STUDY DESCRIPTION AR-6
Special-status Amphibian and Aquatic Reptile eDNA Sampling
~~*August 20, 2026*~~*November 3, 2026*

This Study Description is developed to address information gaps identified by Calaveras County Water District (CCWD) for the relicensing of its North Fork Stanislaus River Hydroelectric Project (Project). [1]

Five special-status amphibian species and one special-status aquatic reptile species have the potential to occur within the proposed Federal Energy Regulatory Commission (FERC) Project Boundary as identified in the Pre-Application Document: southern long-toed salamander (*Ambystoma macrodactylum sigillatum*), Yosemite toad (*Anaxyrus canorus*), foothill yellow-legged frog (*Rana boylii*; South Sierra Distinct Population Segment), California red-legged frog (*Rana draytonii*), Sierra Nevada yellow-legged frog (*Rana sierrae*), and northwestern pond turtle (*Actinemys marmorata*).

This study will use environmental deoxyribonucleic acid (eDNA) sampling to detect special-status amphibian and aquatic reptiles within Project reservoirs and Project-affected reaches. This study will be conducted alongside Study AR-7 (*ESA-Listed Amphibian and Aquatic Reptile Visual Encounter Surveys)* and is intended to supplement the Visual Encounter Survey (VES) results.

STUDY AREA

The Study Area includes safely accessible areas within 0.25 mile of the FERC Project Boundary, which includes Project impoundments (i.e., North Fork Diversion Dam impoundment, New Spicer Meadow Reservoir, Beaver Creek Diversion Dam impoundment, and McKays Point Reservoir impoundment). The Study Area also includes stream reaches downstream of Project dams or other release points that have the potential to be affected by Project activities, including Highland Creek, North Fork Stanislaus River, Beaver Creek, and Stanislaus River. The elevation range of this area is approximately 6,700 feet down to 1,100 feet above mean sea level.

Sampling Locations

eDNA sampling locations are consistent with Study AR-7 and described in Table 1 on the following page.

[1] In addition to the details provided in this Study Description, study implementation will also follow the "*Concepts and Practices Applicable to All Relicensing Studies*" developed by CCWD.

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Table 1. General survey locations for Study AR-6, *Special-status Amphibian and Aquatic Reptile eDNA Sampling*.

Waterbody	General Survey Location (Approximate River Mile[1])	Type	Target Species					
			SLTS	YT	SNYLF	CRLF	FYLF	NWPT
North Fork Diversion Impoundment	Accessible shoreline areas with suitable meadow/ wetland habitat and suitable upland meadow/wetland habitat within the FERC Project Boundary	Reservoir	X	X	X	--	--	--
New Spicer Meadow Reservoir	North Fork Diversion Tunnel outlet channel	Stream	--	--	X	--	--	--
	Accessible shoreline areas with suitable meadow/ wetland habitat and suitable upland meadow/wetland habitat within the FERC Project Boundary	Reservoir	X	X	X	--	--	--
Highland Creek	Downstream of New Spicer Meadow Reservoir (HC 5.5)	Stream	--	--	X	--	--	X
North Fork Stanislaus River	North Fork Stanislaus River or Duck Creek upstream of North Fork Diversion impoundment (NF 33.3)	Stream	X	X	X	--	--	--
	Downstream of North Fork Diversion Dam (NF 32.7)	Stream	--	--	X	--	--	--
	Pioneer Walking Bridge upstream of confluence with Highland Creek (NF 27.8)	Stream	--	--	X	--	--	X
	Wakalu Hep Yo Campground Area (NF 16.2)	Stream	--	--	--	X	X	X
	Upstream of Boards Crossing Road (NF 14.5)	Stream	--	--	--	X	X	X
	Stanislaus River Day Use Area near Lava Bluffs Trailhead (NF 11.2)	Stream	--	--	--	X	X	X
	Downstream of McKays Diversion Dam (NF 7.7)	Stream	--	--	--	X	X	X
McKays Point Reservoir	Accessible shoreline areas with suitable habitat	Reservoir	X	--	--	X	--	X
Beaver Creek Diversion Impoundment	Accessible shoreline areas with suitable habitat	Reservoir	X	--	--	X	--	X
Beaver Creek	Downstream of Beaver Creek Diversion Dam (BC 3.0)	Stream	--	--	--	X	X	X
Stanislaus River	Near confluence of Collierville Powerhouse Tailrace	Stream	--	--	--	X	X	X

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NOTES: CRLF = California red-legged frog; FYLF = foothill yellow-legged frog; SLTS = southern long-toed salamander; NA = not applicable; NWPT = northwestern pond turtle; SNYLF = Sierra Nevada yellow-legged frog; YT = Yosemite toad.

[1] For survey locations in stream reaches, approximate river miles are provided.

STUDY METHODS

Excluding Data Summary Development (i.e., Reporting), the Study consists of two steps: 1) eDNA Sampling; and 2) Analysis. Each of these is described below.

Step 1 - eDNA Sampling

Samples at stream sites will be collected at the downstream end of the site, prior to a VES to reduce the potential for sample contamination. Reservoir or impoundment sites will be evaluated for target species' suitability during a desktop analysis; if suitable, a composite sample will be collected once from the downstream end of the reservoir/impoundment.

For both stream and reservoir survey locations included in Table 1, eDNA samples will be collected once during the second VES (of three VES, described in Study AR-7). This timing is intended to maximize detection probability by targeting periods when most species are likely to be present in aquatic habitats and when streamflows are lower and eDNA may be more concentrated (Bedwell 2018).

Because Yosemite toads may breed earlier than the other target species, surveyors will collect an additional eDNA sample during the first VES at all Yosemite toad survey locations identified in Table 1. Study AR-7 also includes a desktop analysis to identify additional potential Yosemite toad VES locations. If additional locations are identified through this analysis, eDNA samples will also be collected during the first Yosemite toad VES where habitat cannot be fully surveyed.

eDNA samples will be collected following laboratory recommended protocols (e.g., Blankenship and Schumer 2022) and using designated sampling kits.

Site-specific eDNA sample collection design (e.g., the number of samples collected and sampling locations) will be developed based on habitat characteristics, species elevational and habitat range, and laboratory recommendations to maximize the likelihood of detecting species at the sample sites.

Step 2 - Analysis

The collected eDNA samples will be analyzed via quantitative polymerase chain reaction (qPCR) by a qualified laboratory to detect the DNA of specific target species, using environmental samples. Samples will be analyzed using published assays[2] (e.g., Bedwell 2018, Dean 2022, Starks and Schumer 2019).

[2] Because there is very little genetic differentiation among the *Anaxyrus* species, an assay for the western toad complex, which includes *Anaxyrus boreas, canorus, exsul, nelsoni*, will be used in the laboratory analysis and the geographical and elevational location of the sample collection site will be used to determine specific species.

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Laboratory results will be tabulated or summarized as a positive (i.e., DNA of species detected in sample) or negative detection by species and sample location. These data, together with VES data collected during Study AR-7, will inform species' presence in Project reservoirs and Project-affected reaches.

REPORTING

CCWD will analyze the results of Study as described above. Study data will be made available to interested parties and standard GIS shapefiles, including metadata, if developed will be provided.[3]

SCHEDULE

CCWD anticipates the schedule for Study completion as follows:

Step 1 – eDNA Sampling	~~May~~ February – August 2027
Step 2 – Analysis	September – December 2027
Reporting	January – March 2028

REFERENCES

Bedwell, M.E. 2018. Using genetic tools to investigate distribution and connectivity of two Sierra Nevada amphibians, *Rana sierrae* and *Rana boylii*. M.S. Thesis. Washington State University, WA.

Blankenship, S.M. and G. Schumer. 2022. Field collection procedure for aquatic environmental DNA sample collection and analysis. Cramer Fish Sciences/Genidaqs, West Sacramento, CA.

Dean, S. 2022. Western pond turtle (*Actinemys marmorata*) laboratory test (assay) for eDNA samples. Unpublished. Cramer Fish Sciences – Genidaqs.

Starks, H. and F. Schumer. 2019. Foothill yellow-legged frog (*Rana boylii*) laboratory test (assay) for eDNA samples. Unpublished. Cramer Fish Sciences – Genidaqs.

[3] In addition to the details provided in this section, reporting will also follow the "*Concepts and Practices Applicable to All Relicensing Studies*" developed by CCWD.

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