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

Attachment 3: Protocol for Translocation and Reintroduction of *Rana muscosa* and *Rana sierrae*

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Introduction

Endangered species conservation often requires that animals be moved from one site to another, usually in an effort to establish new populations. This includes "translocation" of animals from a natural population (i.e., donor population) to a recipient site, transfer of animals from the wild to a captive rearing facility, and "reintroduction" of captive-reared animals back into the wild. This protocol describes methods for moving mountain yellow-legged frogs (Rana muscosa and Rana sierrae) as part of translocations or reintroductions. In addition to describing these methods, I address several related considerations when planning translocations or reintroductions.

What life stage to translocate or reintroduce?

Numerous factors could potentially influence decisions regarding what mountain yellow-legged frog life stage to move. However, two primary considerations are (1) their availability in the donor populations, and (2) their susceptibility to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; hereafter "Bd"). Adults are generally the best life stage to translocate or reintroduce. Under natural conditions, adults have the highest survival rates of any of the life stages, with 50-90% annual survival being typical (Briggs et al. 2010, Knapp et al. 2011). Survival rates of eggs, tadpoles, and juveniles are typically much lower, often <1%. In addition, when moved to a new site adults have the potential to breed the following year. As such, the population could quickly begin growing. In contrast, given that tadpoles take at least two summers to metamorphose and juveniles require 2-4 years to reach sexual maturity, translocating eggs, tadpoles, or juveniles could result in a 2-6 year delay before any reproduction is possible.

Adults are also the life stage with the lowest potential susceptibility to Bd. Recent laboratory experiments indicate that Bd infection of Bd-naïve adult mountain yellow-legged frogs results in the development of a strong acquired immune response against Bd in the exposed animals (M. Toothman, unpublished data). This immune response is likely the cause of the much lower susceptibility of frogs from "persistent" populations (populations that are persisting despite ongoing chytridiomycosis) than Bd-naïve frogs (C. Briggs et al., unpublished data). Juvenile and tadpole stages appear unable to mount an acquired immune response against Bd and are therefore highly susceptible to chytridiomycosis (the disease caused by Bd).

Despite the advantages to moving adults, in some cases it may be preferable to move other life stages. For example, collecting any adults from small populations should generally be avoided. In these cases, it may be possible to collect small numbers of egg masses, tadpoles, or juveniles, because these life stages typically experience very high natural mortality. Therefore, collection of small numbers will have minimal impact on the donor population. The availability of these life stages differs markedly between sites, and planning collections therefore requires population-specific knowledge. For example, at many sites egg masses can be difficult to find. A site I worked at for over a decade contained >3000 adult *R. sierrae* and yet I never saw a single egg mass during surveys conducted in the breeding period. Based on the behavior of amplexing adults I suspect that females were laying egg masses in submerged rock piles along the lake shore. At other sites, especially those where the shoreline is dominated by mud and logs instead of broken rock, egg masses may be much easier to find. The availability of tadpoles is also highly variable between sites and even seasonally within a single site. In high elevation sites tadpoles typically congregate during mid-day in shallow areas where the water is much warmer than in the rest of the water body. In contrast, in low elevation habitats tadpoles often do not aggregate in shallow water at all, perhaps because the entire water body is relatively warm. In the latter situation, tadpoles may be observed only rarely.

Metamorphs are found most commonly in August and September, but in Bd-positive habitats a high proportion of metamorphs succumb to chytridiomycosis very quickly. As a result, metamorphs seen during any one survey are often those that metamorphosed in the previous few days. Therefore, collecting sufficient numbers of metamorphs will often require multiple collection trips within the time window when tadpoles are metamorphosing.

Given the high Bd-caused mortality of juveniles, translocating or reintroducing tadpoles or juveniles into Bd-positive sites may be insufficient to establish new frog populations. In these situations, the probability of project success may be increased by rearing eggs, tadpoles, or juveniles to adulthood in captivity. Captive-reared adults could then be immunized against Bd prior to being reintroduced into Bd-positive sites in the wild.

How many animals to translocate or reintroduce?

The success of translocations and reintroductions in allowing the establishment of new populations is strongly influenced by the number of animals that are moved. Although increasing the number of animals moved will increase the probability population establishment, this needs to be weighed against impacts of these collections to the donor populations. Given the importance of every remaining population for the long-term viability of *R. muscosa* and *R. sierrae*, it is essential that any collections do not compromise the long-term persistence of the donor populations. When the donor populations are relatively small (<100 adults), ensuring that this is the case will often require detailed monitoring of the donor populations using capture-mark-recapture methods to quantify population size and individual survival through time.

Specifying a specific percentage of a donor population that can be collected is difficult and depends on a number of site-specific considerations, including the current growth rate of the donor population, density of frogs at the donor population, and geographic and/or genetic uniqueness of the donor population. A useful general guideline is that no more than 10% of each life stage be collected from a donor population per year. Given the high reproductive potential of mountain yellow-legged frogs (e.g., a single moderate-sized female can lay 100-200 eggs per year), this 10% rule may be overly conservative. Nonetheless this 10% rule is likely a reasonable starting point when planning collections, especially when collections are anticipated to be made from a donor population during several consecutive years. In large populations in which crowding is reducing frog condition, fecundity, and survival, removal of a larger percentage of frogs is likely possible because such removals could actually benefit the donor population (R. Knapp, unpublished data).

The survival of translocated or reintroduced animals is likely to vary widely between years due to site-specific conditions. Results from previous translocations suggest that favorable conditions for recruitment may occur only rarely. Therefore, to maximize the chances of project success it is advisable to move animals into recipient sites during several consecutive years (one of which may be a year favorable for recruitment), even if this means moving fewer animals per year.

Disease considerations

When moving animals between sites it is important to consider the pathogen status of both the donor and recipient sites. Bd is virtually ubiquitous across the ranges of *R. muscosa* and *R. sierrae* but the presence of multiple strains (Morgan et al. 2007) that likely vary in their pathogenicity (e.g., Berger et al. 2005) suggests caution, particularly when moving animals long distances. Another pathogen of concern is ranavirus. Ranavirus has been detected in mountain yellow-legged frogs and outbreaks can result in very high mortality of tadpoles (R. Knapp, unpublished data). However, the distribution of ranavirus in mountain yellow-legged frog populations remains poorly understood.

Available data suggest the distribution of ranavirus is much more patchy than Bd, arguing for a careful approach when moving animals to ensure that ranavirus is not introduced into areas where it currently does not exist. Concerns regarding pathogens are heightened when reintroducing captive- reared animals into the wild because animals could have acquired novel pathogens whose transfer into the wild must be prevented.

Because of the near ubiquity of Bd, it can generally be assumed that recipient sites are Bd-positive. Adult frogs from Bd-naïve populations are highly susceptible to Bd infection and their survival when exposed to Bd is therefore very low. Adults that were previously exposed to Bd have reduced susceptibility at least in part due to the activation of an effective acquired immune response against Bd (M. Toothman, unpublished data). Such animals are likely to have much higher survival in Bd- positive habitats, and should generally be the focus of translocation efforts. Captive-reared animals will almost always be Bd-naïve and therefore highly susceptible. To improve the survival of these captive animals when released into the wild, these animals will generally need to be exposed to Bd to activate the immune response (and then be cleared of infection) before being reintroduced into the wild.

Translocation/reintroduction methods

Temperature management

The most important consideration when moving mountain yellow-legged frogs is ensuring that animals are kept cold throughout the transport process. This ensures that oxygen demand and production of waste is minimized during transport. Temperatures that exceed the tolerances of the transported animals, even for just a few seconds, can be lethal. For mountain yellow-legged frogs, the lethal upper temperature for adults is only ~30° C (85° F; Bradford 1984). In addition to direct negative effects of temperature, indirect effects can result from increases in metabolic rates with increasing temperature. Increased metabolism causes high oxygen requirements and increased waste excretion rates (especially ammonia by tadpoles), both of which can result in the rapid mortality of animals being transported. At the low end of the temperature scale, temperatures as low as 1° C (34° F) have no negative effects on frogs. However, frogs will die when exposed to temperatures below freezing. Given the paramount importance of keeping animals cold throughout the movement period, it is typically necessary to surround containers holding frogs with snow or ice packs. If snow or ice packs are not available, the only safe alternative is to transport animals when air temperatures are cold, for example at night.

Animals should be transported as soon after collection from the donor population as possible. When immediate transportation is not possible, tadpoles and frogs can be held overnight in large mesh pens anchored in shallow waters of the donor habitat (Figure 1). Use of such pens ensures that captive frogs will not be exposed to excessively high or low temperatures and will be held in small containers for as short a period as possible. To ensure that frogs cannot escape or hide in the substrate and to prevent predation (e.g., by garter snakes), pens should have mesh floors and lids (in addition to walls) and have a mesh size of no larger than ~0.6 cm (0.25"). While in pens, adults and juveniles should be provided with appropriate habitats on which to bask, such as partially submerged rocks. Be careful not to rip holes in the mesh when placing rocks in the pen; it is often safer to drape the bottom of the pen over suitable basking rocks than to place rocks inside the pen.

To avoid undue stress on the animals being moved the duration of movement periods should be kept as short as possible. That said, I have successfully transported adult frogs on foot (in a backpack, surrounded by snow) for more than four hours and in a vehicle for more than six hours without any obvious negative effects on the animals. As long as the animals are kept cold they could presumably be subjected to longer transport times, but additional measures will need to be taken to minimize stress.

Adults and juveniles:

To ensure adequate ventilation for each animal being transported, it is safest to place animals singly into transport containers. Containers must have holes to ensure adequate ventilation. When moving metamorphs or juveniles, placing two animals per container is acceptable but adults should always be held one per container. I typically hold frogs in small Ziploc™ plastic containers, each with several small holes drilled into the lids (Figure 2; drilling from the inside to the outside of the lid will reduce the likelihood of sharp edges on the inside of the lid, where they could harm the animals). To keep animals moist and hydrated during transport, place either a wet paper towel or ~5 mm (0.2") of water into the bottom of each container (avoid use of chlorinated water). While placing animals into containers, always keep the containers in the shade. Regardless of the number of ventilation holes, when in direct sun temperatures inside these containers will increase rapidly to levels exceeding the lethal upper limits of mountain yellow-legged frogs.

When transporting animals on foot, frogs in Ziploc™ containers are typically placed into a backpack. In this situation, it is advisable to package the Ziploc™ containers into crush-proof ventilated tubs. This is important both to ensure adequate ventilation (by keeping containers upright and away from items such as clothing that could restrict airflow) and also to ensure that the lids of containers do no pop off due to being partially crushed in a backpack. My preferred tub is a bear canister. Those made by BearVault™ (model BV500) are better than others due to their relatively large size (11.5 L), wide opening, and translucent plastic walls that allow frogs to be observed without removing them from the bear canister. Frog-containing Ziploc™ containers can be stacked vertically in the canister, with up to 18 per canister. The canister should be placed upright into the backpack and close to the opening of the pack. The lid of the bear canister should be left off or placed onto the canister but not screwed into place, and the top lid of the pack (if such a lid exists) should be left open. This will ensure adequate ventilation for the animals in the bear canister.

However, while in backpacks it is important to avoid direct exposure of Ziploc™ containers to the sun. This can often be best accomplished by placing a piece of closed cell foam (e.g., a sleeping pad) or other insulating material loosely over the top of the canister. An article of clothing (e.g. down jacket) should generally be avoided because it could cut off the flow of air into and out of the canister. After placing the canister into a backpack, the canister should be surrounded with snow or ice packs. It is best to put snow into a large dry bag (20 L; used to keep gear dry during river rafting). Fill the dry bag with about 20 cm of snow, flatten it, and wrap the flattened dry bag/snow partially around the bear canister. Place the dry bag in such a way as to keep the heat of the sun off of the frogs. Snow can be placed on top of the bear canister (instead of insulating foam) but must be held in a watertight container such as a dry bag. Without a watertight container, some of the water dripping out of the melting snow could flood the Ziploc™ containers. When the recipient site is reached, the animals can be released without first acclimating them to the water temperature of the site.

Transporting frogs via helicopter instead of on foot may be advisable when transport times exceed four hours and/or when terrain is such that foot transport would result in continuous jostling of animals for several hours. For helicopter transport, Ziploc™ containers with frogs can be placed into cardboard boxes for transport. Prior to the helicopter flight, keep the frog-holding containers in the shade and surrounded with snow-filled dry bags. The pressure changes that accompany helicopter flights will have no negative effect on the frogs. Unlike the human ear that contains air- filled compartments, the structures that make up the amphibian ear are filled with water. As a result, frogs tolerate helicopter flights without any ill effects. During past helicopter-supported translocations, frogs have been subjected to altitude changes exceeding 2000 m (6000') and experienced no mortality.

Tadpoles

When transporting tadpoles it is critical that they be kept cold. This will reduce both tadpole oxygen demand and waste excretion rates. Reducing excretion rates is particularly important because elevated ammonia concentrations can negatively affect tadpole health and survival. As such, tadpoles are best transported in a cooler. I use a 3-gallon Gatorade cooler that because of its cylindrical shape fits well into a backpack. Fill the cooler with cold lake water and if water is well above 0° C (32° F) cool water further by adding snow or ice (avoid commercially-sold ice – it is generally made with chlorinated water that could harm tadpoles). Leave ~5 cm (2") of airspace at the top of the cooler. After removing any remaining chunks of snow or ice from the water, add up to 100 tadpoles to the cooler just prior to beginning the transport and screw the cooler lid on snugly. To ensure that water remains oxygenated during the transport, use a battery-powered aerator. I thread the aerator hose through the spout at the top of the cooler (remove air stone, thread the hose, then re-attach the stone inside the cooler). Carry an extra set of batteries in the event that the batteries powering the aerator are exhausted during the transport. When the recipient site is reached, tadpoles should be released immediately. Acclimating the tadpoles to the water temperatures of the recipient site is not necessary. I have successfully transported tadpoles for up to six hours. Longer transport times may be feasible, especially if at least some of the water can be exchanged with fresh water during the transport.

Egg masses

As is the case with all other life stages, egg masses must be kept cold during transport. I recommend transporting egg masses in 1-L wide-mouth Nalgene™ bottles. Fill the bottles with cold water and if water is well above 0° C (32 °F) cool the water further using snow or ice (avoid using commercially-sold ice). After ensuring that no chunks of snow or ice remain in the water, collect egg masses by gently cradling them in both hands and sliding them into the bottle. This transfer is facilitated by tilting the bottle to ~45°. Egg masses are best collected and transported within a few days of being laid (identified by their spherical embryos; Figure 3). Older egg masses disintegrate much more easily when handled. Several egg masses can be transported in a single water bottle but egg masses should occupy ≤25% of the water bottle volume. Leave ~3 cm (1") of airspace at the top of the water bottle and screw the lid on snugly. Fill a dry bag ~20% full of snow or ice, place the water bottle(s) upright in the snow/ice, and place the sealed dry bag into a backpack. As with other life stages, gradual acclimation of egg masses to the water temperatures at the recipient site is not necessary.

At the recipient site, egg masses must be held in mesh holding bags until they hatch (Figure 4). Under natural conditions, egg masses are attached to the substrate at the time of laying. This attachment is important for egg mass survival, and egg masses that become unattached experience low survival (Garwood et al. 2007). The holding bag should be anchored in suitable habitat by placing several rocks inside the bag. Place egg masses into the bag and cinch the top closed.

Suitable habitat is typically a warm shallow area ≤30 cm deep (12") and at least partially protected from wave action. The mesh size of the holding bag is small enough to contain individual eggs but large enough to allow hatched tadpoles to swim out of the bag (mesh size = 0.64 cm (0.25")). Water levels can drop substantially during the 2-3 week development times for embryos so be sure to place the mesh bag in sufficiently deep water that the egg masses do not become stranded over time.

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Figure 1. Large mesh pens anchored in shallow areas of a donor site. Pens are 2 m on a side and 0.75 m tall. Access into the pens is via a zipper around the edge of the lid. In each pen, frogs are provided with basking habitat, typically a boulder over which the pen is placed. In the closest pen, frogs can be seen basking on the rock in the right-most corner.

Figure 2. Ziploc™ plastic container used to hold frogs during transport. Holes in the lids ensure adequate ventilation. Container volume is 236 mL (1 cup; 8 oz.).

Figure 3. Mountain-yellow-legged frog egg masses laid in a shallow area of a pond. These masses were recently laid, as indicated by the still-spherical embryos.

Figure 4. Mesh bag used for holding egg masses following translocation. This bag is 35 cm (14") in diameter, 38 cm (15") tall, and has 0.64 cm (0.25") mesh and a floating ring at the top.