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EXPERIMENTAL REPATRIATION OF BOREAL TOAD (*BUFO BOREAS*) EGGS, METAMORPHS, AND ADULTS IN ROCKY MOUNTAIN NATIONAL PARK

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The boreal toad (*Bufo boreas*) is an endangered species in Colorado and is considered a candidate species for federal listing by the United States Fish and Wildlife Service. Boreal toads are absent from many areas of suitable habitat in the Southern Rocky Mountains of Colorado presumably due to a combination of causes. We moved boreal toads from existing populations and from captive rearing facilities to habitat which was historically, but is not currently, occupied by toads to experimentally examine methods of repatriation for this species. Repatriation is defined as the release of individuals into areas currently or historically occupied by that species (Dodd and Seigel, 1991). This effort was in response to one of the criteria for delisting the boreal toad in Colorado stated in the conservation plan and agreement for the management and recovery of the Southern Rocky Mountain population of the boreal toad (Loeffler, 1998:16); "... there must be at least 2 viable breeding populations of boreal toads in each of at least 9 of 11 mountain ranges of its historic distribution." Without moving eggs from established wild populations, or from captivity to historical localities, it is doubtful whether the recovery team will attain this ambitious goal.

Because repatriation and translocation of anurans has had little documented success (Dodd and Seigel, 1991; but see Cooke and Oldham, 1995; Denton et al. 1997), our objectives were to determine methods and optimal life stages for moving boreal toads. Specifically, we translocated egg masses and monitored the hatching success of translocated eggs compared to the hatching success of clutches at the donor site; we released captive-reared, marked metamorphs and monitored their survival over winter; and we released captive-reared, 1 year-old toads and monitored their dispersal and site fidelity for 4 years. We documented monetary costs, because they are important for determining efficacy of species recovery efforts and are of interest to wildlife managers.

All sites are in Rocky Mountain National Park, Larimer Co., Colorado. Both release sites are historic localities for boreal toads (Corn et al., 1997). Lost Lake, the donor site, is a high altitude lake (elevation 3,265 m) in the North Fork drainage of the Big Thompson River (Fig. 1). About half of the shoreline is bordered by a 10 to 20 m wide rocky shelf dotted with small, shallow (3 to 15 cm) pools used for egg deposition by boreal toads. Greenback cutthroat (*Oncorhynchus clarki stomais*) are present in the main portion of the lake but not the shallow pools. Willow (*Salix planifolia*) and bog birch (*Betula glandulosa*) dominate a transition zone between the lake and Englemann spruce (*Picea englemannii*)–subalpine fir (*Abies lasiocarpa*) forest. Late spring thaws and short summers are typical at Lost Lake where the boreal toad population has been monitored since 1991 (Corn et al., 1997). Reproductive success is erratic because tadpoles often fail to metamorphose before the onset of winter, typical of high-altitude populations of boreal toads (Livo and Fetkavich, 1998; E. Muths, pers. obser.).

Horseshoe Park (1995 release site, 2,595 m) is located in the Fall River drainage (Fig. 1) where toads have been absent since 1987 (Corn et al., 1997). Two inactive beaver ponds were used. They are shallow (10 to 30 cm), ca. 50 m apart, with emergent vegetation (grass and *Carex* sp.). Willow (*Salix monticola*) is the dominant vegetation type around the ponds. The total area searched after releasing eggs was ca. 150 m by 75 m.

Glacier Basin (1995 and 1996 release site, 2,560 m) is located in the Glacier Creek drainage (Fig. 1) and is dominated by willow and bog birch. The site is a marshy area of old beaver ponds with Glacier Creek running south to north; brook trout (*Salvelinus fontinalis*) are present. The low area is bordered by dry, Ponderosa pine (*Pinus ponderosa*) forest with granite outcroppings and little undergrowth. A horse and hiking trail runs through the middle of the site and is used heavily from June through mid September. Translocated egg masses were deposited in flooded grass areas that typically stay wet through the middle of June. The area searched after releasing eggs, metamorphs, and toads was ca. 900 m by 100 m. Neither breeding nor adult toads had been observed since 1990 (Corn et al., 1997). However, an unmarked resident female was found at the site after the first experimental toads were moved to the site.

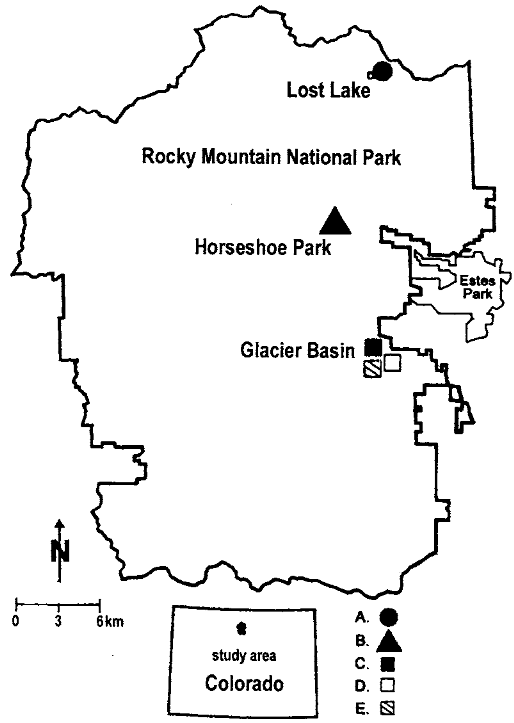

FIG. 1—Map of the study site, Rocky Mountain National Park, Colorado. A) donor site 1995, 1996; B) recipient site 1995 eggs; C) recipient site 1995 metamorphs; D) recipient site 1996 eggs; E) recipient site 1996 adult-sized toads.

Financial costs presented include only large expenditures such as helicopter transportation, not salaries of principal investigators or technicians. Person-hours of effort were recorded for translocations, captive rearing, and on site monitoring and do not include travel to and from field sites. Eggs, metamorphs, and adult toads were searched for simultaneously at the Glacier Basin site.

On 7 July 1995 and 20 June 1996, we collected boreal toad egg masses (5,000 to 10,000 eggs; 8 to 15 h old) from Lost Lake (11 and 6 masses, respectively). To assess hatching success, we placed a small number of eggs (28 to 46, mean = 40) from each egg mass in a covered, ventilated petri dish at each oviposition site. The remainder of the eggs were placed in 4.5 l plastic bags, including about 1.5 l of lake water. Bags were kept shaded in the lake for ca. 2 h, then put on snow in plastic coolers and flown to the helibase at Upper Beaver Meadows (ca. 20 min flight time). Due to heavy snow pack in 1995, the helicopter was unable to land at the collection site and bags with eggs were transported on foot for ca. 25 min before loading. In 1996, we loaded eggs directly from the collection site onto the helicopter.

In 1995, 9 of the 11 egg masses were separated into 2, approximately equal, portions; one half of each portion was distributed in water at Horseshoe Park and monitored once per week. The other portions plus 2 whole clutches were transported by vehicle to the Colorado Division of Wildlife (CDOW) Research Hatchery for captive rearing (Scherff-Norris, 1997).

In 1996, all 6 egg masses were transported, from the helibase, by vehicle to Glacier Basin where they were placed in water. Eggs and subsequent tadpoles were monitored twice weekly; metamorphs were monitored weekly. Upon metamorphosis, 2 person-hours, on 4 separate occasions, were spent locating and capturing metamorphs. Captured metamorphs were given a cohort mark by clipping a single toe and released.

A small number of eggs were separated from each egg mass and placed in petri dishes (1995, Horseshoe Park, n = 10 to 40, mean = 26) or screened boxes (1996, Glacier Basin, n = 20 to 40, mean = 29) next to the respective egg mass to compare hatching success between donor site and release sites. Eggs were protected from predation by dishes or boxes. The proportion of eggs hatching (arcsin transformed) was compared between sites in each year using t-tests with pooled variance due to small sample size. Data loggers to record water temperature were placed ca. 4 cm under water at all sites. Temperatures were recorded every 1.2 h.

Eggs delivered to the CDOW Research Hatchery in July 1995 were reared in modified indoor trout raceways until metamorphosis. Metamorphs (ca. 0.71 g and 15 mm SVL—snout–vent length) were moved to large aquaria or to modified outdoor raceways.

On 25 September 1995, 800 captive reared metamorphs were given a unique cohort mark by clipping a single toe and released at Glacier Basin. Before release, we measured SVL to the nearest 0.1 mm and determined mass to the nearest 0.01 g for a sample of 51 metamorphs. The site was monitored for 1 week after the release. An early snowstorm a week later prevented further monitoring until spring. Approximately 1,000 captive reared metamorphs from the same cohort were housed over winter in aquaria at the CDOW Research Hatchery until July 1996. These animals were kept at constant temperatures (above 20°C) that did not induce hibernation (Scherff-Norris, 1997); therefore after approximately 1 year of captivity, they attained sizes typical of two-year-old adult toads (Table 1). Glacier Basin was searched for metamorphs twice per month in May and June 1996. After June, the site was searched as for the 1996 egg release.

On 19 July 1996, each surviving captive at the research hatchery was uniquely marked by dorsal subcutaneous injection with a passive integrated transponder (PIT) tag. We measured SVL to the nearest 0.1 mm and determined mass to the nearest 0.01 g for each toad, then released them at Glacier Basin (32 males, 32 females, 36 smaller toads of unknown sex). Toads were monitored every other day from 19 July through 13 September, then once weekly through the first week of November. Four toads (3 released males and 1 unmarked female) were fitted with radio transmitters (Holohil model BD-2G) on 19 August, 21 August, 11 September, and 3 October, respectively. Radios, including a 12 cm whip antenna, weighed <2 g and were placed only on toads weighing ≥34 g. Radios were fitted around the toad's waist using small surgical tubing, #9 fly line pins, and Superglue (Bartelt and Peterson, 2000). At least 4 person-hours were spent each visit searching for PIT-tagged toads and locat-

TABLE 1—Characterization of mass and snout–vent length (SVL) of 1 year-old, adult-sized toads released in 1996 at Glacier Basin. Sex determined by size, vocalization, and presence of cornified nuptial pad on thumb. Animals that could not be clearly identified were labeled as unknown.

	Mass				SVL			
Sex (n)	Mean	*SE*	Min	Max	Mean	*SE*	Min	Max
Female (32)	20.91	0.97	12.5	35.1	50.96	0.79	41.9	60.3
Male (32)	30.43	1.00	16.5	40.6	57.50	0.74	46.4	65.0
Juvenile (14)	13.28	0.95	6.3	19.3	44.12	1.40	33.7	51.0
Unknown (22)	19.36	1.78	10.5	52.1	48.50	0.78	39.4	55.7

ing the radio-tagged individuals using a Telonics receiver and a hand held, two element Yagi antenna.

The Glacier Basin site was monitored for 2 to 10 person-hours per visit, 3 days per week from snowmelt (mid-April) through July, and once per week from August through September 1997. In 1998, the site was monitored 2 to 3 days per week from snow melt (mid April) through June, and in 1999, the site was monitored 2 days per week from snowmelt (mid May) through June.

Water temperature at recipient sites in June was 11.56 ± 0.53°C (*SE*), $n = 95$ at Horseshoe Park in 1995; and 25°C, $n = 1$, at Glacier Basin in 1996. Water temperature at the donor site in June was 7°C $n = 1$, in 1995; and 13.5 ± 0.02°C (*SE*), $n > 1{,}200$, in 1996. Water at recipient sites was warmer than at donor sites in all years. There were no differences in hatching success between Horseshoe Park (38%) and Lost Lake (69%) in 1995 ($P = 0.30$). However, only 12 tadpoles were observed at Horseshoe Park 1 week after hatching and none were observed 2 weeks after hatching. No tadpoles, metamorphs, or adults were found in subsequent monitoring through August 1995.

TABLE 2—Number of newly marked metamorphs (hatched from translocated eggs), number of metamorphs recaptured, and number of carcasses found during searches (carcasses were either too decomposed or desiccated to determine if they were marked).

Date (1996)	Newly marked	Recaptured[a]	Carcasses
16 August	100	—	—
21 August	100	13	14
26 August	100	12	—
4 September	33	13	30

[a] Animals had healed toeclips which were distinguishable.

Hatching success did not differ between Glacier Basin (72%) and Lost Lake (64%) in 1996 ($P = 0.59$). Fourteen days after hatching, all screened boxes that had >10% success contained growing tadpoles. Metamorphs (n = 333) were captured and marked at metamorphosis, 100 on each of 3 occasions and 33 on 1 occasion (Table 2). A number of dead metamorphs were discovered during these searches but the cause of death was unknown. Metamorphs reared at the hatchery and released in September 1995 measured 13.0 ± 0.2 mm, mean SVL, and had a mean mass of 0.24 ± 0.01 g. Eighteen of the 800 metamorphs released were found on the single visit to Glacier Basin 1 week later. No marked metamorphs were found during the intensive monitoring May to October 1996, 1997, April to July 1998, and May to June 1999, although unmarked metamorphs were found in 1996 and 1997.

Metamorphs held over winter at the research hatchery suffered approximately 90% mortality prior to release in 1996. Adult-sized toads (n = 100) released at Glacier Basin had a mean mass of 22.55 ± 0.84 g and a mean SVL of 51.6 ± 0.6 mm (Table 2). On the first 13 visits (20 July to 16 August), we found 1 to 56 PIT-tagged toads per visit (mean = 12.9). On the final 17 visits (19 August to 16 October), we found 0 (1 occasion) to 8 PIT-tagged toads per visit (mean = 3.9). Fifty-six of the original 100 toads were captured at least once. We found 38 individuals 2 to 4 times; 15 individuals 5 to 9 times; 3 individuals 10 to 15 times, and 1 unmarked adult female 7 times for 223 total sightings. Recaptured animals appeared healthy and had gained mass.

Released adult-sized toads moved an average distance of 166.5 ± 21.6 m, n = 56, range 10–1,000 m) from the release site on 19 July to other locations by mid September 1996. They were more difficult to find on cool, rainy days, were found mainly in or near the creek, and tended to move downstream (north) from the release site. Only 2 toads were found upstream of the release site.

Two toads were found dead. The first radio-tagged male was found dead on 11 September. It was observed soon after its death on the creek bank just out of the water with a wound on its left side. The exact cause of death was equivocal, but did not appear to be due to chafing or distress from the radio that had been on since 21 August. The second toad was found on 4 September. It was floating in an eddy of the creek and the cause of death was not determined.

The toad radio-tagged on 19 August lost its tag within 3 weeks. This radio was placed subsequently on another male on 11 September. The female tagged on 3 October was not PIT-tagged and was assumed to be a resident toad. Toads tagged on 11 September and 3 October were followed into hibernation in ground squirrel burrows ca. 20 m and 2 m from the creek, respectively. In November, we constructed pens around the putative hibernacula using metal flashing (30 cm high) and wooden stakes. These toads were monitored once monthly through the winter of 1996–1997 until batteries on the radios died in March 1997. These 2 toads came out of hibernation and escaped from the pens before we could locate them.

An unmarked, adult female toad was captured on 7 occasions after the initial translocations in 1996. An adult toad was sighted on 3 separate occasions in 1997. On one occasion, the toad was a female and unmarked (81 g, 86 mm SVL); on the other occasions the toad eluded capture. One unmarked adult toad (female, >100 g, 89 mm SVL) was encountered twice in 1998, and 3 unmarked toads (1 adult female >100 g and two unsexed individuals at 53 g, 76 mm SVL and 40 g, 69 mm SVL) were encountered in 1999. Based on size, we suspect that the large female encountered in 1997, 1998, and 1999, was the same toad. These unmarked toads were probably residents that were missed in earlier searches.

Transportation of egg masses in 1995 cost ca. $700.00 and 60 person-hours of time. This repatriation effort apparently failed. The 1996 transportation of egg masses cost $700.00 and yielded thousands of metamorphs at the repatriation site. One hundred and seventy-six person-hours of time were spent in 1996, 188 person-hours in 1997, 40 person-hours in 1998, and 48 person-hours in 1999.

Rearing and repatriating metamorphs from eggs brought into captivity cost $2,000.00 and 250 person-hours. Rearing and repatriation of adult-sized toads cost $12,000.00 and 1,550 person-hours. Each of these methods had the additional cost of the person-hours listed above for time spent monitoring from 1996 through 1999. Repatriation, reintroduction, or translocation (RRT; Dodd and Seigel, 1991) of amphibians usually involves 1 of 2 methods: transplanting eggs, larvae, or adults from one location to another, or release of offspring from captive breeding. Examples of successful translocations include natterjack toad (*B. calamita*), the common toad (*B. bufo*), and common frog (*Rana temporaria*) in England (Cooke and Oldham, 1995), Hamilton's frog (*Leiopelma hamiltoni*) in New Zealand (Brown, 1994), wood frog (*R. sylvatica*) in Missouri (Sexton and Phillips, 1986) and Illinois (Thurow, 1994), and two-lined salamanders (*Eurycea cirrigera*) in Illinois (Thurow, 1997). Captive breeding is the most common technique used for endangered species, including the Wyoming toad (*B. hemiophrys*; Jennings and Anderson, 1997), Houston toad (*B. houstonensis*; Quinn and Mengden, 1984), Puerto Rican crested toad (*Peltophryne lemur*; Bloxam and Tonge, 1995), and Majorcan midwife toad (*Alytes muletensis*; Bloxam and Tonge, 1995).

Successful repatriation can be defined as the establishment of one or more breeding pairs of animals that produce viable offspring such that a stable population exists at the site. By these criteria, our experiments failed. No tadpoles survived from egg masses translocated to Horseshoe Park in 1995. Except for 2 toads known to be alive in March 1997, none of the adult-sized toads released in Glacier Basin in 1996 or the captive-reared metamorphs released at Glacier Basin in 1995, have been observed since the year of release. Metamorphs resulting from egg masses moved to Glacier Basin in 1996 were observed and several hundred of them were marked in mid-summer 1996, but none have been seen since. It is possible that the released adult-sized toads or the metamorphs from the translocated egg masses have survived undetected and may yet return to breed at Glacier Basin. Natterjack toads (*B. calamita*), marked as metamorphs, were not recaptured as breeding adults until 2 to 3 years later (Sinsch, 1997). Boreal toads require at least 2 to 4 years to reach sexual maturity (Carey et al., in press) but we consider the probability that sufficient numbers of toads have survived and will return to establish a breeding population to be extremely small. In contrast to repatriation, the successful movement (e.g., translocation) of eggs, metamorphs, or adults, can be viewed in the shorter term. In our case, this experiment succeeded in indicating which was the most cost effective life stage of boreal toads to move.

Some reasons for the lack of long term success of this experiment are apparent. Record snowfall occurred in May 1995, delaying breeding by boreal toads at Lost Lake by 3 to 4 weeks. When eggs were finally translocated to Horseshoe Park, they encountered much warmer temperatures than they would have experienced at Lost Lake. The late start meant that metamorphs reared in captivity were not ready for release until late September. Snowfall and low temperatures a week after their release probably doomed these toads. Disappearance of the larger toads released in 1996 is more puzzling. We cannot determine whether these toads died or migrated out of the repatriation area.

Corvids (Corn, 1993; Beiswenger, 1981) and wandering garter snakes (*Thamnophis elegans*; Livo, 1998) are known to prey on boreal toads. We saw more garter snakes at Glacier Basin than at other sites. This site also has a noticeably higher density of corvids because it is located within 200 m of a campground. Although we observed no predation at any sites during this experiment, it should also be considered as a possible factor in our results.

Less than 20% of RRTs involving amphibians and reptiles have been successful (Dodd and Seigel, 1991; Beck et al., 1994); this is about half the rate of equivalent projects involving mammals and birds (Griffith et al., 1989). Dodd and Seigel (1991) and Short et al. (1992) identified lack of clearly defined methods and provisions for long-term monitoring as important factors in failure of RRTs. Our repatriation efforts lacked repeated movements of egg masses for multiple years and used relatively small numbers of adults and metamorphic toads. These factors severely reduced our chances of success. In contrast, successful repatriation of common toads in England required transplanting thousands of adult toads and egg masses over 6 years (Cooke and Oldham, 1995), and Denton et al. (1997) determined that translocation of eggs to the same site, every year for at least 5 years, is required to attempt establishment of a breeding population for the natterjack toad. Large numbers of animals and egg masses are not always required to establish a new population, however. Thurow (1994) translocated 1,000 to 12,000 wood frog eggs during 6 years to establish a new breeding population in western Illinois, outside the known range of this frog. Release of a few thousand captive-reared Wyoming toad tadpoles each year since 1994 has resulted in survival and breeding by toads at one site in Wyoming in 1998 (M. Jennings, pers. comm.).

Moving egg masses appears to have the highest probability of success and to be the most cost-effective method of repatriation for the boreal toad. Many more egg masses could have been moved and monitored without affecting the cost or the effort. In contrast, cost of captive rearing is excessive compared to probability of success.

Boreal toad eggs are able to withstand the trauma involved in collection and air and vehicle transport with minimal or no decrease in hatching success. All but 1 of the 17 clutches moved in 1995 and 1996 produced hatchlings. Although hatching success varied tremendously in transported egg masses (8 to 100%) this range in success rate is mirrored by field observations of control clutches (0 to 100%) and in boreal toad reproduction in general (P. S. Corn, pers. obser.). More than half of the adult-sized toads released were captured at least once and had maintained or increased their mass at release suggesting that adults can survive the transportation process and remain healthy for several months afterwards.

The problem for repatriation efforts with boreal toads is the lack of source material; no existing population in the southern Rocky Mountains can sustain the continued harvest of >10 egg masses each year for several years. Because moving egg masses deposited in the wild to sites within the historical range of the animal may not be an option in repatriating the boreal toad, we suggest that a captive rearing strategy for repatriation should focus on introduction and monitoring of large numbers of early life stages (eggs and larvae) to an historic site for at least 5 years to have a chance of success.

Resumen—Experimentalmente transportamos sapos boreals (ovulos, juveniles, y adultos) de una populacion estable y natural, y de colonias endonde fueron criados, a localizaciones historicas en RMNP, y por cinco anos advertimos la accion de empollar y dispersion. Nuestro objetivo era determinar metodos efectivos para translocaciones de los sapos boreals que tuvieran exito. Aunque esta translocacicion no se puede considerar exito, el experimento indica que translocalizando las masas de ovulos parece ser el mas efectivo metodo de nueva introduccion; exito de incubacion era mas de o iqual al exito de incubacion en el sitio de donado.

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