Appendix N

Amphibian Data and Protocol

Contents

List of Tables

List of Figures

N. AMPHIBIAN DATA AND PROTOCOLS

N.1 Surveyor Qualifications

Individuals trained in herpetology or those who have received training specific to the biology of covered aquatic species from experienced MRC staff will conduct all aquatic surveys. The USFWS and CDFG must approve those who are performing the surveys based on their resume and relevant experience. If MRC trains individuals to conduct survey work, USFWS and CDFG must approve their curriculum and trainer.

N.2 Baseline Distribution of Red-legged Frog Breeding Sites

N.2.1 Establishing baseline distribution

MRC will locate potential breeding sites of red-legged frogs by reviewing existing data, looking at aerial photographs, examining maps, driving roads, and walking over countryside. In addition, we will conduct surveys in and around potential breeding sites, i.e., ponds, marshes, and still-water habitats with water present after June 1.

N.2.2 Characteristics of potential breeding sites

Upon encountering a specific wet-area feature, we will determine whether or not the site could support red-legged frog reproduction. If a site could support reproduction, we will classify it as a potential breeding site for red-legged frogs, using criteria for depth and persistence of water (see Table N-1). A site which is 1.25 ft deep during high water, for example, and which has both hydrophytic vegetation and invertebrate life is a potential red-legged frog breeding site.

Table N-1 Characteristics of Breeding Sites

Characteristics of Potential Red-legged Frog Breeding Sites	
Habitat	Site must have standing, slow, or still water (lentic environment).
Depth	Site, measured during high water conditions, must have water to a depth of 10 in. or more (USFWS 2002).
Persistence of Water	Site must retain water, given average rainfall, until June 1st and meet 1 of the following criteria: 1. Presence of hydrophytic or obligatory wetland plant species and presence of aquatic invertebrate life. 2. Presence of aquatic phases or newly metamorphosed amphibian species which use "pond type" habitats for reproduction (northwestern salamanders, pacific newts, bullfrogs, etc), excluding pacific tree frogs. **NOTE** Pacific tree frogs are not good indicators of water persistence; they often use water puddles to breed which dry up before the larvae complete metamorphosis. 3. Presence of fish species.

N.2.3 Sampling intensity

Upon locating a potential breeding site for red-legged frogs, MRC will determine the sampling intensity. Sampling intensity will depend on habitat complexity and accessibility. In MRC protocol, sites are either "easy to sample" or "difficult to sample" (see Figure N-1). A site is "difficult to sample" if seining, dip-netting, or observing are hard to perform, subsequently reducing the likelihood of detecting red-legged frogs. MRC will expend more effort at such sites. We will conduct surveys at each potential breeding site at least 10 days apart to increase the likelihood of encountering egg masses. Our nocturnal surveys may take place on the same calendar date as a daytime survey.

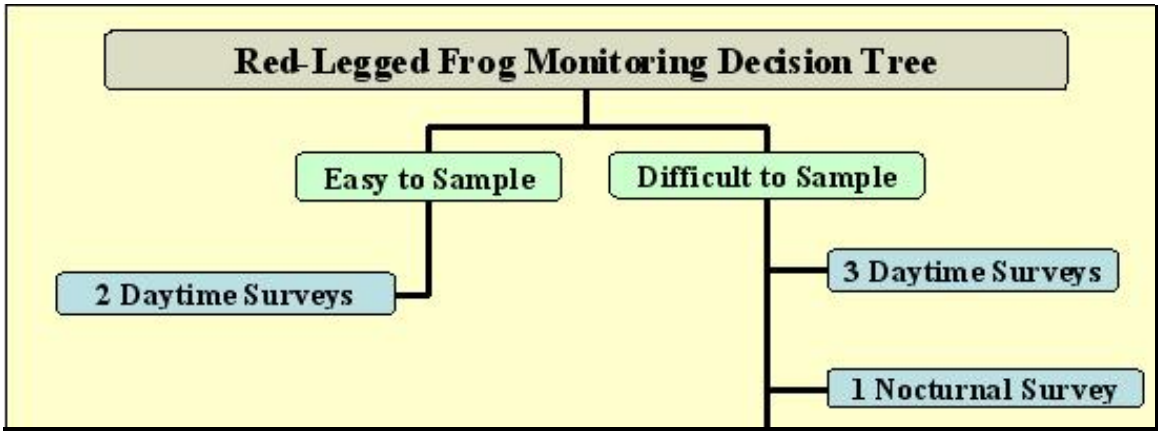

Figure N-1 Decision Tree

MRC will classify a site as "difficult to survey" if it meets 2 or more of the following criteria:

1. Maximum water depth is >6 ft during high water.
2. Floating woody debris covers at least 30% of the water's surface area or submerged vegetation and woody material make seining difficult.
3. Less than 40% of the water's perimeter is accessible due to steep gradient, brush, etc.
4. Water surface area is >1500 ft^2, as measured during high water.

Fish, particularly centrarchids, inhibit the growth and success of red-legged frogs. MRC will survey potential breeding sites of red-legged frogs for fish species at least once in a given monitoring cycle, if the site has sufficient persistence of water to support fish species. Fish surveys will focus on habitat most likely to contain centrarchids, since these amphibian predators are not native; surveys will consist of seining, dip-netting, and hook-and-line sampling.

N.2.4 Surveying potential breeding sites

MRC conducts surveys for red-legged frogs in late winter or early spring (from January 1–May 1). At these times, the species generally congregates at breeding sites to reproduce. Using techniques to detect evidence of reproduction such as tadpoles or egg masses, we search potential breeding sites. As part of the search, we walk the perimeter of the potential breeding site, turn over movable objects, examine vegetation in the water, and look for conspicuous red-legged frog egg masses. We use dip nets and seines to capture larval red-legged frogs and other amphibian species. To survey the entire wetted area, we use small vessels, including kayaks and rafts. While

identifying encountered species to their lowest taxonomic level, we also note their life stage (adult, larval, or embryonic).

N.2.5 Measuring habitat quality at potential breeding sites

Upon surveying a potential breeding site, MRC measures habitat characteristics which we will then monitor over time for our inventory database. An observer must use his or her best judgment to estimate what a feature might look like during high water conditions. Due to variation in rainfall and survey timing, a site's water surface area and maximum depth fluctuate. For standardization, we measure surface area (length x width) and maximum depth during high water conditions, which is often, of course, an estimate.

N.2.6 Documented red-Legged frog breeding site

Potential breeding sites become documented breeding sites when we observe evidence of red-legged frog reproduction, i.e., egg masses or larvae. We assume red-legged frogs are using documented breeding sites for reproduction due to the presence of embryonic or larval life stages. If we do not observe any evidence of red-legged frog reproduction, a site remains a potential breeding site.

N.3 Monitoring Occupancy of Documented Breeding Sites

Every year MRC monitors all documented breeding sites of red-legged frogs identified during either an initial distribution survey or a follow-up survey; we use the survey methodology presented in N.1.4. The intent of these surveys is to assess whether or not red-legged frogs used the site for reproduction during the given season. The presence of adult, or post-metamorphic frogs, does not indicate red-legged frogs used the site for reproduction.

N.4 Re-assessing Distribution and Monitoring Habitat Quality

Every 5 years, MRC will re-visit the potential breeding sites identified during the initial distribution study to (1) assess the persistence of the habitat and (2) evaluate whether or not red-legged frogs are present. At that time, we will take measurements of habitat quality using the methodology presented in N.1.5. Surveys at potential breeding sites follow the methodology presented in N.1.3 and N.1.4.

N.5 Establishing the Baseline Distribution of Coastal Tailed Frogs

During the first 2 years of HCP/NCCP implementation, MRC will establish the baseline distribution of coastal tailed frogs. As of 2009, we have surveyed approximately 75% of the plan area and detected coastal tailed frogs at more than 70 of the 450 sites surveyed. Chapter 13, section 13.5.3.1, outlines our survey methodologies. Maps showing planning watersheds occupied by coastal tailed frogs are in the *HCP/NCCP Atlas,* MAPS 11A-C.

N.6 Distribution and Relative Abundance of Coastal Tailed Frogs

MRC will re-survey approximately 10 streams per year to monitor for the continued presence and relative abundance of coastal tailed frogs. Based upon our current knowledge of the baseline distribution of coastal tailed frogs, there are approximately 70 occupied streams. We must still survey 10 additional planning watersheds for coastal tailed frogs by Year 2 of HCP/NCCP implementation. Based upon the results of the remaining baseline distribution survey work, the averages presented below may increase by 1 year.

On average once every 7-8 years, MRC will monitor all occupied streams identified during baseline distribution surveys, new surveys, or incidental observations throughout the HCP/NCCP term. Monitoring will focus on (1) determining whether coastal tailed frogs continue to remain present in occupied sites and (2) determining the relative abundance of coastal tailed frogs at occupied sites. Sampling will begin in late spring or early summer when flows are low enough for MRC biologists to work efficiently in the stream; they must complete the animal sampling by late July when larvae metamorphose and leave the stream.

N.6.1 Monitoring distribution of coastal tailed frogs

Monitoring surveys will consist of searching upstream using glass bottom viewing boxes and rubble rousing techniques to attempt to locate any life stages of coastal tailed frogs. Surveys will continue upstream until the animals are located or until 1 hour of search time has elapsed. Upon encountering the first coastal tailed frog during a monitoring survey, we will confirm and document presence. We will note the required search time to detect the initial animal and take water quality measurements.

N.6.2 Monitoring relative abundance of coastal tailed frogs

MRC modified the process in the *Green Diamond Aquatic Habitat Conservation Plan* (2006) to determine relative abundance. Measurements and sampling for relative abundance will begin immediately upstream of (and not including) the location of the animal located during the monitoring survey as described in N.2.1. During each subsequent monitoring cycle, the start point of the relative abundance measurements will be variable and based upon where observers locate the initial animal. We decided this approach will focus on areas closest to the animals bred that season, rather than on areas permanently monumented and subjected to chronic disturbance from monitoring efforts.

N.6.2.1 Sampling belt selection

The sampling procedure is as follows:

1. Delineate habitat units by hiking upstream with a hip chain and recording fast and slow-water stream habitat units which are at least 1.0 m in length. Fast-water equates to riffles and cascades; slow-water, to pools and runs. Units less than 1.0 m long are subsumed into the adjacent units (i.e., not distinguished from them).
2. Continue up stream until there is a combined total of at least 150 m of fast water habitat delineated. All fast-water habitat units will be, in theory, placed end-to-end as if contained in one long habitat unit.
3. Choose a random start, labeled m, between 1 and 3.
4. Sample every tenth fast water unit after the m-th unit.
    - Sample the m-th unit from the beginning of the linear assemblage of fast water habitat.
    - Sample the (m+10)-th unit from the beginning of the linear assemblage.
    - Sample the (m+20)-th unit from the beginning of the linear assemblage.
    - Sample until there are a total of 10 sampling belts (a cumulative total of 20 m of fast water habitats).

Each sampling belt is 2 m long and may contain up to 1 m of slow water. If the designated unit is unsearchable due to water depth, organic debris, or excessive gradient, proceed to the next available sampling belt immediately upstream.

N.6.2.2 Animal sampling procedure

The procedure for animal sampling is as follows:

1. Place a blocking net at the upstream and downstream ends of the unit, prior to any disturbance of the sampling belt.
2. Measure the gradient, average depth, and average width of the belt.
3. Remove all the substrate that can be moved by hand within the sampling belt and collect any animals that may be incidentally seen during this process.
4. Complete the first visual search of the sampling belt using a viewing bucket and remove all coastal tailed frogs observed.
5. Place the coastal tailed frogs removed from the viewing bucket in an appropriate container and repeat the visual search 3 additional times.
   NOTE
   MRC will make an effort to keep the frogs in separate, identifiable buckets per pass to allow for an estimate of population size.
6. Check the blocking net after each pass and place any animals encountered with the other animals collected during the search.
7. Perform an additional pass if the number of frogs obtained in a removal pass is at least 50% of the number of frogs obtained in a previous pass.
8. Record the sex (adults only) and developmental stage for each coastal tailed frog captured.
9. Remove the blocking net following the final search.
10. Put the substrate back into the stream.
11. Release the coastal tailed frogs back into the stream.

N.6.2.3 Data analyses

MRC will collect measurement of relative abundance from 10 occupied coastal tailed frog streams every year. In annual reports, we will include the following data:

- Locations of detections.
- Stream characteristics (length, average depth, width, surface area, and volume of water searched).
- Total number of animals collected.
- Estimate of population depletion.
- Number of animals found per unit of water.