TECHNICAL MEMORANDUM (TM-42)

Sierra Nevada Yellow-Legged Frog Surveys along Milk Ranch Conduit Tributaries

DATE: May 8, 2018

TO: Bucks Creek Project Relicensing Participants

FROM: PG&E,[1] City of Santa Clara,[2] and Stillwater Sciences[3]

SUBJECT: Bucks Creek Hydroelectric Project – 2017 Sierra Nevada Yellow-legged Frog Surveys and Environmental DNA sampling in Milk Ranch Conduit Diverted Tributaries: Bear Ravine (Diversion No. 8), South Fork Grouse Hollow Creek (Diversion No. 3), and Milk Ranch Creek (Diversion No. 1)

This updated Technical Memorandum provides additional information since the document was last provided on July 5, 2017. This update presents 2017 survey results and discussion; the July 2017 version included the Introduction, Methods, and Schedule sections only.

This technical memorandum describes the methods and results for Sierra Nevada yellow-legged frog (*Rana sierrae*[4]) surveys and environmental DNA (eDNA) sampling along tributaries to the Milk Ranch Conduit in 2017. The objectives of the surveys and eDNA sampling were to supplement 2015 and 2016 amphibian surveys to determine whether any life stages of Sierra Nevada yellow-legged frogs are present and using habitats within the Project-affected reaches of the Milk Ranch Conduit tributaries, Bear Ravine (Diversion No. 8), South Fork Grouse Hollow Creek (Diversion No. 3), and Milk Ranch Creek (Diversion No. 1). This technical memorandum also includes an evaluation of habitat suitability for various life stages of Sierra Nevada yellow-legged frog (*e.g.*, egg masses, tadpoles, overwintering adults, dispersing adults, *etc.*) in the study area.

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[4] Formerly known as mountain yellow-legged frog [*R. muscosa*], now a complex of two separate species (Vredenburg *et al.* 2007)

1 INTRODUCTION

1.1 Purpose

In 2014, Pacific Gas and Electric Company (PG&E) and the City of Santa Clara (collectively, Licensees) developed a Study Plan to characterize aquatic habitats and gather information on the presence, distribution, and relative abundance of special-status amphibian and aquatic reptile species at, and around, Bucks Creek Hydroelectric Project (FERC Project No. 619) (Project) reservoirs and Project-affected stream reaches (Study RTE-S1, *Special-Status Amphibians and Aquatic Reptiles*) (PG&E 2014). The Study Plan was approved by FERC in their October 27, 2014 Study Plan Determination. Study RTE-S1 included 11 survey sites collaboratively selected by the Licensees and resource agencies targeting Sierra Nevada yellow-legged frog: 7 lotic (stream) survey sites and 4 lentic (still water) survey sites. Diverted tributary streams to the Milk Ranch Conduit were not included among the 11 Study RTE-S1 survey sites for the Sierra Nevada yellow-legged frog.

During the relicensing surveys, three tributary streams to the Milk Ranch Conduit were observed to have year-round water upstream of their diversions: Bear Ravine (Milk Ranch Conduit Diversion No. 8), South Fork Grouse Hollow Creek (Milk Ranch Conduit Diversion No. 3), and Milk Ranch Creek (Milk Ranch Conduit Diversion No. 1). Of those, Bear Ravine is located within the recently defined critical habitat for Sierra Nevada yellow-legged frog (USFWS 2016). The Licensees conducted this supplementary study to inform discussions of potential effects of Project operations on Sierra Nevada yellow-legged frog in these tributary streams to Milk Ranch Conduit.

1.2 Previous Surveys and Nearest Documented Occurrences

No Sierra Nevada yellow-legged frogs were found during the 2015 relicensing surveys in the Bucks Creek Project area, which included Three Lakes, Grassy Lakes, Bald Eagle Lake, tributaries to Bucks Lake, and portions of Grizzly Creek (PG&E 2016a). During 2002 amphibian and aquatic reptile surveys for the Bucks Creek Project, one individual adult Sierra Nevada yellow-legged frog was observed at Bald Eagle Lake (non-Project) during a third and final visit on September 27, 2002 (PG&E 2002a). Bald Eagle Lake is located between Bear Ravine and South Fork Grouse Hollow Creek, perched atop a steep northwest-facing slope of Bald Eagle Mountain, in a granite cirque, located approximately 0.25 miles (mi) southeast of Three Lakes Road. The lake is relatively small, approximately 1.5 acres (ac) in area, and at 6,060 feet (ft) elevation.

Between 2015 to 2017, Sierra Nevada yellow-legged frog surveys were also conducted in South Fork Grouse Hollow Creek and two ponds in the South Fork Grouse Hollow Creek drainage area (Unnamed Pond South and Unnamed Pond North) for PG&E's Milk Ranch Conduit Diversion No. 3 Repair Project (PG&E 2016b, 2016c, 2017); no Sierra Nevada yellow-legged frogs were found at these sites during three separate visits conducted each of those years. While Unnamed Pond South provides suitable physical lentic breeding habitat for Sierra Nevada yellow-legged frog within 0.5 mi of Milk Ranch Conduit Diversion No. 3, the species was not observed there during the 2015–2017 PG&E surveys (PG&E 2016b, 2016c, 2017), or during other surveys in 2015 (USFS 2015) or 2002 (PG&E 2002a).

The closest documented occurrences of Sierra Nevada yellow-legged frog to Bear Ravine (Milk Ranch Conduit Diversion No. 8) prior to 2017 is a reported sighting of three individual adult frogs on a mid-slope north-facing aspect, approximately one mile north of Lower Bucks Lake, and an estimated 1,000 ft south of Diversion No. 8 (CDFW 2017). This observation, recorded in 1993, was not associated with any known water sources (as mapped). Other more recent sightings are in the Cape Lake area, including at the headwaters of Bear Ravine (estimated at a minimum of 0.8 mi upstream from the diversion) and at Cape Lake (estimated 1.2 mi upstream and overland from the diversion) (CDFW 2017). USFS surveys have found Sierra Nevada yellow-legged frogs at these locations in 1994, each year between 2004 and 2009, and 2013 (CDFW 2017). Additionally, Sierra Nevada yellow-legged frogs were documented at Cape Lake during research studies conducted by Washington State University researchers as recently as 2016 (pers. comm. M. Bedwell, Washington State University, and H. Burger, Stillwater Sciences, June 2017). It is unknown from review of the California Natural Diversity Database (CNDDB) attribute data (CDFW 2017) whether breeding was documented.

2 STUDY GOALS AND OBJECTIVES

Within the Project-affected reaches of Bear Ravine, South Fork Grouse Hollow Creek[5], and Milk Ranch Creek in the vicinity of the Milk Ranch Conduit diversions, the objectives of the study are to:

- evaluate habitat suitability for the various life stages of Sierra Nevada yellow-legged frog (*e.g.*, egg masses, tadpoles, overwintering adults, dispersing adults, *etc.*),
- determine if Sierra Nevada yellow-legged frogs are present,
- compare the outcomes of Visual Encounter Survey (VES) and eDNA sampling methodologies, and
- evaluate any potential effects of Project operations on Sierra Nevada yellow-legged frog, if present.

3 STUDY AREA AND STUDY SITES

This study area is in the North Fork Feather River watershed on the Plumas National Forest, northeast of Oroville and west of Quincy near Bucks Lake in Plumas County, California (Figure

[5] South Fork Grouse Hollow Creek and two nearby unnamed ponds were evaluated for habitat suitability in 2015 and surveyed for Sierra Nevada yellow-legged frog in 2015–2016; these sites were resurveyed in 2017 as part of the Milk Ranch Conduit Diversion No. 3 Repair Project.

1). The survey sites are: (1) Bear Ravine, including a 1,300-ft section downstream of its diversion and a 2,600-ft section upstream of its diversion; (2) South Fork Grouse Hollow Creek, including a 1,000-ft section downstream of its diversion and a 2,300-ft section upstream of its diversion, as well as two nearby unnamed ponds, referred to in this technical memorandum as "Unnamed Pond South" and "Unnamed Pond North"[6], (3) and Milk Ranch Creek, including a 1,200-ft section downstream of its diversion and a 1,000-ft section upstream of its diversion (below Three Lakes Dam) (Figure 1).

Additionally, water samples were collected for eDNA analysis from Bear Ravine, South Fork Grouse Hollow Creek, and Milk Ranch Creek. Visual encounter surveys conducted on the same day as eDNA sample collection were shortened due to time constraints associated with collecting and processing the water samples for eDNA. During these abbreviated surveys, Bear Ravine was surveyed from 1,300 ft downstream of the road to 660 ft and 980 ft upstream of the road (during the August and September survey, respectively); South Fork Grouse Hollow Creek was surveyed from approximately 900 ft downstream of the road to 550 ft upstream of the road; and Milk Ranch Creek was surveyed from approximately 400 ft downstream of the road to 330 ft upstream of the road. Specifically excluded from the study area were areas where access was unsafe (*e.g.*, very steep terrain) or infeasible (*e.g.*, overgrown vegetation).

[6] South Fork Grouse Hollow Creek and the two associated unnamed ponds were surveyed annually from 2015 through 2017 as part of the Milk Ranch Conduit Diversion No. 3 Repair Project; survey methods and results for 2017 are summarized in this technical memorandum. More detailed methods and results, including habitat assessments, are reported in PG&E 2016b, 2016c, and 2017.

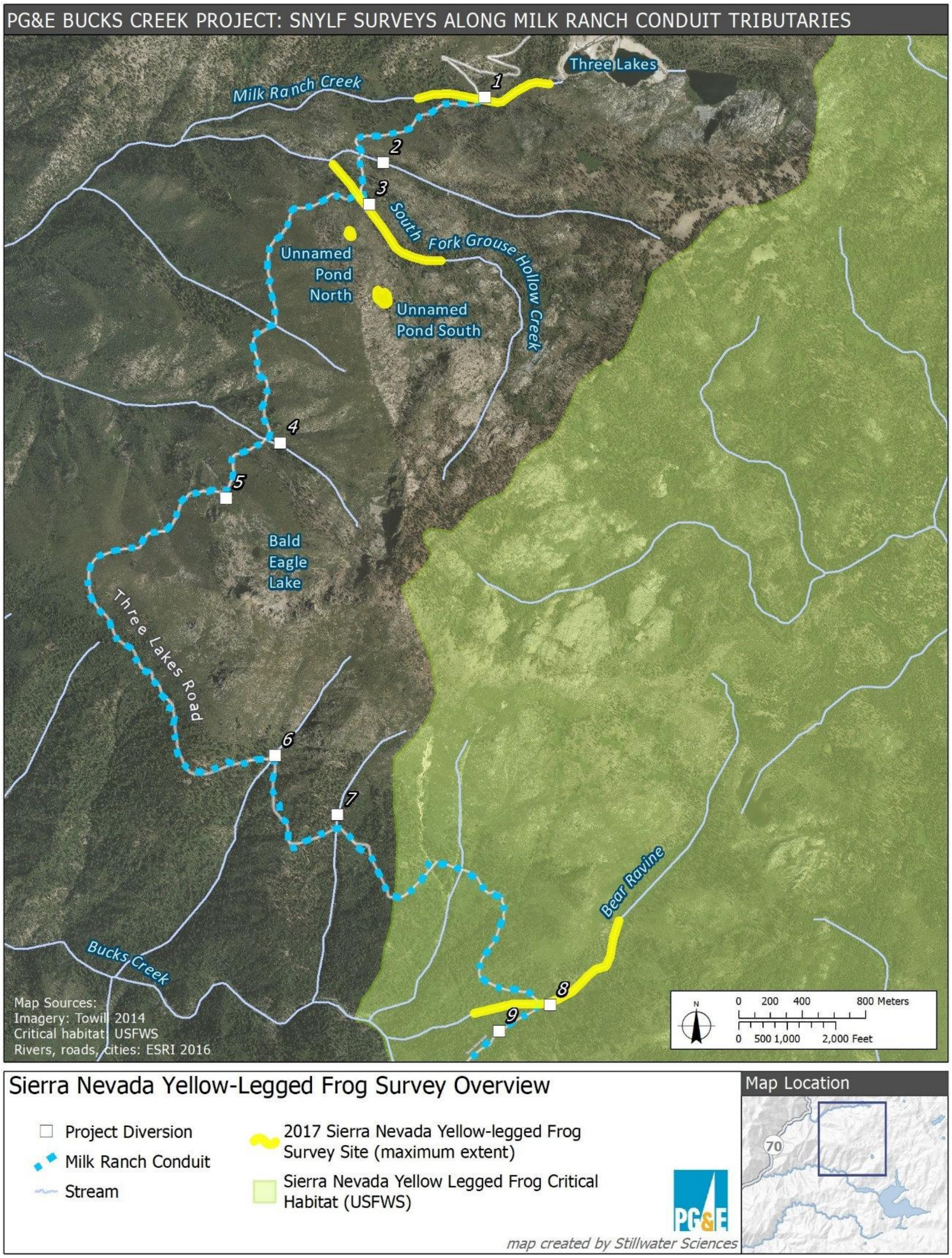

Figure 1. **Sierra Nevada Yellow-legged Frog Study Site Locations along the Milk Ranch Conduit**

4 METHODS

4.1 Visual Encounter Surveys

Three VESs were conducted for Sierra Nevada yellow-legged frogs at each of the sites on July 19–21, August 15–17, and September 6–8, 2017. VESs followed the same protocols employed during 2015 relicensing (Study RTE-S1, *Special-Status Amphibians and Aquatic Reptiles* [PG&E 2014]). The 2015–2017 VESs for the Milk Ranch Conduit No. 3 Project (PG&E 2016b, 2016c, 2017) also followed the same protocols employed during relicensing. The three surveys were staggered during the summer with an early-, mid-, and late-season survey, and occurred during a water year where snowpack was 80 percent greater than normal; these parameters are consistent with USFWS procedures to determine habitat occupancy (USFWS 2014). Breeding and egg mass surveys in 2017 were initiated as late as mid-July because of high flows from an above-average snowpack year. Surveys targeting tadpoles and young-of-year/metamorphic frogs (i.e., a frog that has recently metamorphosed from a tadpole, having just completely absorbed its tail) were thus conducted in August and September, respectively, to accommodate the wet season.

The margins of each stream and pond site were surveyed. Two surveyors worked in tandem to slowly walk the perimeter of the pond site and left and right bank of the stream reach while scanning ahead to count all observed frogs and document their life stage. Surveyors sought to detect post-metamorphic individuals (adults and subadults) on exposed substrate or partially hiding under cover. Stream surveys began at the downstream end of the site and continued upstream, if possible (depending on roughness of terrain and site accessibility). All amphibian and reptile species observed during the surveys were recorded. Water temperatures (°C) and air temperatures (°C) were recorded and amphibian and habitat photos were taken at each site.

Survey datasheets (adapted from Heyer *et al.* 1994; PG&E 2002b, c; Olson *et al.* 1997; and CDFG 2009) were completed for each site and survey effort (Appendix A). The total numbers of individual Sierra Nevada yellow-legged frogs and other incidentally observed amphibians were recorded by species and life stage. Sierra Nevada yellow-legged frog data collection included information specific to each life stage. For post-metamorphs, this included: estimated age class (*i.e.*, adult, subadult, or metamorph), sex, and snout-to-vent length (if captured). An individual was classified as an adult if it was equal to or greater than 40 millimeters (mm) snout-to-vent length (Vredenburg *et al.* 2010), a subadult if it was less than 40 mm snout-to-vent length, and a metamorph if it was a very small subadult that showed evidence of having recently metamorphosed, as indicated by greater than 50% tail resorption. Habitat characteristics, including, air and water temperature, water depth, dominant substrate, associated vegetation and cover, and Universal Transverse Mercator (UTM) coordinates were recorded where Sierra Nevada yellow-legged frogs were observed. Individual Sierra Nevada yellow-legged frogs were photographed, along with the associated microhabitat. Chin patterns—hypothesized to be unique to and persisting throughout the life of each frog (Wengert and Gabriel 2006, as cited in Marlow et al. 2016)—were photographed for each captured individual to use for comparison with future captures, allowing potential identification of individual frogs and tracking of movement.

To minimize the potential to spread invasive species (*e.g.*, New Zealand Mud Snail, quagga/zebra mussel, chytrid fungus [*Batrachochytrium dendrobatidis*], *etc.*), appropriate decontamination protocols[7] were followed prior to each aquatic-based field effort or moving between watersheds. Additional decontamination measures were implemented to limit possible transfer of Sierra Nevada yellow-legged frog DNA between sites.

4.2 Environmental DNA

4.2.1 Field Sampling

Water samples were collected for eDNA analysis at the three lotic sites (Bear Ravine, South Fork Grouse Hollow Creek, and Milk Ranch Creek) and one lentic site (Unnamed Pond South) during the second and third VESs (on August 15, 16, and 17 and September 6, 7, and 8, 2017). Unnamed Pond North was not included for eDNA sampling since the site dries by late summer and is therefore not suitable for breeding. During the August visit, site-specific eDNA sample designs were developed in the field with the guidance of M. Bedwell (Washington State University) to maximize the likelihood of detection within the selected sampling reach while accommodating varying habitat conditions and low surface flow at each site. Field sampling techniques and contamination prevention procedures, as prescribed in Goldberg and Strickler (2017), were used for eDNA sample collection and preservation.

At each lotic site, two approximately 200 m (660-ft) long sub-reaches were established to delineate the source of DNA in the creek relative to each PG&E diversion: one located upstream of the diversion (referred to as the "upper" sub-reach) and one located downstream of the diversion (referred to as the "lower" sub-reach) (Figure 2). If the stream channel immediately below the diversion was dry, then the top of the lower sub-reach was established where the surface flow first resumed (Figure 2). At Unnamed Pond South, the only lentic site, samples were collected at five sample locations evenly distributed along the perimeter of the pond.

Surveyors collected water samples starting at the downstream end of each lotic site and worked upstream. Water samples were collected at two locations approximately 100 m (330 ft) apart within each sub-reach[8]; within the upper sub-reaches, these included the diversion pool and approximately 100 m (330 ft) upstream of the diversion pool (Figure 2) (Table 1) (Section 6, *Results*, has figures showing maps of survey results juxtaposed with sub-reaches and sampling locations). Specific water sample collection locations were chosen based on areas where there was either active flow or pools connected with active upstream surface flows.

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[7] Decontamination protocols followed *Equipment Decontamination Protocol for Field Staff in Sequoia and Kings Canyon National Parks http://www.fws.gov/sacramento/es/Survey-Protocols-Guidelines/Documents/SEKI_DecontaminationProtocol_2014.pdf*

[8] The eDNA "signal" for Sierra Nevada yellow-legged frogs in lotic environments is thought to diminish between 100 meters (m) (330 ft) and 200 m (660 ft) from the source animal upstream (M. Bedwell, pers. communication, 2017).

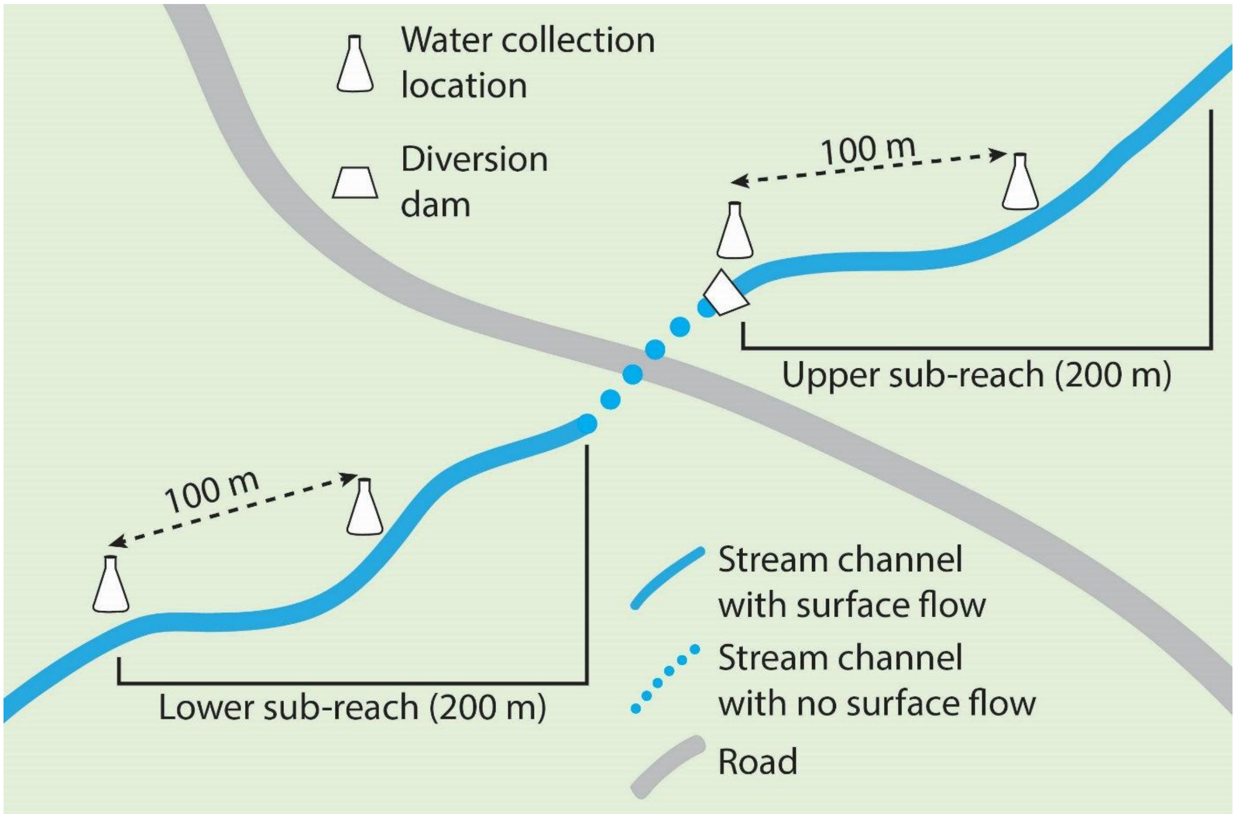


Figure 2. Environmental DNA Field Sampling Design for the Lotic Sierra Nevada Yellow-legged Frog Sites

Table 1. Environmental DNA Water Sample Collection Locations

Site Name	Sub-reach	UTM Downstream Location[a]		UTM Upstream Location[a]	
		Easting	Northing	Easting	Northing
Bear Ravine	Lower	651361	4419898	651460	4419921
	Upper	651624	4419896	651716	4419956
South Fork Grouse Hollow Creek	Lower	650285	4425223	650334	4425110
	Upper	650507	4424936	650536	4424851
Unnamed Pond South	NA	650588	4424275	NA	NA
Milk Ranch Creek	Lower	651075	4425625	651123	4425624
	Upper	651240	4425551	651450	4425486

Notes: NA = Not Applicable
[a] Projection: NAD83 UTM Zone 10 North

Surface water samples were collected in enough 750-milliliter (mL) Whirl-Paks® for a combined minimum of 6 liters (L) of water per sub-reach. Combined water samples from each sub-reach were filtered through cellulose nitrate filters in the field using a handheld vacuum pump. All eDNA samples were collected as three replicates per sub-reach. For eDNA samples processed at lotic sites, each 2 L water sample was filtered through a 0.45-micrometer (µm) pore cellulose nitrate filter. For each eDNA sample processed at the lentic site (Unnamed Pond), the 500-mL water sample was filtered through a 5-µm pore cellulose nitrate filter. Each nitrate filter was folded and placed in a coin envelope and stored in a Ziploc® bag at room temperature with loose silica gel desiccant. Figure 3 shows photos of eDNA collection materials and processing set-up.

Conductivity (microsiemens [µS]), pH (standard units [s.u.]), and temperature [°C]) data were collected for each sub-reach using a YSI Model 600xl or handheld waterproof pocket pH/conductivity tester.

Field negatives were collected at the end of each sampling day to identify if there were potential sources of contamination during sample collection. Field negatives were collected by filtering 250 mL of deionized water through 0.45-µm pore filters.

Figure 3. Environmental DNA Field Sampling Materials and Set-up

4.2.2 Analytical Methods and Calculations

eDNA samples were shipped to, and processed in, a limited-access eDNA lab (Goldberg Lab) at Washington State University and analyzed for the presence of Sierra Nevada yellow-legged frog and foothill yellow-legged frog (*Rana boylii*) DNA using quantitative polymerase chain reaction (qPCR) methods. No PCR products or tissue is handled in this lab and researchers must change clothes and shower after exposure to high quality DNA or PCR product. Filters were cut in half, with one half being used for DNA extraction and the remaining half stored in silica. DNA was extracted using the QiaShredder/Qiagen DNeasy Blood and Tissue extraction protocol (Goldberg *et al.*, 2011).

For DNA analysis, each sample was run using a multiplex of previously designed and validated qPCR assays for Sierra Nevada yellow-legged frog and foothill yellow-legged frog (Bedwell and Goldberg, unpublished data). All filter samples were run in triplicate. Each sample also included an added internal positive control (IC, Qiagen) to test for inhibition. If a sample was inhibited, it was run through a OneStepTM PCR Inhibitor Removal Kit (Zymo, Inc., Irvine, CA).

Results are reported as SQ Average, with a calculation of greater than 1 indicating a positive detection for a given sample. SQ is an index of starting quantity of DNA, calculated by using a regression analysis to compare the field sample with a standard curve. The standard curve is created using known quantities of DNA copies from synthesized genes (gBlocks, Integrated DNA Technologies) for each species (the same gene targeted by the qPCR assay) in a serial dilution[9].

Conductivity, pH, and temperature data were used to qualitatively assess probable eDNA persistence; for example, it has been found that eDNA persists longer in colder, more basic pH water with less organic material (Strickler *et al.* 2015).

[9] Standard wells included 3000, 300, 30, and 3 copies of each gene.

5 RESULTS

5.1 Visual Encounter Surveys

Sierra Nevada yellow-legged frogs were only observed in Bear Ravine (see Section 5.1.1). Table 2 lists VES conditions for each survey, and Appendix B provides representative habitat photos of each site. All three lotic study sites exhibited a decrease in flows as the summer progressed, with surface flow becoming increasingly scattered and isolated pools becoming more frequent below the diversions during subsequent surveys.

Table 2. Visual Encounter Survey Conditions

Site Name	Visit Number	Survey Date (2017)	Survey Length (ft)	Start Time (hrs)	End Time (hrs)	Temperature Range	
						Water Temp. (°C)	Air Temp. (°C)
Bear Ravine	1	7/19	3,900	1145	1630	11	19–22.5
	2[a]	8/15	1,960	1046	1614	12–13	22–24
	3[a]	9/6	2,280	1034	1546	13.5–13	19.5–21.5
South Fork Grouse Hollow Creek	1	7/20	3,300	945	1200	10.5–11.5	12.5–22.5
	2[a]	8/16	1,450	950	1500	13–14.5	17–23.5
	3[a]	9/7	1,450	900	1208	14.5	16–19
Unnamed Pond South	1	7/20	--	1315	1345	23	21.5
	2[a]	8/16	--	1548	1730	24–24.5	22.5–25
	3[a]	9/7	--	1443	1623	21.5–22	16–17
Unnamed Pond North	1	7/20	--	1430	1450	30	23.5
Milk Ranch Creek	1	7/21	2,200	955	1237	10.5	16.5–19.5
	2[a]	8/17	730	925	1250	9.5–14	15.5–22.5
	3[a]	9/8	730	925	1201	10.5–14.5	12–15

Notes: ft = feet, hrs = hours; °C = degrees Celsius
[a] Surveys associated with eDNA sampling
-- = no data

5.1.1 Bear Ravine

Three Sierra Nevada yellow-legged frogs were observed a total of five times during the VESs in Bear Ravine (Table 3, Figure 4); one frog was located upstream of the diversion and the other frogs were observed downstream of the diversion (Figure 5). Two adult females were observed downstream of the diversion in a high gradient, scoured section of the reach. One adult female relocated from a depression in a shallow riffle (in July) approximately 70 ft to a boulder-dominated pool with a maximum depth of 1 ft (in September); the shallow riffle where the frog was found in July was dry during the August survey. This frog was determined to be the same individual because of the same snout-vent length and matching pattern of pigmentation on the underside of the chin (Table 3, Figure 6). The other adult female was found in a boulder/bedrock-dominated pool with a maximum depth of 1.5 ft during both the August and September surveys; this was presumed to be the same adult female from August 15 since the observation was in the same pool and the patterning on the snout as seen on the photos appears to be the same (Table 3, Figure 7). The single subadult frog was observed in 1 ft of water in a cobble-dominant cascade/step complex upstream of the diversion, with a proximal maximum depth of approximately 0.3 ft. All three individuals were observed in locations with dense riparian vegetation dominated by dogwood and alder, though breaks in the canopy provided ample exposure to sunlight for basking.

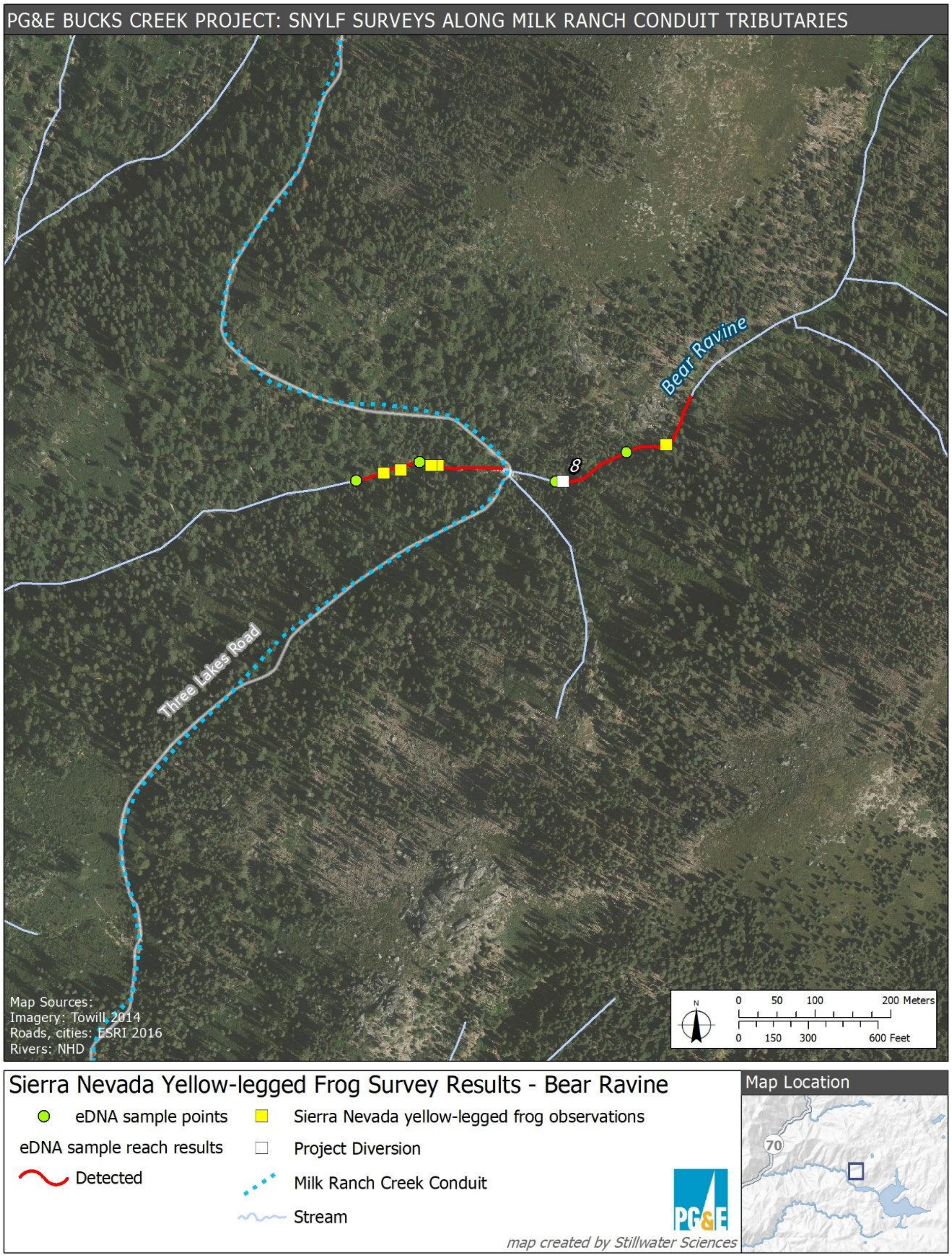
Table 3. Sierra Nevada Yellow-legged Frog Observation in Bear Ravine

Date (2017)	Visit	UTM Coordinates[a]		Sub-reach	Life Stage	Sex	SVL (mm)	Notes
		Easting	Northing					
7/19	1	0651397	4419913	Lower	Adult	Female	71	
7/19	1	0651768	4419950	Upper	Subadult	Unk	29	
8/15	2	0651468	4419923	Lower	Adult	Unk	ND	Escaped capture under large overhanging boulder
9/6	3	0651419	4419917	Lower	Adult	Female	71	Presumed same adult female as found on 7/19 based on SVL and chin pattern
9/6	3	0651459	4419923	Lower	Adult	Female	76	Presumed same adult female as found on 8/15 based on location and general size

Notes: SVL = snout-vent length, mm= millimeters, Unk = unknown, ND = no data
[a] Projection: NAD83 UTM Zone 10 North

The Bear Ravine survey site is characterized by a steep gradient, exposed bedrock, and extensive step/pool/cascade complexes. The dominant substrates are bedrock and boulder, and the average slope is approximately 22 degrees with steeper sections present immediately downstream of the road. Streamflow is intermittent both upstream and downstream of the diversion; however, aquatic habitat is more fragmented below the diversion than above. Downstream of the diversion, the channel is a series of disconnected pools, with approximately 520 ft (during the first VES) to 660 ft (during the third VES) of dry creekbed separating the diversion pool from first observed surface water downstream. Basking sites were available throughout the habitat units, except for near the top of the survey reach where the gradient is lower and dense vegetation was present. Based on surveyed pool depths, pools are presumably deep enough to hold water through summer during drier years.

Figure 4. Sierra Nevada Yellow-legged Frog 2017 Bear Ravine Observations, Clockwise from Upper Left: Subadult from July 19, Adult from July 19, Adult from August 15, and Adult from September 6


PG&E BUCKS CREEK PROJECT: SNYLF SURVEYS ALONG MILK RANCH CONDUIT TRIBUTARIES
Bear Ravine
8
Three Lakes Road
Map Sources:
Imagery: Towill 2014
Roads, cities: ESRI 2016
Rivers: NHD
0 50 100 200 Meters
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Sierra Nevada Yellow-legged Frog Survey Results - Bear Ravine
eDNA sample points
Sierra Nevada yellow-legged frog observations
eDNA sample reach results
Project Diversion
Detected
Milk Ranch Creek Conduit
Stream
map created by Stillwater Sciences
Map Location
70

Figure 5. **Map of Sierra Nevada Yellow-legged Frog Sightings and eDNA Detections in Bear Ravine, 2017**

Figure 6. **Chin Photographs of Sierra Nevada Yellow-legged Frog in Bear Ravine Taken July 19 (Left) and September 6 (Right), 2017**

Figure 7. **Photographs of Sierra Nevada Yellow-legged Frog in Bear Ravine Taken August 15 (Left) and September 6 (Right), 2017**

5.1.2 South Fork Grouse Hollow Creek

No Sierra Nevada yellow-legged frogs were found in South Fork Grouse Hollow Creek (Figure 8). South Fork Grouse Hollow Creek (both the section downstream of Three Lakes Road as well as the section upstream of the road extending to approximately 25 ft above Milk Ranch Conduit Diversion No. 3) is a series of boulder-dominated, high gradient, intermittent cascades with a few isolated pools. The average slope of the survey reach is approximately 18 degrees. The stream banks on either side of this corridor are steep with very little overhanging emergent vegetation. Approximately 25 ft above Milk Ranch Conduit Diversion No. 3 the habitat conditions of the stream corridor transition; the gradient is approximately 5–10 percent and surface flow is more continuous. Habitat in this upper section is dominated by high-gradient riffles and cascades with several standing pools. The substrate is a diverse mix of boulder, cobble, and gravel. Although the riparian corridor is narrow, more overhanging vegetation is present in this upper section. Stream margin vegetation is generally forbs and sedges. On average, the stream bankfull width along the site was 25 ft, with estimated average wetted widths between 6 and 10 ft in spring, and 3 ft in late summer. During the September survey, South Fork Grouse Hollow Creek had fragmented flow below the diversion (which was inactive at the time of the surveys) with a few shallow pools (approximately 0.3 ft to 1 ft in depth) remaining. Surface water was mostly confined to areas of bedrock substrate, with flow frequently disappearing into interstitial spaces between cobble and boulder substrates. Above the diversion, flow was also intermittent with maximum pool depths of approximately 2.6 ft.

5.1.2.1 Unnamed Pond South

No Sierra Nevada yellow-legged frogs were found in Unnamed Pond South (Figure 8). Unnamed Pond South, located roughly 1,800 ft southwest of Milk Ranch Conduit Diversion No. 3 on South Fork Grouse Hollow Creek, is approximately 1 ac in area and is located at 6,127 ft elevation. Access to this site by foot consists of cross-country hiking over and around steep terrain, talus-fields, and through shrubby vegetation. The pond has a maximum depth of over 10 ft, with margin vegetation composed primarily of sedges and forbs. Bedrock and boulders provide basking sites along the pond's perimeter.

5.1.2.2 Unnamed Pond North

No Sierra Nevada yellow-legged frogs were found in Unnamed Pond North. Unnamed Pond North is located approximately 700 ft southwest of Milk Ranch Conduit Diversion No. 3 on South Fork Grouse Hollow Creek (Figure 1). Relatively small, it is approximately 0.2 ac in area and is at 5,670 ft elevation. Access to this site by foot consists of cross-country hiking over and around steep terrain and through shrubby vegetation. Habitat conditions during early season surveys included flooded emergent vegetation with silt/organic substrate. Unnamed Pond North had dried completely prior to the August survey.

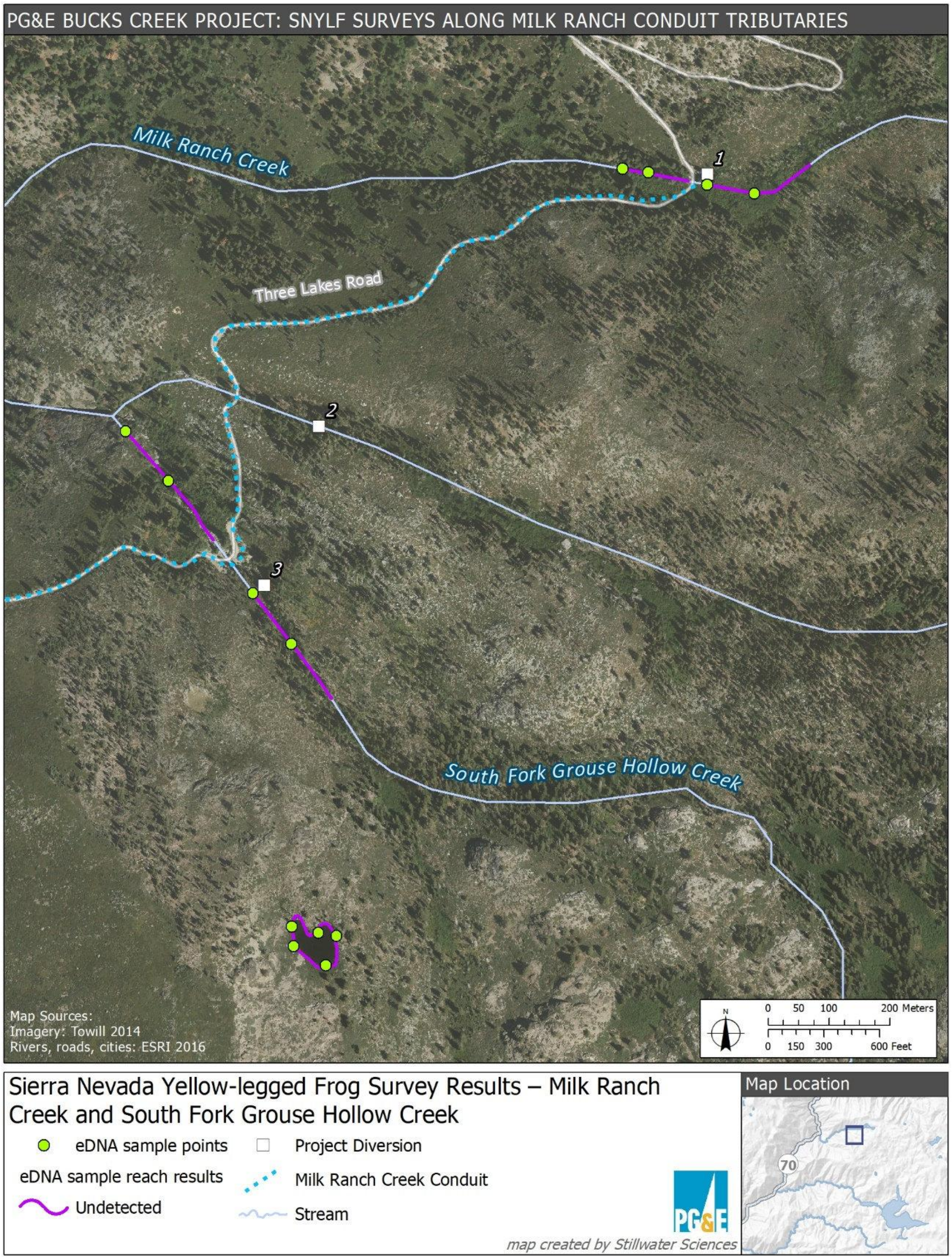

Figure 8. **Results of Sierra Nevada Yellow-legged Frog Surveys in South Fork Grouse Hollow Creek and Milk Ranch Creek**

5.1.3 Milk Ranch Creek

No Sierra Nevada yellow-legged frogs were found in Milk Ranch Creek (Figure 8). This study site is characterized by high-gradient riffles and predominantly cobble/boulder substrate. The average slope in the survey reach is 17 degrees. The surveys followed a period when Three Lakes was flowing over the spillway, and water was observed flowing along the entire study reach upstream of the diversion on all three surveys. The flow directly below the diversion was intermittent, with dry sections (primarily former riffle habitat) increasing as the season progressed. Several tributaries contributed to flow downstream of the diversion, with the first confluence at approximately 330 ft downstream of the road. Riparian vegetation was dense throughout the reach, with dogwood and alder shading nearly the entire channel. Incidental observations included fish upstream of the diversion.

5.2 Environmental DNA Results

The August sampling effort yielded no Sierra Nevada yellow-legged frog DNA detections (Table 4), despite observing one adult frog within the eDNA sampling reach in Bear Ravine downstream of the diversion. The September eDNA sampling effort yielded significant amounts of DNA (SQ average $\geq$ 1) in all three replicate samples downstream of the Bear Ravine diversion, and in one replicate sample upstream of the diversion (Table 4). South Fork Grouse Hollow Creek, Milk Ranch Creek, and Unnamed Pond South yielded no positive detections for Sierra Nevada yellow-legged frog DNA in either month (Table 4).

pH values in the sampling reaches ranged from 7.1 to 8.6 and conductivity values ranged from 4.8 to 42.0 µS (Table 4). All field blank results were negative for Sierra Nevada yellow-legged frog DNA.

No foothill yellow-legged frog DNA was detected at any of the sampling reaches.

5.3 Other Amphibian and Aquatic Reptile Species

Four non-special status amphibian and reptile species were observed in the study area during the VESs, including: southern long-toed salamander (*Ambystoma macrodactylum sigillatum*) larvae, Sierran treefrog (*Pseudacris sierra*) larvae and young-of-year, and a mountain garter snake (*Thamnophis elegans elegans*) at Unnamed Pond North; and a rubber boa (*Charina bottae*) at South Fork Grouse Hollow Creek.

Table 4. Environmental DNA Results

Site Name	Visit	Date (2017)	Sub-Reach	pH (s.u.)	Temp. (°C)	Cond. (µS)	Replicate	SQ Average
Bear Ravine	2	8/15	Lower	--	--	--	1	0
							2	0
							3	0
			Upper	--	--	--	1	0
							2	0
							3	0
	3	9/6[a]	Lower	8.2	12.8	42.0	1	62.16
							2	116.25
							3	23.79
			Upper	7.6	12.3	29.0	1	0
							2	1.74
							3	0
South Fork Grouse Hollow Creek	2	8/16	Lower	8.6	14.2	29.4	1	0
							2	0
							3	0
			Upper	7.5	14.3	28.2	1	0
							2	0
							3	0
	3	9/7	Lower	8.0	14.2	27.0	1	0
							2	0
							3	0
			Upper	7.1	14.6	29.0	1	0
							2	0
							3	0
Unnamed Pond South	2	8/16	NA	7.4	24.5	4.8	1	0
							2	0
							3	0
	3	9/7	NA	7.2	21.5	5.0	1	0
							2	0
							3	0
Milk Ranch Creek	2	8/17	Lower	7.7	11.0	19.2	1	0
							2	0
							3	0
			Upper	7.2	15.1	15.8	1	0
							2	0
							3	0
	3	9/8	Lower	8.0	11.0	15.0	1	0
							2	0
							3	0
			Upper	7.4	13.8	12.0	1	0
							2	0
							3	0

Notes: s.u. = standard units, °C = degrees Celsius, µS = microsiemens, SQ = starting quantity, NA = Not Applicable
[a]pH, temperature, and conductivity were collected in Bear Ravine on 9/7/2017

6 DISCUSSION

6.1 Habitat Suitability of Bear Ravine, South Fork Grouse Hollow Creek, and Milk Ranch Creek

6.1.1 Bear Ravine

Based on survey results and habitat conditions, adult and subadult Sierra Nevada yellow-legged frogs are likely utilizing Bear Ravine within the Project area as post-breeding dispersal and/or foraging habitat, and not for breeding. Furthermore, adults could be seasonally migrating and using the Bear Ravine reach below the diversion as refugia from winter flows upstream of the diversion. Three post-metamorphic Sierra Nevada yellow-legged frogs were observed multiple times within the survey reach on Bear Ravine, both upstream and downstream of the diversion. While sexually mature frogs were identified, no evidence of breeding (*i.e.*, tadpoles or egg masses) was observed; while egg masses for this species are notoriously difficult to find, the surveyors are experienced finding ranid tadpoles in similar conditions, which included habitat with clear water and good visibility. Breeding activities are probably taking place either in the lower-gradient headwaters of the stream or in nearby Cape Lake, where frogs have been documented to occur as early as 1994 (CDFW 2014) (though breeding at Cape Lake has not been reported). Several researchers (Brown *et al.* 2014, Mullally and Cunningham 1956) have hypothesized that Sierra Nevada yellow-legged frogs are unsuccessful reproducing in high-gradient streams, possibly due to lack of refugia for tadpoles from spring runoff events; this is consistent with the lack of detections of tadpoles in the high-gradient sections of Bear Ravine near the diversion. Sierra Nevada yellow-legged frogs have been found to exhibit seasonal migration patterns, dispersing from overwintering sites and breeding habitat early in the active season to a greater number of foraging sites during the remainder of the summer and fall (Matthews and Pope 1999, Matthews and Preisler 2010). Bear Ravine is the only study site hydrologically connected to historical populations; this suggests that proximal source populations may be a factor in determining occupancy status of steep tributary systems along the Milk Ranch Conduit.

6.1.2 South Fork Grouse Hollow Creek

South Fork Grouse Hollow Creek was surveyed nine separate times over three years as part of the Milk Ranch Conduit Diversion No. 3 repair project. Physical habitat conditions, including high stream gradient, lack of deep pools, and lack of consistent surface flow are likely to be contributing factors precluding breeding in this reach (Mullally and Cunningham 1956; R. Knapp as cited in Brown *et al.* 2014). Adult Sierra Nevada yellow-legged frogs could potentially use the Project area for non-breeding activities (*e.g.*, dispersal/migration, feeding, or overwintering). However, there is no source population within dispersal distances cited in the literature (see below under Section 6.1.3) (PG&E 2016b, 2016c, and 2017; PG&E 2016a). The nearest potential source breeding population to South Fork Grouse Hollow Creek is at Cape Lake, approximately 2.75 mi away (overland).

6.1.3 Milk Ranch Creek

Milk Ranch Creek was surveyed three times over the course of the summer with no Sierra Nevada yellow-legged frog detections. Riparian vegetation was dense, limiting the number of suitable basking sites. Downstream of the diversion, surface flow was fragmented, which increased as the summer progressed. Pools were shallow, and unlikely to support multi-year tadpoles. Three Lakes, the source of Milk Ranch Creek, could presumably provide suitable habitat for all life stages of Sierra Nevada yellow-legged frog; however, no Sierra Nevada yellow-legged frogs were found there during 2015 surveys, and the presence of introduced predatory fish likely precludes colonization of frogs in Three Lakes (Bradford, Tabatabai, and Graber 1993). The closest recorded source population is Cape Lake and the headwaters of Bear Ravine, approximately 2.75 mi overland to the southeast.

In lentic habitats, Sierra Nevada yellow-legged frogs generally move over a relatively small area; however, this species is capable of longer distance travel, including over dry land between habitats within lake complexes (Matthews and Pope 1999, USFWS 2016). In Humphreys Basin (Sierra National Forest), frogs dispersed a minimum of 2,900 ft along a stream to recolonize a neighboring lake following fish removal (Knapp *et al.* 2007). There is no direct migration route along drainages from Cape Lake to Milk Ranch Creek or South Fork Grouse Hollow Creek. The farthest reported distance Sierra Nevada yellow-legged frogs have been observed from water is approximately 1,300 ft (Vredenburg *et al.* 2005), though dispersal from Cape Lake would require migrating frogs to travel a minimum of 1,700 ft overland and outside mapped stream channels, after negotiating a complicated route through multiple streams to reach this shortest overland route.

6.2 <u>Visual Encounter and eDNA Survey Methodology Comparison</u>

Environmental DNA monitoring results generally matched the VES results, except for one missed detection in Bear Ravine. No genetic signal for Sierra Nevada yellow-legged or foothill yellow-legged frog was detected in South Fork Grouse Hollow Creek, Unnamed Pond South, or Milk Ranch Creek, which correlates with the negative results of the VESs at these locations. Bear Ravine yielded positive detections in the sub-reaches both above and below the diversion. While the results matched spatially, there was one notable temporal discrepancy: on August 15, an adult Sierra Nevada yellow-legged frog was visually detected within the Bear Ravine eDNA sampling reach, but water samples taken from within the theoretical spatial detection limit of 330 ft contained no identifiable DNA particles. This resulted in a false negative detection for this eDNA survey. Possible explanations for the missed detection may include poor mixing of water within the water column, DNA particles settling out of solution prior to being collected, or insufficient flow for carrying DNA downstream from the occupied habitat unit. It is also possible the individual recently migrated into the area, limiting the amount of DNA shed within the sample reach. Seasonal variability in eDNA detection has been observed for other amphibian species (Buxton *et al.* 2017). Given the strong positive detections during the September sampling effort, it is possible that in August there was less DNA in the water column to detect. A Sierra Nevada yellow-legged frog was observed within one of the September eDNA sampling pools, which also likely contributed to the higher detection results. A lower quantity of DNA

(SQ Average = 1.74) in one of three replicate samples upstream of the diversion was also evident during the September sampling effort. No individuals were observed during the accompanying VES, but a subadult was observed approximately 160 ft upstream of the uppermost sampling location during the July survey.

Using eDNA to detect aquatic herpetofauna presence is a relatively novel approach, with its own unique challenges. The ability to reliably detect rare and cryptic species in remote aquatics systems has obvious benefits. However, as evidenced by the inconsistencies above, the process would benefit from further refinement before it should be relied upon as the sole method of detection. Several modifications to the eDNA survey protocol may have increased the likelihood of detection, especially in intermittent flow streams. Increasing spatial resolution (*i.e.*, a greater density of samples) within sample reaches could possibly reduce effects from surface flow fragmentation. Additionally, improving seasonal timing of sampling could help target periods when waterborne DNA loads are highest and detection is most likely. Until eDNA is proven to be a mature and robust approach to cryptic and endangered species detection, traditional methods such as VESs should be relied upon to inform management decisions in and around suitable amphibian habitat.

6.3 Potential Effects of Project Operations on Sierra Nevada Yellow-legged Frog

6.3.1 Bear Ravine

Since potential for Sierra Nevada yellow-legged frog breeding in the Bear Ravine survey reach is low, potential for entrainment of tadpoles in the diversion is unlikely.

Since current flows are insufficient to provide habitat connectivity downstream of Bear Ravine Diversion (No. 8) during lower flow periods, increasing flows would likely improve aquatic habitat connectivity. However, bypassing all flows at the diversion would alter the hydrograph and could subsequently affect Sierra Nevada yellow-legged frogs using the reach below the diversion by reducing the amount of high winter/spring flow refugia habitat during peak flow events. Stream flows in Bear Ravine that may benefit Sierra Nevada yellow-legged frogs the most would extend the period of filled pools and connected habitat between spring and fall while minimizing high-velocity flows in winter for frogs potentially using the reach below the diversion as refugia.

6.3.2 South Fork Grouse Hollow

There are several factors that likely preclude potential effects of Project operations to Sierra Nevada yellow-legged frog at South Fork Grouse Hollow. First, is the absence of any Sierra Nevada yellow-legged frog found at this tributary, either in historical records or recent surveys. Several VESs between 2015 to 2017 have not documented Sierra Nevada yellow-legged frog in South Fork Grouse Hollow Creek (PG&E 2016b, 2016c, 2017) or nearby suitable habitats (Unnamed Pond South or Unnamed Pond North). While Unnamed Pond South provides suitable physical lentic breeding habitat for Sierra Nevada yellow-legged frog within 0.5 mi of Milk Ranch Conduit Diversion No. 3, the species was not observed there during the 2015–2017 surveys (PG&E 2016b, 2016c, 2017), or in prior surveys in surveys (USFS 2015), or 2002 (PG&E 2002a). eDNA results also did not document Sierra Nevada yellow-legged frog in the tributary or either Unnamed Pond South, or Unnamed Pond North. Second, due primarily to the distance from the nearest source population in Cape Lake (described above), it is highly unlikely Sierra Nevada yellow-legged frog would be able to traverse the approximate 2.75 miles to South Fork Grouse Hollow Creek.

6.3.3 Milk Ranch Creek

Potential effects of Project operations to Sierra Nevada yellow-legged frog at Milk Ranch Creek are the same as for South Fork Grouse Hollow. Sierra Nevada yellow-legged frogs have not been documented in Milk Ranch Creek either in historical records or from recent surveys, including eDNA, and the proximity to the nearest source population is approximately 2.75 miles away in Cape Lake.

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APPENDIX A

SIERRA NEVADA YELLOW-LEGGED FROG SURVEY FORM

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Sierra Nevada Yellow-legged Frog Survey Data Sheet

Project Name: ____ Page _____ of ______

Date:_______________ Location/Reach:_____________________________ Site ID :______________________ VES#: ______ Surveyors:_____________________

Waterbody type: Lake / Pond / Stream / Marsh / Spring Seep / Other __________________ Classification: Perennial / Intermittent

Max Lake/Pond Depth (m)_________ Water Color: Clear / Stained Turbidity: Clear / Cloudy Site Length (m): ___________ Avg. Wetted Width (m):_____________

Start UTM:_______________/________________ Error: _____ Wypt#: ____ End UTM:_______________/________________ Error: _____ WyPnt#: ____ GPS#:_______

Start Time: ________ End Time: ________ Total Survey Time: _________________ Water Temp (C°): Start _______ End_______ Air Temp (C°): Start_______ End _______

Current Wx: (Sky) Clear / Overcast / Rain / Snow (Wind) Calm / Light / Moderate / Strong Survey Methods: Visual / Aural / Dip Net / Seine / Trapped / Hand Collection

Photos: Camera #________ Start:_____________ End: _____________ General: ____

Photo Notes: ____

UTM E	UTM N	GPS error (m)	Lifestage/ Sex[1]	#Obs	Gosner Stage	Length (mm)[2]	Total Depth (m)[3]	EM/Perch Sub[4]	Temp (C°) Water/Air[5]	Nearest Bank[6]	Geom Unit[7]	Dom. Veg. Type[8]	Chytrid[9]	Photos/ Notes
									/				/	
									/				/	
									/				/	
									/				/	
									/				/	
									/				/	
									/				/	
									/				/	
									/				/	

[1] Lifestage/Sex:(E)gg Mass, (L)arvae, (S)ubadult, (AM) adult male, (AF) adult female, (AU) adult unknown; Adult ≥ 40 mm SVL [2] Length in mm: SVL-Snout Vent for A,S; Average total length for tadpoles

[3] Eggs/Tads: Total water depth at obs. location [4] Substrate Attachment/Perch Substrate: MUD, SLT, SND, GRV, COB, BLD, BDX (Bedrock), WOOD, VEG [5] Water and air (1m above water) temperatures at obs. location

[6] Bank nearest obs. location (looking downstream): (RB) right bank, (LB) left bank, (MC) center channel [7] Geomorphic Unit (stream only): RIF, BAR, POOL, STEP, RUN, RAP, BDX

[8] Dominant Vegetation Type: (1) Willow/Alder, (2) Pine/Fir Forest, (3) Grass/Sedges, (4) Bare (no veg.), (5) Other [9] Chytrid sample collected: (Y)es, (N)o / Sample number

Crayfish Present (circle)? Yes / No Fish Present: Yes / No Type: Salmonid Centrarchid Cyprinid Catastomids Other:_________________

Other Herpetofauna: Yes / No ____

Notes: ____

QA/QC (initials):________ Date:________

Page ___ of ___

Incidental Sightings Data Sheet

Date:________________________ Location/Description: ________________________ Site ID:__________________________

Surveyors: ______________________ Camera # ________________ GPS # __________________________

Notes: ____

Row #	Species	Lifestage				Location Description &/or UTM (Optional)	Photos / Notes
		Egg Mass	Larvae	YOY	Juvenile/Adult		
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

Herptofauna Species Codes:

Southern long-toed salamander (AMMA), California slender salamander (BAAT), Sierra Nevada ensatina (ENES), American bullfrog (LICA), Sierran tree frog (PSSI), California red-legged frog (RADR), western toad (ANBO), northern western pond turtle (ACMA), northern alligator lizard (ELCO), sagebrush lizard (SCGR), western fence lizard (SCOC), rubber boa (CHBO), western yellow-bellied racer (COCO), California striped racer (COLA), sharp-tailed snake (COTE), western rattlesnake (CROR), ring-necked snake (DIPU), California nightsnake (HYOC), California kingsnake (LACA), California mountain kingsnake (LACA), Pacific gopher snake (PICA), Sierra garter snake (THCO), mountain garter snake (THEL), valley garter snake (THSI)

Row #	Carapace Length	Carapace Width	Plastral Rings	Total Depth (m)	% Rip. Canopy Cover	Size/Nature of Basking Site	Dominant Riparian Type	Geom Unit

If WPT found, fill corresponding row on auxillary table. See WPT datasheet for column descriptions.

APPENDIX B

REPRESENTATIVE HABITAT PHOTOS

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Figure B-1. Bear Ravine, below Diversion No. 8, 7/19/2017

Figure B-2. Bear Ravine, below Diversion No. 8, 8/15/2017

Figure B-3. Bear Ravine, below Diversion No. 8, 9/6/2017

Figure B-4. Bear Ravine, above Diversion No. 8, 7/19/2017

Figure B-5. Bear Ravine, above Diversion No. 8, 8/15/2017

Figure B-6. Bear Ravine, above Diversion No. 8, 9/6/2017

Figure B-7. South Fork Grouse Hollow Creek, below Diversion No. 3, 7/20/2017

Figure B-8. South Fork Grouse Hollow Creek, below Diversion No. 3, 8/16/2017

Figure B-9. South Fork Grouse Hollow Creek, below Diversion No. 3, 9/7/2017

Figure B-10. South Fork Grouse Hollow Creek, above Diversion No. 3, 7/20/2017

Figure B-11. South Fork Grouse Hollow Creek, above Diversion No. 3, 8/16/2017

Figure B-12. South Fork Grouse Hollow Creek, above Diversion No. 3, 9/7/2017

Figure B-13. Milk Ranch Creek, below Diversion No. 1, 7/21/2017

Figure B-14. Milk Ranch Creek, below Diversion No. 1, 8/17/2017

Figure B-15. Milk Ranch Creek, below Diversion No. 1, 9/8/2017

Figure B-16. Milk Ranch Creek, above Diversion No. 1, 7/21/2017


Figure B-17. Milk Ranch Creek, above Diversion No. 1, 8/17/2017

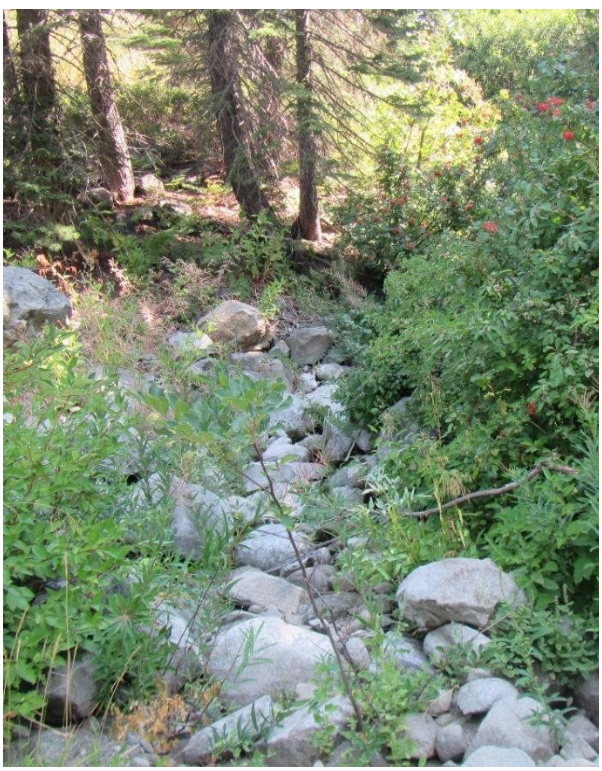
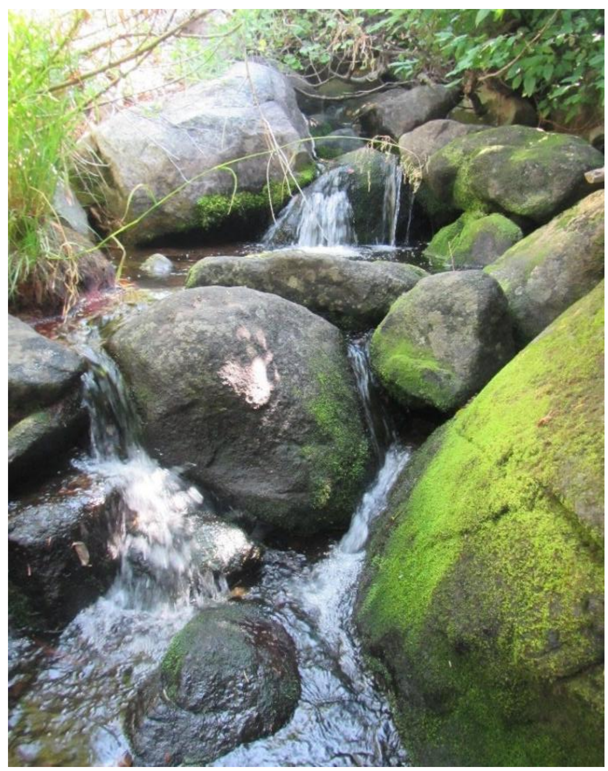

Figure B-18. Milk Ranch Creek, above Diversion No. 1, 9/8/2017

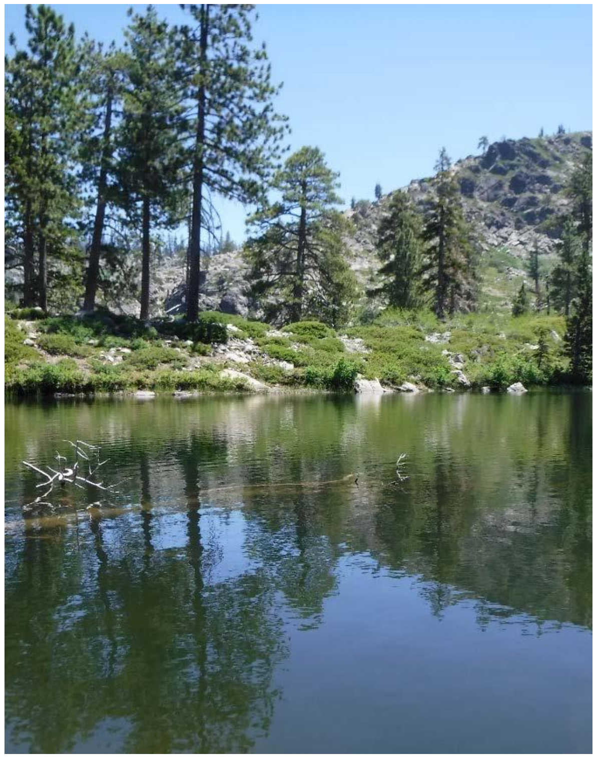

Figure B-19. Unnamed Pond South 7/20/2017

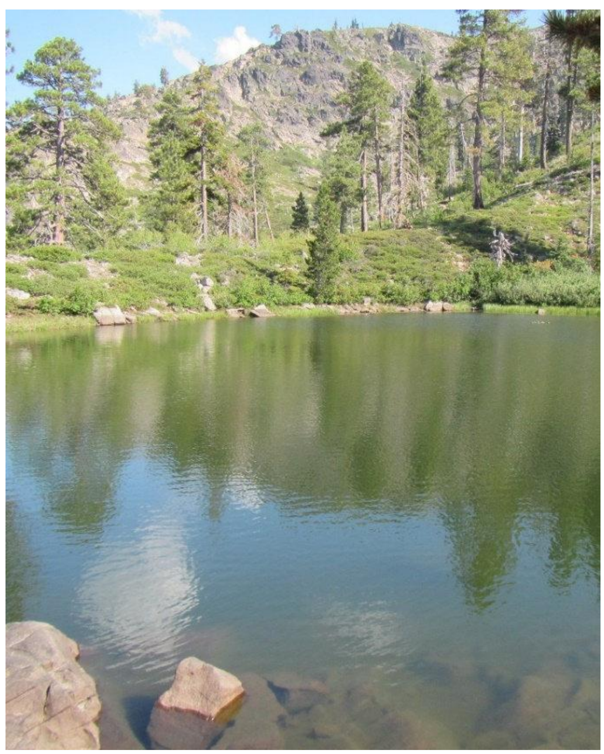

Figure B-20. Unnamed Pond South 8/16/2017

Figure B-21. Unnamed Pond North 7/20/2017

Figure B-22. Unnamed Pond North 8/16/2017