

**San Francisco Estuary
Wetlands Regional Monitoring Program Plan**

**Data Collection Protocol
SURVEY PROTOCOL FOR THE
CALIFORNIA RED-LEGGED FROG
*(Rana aurora draytonii)***

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Introduction

On May 23, 1996 [effective June 24, 1996] the U. S. Fish and Wildlife Service (USFWS) listed the California red-legged frog (*Rana aurora draytonii*) as Threatened, after determining that the animal had disappeared from approximately 70% of its historic range within the State of California (Miller *et al.* 1996). Due to its Threatened status, there is an active, wide-reaching interest in determining and monitoring the current distribution of the California red-legged frog within the species' historic range on state, federal, municipal and private lands. This protocol was developed primarily for sampling

habitats around the San Francisco Bay Area, in order to create a standardized methodology, which will stand up to scientific scrutiny and allow region-wide analysis of data sets obtained through both the regional monitoring program and surveys conducted by individuals from the public and private sectors.

Past methodologies, including the most current protocol required by the USFWS (February 18, 1997), have relied solely upon the detection of juvenile, subadult, or adult California red-legged frogs along riparian zones either during the day, or at night via eyeshine; and this methodology has frequently resulted in false negatives (i.e. the animals were determined to be absent from the site--USFWS 2000a). Terrestrial life stages of California red-legged frogs are not always detected for a variety of reasons, and anurans (including Hylids, Bufonids and other Ranids) are too frequently misidentified by individuals conducting protocol surveys. Observations of larvae and post-metamorphic life stages are often tied to specific periods of time in specific kinds of habitat, which may vary considerably from location to location throughout the species' historic range.

This protocol incorporates methods designed to detect California red-legged frogs at all life stages and thus provide more complete and accurate information on the habitats, distribution and current locations of the California red-legged frog around the San Francisco Bay Area. The sample universe is considered to be the State of California and for the Regional Monitoring Program the sample units are only those counties which make up San Francisco Bay Area (nine counties). As new information from ongoing research becomes available on frog habitat use, this survey protocol may require modification to include local variations in life history traits.

General Site Assessments

Personnel

A trained wildlife biologist must conduct the surveys. Trained volunteers would have to be closely supervised by a professional who is permitted for fieldwork.

Site Assessment Procedure

The following questions should be addressed during a site assessment.

1. Is the site within the known or historic range of the California red-legged frog?

The initial step in assessing the potential for California red-legged frog presence in any given area, is to determine if the site is within the known or historic range of this frog as provided in Stebbins (1985) and Jennings and Hayes (1994) [see also the Draft Critical Habitat Designation and the Draft Recovery Plan for the California red-legged frog as rendered by the USFWS 2000b].¹

2. Are there any records, current or historic, for California red-legged frogs observed within a 5-mile radius of the site under review?

¹ If the site location is outside the present known range of this frog, and frogs are found to be present on the site, the final survey report should provide adequate information to support the finding.

A review of the California Department of Fish and Game's Natural Diversity Data Base (CNDDDB) and a request for information on potential locations from the pertinent field offices of the U.S. Fish and Wildlife Service are essential for beginning to determine if there are any current or historic California red-legged frog records within a 5-mile radius of the site to be surveyed. Some counties, such as Santa Clara County (Jennings *et al.* 1997) may have more current information on the distribution and locations of frogs within their area, than is available through the state and federal agencies. In addition, to these avenues, all available information obtained by biological consulting firms from recent California red-legged frog surveys conducted within the general area around the project site (i.e. a 5-mile radius) should be reviewed and source individuals contacted. Last, and equally as important sources of recent information, local expert herpetologists should be contacted.

Any documented reports of California red-legged frogs occurring on a site within the past 5 years establish the species as present with no further survey work needed-provided no significant habitat alterations have occurred; or if field surveys conducted following this protocol have not found any frogs during the past 3 years, and those three years contained at least one year of "better-than-average" rainfall.

Other reports of California red-legged frogs on-site, such as anecdotal sightings by biologists, the lay public, or other non-specialists, (as well as photographs of potential California red-legged frogs), should be investigated and carefully reviewed. If such observations are not considered valid, a detailed explanation must accompany the survey report.

The final essential step in conducting the background review is to consult maps and recent aerial photographs of the area of interest and the general vicinity surrounding the site, in order to determine if aquatic habitat is present on or adjacent to, the site. If aquatic habitat is not present on-site, but is present nearby, there is a potential for California red-legged frogs to still occur within the survey site during the wet, winter, rainy season. Knowledge of the locations of aquatic habitat, in and near the survey site, is crucial information in determining whether or not potential habitat for California red-legged frogs is present.

Habitat Assessment

Personnel Required

Lead Biologist: Wildlife biologist in possession of a current Federal Endangered Species Take Permit for California red-legged frogs from the USFWS and a current Scientific Collecting Permit from the California Department of Fish and Game. At certain sites, and according to specific permit requirements, a larval, juvenile, or adult voucher specimen deposited in a recognized institution (e.g., California Academy of Sciences, San Francisco, California), or a photograph of a captured frog may be requested by the USFWS, to

verify identifications and, in the former case, to make tissue available for future research on Ranid genetics and potential disease pathogens.²

Assistants (if needed): must be working towards, or possess a university-level biology degree, have worked a minimum of 100 hours in the field and have demonstrated the ability to locate and identify different species of local treefrogs (*Hyla* spp.), toads (*Bufo* spp.), and true frogs (*Rana* spp.) in the field before conducting any formal survey work.

Habitat Assessment Procedure

The following questions should be addressed during a habitat assessment.

Does the site have potential habitat for California red-legged frogs?

If the background review determines that California red-legged frogs could potentially occur at the survey site due to known records in the vicinity, the next step is determining whether or not potential habitat for the frog is currently present on site. An initial site visit is needed to make this determination and the site assessment can be conducted during the day at most times of the year. Written permission must be obtained from the necessary landowner(s) before conducting any fieldwork. California red-legged frogs occupy a wide range of aquatic and terrestrial habitats, which may or may not appear to humans to meet the standard criteria³ for “suitable” California red-legged frog habitat. If potential habitat is present on site, then field surveys must be conducted for all pertinent life-stages of California red-legged frogs in all aquatic and terrestrial habitats.

The absence of a waterbody on the site does not automatically mean that California red-legged frogs do not occur there. Frogs may breed off-site on an immediately adjacent property if a waterbody is present, and during the rainy wet season may use the survey site for foraging, or, as a movement corridor between two waterbodies.

During the habitat assessment field visit, examine the entire site to note proximity to any waterbodies on adjacent properties. It is also important at this time, to take note as to which potential physical hazards to avoid (or consider) when conducting nocturnal surveys, as well as the locations of potential frog habitat.

Habitats Utilized by California red-legged frogs: California red-legged frogs have been observed in a number of aquatic and terrestrial habitats throughout the species’ historic range. Within the state of California, frogs have been found at elevations from just above sea level (in lagoons and ponds at the edge of the beach just above the high tide line), to around 4,500 ft. in the Sierra Nevada foothills, and

² All vouchered specimens and tissue samples should be properly preserved and stored using standard museum techniques (Pisani 1973, Simmons 1987).

³ Deep freshwater ponds with emergent vegetation and surrounded by tules (*Scirpus* spp.) and willows (*Salix* spp.) [Hayes and Jennings 1988].

in southern California, south of the Tehachapi Mountains, to around 5,000 ft. (Jennings and Hayes 1994). Larval, juvenile, and adult frogs have been collected in natural lagoons, dune ponds, pools in or next to streams, streams, marshlands, sag ponds, and springs, as well as human-created stockpools, secondary and tertiary sewage treatment ponds, wells, canals, golf course ponds, irrigation ponds, sand and gravel pits (containing water), gold dredge ponds, and large reservoirs (Storer 1925, Wright and Wright 1949, Stebbins 1951, Hayes and Jennings 1988, Jennings 1988). Juvenile and adult frogs have also been observed in riparian zones next to aquatic habitats and on low tree limbs [e.g., willows (*Salix* spp.)] over the surface of the water. Additionally, during the storm season, juvenile and adult frogs have been recorded foraging and moving overland in upland habitats up to 300 feet away from riparian zones.

The habitats found to contain the largest densities of California red-legged frogs are usually associated with deep-water pools (>2 ft. deep) with dense stands of overhanging willows (*Salix* spp.) and an intermixed fringe of cattails (*Typha latifolia*), tules (*Scirpus* spp.), or sedges (*Carex* spp.) (Hayes and Jennings 1988). However, California red-legged frogs have also been observed to inhabit stock ponds and artificial (e.g., concrete) pools completely devoid of vegetation (Storer 1925).

Water temperature does not seem to be a limiting factor for the successful reproduction of California red-legged frogs in California. One cannot automatically exclude the possible presence of frogs from an aquatic habitat unless the natural water temperature exceeds 85°F for the entire system (such as with a hot spring).

Unpublished field and laboratory observations indicate that California red-legged frogs cannot successfully reproduce at salinities >4.5% (Jennings and Hayes 1990). Larvae cannot survive in salinities >7.0% (Jennings *in litt.* as cited in Miller *et al.* 1996). Juvenile and adult frogs seem to avoid salinities >9.0% (Jennings and Hayes 1990) and will move significant distances to escape from areas that become too saline (Rathbun *et al.* 1993). For these reasons, California red-legged frogs are largely restricted to freshwater and slightly brackish water habitats. For lagoon habitats such as Pescadero Marsh in Santa Cruz County, California red-legged frogs will be present only during periods when the salinities of the lagoons are within the range tolerated by these animals.

Field Surveys

Field surveys to determine the presence of California red-legged frogs include sampling all potentially suitable aquatic, terrestrial and riparian habitats over time. The four life stages of the California red-legged frogs--adult, juvenile, larval, and egg masses--require different survey protocols, which are outlined below. The positive finding of any one of these life stages establishes the presence of the California red-legged frog on-site.

If, over a 3-year period when 1 year contains above-average-rainfall, no larval, juvenile, or adult frogs are detected following this protocol, the absence of the California red-legged frog is conceded for that site.

This protocol requires four daytime and four nighttime aquatic and terrestrial habitat surveys for juvenile and adult frogs. In addition, if a waterbody

is present on-site, four seining events for larval California red-legged frogs and four surveys for egg masses are required.

Nocturnal Surveys for Juvenile and Adult Frogs

Equipment: Light source not to exceed 50,000-candle power

Surveying for juvenile and adult frogs using eye-shine to locate individuals at night requires the proper equipment and technique. The goal of the technique is to minimize the presence of background light (such as moonlight), while maximizing the reflection of the light source from a frog's retina to the eyes of the surveyor. To aid in maximizing detection abilities, nocturnal surveys must therefore be conducted when the phase of the moon is less than two-thirds full and in addition, surveyors must learn to position themselves properly (usually at distances >20 ft. away) in order to best observe reflected eye-shines. All nocturnal surveys are conducted with the use of a bright, narrow, beam of light held at or near eye level (e.g., see the account in Stebbins 1985). Some individuals prefer the use of a light attached to their head (with a headband or hat) just above the eyes because it leaves both hands free. Other individuals prefer to hold the light with one hand next to or just above or below their eyes because they feel that headlamps cause eye muscles to easily tire. Whatever method is used, surveyors must learn and practice the proper technique of locating the eye-shines of organisms before conducting any official field surveys. A number of 3-6 volt flashlights rated at 10,000-50,000 candlepower can be used as a light source, but a rechargeable miner's headlamp (rated at 15,000/45,000 candle power for low and high settings) has been found to give the best results because you can easily focus its light beam for distances up to 60 ft. (where one often first sees a frog's eye shine) and it also provides a consistent amount of light intensity for about 6-8 hours. Under no circumstances should excessively bright lights of >50,000 candle power (e.g., "Q-Beams", spotlights, etc.) or lights with unfocused beams be used, as this will result in excessive illumination of areas examined, poor reflection of light from the frog's retina and thus a false negative for the survey.

Nocturnal surveys are conducted at any time after the onset of total darkness and usually take several hours to complete. It is very important that the surveyor's light source does not fade or change during the survey period. Additionally, observer fatigue usually becomes a factor after about 4 hours of surveying, so individuals should not exceed this amount of survey time per night.

Surveyors look for frog eye-shines by systematically shining their light along the shoreline, the surface of the water, riparian vegetation, and the bank within about 4 ft. of the water's edge. When searching for frogs, it is important to constantly change the position of your body, head, and light beam in order to put yourself in the proper alignment with a frog's potential eye shine. The best methodology is to scan about 50-60 ft. ahead of you to look for eye shins before searching the closer habitat in detail- then move on and repeat the procedure. The eye-shines of frogs <20 feet away often become indistinguishable from the background as you move closer to them.

Once a frog (or its eye-shine) is sighted, keep the light on the frog's eyes and slowly move in close enough to be able to see part of its head or upper body and to be able to correctly identify it.

Every surveyor should know the key characteristics of all local aquatic amphibians before they start surveying for California red-legged frogs in the field. Such characteristics include small size, toe pads and black eye stripes for Pacific treefrogs (*Hyla regilla*), short snouts, short legs and large parotid glands for toads, large size, dark snouts and dorsolateral folds for California red-legged frogs, and large size, green snouts, "eyes-on-top-of-the-head", yellowish throats, large tympanums and the lack of dorsolateral folds for bullfrogs (*Rana catesbeiana*) [Stebbins 1985]. If the Lead surveyor is still uncertain as to the identity of the anuran under observation, capturing the animal may be the only option to accurately identify it and thereby prevent a false negative of frog occurrences. Only the permitted Lead biologist is allowed by Federal and State law to handle (i.e., "take") California red-legged frogs. Surveyors should note the number and species of any anurans (e.g., bullfrogs) and potential predators (e.g., herons, raccoons and fishes) they observe during their field surveys.

Eyeshine alone does not determine species identity. The color of eye-shines for California red-legged frogs, bullfrogs, and other creatures vary considerably amongst individual observers. Surveyors should practice in the field until they can easily discern what is an anuran, raccoon (*Procyon lotor*), insect, spider, or inanimate object before conducting actual surveys. Such efforts will greatly reduce the amount of time lost examining non-anuran eye shines. In small stream situations, a single Lead surveyor can easily survey the stream for California red-legged frogs by systematically working upstream along the bank or in the creek itself and checking both banks and the stream-proper for frogs. Upon reaching the end of the survey area, the surveyor should repeat the same survey procedure downstream. If the stream is over 10 ft. wide, then two surveyors, one Lead and one Assistant, (one person for each bank) should be used.

In pond or marsh situations, a Lead surveyor and one or more assistants should survey for California red-legged frogs by systematically working along the edge of the pond for at least two complete circuits. In larger waterbodies, a small kayak, float tube, or wet suit can be used to efficiently gain access to all potential frog habitat. In large areas of habitat that cannot be completely covered by surveyors in one night, surveys should be conducted for 4 hours each night for as many consecutive nights as is needed to survey the entire area (this will equal one complete survey event and this effort will have to be replicated three more times to fulfill the requirement of four nocturnal surveys). During all surveys, surveyors should be careful not to make any loud noises or create any large water disturbances, as this can alarm frogs and cause them to jump into the water before they can be identified.

Surveys should be completed on a minimum of four separate nights at any time during the months of January-May (in the San Francisco Bay area) with no more than one survey during any given month in order to detect the presence of adult frogs.

Frog Calls

Adult California red-legged frog males, like many other anurans, vocalize to attract a female during the breeding season. The call of this frog is very low and short (Davidson 1995) and generally one must be fairly near the source to be able to detect the call of a single male, particularly if other Ranids are calling as well. Although listening for a calling male can aid in locating an individual and in species identification, counting calls is not a reliable method for determining population size. Rather, the counting of egg masses gives a much more reliable method of determining the number of breeding adults (see the account below for frog egg mass surveys).

Diurnal Surveys for Adult and Juvenile Frogs

Equipment: binoculars

Surveys for juvenile California red-legged frogs are best conducted during daylight hours, although juveniles can be observed at night using the same survey techniques for adult frogs. Juvenile frogs can often be seen sunning themselves on shore near the water's edge during the warmer parts of the day. Surveying for juvenile frogs during the day follows the same techniques as searching for frogs at night except that one relies upon finding frogs with the naked eye or the aid of binoculars rather, than with the use of a headlamp and eye-shines. As with adult frog surveys, small stream situations require one Lead surveyor, large stream situations require one Lead surveyor and one assistant, and pond or marsh situations require one Lead surveyor and one or more assistants. Surveys should be completed on a minimum of at least four separate days at any time during the months of June through September with no more than one survey during any given month in order to detect juvenile frogs. In large areas of habitat that cannot be completely covered by surveyors, surveys should be conducted for a minimum of 4 hours each day for as many consecutive days as is needed to survey the entire area (this will equal one complete survey event and this effort will have to be replicated three more times to fulfill the requirement of four diurnal surveys).

Surveys for Larval Frogs

Equipment: Current USFWS Endangered Species Take Permit, California Scientific Collecting Permit, 0.125"-mesh dip nets, 0.25"-mesh two-pole seines

It is mandatory to this protocol, that 1-5 voucher specimens of larval anurans be deposited at the California Academy of Sciences (San Francisco, California) for species verification by experts familiar with tadpole identification. These voucher specimens will also provide genetic material for future research as well as tissues for pathologic examination. Any larvae collected for this purpose must have the tail tips removed and preserved in alcohol to allow for future tissue sampling, and the rest of the specimen must then be preserved in 10% buffered formalin (see Simmons 1987).

Surveys for larval stages of California red-legged frogs are conducted during the daytime hours by using 0.125"-mesh dip nets and 0.25"-mesh two-pole seines. Larval surveys should be conducted by carefully netting the bottom of the pond or by rooting around aquatic vegetation with dip nets or two-pole seines and examining any anuran

larvae captured. All potential aquatic habitats (especially the deeper sections of pools) should be intensively sampled.

All larvae captured should be examined visually while holding the seining net just under water. Animals, which need to be examined closely for accurate identification, should be handled *only with wet hands* and should be handled for as short a time as possible. Irrigate the animal with site water frequently. All organisms should be returned to the water **as soon as possible**, except for individuals being preserved as voucher specimens. Surveys should be conducted a minimum of four times between the months of January and September, with no more than one survey in any given month.

At no time should minnow traps be used to survey for California red-legged frogs. The use of minnow traps is known to result in mortality for many captured organisms--either by suffocation or predation. Individuals who use minnow traps have no way of knowing how many animals have been preyed upon, as the evidence of the organisms' presence is gone once it has been consumed. In such close quarters, organisms captured in traps can prey upon each other (at times leaving one animal in the trap) and animals such as San Francisco garter snakes (*Thamnophis sirtalis tetrataenia*) can easily enter the trap, consume the trapped organisms and exit the trap-- leaving the unsuspecting surveyor to pull up an empty minnow trap and (if frogs were not detected during terrestrial surveys) to subsequently conclude that California red-legged frogs are absent from the site. Since minnow traps are not particularly selective in the organisms that become trapped in them, surveyors can find themselves in a detrimental legal situation when other listed animals for which the Lead surveyor is not permitted to "take", are killed in the minnow traps.

Diurnal Surveys for Frog Egg Masses

Egg masses laid by California red-legged frogs are usually composed of 2,000-6,000 eggs ranging from 2.0-2.8 mm in diameter. Following egg deposition and after several days in the water, individual egg masses swell to about the size of a softball. Egg masses are normally laid at night near or at the surface of the water and are attached to some type of vegetation brace; they are rarely attached to rocks in the slack water of pools. Embryos normally hatch after an incubation of 6-14 days, so egg masses are probably present at any one locality for a period of about 2-5 weeks.

Surveys for egg masses should occur during the months of December-February in the San Francisco Bay region. Surveys should be conducted during daylight hours once every 2 weeks for 2 months (a minimum of four survey events) by walking the area and carefully checking places with emergent vegetation near the edge of the water. Calling male frogs are often heard during the night (and sometimes during the early evening or morning hours) during this same season and the calls can often provide clues as to where to conduct searches for potential egg masses. Thus, surveyors may want to combine adult frog surveys at night with egg mass surveys during the day. Counting egg masses is the most accurate method to determine breeding population size. A cassette tape of the

weak-volumed breeding call of the California red-legged frog and other anurans of California is available (Davidson 1995).



Data Sheets

Date:	Begin Time:	End Time:	Site Visit #: 1 2 3 4 5	Observers:
Locality:				
County:	Landowner:	UTM (or Lat/Long):		Elevation: m ft
USGS Topo 7.5" Quad Name:		Township:	Range:	Section:
Section Description:				
Purpose for Survey: <input type="checkbox"/> Private Research <input type="checkbox"/> Agency Research <input type="checkbox"/> Regional Monitoring <input type="checkbox"/> Private Consulting <input type="checkbox"/> Other (Please fill in):				
Current Land Use:		Potential Threats to Habitat:		
Physical Environment (optional-space provided for other potential variable)				
Weather: Clear Snow Rain Drizzle Fog Overcast Pt. Cloudy				Wind: Calm Light Strong
Water Temp: °C °F	Air Temp: °C °F	Water Turbidity: Clear Cloudy	Water Color (fill in):	
Weather Conditions 24 hours prior:		pH:	Conductivity:	
Site Description- Fill this out only for the first visit.				
Habitat Type: Mixed Evergreen Oak Woodland Chaparral Grassland Riparian Ruderal Urban Landscaped Park Golf Course Other (Please fill in).				
Description: Lake/Pond Marsh/Bog River/Stream/Creek Spring/Seep Other				
Origin: <input type="checkbox"/> Natural <input type="checkbox"/> Man-made		Drainage: <input type="checkbox"/> Perennial <input type="checkbox"/> Seasonal <input type="checkbox"/> Intermittent <input type="checkbox"/> Temporary		
Site Length (m):	Site Width (m):	Maximum Depth: < 1m 1-2m >2m		
Stream Order (if applicable):	1	2	3	4 5
Primary Substrate:	Silt/Mud	Sand/Gravel	Cobble	Boulder/Rock Other
% Canopy Cover:				
Canopy Cover Species (List order of abundance)				
% Waterbody Margins with Emergent Vegetation				
Emergent Vegetation Species (List order of abundance)				
Submergent Vegetation Present? Yes No Choked Extensive Moderate Little None (water "crystal clear")				
Algae level: Choked Extensive Moderate Little None (water "crystal clear")*				

Amphibian and/or reptile (e.g. garter snake) species present (indicate numbers when possible).								
Species	Adult	Calling?	Sub-Adult/Juvenile	Metamorph	Larvae	Egg Masses	Survey Method (Circle one)	How was species ID determined and by whom?
							Visual Dip Net/Seine Hand Collected Voucher Specimen Collected? Yes No Photograph? Yes No	
							Visual Dip Net/Seine Hand Collected Voucher Specimen Collected? Yes No Photograph? Yes No	
							Visual Dip Net/Seine Hand Collected Voucher Specimen Collected? Yes No Photograph? Yes No	
							Visual Dip Net/Seine Hand Collected Voucher Specimen Collected? Yes No Photograph? Yes No	
							Visual Dip Net/Seine Hand Collected Voucher Specimen Collected? Yes No Photograph? Yes No	
							Visual Dip Net/Seine Hand Collected Voucher Specimen Collected? Yes No Photograph? Yes No	
Predators : Indicate numbers when possible, otherwise use + to indicate presence.								
Bullfrogs	Mosquito fish			Crayfish		Other introduced fishes or invertebrates (species if known)		

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