INTERAGENCY CONSERVATION STRATEGY FOR MOUNTAIN YELLOW-LEGGED FROGS IN THE SIERRA NEVADA

Attachment 5: Captive Rearing and Propagation and the Interagency Process for Permitting Salvage and/or Translocation

Laura Patterson, Cathy Brown, and Steven Detwiler
California Department of Fish and Wildlife,
U.S. Forest Service,
U.S. Fish and Wildlife Service

Version 1.0

The Role of Translocation, Salvage, and Captive Husbandry in the conservation of Mountain Yellow-legged Frogs

The Conservation Strategy recognizes the significant potential conservation benefits available via the salvage of populations at high risk of extirpation due to drought, disease, or other catastrophic events. Translocation of at risk individuals into other available (stable) habitat, when the loss of such populations is deemed highly probable by field monitoring and expert consensus, is likely to be a net benefit overall to metapopulation resilience within each FCA.

In times of severe drought stress, it is possible that frog populations are lost when their aquatic habitat will completely dry out. More monitoring and study should be done to address the degree of this extant threat, especially in more ephemeral stream and meadow populations. In some circumstances, such as the recent Tyndall and Sixty Lake Basin tadpole/metamorph collections from Sequoia/Kings Canyon National Park Bd-positive populations for transport to the San Francisco and Oakland Zoos, population demographics and chytrid may indicate bringing affected late-stage tadpoles and metamorphs into captivity is the prudent action. At the zoos, these individuals, which would otherwise suffer very high field mortality rates, were cleared of the chytrid fungus using itraconazole, and quickly metamorphosed. Rearing these juveniles for release back into their native ponds is expected to yield a large number of individuals that otherwise would have been lost to disease at the most vulnerable life stage. The survival rates provided in captive settings far exceed those observed in their natural habitat; hence this management tool is a powerful conservation measure when available. The expectation is that repeated "challenge," the successive infection and then clearing of Bd from the frogs, will both render them Bd-tolerant, as well as carry them through the most critical (chytrid-sensitive) life stage.

In some cases, for example, when surveys indicate a surviving population is at extremely low abundance with possible increasing extant threats to the native habitat, translocations of adults may be warranted. In the absence of immediate threats, circumstances such as repeated surveys that indicate there are no breeding age individuals of the opposite sex in a repeatedly occupied water body (including no eggs/tadpoles observed for multiple years) would indicate a need for adult translocations. Individuals of the absent sex, if available, might be introduced to augment the population. It is also possible that adults from highly endangered clades might be brought into captivity for captive breeding; however this scenario is likely a last resort consideration. All such actions will be coordinated within an interagency technical team (the Conservation Strategy Translocation Subteam). Technical guidance for translocation is provided in Attachment A.

Captive Husbandry, Breeding and Rearing

Research on captive husbandry, breeding, and rearing of mountain yellow-legged frogs was initiated in response to the continued decline and extirpations of the few small and isolated populations of the Southern DPS of *R. muscosa*. In most cases, source frogs were opportunistically salvaged from natural catastrophes. In 2007, the USFWS officially approved an experimental captive breeding, reintroduction, and monitoring program (USFWS 2007). Thus far, that program has maintained captive populations at the Los Angeles Zoo (LA Zoo) and the San Diego Zoo's Institute for Conservation Research (SD Zoo ICR). This program has allowed for the breeding of individuals in captivity, with concurrent research on the biological requirements of propagating and rearing mountain yellow-legged frogs, and has also conducted the first reestablishment efforts at Indian Creek in Hall Canyon and Fuller Mill Creek in the San Jacinto Mountains (Backlin pers. comm., USFWS 2012). Additionally, the San Francisco Zoo (SF Zoo) began experimenting with captive breeding and rearing *R. sierrae* in 2013, and the northern California captive rearing effort was bolstered by inclusion of facilities at the Oakland Zoo a year later.

For a decade, researchers have been working to improve mountain yellow-legged frog captive breeding and rearing success. In 2003, the first frogs were emergency-salvaged from the San Bernardino Mountains after the Old Fire destroyed their habitat. They were taken to the SD Zoo ICR, but were infected with both Bd

and mycobacteriosis, and in spite of treatment with itraconazole, they eventually died from mycobacteriosis (USFWS 2012). In 2006, 84 first-year tadpoles were emergency-salvaged from drying habitats in the San Jacinto Mountains and taken to the SD Zoo ICR, and these animals became the first captive breeding colony (Hitchcock et al. 2007).

In addition to challenges from disease, water mold and suspected water quality issues have impacted *R. muscosa* egg and tadpole survival. A clutch laid in 2008 was overcome by *Saprolegnia*, and only three tadpoles survived to produce one frog (Backlin pers. comm., USFWS 2012). Experiments with incubation temperature were conducted to determine if it affected *Saprolegnia* growth, but results were inconclusive. In 2009, 106 first-year tadpoles were emergency-salvaged after the Station Fire in the San Gabriel Mountains and taken to the Fresno Chaffee Zoo (Backlin et al. 2009). Many survived to the juvenile stage, but they all eventually died in 2011. An increase in phosphate in the municipal water system was suggested as a possible cause, but this remains unsubstantiated since none of the other amphibians that were exposed showed any negative effects (USFWS 2012). In 2013, a 24-hour spike in ammonia occurred at the SF Zoo because the tadpole waste output was greater than the filters could handle, resulting in a die-off (Mutlow pers. comm.). Necropsy results of the deceased tadpoles identified ammonia toxicity accompanied by secondary bacterial and fungal infections (Bushell pers. comm.). Moribund tadpoles were treated with antibiotics and calcium supplementation to the water systems, but this treatment met with limited success.

The first successful captive breeding occurred in 2010 after experimentally placing some of the frogs into hibernation first. Some of those progeny were moved to the LA Zoo to ensure redundancy in the program in case of a catastrophic event at one of the facilities. In 2011, all frogs at SD Zoo ICR and LA Zoo were placed into hibernation, and reproductive output was substantially higher. In 2012, despite imposed the hibernation step, reproductive output was lower.

To try to improve fertility, hormone treatments were administered to captive frogs at the LA Zoo in 2012. This resulted in an increase in reproductive behaviors such as male advertisement calls and longer periods of amplexus; however, these did not result in greater reproductive output (Rechhio pers. comm.). In 2013, this experiment was repeated at the SD Zoo ICR (Santana pers. comm.). Males and females that did not lay eggs were injected with reproductive hormones, but this treatment had a very limited effect on amplexus and advertisement calling rates and did not result in the production of any eggs. It is important to note that hormones were utilized two months after the frogs' natural breeding season in order to give them the opportunity to breed naturally first. In 2014, SD Zoo ICR conducted an experiment using hormone treatments using two groups of eight pairs of frogs, hormone eligible and ineligible (Santana pers. comm.). Five of the eight hormone eligible pairs did not lay within a week and were administered hormones on the eight day. Four of the five pairs subsequently laid eggs. Seven of the eight hormone ineligible pairs did not lay eggs, so after two weeks females were administered hormones. Two of these females, whose mates had stopped amplexing, laid eggs seven days after the treatment, demonstrating this species is capable of spontaneous oviposition, which affords an opportunity for in vitro fertilization using spermic urine if necessary (Santana pers. comm.).

In 2015, another hormone experiment using Amphiplex was conducted at SD Zoo ICR, with additional complementary measures. Males came out of hibernation about a month before females and started showing reproductive behavior as the month progressed (Calatayud pers.comm.). Mating pairs were not assigned this time, and females were amplexed within 1 to 2 hours of being placed with males. Pairs were partitioned into separate enclosures once they'd picked a mate. As the atmospheric temperature rose, water temperature was kept low, which reduced the incidence of water molds. Fertilization rate was the highest recorded in this population so far, indicating there may be an advantage to allowing males to come out of hibernation before females and to allow mate selection; however, preliminary data suggest the treatments did not result in an increased number of eggs oviposited, spermiation or the number of males' amplexing (Calatayud pers. comm.). There are alternative hormone treatments that may prove more advantageous. Additionally, closer

analysis of individual female reproductive histories from the San Jacinto populations suggests this species may not experience annual breeding (Calatayud pers. comm.). Over the last 5 years, the percentage of females that have oviposited has decreased from 80% in 2011 to 28% in 2014. It is unclear if changes in reproduction are related to husbandry or a lack of information regarding the natural reproductive cycles of these animals.

Progeny from captive breeding efforts of the San Jacinto Mountains frogs have been used in several re-introduction attempts. Some tadpoles were re-introduced at Hall Canyon in 2011, using an experimental design that tested caged (soft) and non-caged (hard) releases to determine whether protecting tadpoles increased their survival rates. Two late-stage tadpoles were observed in 2013 that are suspected to be from this re-establishment effort. To test whether release of later life stages increased survival rates, all surviving tadpoles from the 2012 cohort were reared in captivity to juveniles. Some of these juveniles were released in 2013 using another hard versus soft release experiment. Preliminary results suggest no added benefit of caged releases, and in fact some problems were associated with flash flooding; thus, cages are no longer proposed (Santana pers. comm.). Additional releases of juveniles (2014) and tadpoles and juveniles (2015) of captive bred individuals have taken place in the San Jacinto Mountains.

In addition to the initial San Jacinto frogs, a second captive breeding colony was established as a last resort in response to a population that appeared on the brink of extirpation (Backlin pers. comm., USFWS 2012). In 2011 and 2012, eight adults and six juveniles were collected from the only known population in the San Bernardino Mountains and taken to the SD Zoo ICR. A subset of the juvenile frogs tested positive for Bd and were treated with itraconazole, but they were not successfully cleared after the first treatment. A second treatment was applied, but the frogs began to die soon after of unknown causes; however, water quality issues or exposure to a skin irritant is suspected (USFWS 2012). Two adult females and three adult males survived, and in 2013 and 2014, only one pair each year produced a small fertile clutch. The two wild-caught females died in 2015, and only seven offspring (four males, three females) from the two clutches remain (Gardner pers. comm.). Based on the low number of animals and limited reproductive success, the decision was made to collect any other adults from the area to augment the captive breeding population, and discussion has begun on whether this population requires rescue through interbreeding with San Jacinto Mountains frogs. In September 2015, the only known wild frog left from this population, an adult male originally captured in 2012, was taken into captivity (Gallegos pers. comm.).

A third captive colony was established at the L.A. Zoo in 2014. As wild population levels started rapidly declining in parts of the San Gabriel Mountains, 25 tadpoles each from Little Rock and Big Rock creeks, sites with relatively large healthy populations, were collected and transported to the L.A. Zoo to ensure some of the genetic diversity from this area was conserved. As of October 2015, there are 18 and 23 frogs, respectively, that survived through metamorphosis.

Prior to federal listing in 2014, SF Zoo began captive breeding and rearing experiments with Sierra Nevada populations. Using *R. sierrae* collected from Marmot Lake during a previous research study, SF Zoo hibernated and attempted to breed three females (Bushell pers. comm.). Fertility of eggs was low but not unexpected from an initial breeding attempt after years of immunization experimentation in captivity, and mortality was high in the tadpole stage. Some of the surviving tadpoles showed signs of scoliosis, so SF Zoo increased the water hardness and temporarily added Vitamin B, which has been successful with other amphibians to avoid such deformities. As of October 2015, four juveniles survive and are nearly adult size. Some of the adult frogs showed mild to moderate keratopathy, a common ailment of amphibians in captivity fed an all cricket diet (Mutlow pers. comm.). While not reversible, its progress has been slowed by feeding the frogs a more varied diet. In addition to the experiments with research frogs, a group of small *R. sierrae* egg masses, thought to be from several females, was opportunistically collected from El Dorado National Forest (ENF). This egg group had a much higher (89%) fertility rate; however, tadpole and metamorph survival was low, and only two survived.

In addition to captive breeding, SF Zoo also began captive rearing *R. sierrae* in 2013. Thirty-four juvenile frogs from a Bd-positive area in the ENF were brought in to treat for disease and to rear for release following year. All of them survived their itraconazole and Bd treatments, and 33 were released in June 2014 along with one egg group frog (the other egg group frog was too small and was retained till 2015. In 2014, approximately 200 eggs and 50 juvenile frogs were brought in from ENF and reared at the SF Zoo (Bushell pers. comm.). By April 2015, 135 frogs had metamorphosed from the egg masses, and 54 were large enough to undergo immunization treatment prior to release along with 49 juvenile frogs and the frog held over from 2013. Five frogs died during treatment from apparent complications from Bd, and 97 were released (2 were retained for health concerns). Also in 2014, 22 tadpoles from Big Pine Lakes were salvaged from a population crashing due to recent Bd-infection and taken to the Oakland Zoo. This group suffered a high proportion of mortality from unknown and seemingly unrelated reasons throughout year they've been in captivity, and as of October 2015, only four successfully metamorphosed and survive (Alm pers. comm.). As mentioned earlier, in late-August 2015, tadpoles and recent metamorphs from Sequoia/Kings Canyon National Park were also salvaged from two areas undergoing a Bd-outbreak and taken to SF and Oakland zoos for rearing and immunization treatment.

While a number of important successes have been attained and lessons learned over the past decade of captive rearing and breeding mountain yellow-legged frogs, further research is needed to increase fertility and survival in captivity and to improve efficiency and effectiveness of post-release monitoring. Experimental hormone treatments along with changes in hibernation regimes and possibly outbreeding are options for increasing reproductive success. Improved ability to determine causes of death in captive bred and reared frogs is vital, so adjustments can be made that increase survival. In terms of re-introduction strategies, releasing tadpoles is much cheaper because they do not need to be housed in captivity as long and with as much individual space; however, tadpoles are difficult to relocate in the field to determine survival success. Juvenile frogs can be identified by pattern, but this requires frequent recapture. Radio telemetry can also be undertaken to determine the short-term fate of the juvenile frogs, but this is both labor intensive and of somewhat high risk to the frogs. PIT tagging has been the standard marking technique to assess the survival of captive reared or bred frogs in the wild; however, that requires they be raised to nearly adult size before release. SD Zoo ICR is investigating the efficacy of using p-chips, which are only 500 x 500 microns and nominally 100 microns thick, in smaller animals (Calatayud pers. comm.). Future research will help evaluate the relative costs and benefits of releasing different life stages and using different post- release monitoring methods. Additional considerations involve a secure funding source for both breeding and post-release monitoring as well as timely regulatory and land management agency approval for both activities.

Currently, per our interagency coordination process, we have identified individuals from the following clades as highest priority populations for captive rearing/breeding efforts: Clade 1, northern Clade 2 (northern *Rana sierrae*), Clade 4 and Clade 5 (*R. muscosa*). Future efforts will go through environmental compliance and public review, as appropriate, and will be under the permitting authority of the California Department of Fish and Wildlife and U.S. Fish and Wildlife Service, as described herein.

State and Federal Permitting and Interagency Coordination

For purposes of interagency coordination, in addition to assisting field personnel with translocation and salvage decisions within the context of the range wide Strategy, the participating agencies will convene calls to discuss ongoing salvage situations. Such discussions, owing to the unpredictability of such events, will necessarily be impromptu, on an as-needed basis. USFWS will facilitate the calls. In general, annual priorities and coordination will happen during the fall annual Mountain Yellow- Legged Frog Conservation Strategy Team meetings. However, the possible engagement of limited captive rearing facilities will require real-time coordination with facility managers.

In the case of dire emergencies (i.e., field crews discover and anticipate individuals will perish within a couple days), local translocations may be conducted without notice, provided the translocation area is within the same clade as the salvage population, or in uninhabited areas of high habitat value pre-designated for emergency translocation by prior arrangement through the Strategy team. This emergency decision and the translocation itself must be made by a 10(a)1(A) and CESA MOU permitted biologist for the mountain yellow-legged frog, and both the State and Federal permitting biologists (in the Sacramento, or Reno offices) will be notified at the earliest convenience that such translocations have been conducted (preferably within a month, but no later than the end of the calendar year). Under no circumstances should individuals be brought into captivity without prior notice and arrangement with the receiving entity (a permitted zoo facility). Appropriate notice (to USFWS and CDFW) and tracking (State chain of custody forms) will be conducted.

An example copy of the State of California chain of custody form appears as Figure 1, below. The decision tree covering the procedure for convening Translocation Subteam calls and contacts with permitting agencies appears as Figure 2, below.

State of California - Natural Resources Agency
DEPARTMENT OF FISH AND WILDLIFE
Wildlife & Fisheries Division / Marine Region

EDMUND G. BROWN JR., Governor
CHARLTON H. BONHAM, Director

CHAIN OF CUSTODY

Fish & Game Code 1002, 1002.5, Title 14 § 650

Original Collector:

Name: _____
Address: _____
City/State/Zip: _____
Phone No.: _____
e-mail: _____

Authority of Original Collection (State and Federal Permits - if Applicable):

10(a)(1)(A) or 4(d): _____
CESA MOU: _____
Scientific Collecting Permit: _____
Biological Opinion: _____
Other: _____

Sample(s), Salvaged Animal(s), and/or Parts Thereof Information:

Line No.	ID(s)	Species	Collection: Date(s)	Collection: Location	Collection: Method	Sample Type	Preservation Type	No. of Samples

Transfer No. 1 -

Split Batch ☐ Yes ☐ No → If Yes, No. of Samples _____ ID(s) _____
Split Sample ☐ Yes ☐ No → If Yes, No. of Samples _____ ID(s) _____

Reliquished By Original Collector (Signature)	Print Name	Affiliation	Date
Recieved By (Signature)	Print Name	Affiliation	Date

Disposition of Samples:

Page ____ of ____

Figure 1. Example State of California Department of Fish and Wildlife Chain of Custody Forms for Mountain Yellow-legged Frog Lifestages.

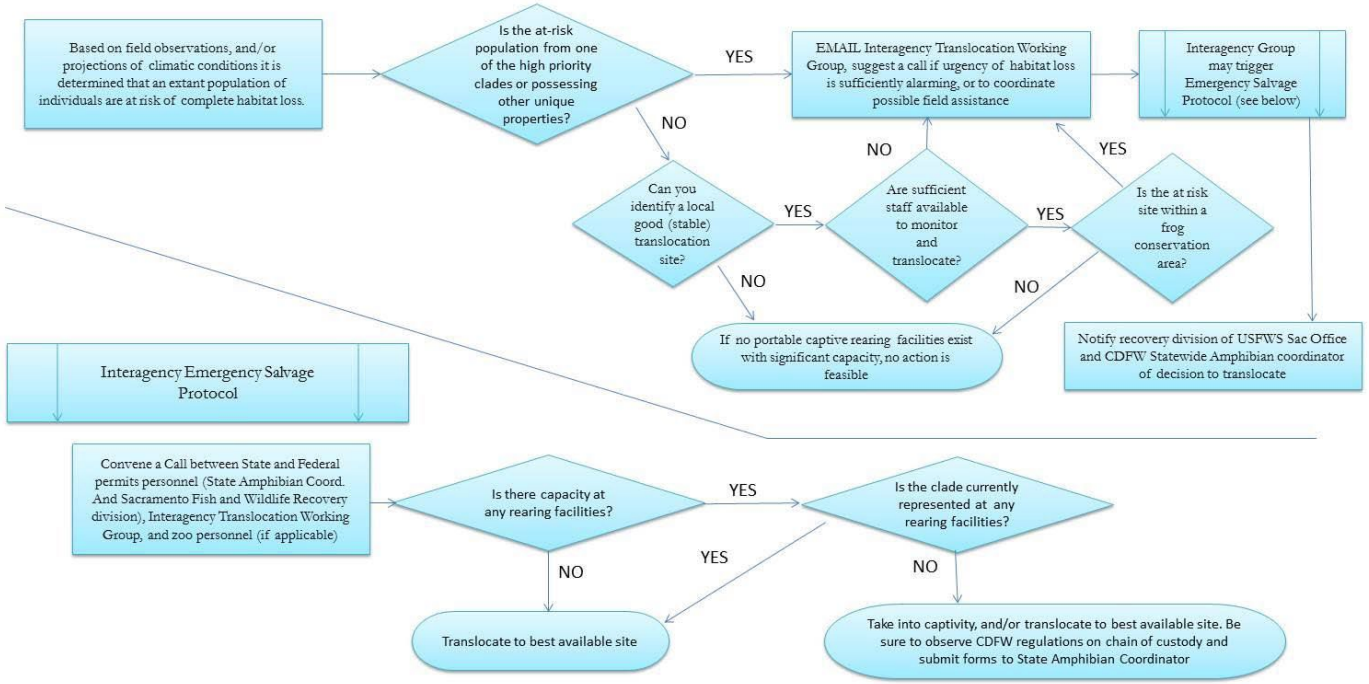

Figure 2. Process Diagram for Interagency Coordination on Salvage Decisions.

Literature Cited

Backlin, A.R., E. Gallegos, S, Shuster, and R. N. Fisher. 2009. Emergency salvage of mountain yellow-legged frog (*Rana muscosa*) tadpoles from Devil's Canyon, Los Angeles County, California: 7 Day Recovery Permit Action Report. Prepared for the United States Department of the Interior Fish and Wildlife Service and California Department of Fish and Game. U.S. Geological Survey, Western Ecological Research Center. San Diego, CA. 15 pp.

Hitchcock, C. J., A. R. Backlin, and R. N. Fisher. 2007. Using experimental translocation as a last resort for the recovery of the mountain yellow-legged frog (*Rana muscosa*) in southern California. U.S. Geological Survey, Western Ecological Research Center. San Diego, CA. 12 pp.

USFWS. 2007. Memorandum: Captive Breeding and Reintroduction/Population Augmentation Program for the Federally Endangered Mountain Yellow-legged Frog in Southern California. FWS-CWFO-4995.2. 4 pp.

USFWS. 2012. Mountain yellow-legged frog (*Rana muscosa*) Southern California Distinct Population Segment 5-yr Status Review: Summary and Evaluation. Carlsbad Fish and Wildlife Office. Carlsbad, CA. 78 pp.