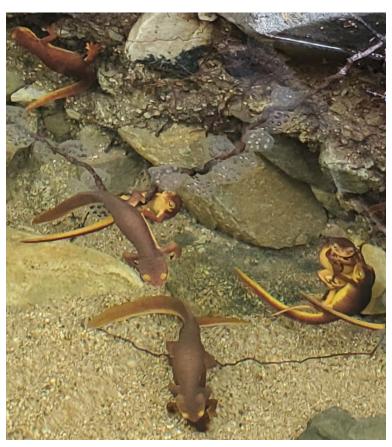
Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP) Biological Monitoring Program

Stream Survey Report 2020



June 2021

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NOTE TO READER:

This report is an account of the survey activities conducted by the Biological Monitoring Program for the Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP). The MSHCP was permitted in June 2004. Reserve assembly is ongoing and is expected to take 20 or more years to complete. The Conservation Area includes lands acquired under the terms of the MSHCP and other lands that have conservation value in the Plan Area (called public or quasi-public lands in the MSHCP). In this report, the term "Conservation Area" refers to these lands as they were understood by the Monitoring Program at the time the surveys were conducted.

The Monitoring Program monitors the status and distribution of the 146 species covered by the MSHCP within the Conservation Area to provide information to Permittees, land managers, the public, and Wildlife Agencies [i.e., the California Department of Fish and Wildlife (CDFW, formerly California Department of Fish and Game) and the U.S. Fish and Wildlife Service]. Monitoring Program activities are guided by defined conservation objectives for each Covered Species, other information needs identified in MSHCP Section 5.3 or elsewhere in the document, and the information needs of the Permittees. A list of the lands where data collection activities were conducted in 2020 is included in Section 7.0 of the Western Riverside County Regional Conservation Authority (RCA) Annual Report to the Wildlife Agencies.

The primary authors of this report were Field Biologists, Amanda Leach and Nathan Pinckard, and the 2020 Herpetofauna Program Lead, Robert Packard. This report should be cited as:

Biological Monitoring Program. 2021. Western Riverside County MSHCP Biological Monitoring Program Stream Survey Report 2020. Prepared for the Western Riverside County Multiple Species Habitat Conservation Plan, Riverside, CA. Available online: http://www.wrc-rca.org/monitoring/monitoring-surveys/.

While we have made every effort to accurately represent our data and results, the reader should recognize that data management and analysis are ongoing activities. Readers wishing to make further use of the information or data provided in this report should contact the Monitoring Program to ensure that they have access to the best available or most current data. Please contact the Monitoring Program Administrator with questions about the information provided in this report. Questions about the MSHCP should be directed to the Executive Director of the RCA. Further information on the MSHCP and the RCA can be found at www.wrc-rca.org.

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INTRODUCTION

The Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP) includes five covered amphibian species that inhabit stream environments in Southern California; arroyo toad (formerly Bufo californicus; currently Anaxyrus californicus; Frost et al. 2006), coast range newt (formerly Taricha torosa torosa; currently *Taricha torosa*; Kuchta 2007), California red-legged frog (formerly *Rana* aurora draytonii; currently Rana draytonii; Shaffer et al. 2004), Southern mountain yellow-legged frog (Rana muscosa), and western spadefoot (formerly Scaphiopus hammondii; currently Spea Hammondii; Wiens and Titus 1991). The Biological Monitoring Program has collaborated with the Western Ecological Research Center, U.S. Geological Survey (USGS) and the U.S. Forest Service (USFS) in conducting amphibianstream surveys since 2004 to prevent overlapping survey efforts and maintain consistent data collection methodology. The purpose of the stream surveys is to assess the habitat suitability and document breeding evidence and locations of the MSHCP covered amphibian species in streams and/or their tributaries in species-specific Core Areas. Focal species are different year to year and depend on the efforts of collaborating agencies and information needs. The target species for the stream surveys in 2020 was the California newt (*Taricha torosa*), formally known as the coast range newt (*Taricha torosa torosa*).

The coast range newt was originally thought to be a subspecies of *T. torosa* based on geographic distribution and coloration. However, recent phylogeographic work on *T. t. torosa* and *T. t. sierrae*, has shown that the two subspecies constitute distinct evolutionary lineages justifying recognition as separate species (Crother 2017; Kuchta 2007). The newly named California newt (*Taricha torosa*) is a species of special concern in California (CDFG 2019). The Monitoring Program will follow the currently accepted taxonomy and refer to this species as the California newt (*T. torosa*) going forward.

The California newt requires specific breeding habitat conditions restricted to "pools and runs" stream configurations adjacent to woodland and chaparral habitats where suitable refugia exists, which includes logs, leaf litter, and burrows (Gamradt and Kats 1997, Riemer 1958, Storer 1925). The known distribution of the California newt within the Plan Area is restricted to the Santa Ana Mountains Bioregion, which includes creeks, streams, ponds, and other wetland habitat in the Cleveland National Forest (Santa Ana Mountains Bioregion) and Santa Rosa Plateau (including portions of Cole Creek). Due to the species' narrow habitat requirements and limited known distribution, the California newt requires site-specific consideration, habitat protection, and species-specific conservation mandates.

A primary species objective for the California newt (Objective 5) is to maintain occupancy of at least 75 percent of the occupied California newt habitat and determine if successful reproduction is occurring within the MSHCP Conservation Area as measured by the presence/absence of larvae or egg masses once a year for the first five years after permit issuance and then as determined by the Reserve Management Oversight Committee as described in *Section 6.6, MSHCP Volume I* (but not less frequently than every 8 years; Dudek and Associates 2003).

Yearly stream surveys were conducted from 2005-2008 throughout the Santa Rosa Plateau, and intermittently at easily accessible stream segments in the Santa Ana Mountains from 2005-2007. The coverage of the Santa Rosa Plateau has been thorough in previous surveys, but many of the drainages in the Santa Ana Mountains are difficult to access and were avoided to maximize survey efficiency. In 2009, the Monitoring Program conducted more extensive stream surveys targeting California newt and California red-legged frog in the Santa Ana Mountains Bioregion and detailed a more thorough baseline of breeding habitat in the Santa Ana Mountains, which we used to determine our survey efforts in 2020. However, more remote and inaccessible drainages of the Santa Ana Mountains may support additional populations of California newt and other covered amphibians.

In March 2020, a global Covid-19 pandemic was declared, resulting in strict state-wide work restrictions and social-distancing. The 2020 Stream Survey Protocol was modified accordingly from previous versions to accommodate the novel work-place restrictions due to Covid-19.

Our efforts in 2020 were focused on locating adult California newts to determine occupancy and to document breeding evidence in as many stream reaches in the Santa Ana Mountains and Santa Rosa Plateau as possible, given available personnel and resources. In addition to delineating California newt occupancy, our intent was to characterize environmental and water chemistry conditions at each stream reach. Furthermore, all aquatic reptile and amphibian species (Covered and non-covered) were considered target species for the 2020 Stream Surveys, including California red-legged frog, mountain yellow-legged frog, western pond turtle (*Actinemys marmorata*), and invasive aquatic species. Invasive plant species encountered along stream segments were also recorded. The overall 2020 stream survey goals and objectives were as follows:

Goals and Objectives

- 1) Delineate presence and distribution of California newt across the Santa Ana Mountains and Santa Rosa Plateau to determine occupancy and reproduction.
 - a. Conduct visual-encounter and dip-net surveys within accessible drainages, recording all amphibian and aquatic species observed.
 - b. Document the presence of aquatic and riparian invasive species along each stream segment.
- 2) Work in collaboration with USGS to collect genetic material for on-going population studies of amphibians and reptiles in southern California.

METHODS

Protocol Development

The Monitoring Program began conducting stream surveys in 2005 following the USGS Aquatic Species and Habitat Assessment Protocol for Southcoast Ecoregion Rivers, Streams, and Creeks (USGS 2005). This protocol describes both a visual

encounter and dipnet survey methods for detecting all life stages of amphibians and an assessment of habitat characteristics. Additionally, the streams were divided into 250-m reaches, and labeled following the naming convention described in the USGS (2005) protocol.

In 2009, the protocol was modified to better address MSHCP species-specific objectives for the target species; California red-legged frog and California newt. Modifications included using the Sawyer and Keeler-Wolf (1995) vegetation classifications for characterizing the surrounding landscape, collecting additional water chemistry data, recording weather data at the end of the survey, and the change and addition of characteristics recorded for animal records. Furthermore, we began recording animal abundance, shallow pools, medium pools, and deep pools as continuous rather than categorical values.

The 2020 Stream Survey was revised further to maximize survey efforts with limited personnel and novel work-place procedures implemented in response to Covid-19. These modifications included reducing collection of habitat metrics and increasing efforts on monitoring for target species presence and reproduction. Observing breeding evidence fulfills the species-specific objective of the MSHCP, therefore if larva or egg masses were encountered during surveys, the observation was documented, and surveyors moved forward to the next stream segment. Similarly, surveyors stopped determining abundance estimates of adult newts. Lastly, water chemistry data was limited to two variables, velocity (m/s) and temperature (°C), and all other attributes from previous surveys were not assessed (i.e., pool size and abundance, water transparency, pH, dissolved oxygen, etc., Appendix A).

Tissue samples were collected from those USGS Target Species captured following a standardized protocol developed by Monitoring Program staff (Appendix B). We also opportunistically swabbed captured amphibians and saved the swabs for USGS to determine the potential presence of chytrid fungus (*Batrachochytrium dendrobatidis*).

Personnel and Training

In 2009, the Biological Monitoring training familiarized staff with species identification, field methods, and data collection techniques. The Program Lead was originally trained prior to the 2009 stream surveys by a staff member that attended a USGS training (29 March 2006) that covered the use of the USGS stream-survey protocol and anuran (frog and toad) and fish identification in the region. The Program Lead also examined tadpole specimens at the USGS San Diego Field Office (5 March 2009) and at the Los Angeles County Natural History Museum (17 Feb. 2009) prior to conducting surveys. Field methodology for collecting tissue samples was demonstrated using live animals and specimens at the USGS San Diego Field Office (5 March 2009).

In 2020, training techniques were modified from the 2009 surveys to comply with state-wide social distancing mandates in response to Covid-19 safety procedures. The Program Lead and another biologist trained during the 2009 survey participated in the 2020 stream surveys. The Program Lead provided all participating biologists with virtual training material equivalent to the 2009 training in the form of protocols, PowerPoint presentations, specimen photos, and personal communications via phone and email.

Training material included information to accurately identify all California newt age classes by sight and sound (for adults), as well as training on the identification of co-occurring aquatic wildlife and invasive plant species. Surveyors were also instructed on proper field equipment sterilization techniques, survey methods and data collection techniques, and provided with field guides and keys to aid in species identification. The following staff conducted stream surveys in 2020: Project Lead, Bob Packard and Field Biologists, Amanda Leach, Esperanza Sandoval, Taylor Zagelbaum, and Tara Graham.

Study Site Selection

The Monitoring Program conducted stream surveys in 2020 by geographically targeting California newts at select sites based on historic occurrences of adults or breeding evidence, specifically from the 2009 stream surveys. In 2009, drainages were selected by constructing an accessibility model using Arc GIS v.9.2 Global Information System (GIS) software (Esri 2006) and GIS-based vegetation (CDFG et al. 2005) and slope (USGS 2006) layers. Landscape was considered inaccessible if it had a slope > 25 degrees or consisted of chaparral with cover density > 40 % (unless within 50 m of a road or trail). The accessibility model was field verified in 2009 and used Arc GIS v.9.2 to identify drainage-access points no more than 1600 m from roads and with continuous traversable landscape leading to the stream channel. Streams at the boundary of conserved land were also selected as potential access points if they passed through traversable landscapes, and if the drainage could be accessed without passing through private lands. In 2020, the Monitoring Program surveyors entered drainages at these modeled access points in reaches where California newts were observed breeding in previous years and surveyed as many 250-m segments as possible in 8- and 10-hour days. We also surveyed a few additional conservation areas added to the Core Area since 2009. These new streams were surveyed for California newt if determined to have suitable habitat and access.

Survey Methods

Survey methodology was adapted from the *USGS Aquatic Species and Habitat Assessment Protocol for Southcoast Ecoregion Rivers, Streams, and Creeks*, written and distributed by USGS (USGS 2005). Modifications of the protocol were made to better suit the species-specific objective needs of the MSHCP's Monitoring Program.

Visual-encounter surveys (VES) and dipnet surveys began on March 24 along 250-m reaches in Core Areas with known California newt range occupancy. All surveys were conducted in daylight hours (0800 h - 1700 h) from downstream to upstream as outlined in the USGS protocol (USGS 2005). At the start of each stream segment, we took an upstream photograph, and collected the following variables: station ID, creek name, date, start time, site photo, observers, sky condition (0 = clear or few clouds, 1 = party cloudy or variable, 2 = cloudy or overcast, 3 = fog, 4 = mist or drizzle, 5 = showers or light rain, 6 = heavy rain, 7 = sleet or hail, 8 = snow), ambient air temperature (C) in shade at 1m above ground, and average wind speed (km/h), water temperature (C), and water velocity (m/s). At the end of each stream segment, we recorded the presence and abundance of exotic plant species, percent wet length, abundance of additional target species observed, and the presence, type, and level of recent disturbances.

To increase the likelihood of documenting reproductive success, repeat VES were conducted in stream segments where adults were initially observed in amplexus (anuran mating position), but no egg masses or larvae were documented. Furthermore, several stream reaches where egg masses were observed prior to the uncharacteristic heavy rains in April 2020, ranging between 275-500% of normal, (NOAA 2021), were revisited once water-levels subsided to ensure egg masses had not been washed away.

Covered Species

Each group of covered species were recorded as separate clusters, at the same location, by life stage. We recorded the following information for covered species: location, quantity, UTM coordinates, species code, age (e.g., adult, larvae, juvenile, or egg mass), detection method, number of photos taken, photo ID, and any relevant notes. If California newt breeding evidence, egg masses or larvae, was observed the biologist ended the survey and moved on to the next stream segment. For the purpose of analysis, adult presence was assumed even if not recorded in surveys that ended prematurely because breeding evidence was observed.

Non-covered Species

We documented one animal record for each non-covered species, from each age class (e.g., larvae, juvenile, egg mass, etc.) encountered per reach. At the end of each segment, the abundance of each non-covered species life stage was recorded. Apart from GPS coordinates, all information listed above for covered species was also collected for non-covered species.

RESULTS

The 2020 Monitoring Program surveyed 135 (33.75 km, repeated segments, n=14) stream segments in 25 drainages in the Santa Ana Mountains and Santa Rosa Plateau with historic California newt presence. Several streams from previous surveys were not monitored in 2020 due to limited resources, including Nickel Creek, Lucas Creek, both occupied by California Newt in 2009 (Biological Monitoring Program 2009), and portions of Bluewater Creek and San Mateo Creek (Figure 1).

We found very few juvenile or larval California newt (n=3) however, adults and egg masses were detected in abundance in the Core Areas surveyed. This is likely due to survey timing. Precise abundances could not be calculated due to adaptations in the protocol, but we recorded egg masses in 56 segments (46.3%), and adults in 85 segments (70.2%). Among the 25 drainages surveyed, we recorded, at minimum, 371 egg masses across 17 drainages and 444 adults across 23 drainages. Twenty-three of the 25 drainages surveyed (92%) were occupied by California newt, including four new drainages that were surveyed in the Santa Ana Mountains; Bedford Wash, De Luz Creek-West Fork,

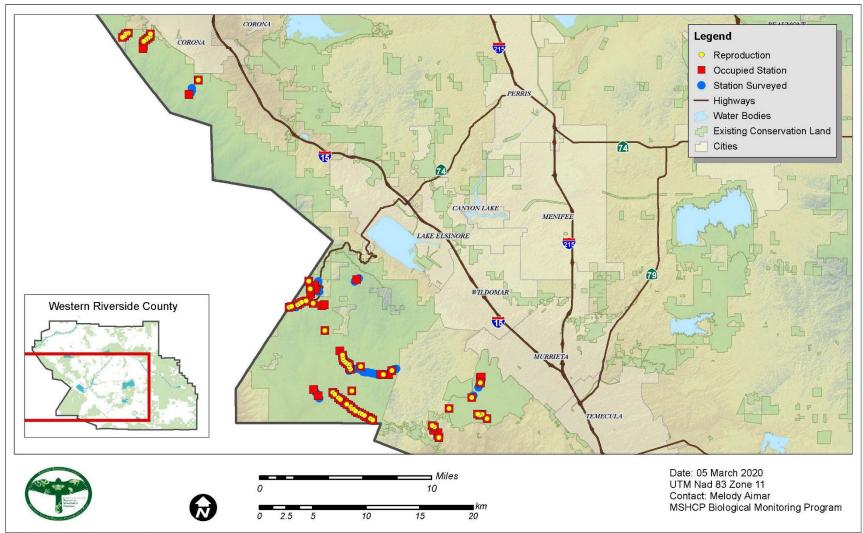


Figure 1. California newt 2020 survey results.

San Mateo Creek Trib 10a, and Murrieta Creek Trib 7 (Figure 1). All of these new drainages, except for Murrieta Creek Trib 7, contributed to new breeding grounds located this year and will be added to the baseline for future surveys (Table 1).

Table 1. Estimates of California newt, *Taricha torosa*, detected in 2020 Stream Surveys in reaches containing habitat. Streams containing an X indicate where adult presence was assumed based on the presence of egg masses or larvae.

Stream Name	Adult	Egg Mass	Juvenile	Larvae
Adobe Creek	22	14-23	0	0
Bedford Wash	6	5	0	0
Bluewater Creek	2	0	0	0
Cole Creek	8	3	0	0
Cole Creek Trib 6	X	2	0	0
De Luz Creek	3	7-11	0	0
De Luz Creek, W.Fork	165-202	71-99	0	0
Decker Creek	0	0	0	0
Hagador Creek	35	88-128	0	0
Long Creek (in Trabuco)	5	0	0	0
Los Alamos Canyon	0	0	0	0
Los Alamos Creek	6	3	1	0
Morrell Creek	1	0	0	0
Murrieta Creek Trib 7	5	0	0	0
San Juan Creek	27-30	8-11	0	0
San Juan Creek Trib 1	2	0	0	1
San Juan Creek Trib 2	X	3	0	0
San Juan Creek Trib 2a	3	0	0	0
San Juan Creek Trib 3	3	3	0	0
San Mateo Creek Trib 10	29-45	75-116	1	0
San Mateo Creek Trib 10a	4-10	2	0	0
San Mateo Creek Trib 9	31	30-43	0	0
Tenaja Creek	51-89	50-62	0	0
Tin Mine Creek	33-39	6	0	0
Wildhorse Creek	3	1	0	0
Grand Total	444-550	371-521	2	1

Other covered species recorded during stream surveys included southwestern pond turtle (formerly *Clemmys marmorata pallida;* currently *Actinemys pallida,* Spinks et al 2016; n = 2), granite spiny lizard (*Sceloporus orcutti*, n = 2), and arroyo toad (*Anaxyrus californicus*, n=51 tadpoles). We also detected five non-covered species during the surveys, including Baja California and California chorus frogs (*Pseudacris hypochondriaca* and *P. cadaverina* respectively) and two-striped garter snakes (*Thamnophis hammondi*, n=4; Appendix C). Invasive

aquatic species detected include Bullfrog (*Lithobates catesbeianus*). Invasive plants were found in most of the larger drainages such as Cole Creek and Morrell Creek, but smaller drainages had fewer invasive plants (Appendix D).

DISCUSSION

Our survey goals in 2020 focused on MSHCP species Objective 5; to maintain occupancy in 75% of the known occupied California newt habitat and determine if successful reproduction is occurring within Core Areas. Adult California newt were detected in 92% of 25 streams surveyed (n=23) and evidence of breeding was detected in 72% of those streams (n=18) in 2020. Two of the drainages that were considered as baseline locations in 2009 (Nickel Creek and Lucas Creek) were not surveyed in 2020 due to staff limitations. Although both of those locations were occupied with California newt in 2009 (Biological Monitoring Program 2009), occupation was not verified in 2020. Therefore, we calculate the occupied drainages as 23 of 27 (85%), exceeding the occupancy objective of 75%. Additionally, successful reproduction was documented as occurring within the MSHCP Conservation Area, which if our interpretation is correct, the reproductive metric in Objective 5 has been met in 2020. The Monitoring Program will continue to conduct stream surveys for California newt and hopefully add to the known habitat baseline, as determined necessary by Reserve Management Oversight Committee, but not less frequently than every 8 years.

California newt exhibit strong breeding site fidelity and are known to migrate long distances between breeding pools and aestivation and/or brumation refugia (Endler 1970, Trenham 1998). These traits, in conjunction with narrow breeding habitat conditions, result in variable population estimates year to year. Moreover, California newt survival is highly dependent on environmental factors, primarily precipitation, which may cause California newt populations to become critically endangered in response to sustained severe drought, but they could rebound if dry periods are considerably short (Jones et al. 2017). The Santa Ana Mountain Bioregion experienced an approximately 275-500% increase in precipitation for the month of April 2020 (NOAA 2021) as compared to previous years, which may have influenced the increase in California newt detections this year and in previous surveys. Therefore, these high California newt detections from this and previous surveys may be a result of optimal environmental conditions, and Refuge Managers should be cautious when using these findings to inform management decision. We suspect that the species will continue to thrive in protected watersheds within this Core Area, given sufficient rainfall, no major change to hydrology that would result in fewer medium and deep pools, and continued management of other threats to the species (e.g., habitat loss and alteration, wildfires, and aquatic invasive predators such as bullfrog and crayfish).

Recommendations for Future Surveys

Stream reaches should continue to be visually assessed for suitable habitat of California newt and followed by detailed stream surveys where suitable habitat exists (e.g., presence of water, or potential pooling). Reaches not surveyed in detail can be addressed at a later date, but initial efforts should be focused on describing habitat in the Plan Area most likely to be suitable for this species. More robust surveys that address detectability and percent area occupied can then be possible on reaches with relevant species-specific habitat values (Dudek & Associates 2003).

Species Objective 5 for California newt requires that occupancy be maintained on at least 75% of occupied newt habitat. We interpret this to mean that some baseline estimate of occupancy be measured across suitable habitat in the Core Area, and that species presence be maintained across 75% of that area. Distribution of California newt likely fluctuates with yearly precipitation and the availability of pooled water in drainages. It is therefore difficult to assess when a meaningful baseline measure of occupancy should be recorded. Moreover, much of the suitable California newt habitat in the Santa Ana Mountains cannot be realistically surveyed given issues of safe access. Future surveys should address Species Objective 5 by drawing inferences of occupancy from accessible California newt habitat, based on the accessibility model and documented habitat. Extrapolating estimates to areas that cannot be surveyed is inappropriate and can lead to unknown bias in the estimate. Therefore, the baseline for occupied California newt habitat may continue to increase until all accessible areas containing habitat in the Santa Ana Mountains have been identified and assessed for qualification as California newt habitat. Upon completion of this assessment, a relevant baseline can be determined and purposed to analyze all future survey efforts.

Remote and inaccessible areas will continue to present obstacles to future surveys. We should explore utilizing different strategies for accessing these areas. We should also continue to refine our accessibility model by attempting to access areas identified as inaccessible, given available field personnel. Streams not monitored in 2020 due to crew size constraints and access issues should take priority in future survey efforts and alternative access modes should be considered where appropriate.

Refuge managers should consider the removal of invasive aquatic species, such as bullfrog, crayfish, and predatory fish species from the watersheds in their respective areas of management. Invasive plants may also alter habitats for native species and should be controlled in these watersheds (Appendix D).

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APPENDIX A

2020 TATO Survey Data Sheet

Begin Survey:						2020 Stre	am Survey (DAY)	Oh		Entered by:
StationID (start v Creek Name	vaypoint)				Date Start Time				Observer 1 Observer 2		-
Site Photo	#				End Time						-
Weather: START: END:	Air Temp			km/h km/h	Sky	0=dear o	y conditions: r few clouds, 1= drizzle, 5=show	party cloudy overs or light ra	or variable, 2	=cloudy or overcast, 3= rain, 7=sleet or hail, 8=	=fog esnow
Water (taken wit Water Temp	℃	ntered):	End Survey % Wet Leng	: th (range)	51-75%, 76-100%, 1	Recent I Type (Inte	Disturbance: nsity): hiking(L,	M, H), fishii	ng(L, M, H).	trash(L, M, H), OR\	/ use(L, M, H), wading/bathing(L, M, s flow(L, M, H), other(L, M, H), none
Location #	es: UTM E (s	tarts 0.4.5)	I UTM N	(starts 3)	Species Code	Age	Abundance	Cue (V,A)		Photo#	Notes
ex. 1											
Non-covered Sp					Di1#			Neteri		i	
Species Code	Age	Abundance	Cue (V,A)		Photo #			Notes			
Age : egg mass, ha Abundance can be	atchling, tadpole, 2 an exact number	nd year tadpole or a range. Ran	, metamorph, ji ge values: 1, 2	ivenile, adult, -5, 6-10, 11-20	unknown), 21-30, 31-50, 51-1	100, 101-200,	201-500, 501-10	00, 1000+			
Invasive Plant S	Species	Size Class (few, small scat	ered patches,	large contiguous st	ands)	Invasive Plan	nt Species	Size Clas	SS (few, small scattered p	atches, large contiguous stands)

Western Riverside County MSHCP Biological Monitoring Program

NOTES:

APPENDIX B

Western Riverside County MSHCP Biological Monitoring Program Protocol for Reptile Tissue Sampling, March 2009

Tissue sampling has been shown to be a valuable component of scientific and genetic studies. Many genetic studies have revealed important results about local populations (Richmond, Jockusch 2007; Wood et al. 2008), and tissue sampling allows for analyses of population genetics to be conducted without killing individuals in the population. Reptiles generally recover quickly from injuries sustained during acquisition of a small tissue sample, and the resulting scars can be used to aid in recapture identification analysis. Scale clipping and taking tail tips rarely draws blood, and the application of a tissue adhesive (e.g., New Skin) will speed the healing process and stem any blood loss. The tissue adhesive should also help minimize the risk of bacterial infection, although this is a possible deleterious side-effect. Some species of lizards also readily shed their tails as a defense mechanism and although care will be taken to process all animals as quickly and carefully as possible it is likely that a small number of individuals will lose their tails during handling. Although there are some risks associated with tissue sampling, this method should have less impact on target populations than taking specimens for vouchering and still provide valuable monitoring data.

The protocol outlined below will be followed by Monitoring Program staff processing reptiles in the field. All current herpetological personnel were trained in taking tissue samples by a USGS biologist at the USGS office in San Diego on March 5, 2009 or trained by those who attended said training. Tissue samples were taken by all crew from dead specimens; however, a live specimen was used for demonstrating handling techniques while taking tissue samples. Future personnel will be trained by our crew on live specimens in the field. All tissue samples will be temporarily stored in refrigeration at the MSHCP's Biological Monitoring Office at 1835 Chicago Ave, Suite C, Riverside, CA, and then transported to the USGS Western Ecological Research Center's San Diego Field Office at 4165 Spruance Road, San Diego, CA for genetic analysis.

USGS TARGET SPECIES Processing Methods

- 1. Gender/Age
 - a) Male, female or unknown
- 2. Measurements
 - a) Using metric ruler
 - i. Snout-Vent length (mm)
 - ii. Tail length (mm)
 - b) Using Pesola scale
 - i. Weight (g): <u>tare scale first with sampling bag</u>, then place animal in bag.
 - 1. Use the smallest scale possible for the most accuracy.
- 3. Take tissue sample (y/n) (Do not take a sample if the animal is too small to safely do so)

- a. Label micro-centrifuge tubes with sample # [date, full board name(site#-board#),
 4-letter species code, and individual sequential # (ex. 20091125_MS12-02 EUSK 1)]
- b. Sterilize scissors with alcohol.
- c. For larger snakes: Take three ventral scale clips from the largest midbody scales, the three samples not from adjoining scales. The clip should be ~ 1 mm x ~ 3 mm, but try to clip all the way across each scale, and try to get some of the pigmentation of each scale.
- d. For small snakes and lizards: Snip ~3 mm of the tail tip with scissors into centrifuge tube.
 - i. Place a drop of tissue adhesive (New Skin) on cut, allow to air dry.
 - ii. Place micro-centrifuge tube in designated container in specimen freezer at the office.
- 4. Take photos (Optional except for Mt. Kingsnakes and Rubber Boa)
 - a. Minimum of 3 (1 dorsal, 1 ventral, 1 close-up of dorsal portion of head).
 - i. Place, in each photo, ruler and tape with date and specimen # (corresponding to order entered on datasheet).
 - ii. Label the photos with photo #s [date, photographer initials, and photo file number (ex. 20091125 SLP 362)].
- 5. Notes Record unusual morphology
 - a. Take notes on any unusual characteristics of the animal (e.g., coloration, injuries, regrown tail, etc.).
- 6. Return animal to exact location where found.

Non-Target Species Processing Methods (DO NOT PROCESS ANY VENOMOUS REPTILES!)

- 1. Gender/Age
 - a. Male, female or unknown
- 2. Measurements
 - a. Using metric ruler
 - i. Snout-Vent length (mm)
 - ii. Tail length (mm)
 - b. Using Pesola scale
 - i. Weight (g): tare scale first with sampling bag, then place animal in bag.
 - 1. Use the smallest scale possible for the most accuracy.
- 3. Take photos (optional)
 - i. Record photo #s on datasheet.
 - ii. Label the photos with photo #s [date, photographer initials, and photo file number (ex. 20091125 SLP 362)].
- 4. Return animal to exact location where found.

REFERENCES

Richmond JQ, Jockusch EL 2007. Body size evolution simultaneously creates and collapses species boundaries in a clade of scincid lizards. Proc. R. Soc. Lond. B. 274:1701–1708.

Wood DA, Fisher AN, Reeder TW 2008. Novel patterns of historical isolation, dispersal, and secondary contact across Baja California in the Rosy Boa (*Lichanura trivirgata*). Molecular Phylogenetics and Evolution.; 46:484–502.

APPENDIX C.Target Species Detected During 2020 Stream Surveys in Reaches with Historical California Newt Occurrence

Area Name	Common Name	Scientific Name	Covered
Adobe Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
	Southwestern pond turtle	Actinemys pallida	Yes
Bedford Wash	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
Bluewater Creek	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
Cole Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
	Western toad	Anaxyrus boreas	No
Cole Creek Trib 6	Baja California chorus frog	Pseudacris hypochondriaca	No
	California newt	Taricha torosa	Yes
	Two-striped garter snake	Thamnophis hammondii	No
De Luz Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	California newt	Taricha torosa	Yes
De Luz Creek, West Fork	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
Hagador Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
Long Creek (in Trabuco)	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
	Unidentified lizard	Squamata	No
Los Alamos Canyon	California chorus frog	Pseudacris cadaverina	No

Los Alamos Creek	Baja California chorus frog Bullfrog California chorus frog Chorus frog California newt Southwestern pond turtle Toad Two-striped garter snake Western toad	Pseudacris hypochondriaca Lithobates catesbeianus Pseudacris cadaverina Pseudacris Taricha torosa Actinemys pallida Anaxyrus Thamnophis hammondii Anaxyrus boreas	No No No No Yes Yes No No
Morrell Creek	Arroyo toad Baja California chorus frog California chorus frog California newt Frog or toad Western toad	Anaxyrus californicus Pseudacris hypochondriaca Pseudacris cadaverina Taricha torosa Anura Anaxyrus boreas	Yes No No Yes No No
Murrieta Creek Trib 7	Baja California chorus frog	Pseudacris hypochondriaca	No
	California newt	Taricha torosa	Yes
	Western toad	Anaxyrus boreas	No
San Juan Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	Bullfrog	Lithobates catesbeianus	No
	California chorus frog	Pseudacris cadaverina	No
	Chorus frog	Pseudacris	No
	California newt	Taricha torosa	Yes
San Juan Creek Trib 1	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
San Juan Creek Trib 2	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
San Juan Creek Trib 2a	Baja California chorus frog	Pseudacris hypochondriaca	No
	California newt	Taricha torosa	Yes
San Juan Creek Trib 3	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
San Mateo Creek Trib 10	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes

San Mateo Creek Trib 10a	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes
San Mateo Creek Trib 9	Baja California chorus frog	Pseudacris hypochondriaca	No
	California newt	Taricha torosa	Yes
Tenaja Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	Bullfrog	Lithobates catesbeianus	No
	California chorus frog	Pseudacris cadaverina	No
	Chorus frog	Pseudacris	No
	California newt	Taricha torosa	Yes
	Two-striped garter snake	Thamnophis hammondii	No
Tin Mine Creek	California newt	Taricha torosa	Yes
Wildhorse Creek	Baja California chorus frog	Pseudacris hypochondriaca	No
	California chorus frog	Pseudacris cadaverina	No
	California newt	Taricha torosa	Yes

APPENDIX D.Invasive Plant Species Detected During 2020 Stream Surveys

^{*} Indicates ambiguity of whether species recorded was native variety or non-native variety.

Area Name	Common Name	Scientific Name	Exotic
Adobe Creek	Blackberry	Rubus	*
	Indian sweet-clover	Melilotus indicus	Yes
	Pecan	Carya illinoinensis	Yes
	Plantain	Plantago	*
Bedford Wash	Brome	Bromus	Yes
	Erodium	Erodium	Yes
Cole Creek	Brome	Bromus	Yes
	Bur clover	Medicago	Yes
	Erodium	Erodium	Yes
	Indian sweet-clover	Melilotus indicus	Yes
	Italian thistle	Carduus pycnocephalus	Yes
	Lettuce	Lactuca	*
	Mustard	Brassica	Yes
	Plumeless thistle	Carduus	*
	Rabbit's foot grass	Polypogon	Yes
	Scarlet pimpernel	Anagallis arvensis	Yes
	Shortpod mustard	Brassica nigra	Yes
	Shortpod mustard	Hirschfeldia incana	Yes
	Sow-thistle	Sonchus	Yes
	Storksbill	Erodium cicutarium	Yes
	Sweetclover	Melilotus	Yes
	Toothed medick	Medicago polymorpha	Yes
	Vetch	Vicia	*
	Wild lettuce	Lactuca serriola	Yes
	Wild oats	Avena	Yes
	Winter vetch	Vicia villosa	Yes
	Yellow dock	Rumex crispus	Yes
De Luz Creek	Large periwinkel	Vinca major	Yes
	Winter vetch	Vicia villosa	Yes
De Luz Creek, West Fork	Erodium	Erodium	Yes

Hagador Creek	Erodium	Erodium	Yes
	Mustard	Brassica	Yes
	Sow-thistle	Sonchus	Yes
	Wild oats	Avena	Yes
	Winter vetch	Vicia villosa	Yes
Long Creek (in Trabuco)	Brome	Bromus	Yes
	Erodium	Erodium	Yes
	Hedge mustard	Sisymbrium	Yes
	Indian sweet-clover	Melilotus indicus	Yes
	Large periwinkel	Vinca major	Yes
	Scarlet pimpernel	Anagallis arvensis	Yes
	Sow-thistle	Sonchus	Yes
	Spanish broom	Spartium junceum	Yes
	Spiny-leaf sow-thistle	Sonchus asper	Yes
	Toothed medick	Medicago polymorpha	Yes
	Wild oats	Avena	Yes
	Winter vetch	Vicia villosa	Yes
	Woodsorrel	Oxalis	*
Los Alamos Creek	Indian sweet-clover	Melilotus indicus	Yes
	Scarlet pimpernel	Anagallis arvensis	Yes
Morrell Creek	Bur clover	Medicago	Yes
	Clover	Trifolium	*
	Erodium	Erodium	Yes
	Indian sweet-clover	Melilotus indicus	Yes
	Italian thistle	Carduus pycnocephalus	Yes
	Miner's lettuce	Montia	*
	Mustard	Brassica	Yes
	Rabbit's foot grass	Polypogon	Yes
	Scarlet pimpernel	Anagallis arvensis	Yes
	Sow-thistle	Sonchus	Yes
	Sweetclover	Melilotus	Yes
	Toothed medick	Medicago polymorpha	Yes
	Trefoil	Lotus	*
	Wildrye	Elymus	*
	Winter vetch	Vicia villosa	Yes

Murrieta Creek Trib 7	Mustard	Brassica	Yes
	Sweetclover	Melilotus	Yes
San Juan Creek	Bur clover	Medicago	Yes
	Dock species	Rumex	*
	Giant reed	Arundo donax	Yes
	Indian sweet-clover	Melilotus indicus	Yes
	Italian thistle	Carduus pycnocephalus	Yes
	Mustard	Brassica	Yes
	Rabbit's foot grass	Polypogon	Yes
	Scarlet pimpernel	Anagallis arvensis	Yes
	Sow-thistle	Sonchus	Yes
	Spanish broom	Spartium junceum	Yes
	Sweetclover	Melilotus	Yes
	Vetch	Vicia	*
	Washington fan palm	Washingtonia robusta	Yes
	Yellow dock	Rumex crispus	Yes
San Juan Creek Trib 1	Brome	Bromus	Yes
	Wild oats	Avena	Yes
San Juan Creek Trib 3	Bur clover	Medicago	Yes
	Indian sweet-clover	Melilotus indicus	Yes
	Plantain	Plantago	*
	Sow-thistle	Sonchus	Yes
Tin Mine Creek	Erodium	Erodium	Yes
	Giant reed	Arundo donax	Yes
	Mustard	Brassica	Yes
Wildhorse Creek	Indian sweet-clover	Melilotus indicus	Yes
	Sow-thistle	Sonchus	Yes