	18.0	<5.0	<5.0	10.8
SNAKE RIVER SR3	06/25/98					14	<10	<0.20	2.4	18	15.9	<5.0	<5.0	110.9
SODA CREEK	06/09/97	21.5	20	7.17	7.4	174	<10	<0.20	2.1	110	9.5	<5.0	<5.0	7.4
SODA CREEK	05/24/99		018	4.5	8.4	132	<15	<0.15	<1.0	70	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD	06/23/00		92	7.04	42.8	57	<15	<0.15	1.2	230	10	<2.0	<2.0	18
SOUTH COTTONWOOD CHAFFEE CO	09/04/99		152	7.78	99.4	32	<15	<0.15	1.9	218	<10	<2.0	<2.0	<10
SOUTH COTTONWOOD CHAFFEE, CO	06/01/98		109.7		57.6	66	<10	<0.20	1.4	221	5.7	<5.0	<5.0	<5.0
SOUTH COTTONWOOD CHAFFEE, CO	09/07/98		106.1		55.6	84	<10	<0.20	2.6	463	7.6	<5.0	<5.0	<5.0
SOUTH COTTONWOOD CHAFFEE, CO	06/03/99		63	6.99	28.2	48	<15	<0.15	5.1	129	<10	<2.0	<2.0	<10

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
SOUTH COTTONWOOD CHAFFEE, CO	06/07/99					41	<15	<0.15	2.5	1094	43	<2.0	<2.0	<10
SOUTH COTTONWOOD WEST CHAFFEE	08/30/98					24	<10	<0.20	<1.0	1800	109.5	<5.0	<5.0	<5.0
SOUTH COTTONWOOD WEST CHAFFEE	09/04/99		136	7.59	75.8	26	<15	<0.15	1.6	887	52	<2.0	<2.0	<10
SOUTH FORK BIRD CREEK	07/02/99					91	<15	<0.15	2.4	799	120	<2.0	<2.0	13
SR6	06/08/99					414	<15	2.55	1.5	515	428	8.7	<2.0	2021
SR7	07/16/99		102	7.05	7.2	197	<15	0.72	3.5	875	265	3.3	<2.0	211
STAIRWAY	07/14/00		23	6.30	9.6	46	<15	<0.15	<1.0	225	13	<2.0	<2.0	<10
STAIRWAY POND 1	08/03/00		21	5.79	9.8	113	<15	0.54	7.9	270	15	<2.0	<2.0	24
STAIRWAY POND 3	06/29/00		27	5.05	3.2	38	<15	<0.15	<1.0	139	<10	<2.0	<2.0	<10
STAIRWAY ROUTT CO.	06/20/00		14	7.43	6.0	20	<15	<0.15	<1.0	58	<10	<2.0	<2.0	<10
STAIRWAY ROUTT N. FOREST	08/04/99		020	4.5	10.6	48	<15	<0.15	1.5	120	<10	<2.0	<2.0	<10
STAIRWAY ROUTT N. FOREST	08/23/99		018	5.0	9.4	49	<15	<0.15	1.9	184	<10	<2.0	<2.0	<10
STAIRWAY SITE ROUTT CO.	06/12/00		16	6.81	7.6	24	<15	<0.15	<1.0	50	<10	<2.0	<2.0	<10
STRAIT CREEK	06/26/98					38	<10	<0.20	3.7	81	10.3	<5.0	<5.0	<5.0
TA BRAGG	08/05/99					97	<15	<0.15	3.0	649	44	<2.0	<2.0	21
TEXAS CREEK GUNNISON CO	08/21/99		042	7.65	23.8	88	<15	<0.15	1.1	364	13	<2.0	<2.0	<10
TRIANGLE PASS	06/29/98	11.2	23.4	8.39	5.0	54	<10	<0.20	<1.0	217	4.7	<5.0	<5.0	<5.0
TRIANGLE PASS	07/27/98	12.0	29.0	7.56	3.6	137	<10	<0.20	1.1	373	19.3	<5.0	<5.0	<5.0
TRIANGLE PASS	08/24/98		40.5		19.8	83	<10	<0.20	1.6	287	23.2	<5.0	<5.0	<5.0
TRIANGLE PASS	09/30/98		55.1		28.6	67	<10	<0.20	1.6	464	17.3	<5.0	<5.0	6.9
TRIANGLE PASS	06/23/99		72	7.74	46.4	78	<15	0.23	4.1	341	25	<2.0	<2.0	<10
TRIANGLE PASS	08/12/99		031	7.75	15.4	108	<15	<0.15	1.6	463	30	<2.0	<2.0	<10
TRIANGLE PASS	09/02/99		021	7.61	34.8	<15	<15	<0.15	<1.0	<10	<10	<2.0	<2.0	<10
TRIANGLE PASS	06/09/00		50	7.40	25.4	183	<15	<0.15	<1.0	615	33	<2.0	<2.0	<10
TRIANGLE PASS	07/20/00		42	6.49	16.6	24	<15	<0.15	<1.0	657	35	<2.0	<2.0	<10
UPPER BUCK MTN POND #1	08/07/00		37	6.73	15.2	105	<15	<0.15	3.2	4220	25	<2.0	<2.0	<10
UPPER BUCK MTN POND #2	08/07/00		34	6.75	16.0	52	<15	<0.15	6.3	553	<10	<2.0	<2.0	25
UPPER N. FORK SNAKE	05/23/00		96	7.09	36.2	36	<15	<0.15	<1.0	81	<10	<2.0	<2.0	<10
UPPER N. TENMILE	06/09/00		59	6.86	32.0	60	<15	<0.15	<1.0	99	<10	<2.0	<2.0	<10

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
URAD-HENDERSON 1-POND	08/19/99		200	7.18	70.8	99	<15	0.15	3.3	101	879	<2.0	<2.0	65
URAD-HENDERSON 2 POND	05/14/97		1128	7.68	268.0	3111	<10	1.89	5.4	1055	<1.0	7.7	6.3	1284.6
URAD-HENDERSON 2 POND	07/22/98	14.2	181.3	7.20	51.2	306	<10	0.78	3.1	44	1484.2	<5.0	<5.0	225.8
URAD-HENDERSON ANN'S POND	06/10/97					2553	<10	2.21	13.2	1706	1264.6	57.8	<5.0	412.5
URAD-HENDERSON ANN'S POND	05/27/98	10.9	112.6	5.8	5.6	2856	<10	7.28	20.2	292	4344.5	14.6	<5.0	1225.5
URAD-HENDERSON ANN'S POND	07/08/98	12.3	39.7	7.04	14.0	537	<10	0.26	8.0	545	278.8	20.5	<5.0	48.5
URAD-HENDERSON ANN'S POND	07/13/99		44	7.40	13.4	337	<15	0.16	4.9	182	103	3.7	<2.0	37
URAD-HENDERSON DONUT	05/27/98	10.8	57.1	7.28	4.8	372	<10	0.72	3.2	139	1549.6	6.1	<5.0	185.3
URAD-HENDERSON DONUT	07/07/98	13.3	57.1	7.19	15.8	212	<10	<0.20	2.9	84	148.1	<5.0	<5.0	41.0
URAD-HENDERSON DONUT	07/13/99		51	7.48	15.8	239	<15	<0.15	3.0	45	112	<2.0	<2.0	17
URAD-HENDERSON DONUT	08/19/99		55	7.42	17.8	1155	<15	0.21	5.8	923	346	7.4	<2.0	61
URAD-HENDERSON DONUT	05/16/00		49	6.52	4.4	390	<15	0.21	2.8	410	795	15.3	<2.0	115
URAD-HENDERSON DONUT	07/13/00		63	7.62	20.0	133	<15	<0.15	<1.0	96	136	3.6	<2.0	17
URAD-HENDERSON ECLAIR	10/02/97	11.5	1154	3.2		34710	18	13.38	31.9	<10	8850.7	55.0	5.9	<5.0
URAD-HENDERSON ERIN'S POND	05/15/97		307	7.04	14.0	1492	<10	0.93	9.8	937	2612.6	12.0	<5.0	330.5
URAD-HENDERSON HESBO	05/15/97		80	7.72	22.8	421	<10	<0.20	1.5	221	244.4	<5.0	<5.0	8.2
URAD-HENDERSON HESBO	05/28/98	11.5	102.6	7.45	44.4	106	<10	<0.20	1.8	75	258.1	<5.0	<5.0	9.7
URAD-HENDERSON HESBO	07/21/98	14.3	100.0	6.94	99.4	284	<10	<0.20	3.6	250	2397.9	<5.0	<5.0	38.4
URAD-HENDERSON HESBO	07/09/99		179	7.18	68.8	50	<15	<0.15	1.1	46	672	<2.0	<2.0	<10
URAD-HENDERSON HESBO	08/19/99		184	7.04	71.2	31	<15	<0.15	1.8	29	401	<2.0	<2.0	<10
URAD-HENDERSON HESBO	05/16/00		103	7.7	39.6	223	<15	<0.15	<1.0	193	405	<2.0	<2.0	12
URAD-HENDERSON HESBO	07/13/00		280	7.33	62.6	242	<15	<0.15	<1.0	208	740	2.2	<2.0	21

Table 5. Water quality data from boreal toad sites continued

SITE	DATE	TEMP	COND	PH	ALKALINITY	AL	AS	CD	CU	FE	MN	PB	SE	ZN
URAD-HENDERSON JS POND	08/19/99		169	7.09	72.4	122	<15	<0.15	7.2	866	3044	<2.0	2.0	15
URAD-HENDERSON JS POND (STMNT)	07/22/98	14.2	181.3	7.20	51.2	262	<10	0.33	17.5	601	3437.9	5.7	<5.0	32.1
URAD-HENDERSON POWER ALLEY	05/15/97		64	7.79	2.04	1112	<10	<0.20	2.4	606	49.0	<5.0	<5.0	28.6
URAD-HENDERSON POWER ALLEY	05/28/98	11.1	102.6	7.40	20.6	142	<10	<0.20	2.4	129	55.7	<5.0	<5.0	30.3
URAD-HENDERSON POWER ALLEY	07/08/98	13.1	58.8	6.98	25.6	23	<10	<0.20	2.0	236	150.3	<5.0	<5.0	32.1
URAD-HENDERSON TREATMENT	05/28/98	11.7	100.0	7.23	35.8	176	<10	0.35	5.6	77	279.0	<5.0	<5.0	24.9
URAD-HENDERSON TREATMENT	07/08/98	13.2	100.0	6.75	97.2	29	<10	1.27	8.6	160	2662.7	<5.0	<5.0	52.3
URAD-HENDERSON TREATMENT	07/09/99		1087	7.26	60.0	74	<15	0.88	6.1	45	357	<2.0	<2.0	50
URAD-HENDERSON TREATMENT	08/19/99		836	7.16	93.4	132	<15	0.50	7.3	194	294	<2.0	<2.0	54
URAD-HENDERSON UPPER URAD	06/25/97					553	<10	1.14	6.1	239	<1.0	<5.0	<5.0	468.2
URAD-HENDERSON UPPER URAD	07/08/98	15.4	100.0	6.66	11.8	390	<10	0.62	5.9	143	<1.0	5.9	10.7	426.9
URAD-HENDERSON UPPER URAD	07/13/99		546	6.75	12.0	559	<15	1.99	16.8	133	33800	3.1	4.3	788
URAD-HENDERSON UPPER URAD	06/07/00		549	6.72	10.0	1453	<15	1.49	2.7	75		<2.0	5.8	545
VINTAGE BELOW HEND CLEAR CREEK	05/15/97		135	7.41	20.2	267	<10	0.43	3.5	209	218.9	<5.0	<5.0	144.8
WEST BRUSH CREEK	06/24/99		32	6.58	9.8	104	<15	<0.15	1.7	433	17	<2.0	<2.0	<10
WEST BRUSH CREEK	08/31/99		185	7.87	133.8	17	<15	<0.15	1.2	558	45	<2.0	<2.0	<10

Chapter 16 Supplier information

Food sources

Tadpole feed:
Mazuri Amphibian & Carnivorous Reptile Gel Test Unit
Phone: (765) 966-1885

Tadpole feed:
Hikari algae wafers (item #21328)
Kyorin Co., Ltd. 9, Minami-machi
Himeji, Japan
Available at Wal-Mart or aquarium store.

Tadpole feed:
Spirulina Aquarium Flake Food and shrimp flakes
O.S.I. Marine Lab., Inc.
Hayward, CA 94545
Available at wholesale pet distributor, Payless Cashways, Denver.

Vitamin and mineral supplements for dusting on fruit flies & crickets:
Repta-calcium, 4 oz. & Repta-vitamin, 4 oz.
Fluker Laboratories
1333 Plantation Rd.
Port Allen, LA 70767
Phone: (800) 546-2847

Wingless fruit flies (*Drospohila melanogaster*) culture (item #RG-17-2910):
Carolina Biological Supply Company
Burlington, NC 27215
Phone: (800) 334-5551
Web site: www.carolina.com

Mold inhibitor for fruit flies:
Methylparaben powder (item #RG-87-6161)
Carolina Biological Supply Company
Burlington, NC 27215
Phone: (800) 334-5551
Web site: www.carolina.com

Cricket water product:
Hydrosource (50 pound bag)
Colorado Seed Solutions, 195 North Washington, Monte Vista, CO 81144
Phone: 719-852-3505
email: montevista@seedsolutions.com
or
Cricket Water Bites (much more expensive than Hydrosource)
Nature Zone
Paradise, CA 95969
Web site: www.naturezone.fauna.com

Crickets & waxworms:
Bassets Cricket Ranch
365 S. Mariposa Ave.
Vasalia, CA 93292-9729
Phone: (800) 634-2445

Construction/housing products

Toadlet and toad fiberglass tanks:
Gemini Fiberglass Products, Inc.
14224 West First Drive
Golden, CO 80401
Phone: (303) 278-0033

Lighting for enclosures:
Reptisun 5.0 UVB fluorescent bulb (48-inch) (item #FS-48) and Zoomed incandescent daylight blue reptile bulb (item #DB-100)
ZooMed
3100 McMillan Road
San Luis Obispo, CA
Phone: (805) 542-9988
FAX: (805) 542-9295
email: zoomed@zoomed.com

Aquarium sealant (for tadpole tanks):
Perfecto Manufacturing Inc.
20975 Creek Road
Noblesville, IN 46060
Available at local pet stores

Plastic for “ramp” in tanks:
24” x 48” polypropolene perforated sheets (item #42561)
United States Plastics Corporation
1390 Neubrecht Road
Lima, OH 45801
Phone: (800) 537-9724

Heater to form “ramps” in tanks:
EMX strip heater (item #42037)
United States Plastics Corporation
1390 Neubrecht Road
Lima, OH 45801
Phone: (800) 537-9724

Plastic to prevent toadlets from escaping from tanks:
24” x 48” gray 1/16” thick PVC sheets (item #45084)
United States Plastics Corporation
1390 Neubrecht Road
Lima, OH 45801
Phone: (800) 537-9724

Drain pan for individual hibernacula (item #56981):
United States Plastics Corporation
1390 Neubrecht Road
Lima, OH 45801
Phone: (800) 537-9724

Moss used for hibernacula:
Green moss, 2.5 cubic foot box, 7 pounds (item #GM-104)
Forest Products Packaging Co.
1265 Stryker Rd.
Independence, OR 97351
Phone: (503) 606-9990
Fax: (503) 606-9993
Email: info@mossproducts.com
Web site: www.mossproducts.com

Medical products

Hormone for breeding toads:
LHRH (des-Gly10(D-ALA6) Leutenizing Hormone Releasing Hormone Ethylamide (item #L4513)
Sigma Scientific Company
3500 Dekalb St.
St. Louis, MO 63118
Phone: (314) 771-5765

MS-222 (Tricaine Methanesulfonate, or FINQUEL®) (item #A-5040):
Sigma Scientific Company
3500 Dekalb St.
St. Louis, MO 63118
Phone: (314) 771-5765

For treatment of prolapse:
7.2% hypertonic saline
Phoenix Pharmaceuticals
St. Joseph, MO 64506
Available from local veterinarian

Chapter 17 Datasheets

Tank data sheet

	# OF TOADS August 2002	Initials
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Comments:

Invertebrate Rearing Unit Check List

1. Mist all cricket nest boxes and check all feed and Hydrosource.
   a. Feed Spirulina Aquarium Flake Food and shrimp flakes to the feeder crickets & pinheads.
   b. Feed rabbit pellets to the adults.

2. Mist fruit flies every other day. Only use a light spray.

3. Fill the humidifier daily. Maintain building humidity at 50-80 percent.

4. Maintain building temperature at 80-85ºF.