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

Artificial Cover Survey Report 2010

23 March 2011

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NOTE TO READER:

This report is an account of 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. The Monitoring Program monitors the distribution and status of the 146 Covered Species within the Conservation Area to provide information to Permittees, land managers, the public, and the Wildlife Agencies (i.e., the California Department of Fish and Game and the U.S. Fish and Wildlife Service). Monitoring Program activities are guided by the MSHCP species objectives for each Covered Species, the information needs identified in MSHCP Section 5.3 or elsewhere in the document, and the information needs of the Permittees.

MSHCP reserve assembly is ongoing and it is expected to take 20 or more years to assemble the final Conservation Area. The Conservation Area includes lands acquired for conservation 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 the Conservation Area as understood by the Monitoring Program at the time the surveys were planned and conducted.

We would like to thank and acknowledge the land managers in the MSHCP Plan Area, who in the interest of conservation and stewardship facilitate Monitoring Program activities on the lands for which they are responsible. A list of the lands where data collection activities were conducted in 2009 is included in Section 7.0 of the Western Riverside County Regional Conservation Authority (RCA) Annual Report to the Wildlife Agencies. Partnering organizations and individuals contributing data to our projects are acknowledged in the text of appropriate reports.

While we have made every effort to accurately represent our data and results, it should be recognized that data management and analysis are ongoing activities. Any reader 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.

The primary preparer of this report was the 2010 Herpetofauna Program Lead, Robert Packard. If there are any questions about the information provided in this report, please contact the Monitoring Program Administrator. If you have questions about the MSHCP, please contact 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

Many reptile species commonly use microhabitats under natural objects such as rocks and heavy vegetation. Placing artificial cover (A/C) objects in the vicinity of areas typically occupied by reptiles allows surveyors to increase opportunities for species detection without disturbing the natural habitat. The Western Riverside County MSHCP Biological Monitoring Program began using A/C survey methods in the fall of 2008 as part of targeted searches for covered reptile species.

Artificial cover survey efforts from 2008 to 2010 focused on 3 species (including 2 subspecies) of reptiles covered by the MSHCP. Target species were southern sagebrush lizard (*Sceloporus vandenburgianus*), southern rubber boa (*Charina umbratica*) and 2 subspecies of the mountain kingsnake: San Diego mountain kingsnake (*Lampropeltis zonata pulchra*) and San Bernardino mountain kingsnake (*Lampropeltis zonata parvirubra*). The species-specific conservation objectives for all 4 reptiles require the Biological Monitoring Program to document the continued use of 75 % of Core Areas listed in the MSHCP at least once every 8 years (Dudek & Associates 2003). Although target species have different distributions within the Plan Area, they share similar habitat preferences, making a combined survey effort more efficient.

All subspecies of the California mountain kingsnake have recently been eliminated from the taxonomic classification accepted in the scientific literature, and they are all now known as simply *Lampropeltis zonata* (Rodríguez-Robles et al. 1999, Collins and Taggart 2002). The 2 former subspecies have different Core Areas and habitats in the Plan Area, and will be monitored accordingly.

The 2010 A/C survey effort was an extension of surveys that began in the fall of 2008. The 2008 survey effort was limited and no report was written. The 2009 Artificial Cover Report can be found at the Western Riverside County Regional Conservation Authority website (http://www.wrc-rca.org/library.asp#id162). The data presented here reflect our survey effort from the fall of 2008 through the fall of 2010. Artificial cover surveys by Monitoring Program biologists from 2008 to 2010 focused on documenting target species presence within species-specific Core Areas in the Conservation Area. The following were the overall survey goals and objectives for 2008 to 2010:

Goals and Objectives

1. Document the presence of southern sagebrush lizard, San Diego mountain kingsnake, San Bernardino mountain kingsnake, and southern rubber boa within as many species-specific Core Areas as possible.

    a. Distribute A/C objects in appropriate habitat in target species Core Areas.

b. Check A/C on a regular schedule during optimal times for utilization by target species.

2. Qualitatively evaluate the effectiveness of A/C surveys to detect target species.

   a. Determine whether target species utilize A/C, which type of A/C (plywood or carpet) is preferred, and what environmental conditions produce the most target species observations.

3. Qualitatively evaluate the effectiveness of natural cover checks during A/C surveys to detect target species.

   a. Determine whether natural cover checks can increase detections of target species during A/C surveys.

4. Work in collaboration with the U.S. Geological Survey (USGS) to collect genetic material for an ongoing population study of reptiles in southern California (Appendix A).

   a. Retrieve tissue samples from USGS target species and transport to USGS for genetic analysis.

All target species are known to use rock outcrops and rock crevices as wintering hibernacula, and can often be found in these areas as they travel to and from them in spring and fall (Behler and King 1979, Lemm 2006). The species-specific conservation objectives, suitable habitat, and MSHCP-identified Core Areas for each species are briefly described below.

Southern Sagebrush Lizard (*Sceloporus vandenburgianus*)

The southern sagebrush lizard is not listed by the state or federal governments. It is usually found above 1500 m in open areas with sparse vegetation. It is primarily found in hardwood and conifer forests, woodlands, and juniper woodlands (Dudek & Associates 2003). The species objectives for southern sagebrush lizard require the conservation of 2 Core Areas within the Conservation Area: the San Jacinto Mountains and the Santa Rosa Mountains (Dudek & Associates 2003).

Southern Rubber Boa (*Charina umbratica*)

The southern rubber boa is a California Species of Special Concern. It is found above 1500 m in deciduous and coniferous forests, chaparral, and grasslands. It is usually found under debris or in rock piles and rock crevices (Dudek & Associates 2003). This species has been described as secretive and very difficult to observe (Hoyer and Stewart 2000). The species objectives for southern rubber boa require the conservation of 1 Core Area within the Conservation Area: the San Jacinto Mountains (Dudek & Associates 2003).

San Diego Mountain Kingsnake (*Lampropeltis zonata*)

The San Diego mountain kingsnake is a California Species of Special Concern. In the Plan Area it is expected to occur within the Santa Ana Mountains, Agua Tibia Mountains, and Desert Transition Bioregion near Beauty Mountain and Tule Peak, from 500 to 5500 m in elevation (Fisher and Case 1999, Hubbs 2004). The San Diego mountain kingsnake is restricted to rock outcrops, talus, and steep shady canyons within coniferous and mixed coniferous, hardwood, or riparian woodlands and other edge habitats when associated with coniferous habitat (Hubbs 2004). The species objectives for San Diego mountain kingsnake require the conservation of 3 Core Areas within the Conservation Area: the Santa Ana Mountains, Agua Tibia Mountains, and Desert Transition Bioregion (Dudek & Associates 2003).

San Bernardino Mountain Kingsnake (*Lampropeltis zonata*)

The San Bernardino mountain kingsnake is also a California Species of Special Concern. Within the Plan Area, San Bernardino mountain kingsnake is found only in the San Bernardino and San Jacinto Mountains above 1500 m (Fisher and Case 1999). The San Bernardino mountain kingsnake occurs in rocky areas, talus slopes, and deep shaded canyons in conifer and mixed conifer, hardwood, or riparian forest and edge habitats (Dudek & Associates 2003). They use the rocks and crevices for refuge, basking, hibernating and foraging, and as oviposition sites (Jennings and Hayes 1994, Holland and Goodman 1998). Downed logs, usually of large conifers, are also an important resource in many areas (Holland and Goodman 1998). The species objectives for San Bernardino mountain kingsnake require the conservation of 2 Core Areas within the Conservation Area: the San Jacinto Mountains and the San Bernardino Mountains (Dudek & Associates 2003).

METHODS

Protocol Development

The Biological Monitoring Program protocol for artificial cover surveys was developed by the Herpetofauna Program Lead, Robert Packard (Appendix B). Artificial cover has only been in use by researchers as a method for surveying for reptiles and/or amphibians since the 1980s (Heyer et al. 1994). Moreover, most of the studies using A/C have either been used for detecting amphibians, or placed in very different habitats than those found in southern California. Our survey protocol was developed using current literature and with extensive assistance from Richard Hoyer, an independent herpetologist, who has had considerable success using A/C (carpet and plywood) to detect southern rubber boa and San Bernardino mountain kingsnake in and around rock outcrops in the San Bernardino Mountains and in Oregon (Hoyer 2007, Hoyer and Stewart 2000). Sites for A/C placement were not selected randomly, due to the localized nature of suitable habitat and the difficulty accessing remote areas while hauling artificial-cover objects. Rather, survey locations were selected from within Core Areas

based on: known species-habitat associations; balancing the logistical difficulties of transporting A/C objects far from existing trails; and weighing the threat of vandalism or unauthorized use. The protocol focused on documenting the presence of targeted Covered Species in their respective Core Areas. A summary of survey methods is provided below.

Personnel and Training

Crew members were trained by the Herpetofauna Program Lead on survey techniques as well as species identification. Monitoring Program personnel were funded by the California Department of Fish and Game or the Regional Conservation Authority; volunteers are noted. The following Monitoring Program biologists and volunteers conducted artificial cover surveys in 2008, 2009, or 2010:

- Robert Packard (Herpetofauna Program Lead, Biological Monitoring Program)
- Adam Malisch (Biological Monitoring Program)
- Ana Hernandez (Biological Monitoring Program)
- Ariana Malone (Biological Monitoring Program)
- Ashley Ragsdale (Biological Monitoring Program)
- Betsy Dionne (Biological Monitoring Program)
- Dustin McLain (Volunteer, Riverside County Parks)
- Elise Hinger (Biological Monitoring Program)
- Esperanza Sandoval (Biological Monitoring Program)
- Joanna Gibson (Biological Monitoring Program)
- Jonathan Reinig (Biological Monitoring Program)
- Joseph Sherrock (Biological Monitoring Program)
- Julie Golla (Biological Monitoring Program)
- Laura Magee (Biological Monitoring Program)
- Lauren Ross (Biological Monitoring Program)
- Lynn Miller (Biological Monitoring Program)
- Masanori Abe (Biological Monitoring Program)
- Melody Aimar (Volunteer, Santa Ana Watershed Association)
- Michael Robinson (Biological Monitoring Program)
- Michael Zerwekh (Biological Monitoring Program)
- Misty Gray (Biological Monitoring Program)
- Nate Zalik (Biological Monitoring Program)
- Nicholas Peterson (Biological Monitoring Program)
- Nicole Housel (Volunteer, Santa Ana Watershed Association)
- Nydia Celis (Biological Monitoring Program)
- Rika Setsuda (Biological Monitoring Program)
- Ryann Loomis (Biological Monitoring Program)
- Samantha Treu (Biological Monitoring Program)
- Sloane Seferyn (Biological Monitoring Program)
- Tara Graham (Biological Monitoring Program)

Study Design

We used Google Earth imagery to identify rock outcrops and appropriate habitat in Core Areas that could be safely and/or practicably accessed (San Bernardino National Forest, Cleveland National Forest, Iron Springs, Santa Ana Mountains, Agua Tibia Mountains, and Santa Margarita Ecological Reserve) (Figure 1). We selected artificial cover sites in the field by identifying rock outcrops that were at least 100 m away from the nearest road or trail, at least 20 m by 20 m in size, and in at least 1 of the target species' Core Areas within the existing MSHCP Conservation Area. Sites with a south-facing aspect and sites located near or above riparian areas were selected preferentially. Each site was selected to have exposure to the sun for a good portion of the day, preferably in the morning hours. Sites were chosen that would not be too difficult to access due to steep terrain or dense vegetation.

We installed A/C in the San Jacinto Mountain/Banning Bench/Iron Springs/Santa Margarita Ecological Reserve sites (AREA 1) between 11 August and 27 October 2008. We installed A/C in Cleveland National Forest sites (AREA 2) between 8 October and 26 October 2009 (Figure 1). We were not allowed to install A/C in the Wilderness Areas of Cleveland National Forest. A/C survey areas were generally sampled for approximately 16-18 months as a way to balance a potential increase in detecting species, due in part to leaving A/C objects in place for extended periods of time, with the need to rotate survey areas.. We removed all artificial cover from AREA 1 between 29 April 2010 and 2 June 2010, except for the cover at Iron Springs, which will remain in place until the spring of 2011. The Iron Springs site will be left in place because it is in the San Diego mountain kingsnake Core Area, and there are no objections from Bureau of Land Management managers to leaving it in place.

Monitoring Program biologists distributed 4 pieces of 2 ft x 4 ft plywood and 4 pieces of 2 ft x 4 ft carpeting at each selected survey site, spread out on and around selected rock outcrops. The location of each piece of board or carpet was recorded with a GPS unit.

We placed plywood cover on soft substrate along the edges or in the middle of the outcrop. We chose locations that were fairly level and inconspicuous, but with some sun exposure. We placed plywood sheets on the soft substrate near selected outcrops, leaving a small gap underneath, but with at least 3 sides covered with soil or leaf litter. We weighed down the plywood sheets with rock to keep them from moving during high winds. Each board was numbered from 1 to 4 at each site and labeled with the unique site code.

For the more malleable carpet cover, we chose locations on the rock outcrops themselves, on thin soil, or on low rocks with some sun exposure. We placed the carpet so that it was uneven enough to allow snakes to enter, or placed a few rocks or sticks underneath to facilitate use by target species. We weighed down the carpet with a rock at each corner to keep the carpet from blowing away or flapping in the wind. Each piece of

carpet was numbered from 5 to 8 and labeled with the unique site code. We took at least 1 photo at each A/C site, oriented to characterize the rock outcrop as well as possible.

Survey Methods

At least 2 surveyors checked artificial-cover stations bi-weekly, beginning at least 14 days after the initial set-up of the A/C. We visited sites on a 14-day rotation to allow animals to reacclimatize to cover after being disturbed. Surveys were conducted between 0813 h and 1607 h when there was no precipitation and temperatures were deemed warm enough to allow for thermoregulation (generally between 10 and 20 degrees C for these species) (Stebbins 1985, Hubbs 2004). Survey time per site varied from 7 to 78 min ($\bar{x}$ = 23.0 min), depending on site characteristics (e.g., number of rocks on outcrop) and presence and abundance of animals detected and/or tissue sampled.

Upon arriving at a given survey site, surveyors recorded the date, observers, location, A/C object number, percent sunlight falling on each A/C object, and general weather description. In addition, surveyors noted the ambient air temperature and average wind speed at the start and end of each survey. We attempted to identify all reptile species observed under A/C and recorded observations for each individual of any Covered Species encountered.

We conducted A/C checks in AREA 1 twice between 3 November 2008 and 12 January 2009, 4 times between 9 January and 27 May 2009, twice between 27 October and 16 December 2009, and 3 times between 22 March and 2 June 2010. All A/C was removed from AREA 1 in 2010 except at Iron Springs, which will remain in place until the spring of 2011 (Table 1).

In AREA 2 we conducted checks twice between 16 November and 15 December 2009. In 2010, after adding natural cover checks, we conducted surveys 3 times between 22 March and 2 June, and 4 times between 21 October and 27 December (Table 1). Surveys in the fall of 2010 also included checks at the Iron Springs location. We did not check any stations in the summer, as temperatures are generally too high for target species to use A/C for thermoregulation. We will continue to conduct surveys in AREA 2 and Iron Springs until late spring of 2011.

In 2010 we modified the protocol to include checking natural cover in the vicinity of A/C sampling stations, due to the limited success of detecting target species with A/C in previous seasons. Beginning in the spring of 2010, we checked under all natural cover at A/C sites, carefully lifting and replacing any easily movable rocks, logs, or trash. We also checked crevices in rocks or logs with hand-held mirrors or flashlights.

Additionally, we conducted focused searches for target species in the Banning Bench area and the Santa Ana Mountains when time and personnel were available in 2010. We conducted 2 searches in the Banning Bench area on 13 September and 1 in the Santa Ana Mountains on 13 November. These surveys consisted of searching natural

cover and attempting to opportunistically locate target species within areas with apparently suitable habitat.

Table 1. Survey areas, number of sites per area, and number of survey checks per year.

Survey locations	Number of A/C sites	Number of checks in 2008	Number of checks in 2009	Number of checks in 2010
AREA 1				
Fern Valley	4	2	6	3
May Valley	1	2	6	3
Black Mountain	2	2	6	3
Dark Canyon	2	2	6	3
Thomas Mountain	2	2	6	3
Thousand Trails	4	2	6	3
Suicide Rock	1	2	6	3
Banning Bench	4	2	6	3
Iron Springs	2	2	6	7
Santa Margarita Ecological Res.	4	2	6	3
AREA 1 total	26	20	60	34
AREA 2				
Indian Truck Trail	8	N/A	2	7
Ortega Highway	6	N/A	2	7
Agua Tibia	2	N/A	2	7
AREA 2 total	16	N/A	6	21

We collected a small tissue sample from each USGS target species captured, and delivered these samples to the USGS Western Ecological Research Center in San Diego in collaboration with their ongoing study of reptiles in southern California (Appendix A). Detailed survey methods can be found in *Western Riverside County MSHCP Biological Monitoring Program Protocol for Artificial Cover Check Protocol* (Appendix B).

RESULTS

We checked 336 independent A/C objects between 9 to 11 times each from the fall of 2008 to the winter of 2010 (Table 1). We detected 134 individual reptiles of 13 different species and 5 individual amphibians of 2 species (Appendix C). We found that 57.5% of these detections were under plywood and 42.5% were under carpeting. Additionally, 57% of detections were under cover with 100% sun exposure, 14 % with 75% exposure, 8% with 25% exposure, and 22% with 0% exposure. The sun exposure percentages exclude amphibians, which we assume use the cover as shelter rather than for thermoregulation.

Beginning in the spring of 2010 we added natural cover checks at each A/C site. These checks resulted in 63 detections of 11 reptile species and 1 amphibian species (Appendix C). This addition resulted in slightly more time per survey, with an average of 28 min.

The only target species we have found under artificial cover thus far is the southern sagebrush lizard. All sagebrush lizards found under cover were in the San Jacinto Mountains, with none being found in their other Core Area, the Santa Rosa Mountains (Figure 2). We did not find any mountain kingsnake of either subspecies or southern rubber boa under our A/C in 2008, 2009, or 2010. We did detect 2 San Bernardino mountain kingsnakes under natural cover or in crevices in 2010.

No target species were observed during the limited focal searches conducted in the Banning Bench area and the Santa Ana Mountains (Figure 1).

Incidental observations were reported at 3 Core Areas (Figure 1) Two San Bernardino mountain kingsnakes were incidentally observed in the San Jacinto Mountains (San Bernardino National Forest) by Bureau of Land Management employee James Gannon in 2009 (personal communication). One of these snakes was found under artificial cover. This A/C was plywood, was not marked in any way, and it is unknown who placed it in the National Forest. These boards appear to be considerably older than the A/C distributed by Monitoring Program biologists in 2008 and 2009. We have checked these boards periodically since their initial discovery, but have not found any reptiles underneath them as of December 2010. One San Bernardino mountain kingsnake was found by U.S. Forest Service employees Kathie Meyer and Kim Boss (personal communicaton) in the San Bernardino Mountains Core Area in 2010. These observations of mountain kingsnake occurred from 1745 m to 2009 m in elevation. One southern rubber boa was found in Fuller Mill Creek in the San Jacinto Mountains at 2087 m by a USGS mountain yellow-legged frog survey crew in 2007. A Monitoring Program employee was also present during this observation.

We have collected tissue samples from 19 individuals of 5 species during A/C surveys from 2008 to 2010 and delivered these samples to USGS in support of their ongoing population genetics study of reptiles in southern California (Appendix A).

DISCUSSION

Artificial cover surveys are a simple technique for detecting secretive and/or nocturnal animals that spend the majority of their time on the surface of the ground. Because the target species' monitoring objectives only require documentation of presence within listed Core Areas, these initial surveys did not employ more complex designs such as time-constrained sampling (Crump and Scott 1994) or repeat visits to the same sites in an occupancy framework (MacKenzie et al. 2006).

Artificial cover surveys from 2008 to 2010 have detected few of our target species. The 2 target species detected during these surveys were southern sagebrush lizard and San Bernardino mountain kingsnake, both in the San Jacinto Mountains. Both mountain kingsnakes were found while checking natural cover, and not under A/C, 1 under a large rock at Dark Canyon, and 1 in a rock crevice in Fern Valley. Totaling all reptile species, we found 134 individuals of 8 species, and 5 amphibians of 2 species (Appendix C). As discussed below, the time elapsed between distributing and checking artificial cover can be a significant factor affecting the success of finding animals under A/C (Grant et al.1992, Monti et al. 2000).

We detected southern sagebrush lizard in the San Jacinto Mountains Core Area but not the Santa Rosa Mountains Core Area (Figure 2). Southern sagebrush lizards were also documented via incidental observation in the San Jacinto Mountains at elevations from 944 m to 2068 m. In contrast, portions of the Santa Rosa Mountains that occur within the Plan Area (i.e., eastern end of Existing Core L) consist of desert transition habitats that mostly lie below the stated elevation minimum of 1524 m for this species (Dudek & Associates 2003). The A/C in this area (Iron Springs) is well below this elevation (1358-1458 m). Lemm (2006) states that southern sagebrush lizard is found from 150 m to 3200 m, but we have no records of the species below 944 m. Furthermore, USGS reptile sampling data in the Plan Area includes no record of southern sagebrush lizard below 1524 m (Fisher and Case 1999), and MSHCP historical records are all in the San Bernardino National Forest above 1676 m. The historical locations reported in the MSHCP species account for this species are also all at least 12 km from Existing Core L. We therefore recommend eliminating the Santa Rosa Mountains (Core L) as a listed Core Area for this species.

The incidental observations and survey detections for San Bernardino mountain kingsnake minimally fulfill the species objective for this species for the current reporting period in both Core Areas. The southern rubber boa observation occurred in the only listed Core Area, and this also minimally fulfills the species objective for this species for the current reporting period.

There were several problems with the artificial cover, especially the carpeting. Often, due to the strong winds in southern California, the carpet would get blown away or folded over. Also on a few occasions mice chewed up significant portions of the carpet. Some of the carpet deteriorated in the sun, and some was dragged around by animals. For both types of cover, there were occasions when GPS reception was poor and field crew members could not relocate A/C objects, or the A/C was covered in snow, frozen to the ground, or moved by hikers. On 1 occasion a logging operation was started at an A/C site resulting in some of the cover being buried under tree branches. For plywood cover these issues were only a minor problem, but for carpeting it was a significant issue, as any carpeting that was blown around or folded over would fail to offer the desired microhabitat to make them attractive to reptiles. It is difficult to judge whether or not these issues contributed to the plywood A/C returning more species

detections than the carpet (57.5% vs. 42.5% of total observations, respectively). However, for whatever reason, the plywood has been the superior A/C material during this effort. We also learned that A/C objects placed in full sun were more efficient at producing species observations than those in partial sun or shade.

Although it is also difficult to quantify the effort expended during natural-cover checks, this method seemed more efficient than using A/C, especially considering that there is no time spent distributing natural cover, while distributing A/C objects takes considerable time and personnel. Checking natural cover must be done with extreme care not to damage the habitats of target and non-target species but at the very least it is a worthwhile addition to checking A/C at survey areas.

Recommendations for Future Surveys

Results from conducting artificial cover surveys from 2008 – 2010 have not proven this method to be an efficient means of detecting target species, except sagebrushy lizard. Barring significantly improved results in the near future (spring of 2011), additional survey methods may be necessary to supplement the minimal data gathered thus far through A/C and incidental species observations. Because it can take up to a year or more for snakes to find and utilize artificial cover objects as thermoregulation sites (Grant et al.1992, Monti et al. 2000) we may have needed more years of data before a complete assessment of the use of A/C in detecting target species was possible. However, the disappointingly scant results to date, along with the need to survey such a large area (app. 67,000 acres) with limited personnel necessitated rotating sampling locations after approximately 1.5 years of sampling each survey area. Ultimately, there may be no truly efficient means to reliably detect these target snake species as they are highly secretive and not typically found in high numbers. We will continue to stress the importance of collecting incidental observations both from Biological Monitoring Program biologists and from partnering agencies as conducting focused surveys for these species returns little data per unit effort. A significant increase in the number of A/C sites and/or number of A/C objects per site may be considered in the future, along with continuing to check natural cover at and outside of survey sites.

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Appendix A. 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, Fisher, Reeder 2007), 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 4500 Glenwood Drive, 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

Target Species include: Gilbert's skink (*Plestiodon gilberti*), western skink (*P. skiltonianus*), rosy boa (*Lichanura trivirgata*), southern rubber boa (*Charina umbratica*), glossy snake (*Arizona occidentalis*), shovel-nosed snake (*Chionactis occipitalis*), San Diego mt. kingsnake (*Lampropeltis zonata pulchra*), San Bernardino mt. kingsnake (*L. z. parvirubra*), red coachwhip (*Masticophis flagellum*), striped whipsnake (*M. lateralis*), red-sided garter snake (*Thamnophis sirtalis infernalis*), two-striped garter snake (*T. hammondii*), southwestern blind snake (*Leptotyphlops humilis humilis*) San Diego banded gecko (*Coleonyx variegatus abbotti*), western banded gecko (*C. v. variegatus*), granite night lizard (*Xantusia henshawi henshawi*), and sagebrush lizard (*Sceloporus vandenburgianus*).

1. Gender/Age
    - Male, female or unknown
2. Measurements
    - Using metric ruler
        i. Snout-Vent length (mm)
        ii. Tail length (mm)
    - 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 tissue sample (y/n) (Do not take a sample if the animal is too small to safely do so)
        i. 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)]
    - Sterilize scissors with alcohol.
    - 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.
    - For small snakes and lizards: Snip ~3 mm of the tail tip with scissors into centrifuge tube.

      Place drop of tissue adhesive (New Skin) on cut, allow to air dry. Place micro-centrifuge tube in designated container in specimen freezer at the office.
4. Take photos (Optional except for Mt. Kingsnakes and Rubber Boa)
    - 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
    - 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

   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 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 B. Western Riverside County MSHCP Biological Monitoring Program Artificial Cover Check Protocol 2010.

Goals

To locate southern sagebrush lizard (*Sceloporus vandenburgianus*), southern rubber boa (*Charina umbratica*), San Bernardino mt. kingsnake (*Lampropeltis zonata parvirubra*) and San Diego mt. kingsnake (*Lampropeltis zonata pulchra*) in their respective Core Areas and other areas of suitable habitat in the MSHCP Conservation Area.

Procedure

Artificial cover should be checked in the spring and fall when the temperature is between 10 and 20 °C (50-65 deg. F) and when there is minimal snow cover. First find or print the appropriate maps and download the GPS points of the artificial cover objects. When within 100 m of the site, record the sky code, wind, snow cover, and temperature. When approaching the site, try to be as quiet as possible, and keep an eye on the artificial cover as you approach to observe any animals escaping. Sites must be checked with two people. One person should slowly lift the cover object while the other is observing what is underneath. Be very careful not to leave any part of your body exposed to any rattlesnakes, etc. that may be lurking underneath. If any lizards are present, try to capture them before they escape for positive identification, but only after determining that no snakes are under the cover. After checking all of the artificial cover, start checking all natural cover on the outcropping that can be turned over safely and still be allowed to return to its original state. We will look under each piece of natural cover that occurs along transects and can be manipulated without permanently altering the ability of the object to provide refuge. Animals will be captured and identified in hand whenever possible, and we will record the same information as taken for MSHCP Covered Species and USGS Target Species found under carpet pieces at artificial-cover stations. We will also record a waypoint with Garmin GPS units for each MSHCP Covered Species and USGS Target Species. We will survey natural-cover transects concurrently with artificial-cover stations, and adhere to the following rules when lifting natural cover:

- Objects will be lifted by tilting a portion off of the ground, and replaced in their exact position.
- Any object occurring in a position that will not allow for it to be returned to its original position (e.g., steep slope, possible backfilling of dirt) will not be checked.
- No rock slabs or flakes will be forced off of larger rocks.
- Cover objects will be elevated for only as long as it takes to identify presence of animals and identification of species.

- Any animals encountered should be released in a way that minimizes stress and/or injury to them.
- If the animal was in a natural cavity under the cover, then it will be returned to the natural cavity, and the cover object should be gently returned to its original position. If no obvious cavity exists, the the cover object should be returned to its original position and the animal released next to it.

All reptiles, amphibians, and mammals should be recorded, along with their age and sex, if known. They will be released after positive identification is made. If it is an unusual animal, take a photograph. Any skins or dead reptiles found under the cover should be put in a plastic bag, labeled, and brought back to the office for identification. A record must be made for each cover object. Make sure to record "None" for all cover with no animals underneath. Record all herps seen in the area and during the hike to and from the site under "Notes". Take a photograph of any unusual specimens and put them at Projects\Herps\ArtificialCover\MiscPhotos. Label the photos with photo #s [date, photographer initials, and photo file number (ex. 20101125_JMG_362)].

If any target species is found under the artificial cover, they must be captured and processed. These species should have weight, snout to vent length, tail length, sex, age and any irregularities recorded. For larger snakes take 3 ventral scale clips from the largest midbody scales, the 3 samples not from adjoining scales. Each 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. For small snakes and lizards snip ~3 mm of the tail tip with scissors into a centrifuge tube (For a full target species list and detailed processing checklist, see the Artificial Cover Processing Methods below).

Samples should be labeled with the same unique identifier as the photographs described above. Scissors should be wiped with ethanol afterwards. Photographs of each animal should be taken of the head and dorsal and ventral surfaces. Label photos as above. After processing the animal should be released unharmed exactly where it was found.

Equipment

- Datasheets
- Maps
- Access permits
- Micro-centrifuge tubes with ethanol
- Surgical scissors
- New Skin adhesive
- GPS unit with points downloaded
- Extra batteries
- Pen or pencil
- Camera
- Kestrel
- Gloves
- Water bottle
- Metric ruler
- Sampling bags
- Snake stick
- Snake gaiters (optional)
- Field guides

Appendix C. Species detected at artificial cover stations from 2008 through 2010. (Target species in red) (MSHCP Covered Species in blue).

Site	Common Name	Scientific Name	Totals for 2008-2010	2008	2009	2010
Fern Valley (4 arrays)	Side-blotched lizard	*Uta stansburiana*	2	1	1	0
	Western fence lizard	*Sceloporus occidentalis*	3	0	1	2
	Southern sagebrush lizard	*Sceloporus vandenburgianus*	16	0	5	11
	Unknown lizard		2	0	0	2
	San Diego alligator lizard	*Elgaria multicarinata webbii*	1	0	0	1
	Gilbert's skink	*Plestiodon gilberti*	1	0	0	1
	Western skink	*Plestiodon skiltonianus*	1	0	0	1
	San Bernardino mountain kingsnake	*Lampropeltis zonata parvirubra*	1	0	0	1
	Southern Pacific rattlesnake	*Crotalus helleri*	3	0	0	3
May Valley (1 array)	Side-blotched lizard	*Uta stansburiana*	11	1	2	8
	Unknown lizard		2	0	0	2
	Western fence lizard	*Sceloporus occidentalis*	5	0	0	5

Appendix C cont.

Site	Common Name	Scientific Name	Totals for 2008-2010	2008	2009	2010
Black Mountain (2 arrays)	Western fence lizard	*Sceloporus occidentalis*	4	0	2	2
	Western skink	*Plestiodon skiltonianus*	2	0	1	1
	Unknown lizard		1	0	0	1
	Southern sagebrush lizard	*Sceloporus vandenburgianus*	6	0	0	6
	Gilbert's skink	*Plestiodon gilberti*	1	0	0	1
	Granite night lizard	*Xantusia henshawi*	5	0	0	5
	Southern Pacific rattlesnake	*Crotalus helleri*	1	0	0	1
Dark Canyon (2 sites)	Granite spiny lizard	*Sceloporus orcutti*	1	0	1	0
	Southern sagebrush lizard	*Sceloporus vandenburgianus*	3	0	0	3
	Granite night lizard	*Xantusia henshawi*	1	0	0	1
	San Bernardino mountain kingsnake	*Lampropeltis zonata parvirubra*	1	0	0	1
Thomas Mountain (2 sites)	Western fence lizard	*Sceloporus occidentalis*	8	0	1	7
	Side-blotched lizard	*Uta stansburiana*	2	0	1	1
	Western skink	*Plestiodon skiltonianus*	2	0	1	1
	Ringneck snake	*Diadophis punctatus*	2	0	1	1
	Unknown lizard		1	0	0	1
	San Diego alligator lizard	*Elagaria multicarinata webbii*	5	0	0	5
	Coast horned lizard	*Phrynosoma blainvillii*	1	0	0	1
	Granite spiny lizard	*Sceloporus orcutti*	1	0	0	1
	Gopher snake	*Pituophis catenifer*	1	0	0	1
	Two-striped garter snake	*Thamnophis hammondi*	1	0	0	1

Appendix C cont.

Site	Common Name	Scientific Name	Totals for 2008-2010	2008	2009	2010
Thousand Trails (4 sites)	San Diego alligator lizard	*Elagaria multicarinata webbii*	4	0	1	3
	Southern sagebrush lizard	*Sceloporus vandenburgianus*	6	0	2	4
	Western skink	*Plestiodon skiltonianus*	2	0	1	1
	Side-blotched lizard	*Uta stansburiana*	1	0	0	1
	Unknown lizard		1	0	0	1
Suicide Rock (1 site)	Side-blotched lizard	*Uta stansburiana*	2	0	1	1
	Gilbert's skink	*Plestiodon gilberti*	1	0	0	1
	Western skink	*Plestiodon skiltonianus*	1	0	0	1
Banning Canyon (4 sites)	San Diego alligator lizard	*Elagaria multicarinata webbii*	2	1	0	1
	Western fence lizard	*Sceloporus occidentalis*	4	0	0	4
	Gilbert's skink	*Plestiodon gilberti*	1	0	0	1
Indian Truck Trail (8 sites)	Blackbelly slender salamander	*Batrachoseps nigriventris*	1	-	0	1
	Granite spiny lizard	*Sceloporus orcutti*	4	-	1	3
	San Diego alligator lizard	*Elagaria multicarinata webbii*	2	-	0	2
	Western fence lizard	*Sceloporus occidentalis*	6	-	0	6
	Western skink	*Plestiodon skiltonianus*	2	-	0	2
	Side-blotched lizard	*Uta stansburiana*	4	-	0	4
	Speckled rattlesnake	*Crotalus mitchellii*	2	-	0	2
	Unknown lizard		1	-	0	1

Appendix C cont.

Site	Common Name	Scientific Name	Totals for 2008-2010	2008	2009	2010
Ortega Highway (6 sites)	California chorus frog	*Pseudacris cadaverina*	1	-	0	1
	Slender salamander	*Batrachoseps sp.*	1	-	0	1
	Unknown lizard		1	-	0	1
	San Diego alligator lizard	*Elagaria multicarinata*	2	-	0	2
	Western fence lizard	*Sceloporus occidentalis*	4	-	0	4
	Granite spiny lizard	*Sceloporus orcutti*	2	-	0	2
	Western skink	*Plestiodon skiltonianus*	3	-	0	3
	Speckled rattlesnake	*Crotalus mitchellii*	2	-	0	2
	Ringneck snake	*Diadophis punctatus*	1	-	0	1
	Two-striped garter snake	*Thamnophis hammondi*	1	-	0	1
Agua Tibia (2 sites)	Side-blotched lizard	*Uta stansburiana*	4	-	1	3
	Unknown lizard		2	-	0	2
	Western skink	*Plestiodon skiltonianus*	2	-	0	2
	Unknown whiptail sp.	*Aspicocelis sp.*	1	-	0	1
	Orangethroat Whiptial	*Aspicocelis hyperthra*	1	-	0	1
	Speckled rattlesnake	*Crotalus mitchellii*	1	-	0	1