ANGELS PROJECT
FERC PROJECT NO. P-2699

UTICA PROJECT
FERC PROJECT NO. P-2019

DRAFT
FOOTHILL YELLOW-LEGGED FROG (*RANA BOYLII*) AND NORTHWESTERN POND TURTLE (*ACTINEMYS MARMORATA*) STUDY PLAN

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Abbreviations/Acronyms

CDFW	California Department of Fish and Wildlife
CNDDB	California Natural Diversity Database
CRLF	California red-legged frogs
eDNA	environmental DNA
FERC	Federal Energy Regulatory Commission
FYLF	Foothill yellow-legged frog
PG&E	Pacific Gas and Electric Company
Project	Angels and Utica Hydroelectric Project
TSM	Technical Study Memorandum
UPA	Utica Power Authority
USGS	U.S. Geological Survey
UWPA	Utica Water Power Authority
VES	Visual encounter survey
WPT	Western pond turtles

1.0 INTRODUCTION

On March 25, 2024, the California Department of Fish and Wildlife (CDFW) requested that Utica Water and Power Authority (UWPA) survey the Angels and Utica project areas to characterize populations of fishes, amphibians, benthic macroinvertebrates, aquatic invasive species, and terrestrial wildlife. This request was provided in comments to UWPA's Federal Energy Regulatory Commission (FERC) *Draft Applications for Small Conduit Exemption and Surrender of Licenses.* CDFW stated that the information was needed to determine the fish and wildlife species that could be affected by the continued operation and maintenance of the powerhouses and water delivery systems if FERC approves UWPA's applications. This study plan addresses the collection of data primarily related to foothill yellow-legged frogs (FYLF) and northwestern pond turtles (WPT) in the project area.

2.0 POTENTIAL RESOURCE ISSUE(S)

- Status of FYLF and WPT in affected stream reaches of the UWPA's Angels and Utica Hydroelectric projects.
- Effects of Project operation on FYLF and WPT in affected stream reaches of the Angels and Utica Hydroelectric projects.
- FYLF is federally listed under the Endangered Species Act (ESA) as Endangered and listed by California under the California Endangered Species Act (CESA) as Endangered.
- WPT is a federal candidate species for listing under the ESA and a California species of special concern.

3.0 RELEVANT INFORMATION

Historical observations of FYLF have been reported in the California Natural Diversity Database (CNDDB 2024). FYLF were observed in Angels Creek downstream of Angels Diversion and in a tributary to Mill Creek upstream of Hunters Reservoir in 1953. Studies conducted by Pacific Gas and Electric Company (PG&E) and Utica Power Authority (UPA) in the 1990s and 2000s for FYLF and California red-legged frogs (CRLF) did not locate either species and suggested a lack of suitable habitat for CRLF (Stevens 2007) and the presence of bullfrogs in otherwise suitable habitat for FYLF (Ogden Environmental and Energy Services 1992). WPT were incidentally observed below Ross Reservoir along with numerous bullfrogs (Stevens 2007).

The following information is available and was reviewed to assist in determining special-status amphibian and aquatic reptile study needs:

- CDFW's CNDDB (CNDDB 2024);
- Utica Project, FERC Project No. 2019; Draft Application for Small Conduit Exemption and License Surrender for Hydroelectric Facility Exhibit E – Environmental Report, December 2023 (UPWA 2023a);

- Angels Project, FERC Project No. 2699; Draft Application for Small Conduit Exemption and License Surrender for Hydroelectric Facility Exhibit E – Environmental Report, December 2023 (UPWA 2023b);

- Final PG&E Angels Project Special Status Wildlife Surveys Report, FERC 2699 (Ogden Environmental and Energy Services 1992);

- Amended Application for a Subsequent License for a Minor Water Power Project Angels Hydroelectric Project (FERC Project No. 2699), Exhibit E Environmental Report, June (UPA 1997);

- California Red-legged Frog Habitat Assessment, Utica Power Authority Hydroelectric System Angels Creek, French Gulch Creek, Mill Creek Eastern Calaveras County, California (Stevens 2007);

- A Standardized Approach for Habitat Assessments and Visual Encounter Surveys for the Foothill Yellow-Legged Frog (*Rana boylii*) (Seltenrich and Pool 2002); and

- Visual Encounter Survey Protocol for *Rana Boylii* in Lotic Environments (Peek et al. 2017).

4.0 POTENTIAL INFORMATION GAPS

The following information gaps were identified in the existing data:

- Current status and distribution of FYLFs and FYLF reproduction in Angels Creek and Mill Creek in Project-affected waters; and

- Current status and distribution of WPTs in Project-affected waters.

5.0 PROPOSED STUDY OBJECTIVES AND ANALYSES TO ADDRESS IDENTIFIED INFORMATION GAPS

The following data collection activities will be used to augment existing information:

- Identify and characterize potential habitat for FYLF in the surveyed reaches of Angels and Mill creeks.

- Document the distribution of FYLF populations, if found:

  - Document habitat conditions including water levels and velocities in the vicinity of active FYLF breeding sites, if found.

  - Document the presence of FYLF tadpoles and metamorphs.

- Document the presence of WPTs during FYLF surveys and document visual encounters of WPTs at Project waters, primarily forebays.

- Sample environmental DNA (eDNA) for FYLF, CRLF, and WPT in reaches surveyed for FYLF to facilitate documenting the presence and distribution of these species.

- Record the presence of bullfrogs if encountered.

6.0 EXTENT OF STUDY AREA[1]

The study area for FYLF and WPT includes the following:

- Accessible sections of Angels Creek from upstream of Murphys to the Angels Powerhouse tailrace.

- Accessible portions of Mill Creek from Hunter Reservoir to below Candy Rock Road.

The study area for WPT also includes Project forebays and reservoirs.

7.0 FOOTHILL YELLOW-LEGGED FROG

7.1 METHODS

The following describes the approach to meet each of the study objectives:

1. Determine access and characterize habitat conditions in study reaches;

2. Determine the present distribution and abundance of FYLF in study reaches; and

3. Characterize FYLF breeding and breeding success in study reaches.

7.1.1 Characterize Access and Habitats and Determine Study Reaches

In order to determine the distribution and abundance of FYLF within the study area, a reconnaissance was necessary to determine access and identify locations where private landowners or unsafe conditions may limit access. It also provided the opportunity for potentially encountering FYLFs prior to the breeding survey. The reconnaissance took place on May 6 and 7, 2024. Based on the results of the reconnaissance survey, study reaches were identified. Figures 1 and 2 present the approximate locations of reaches that were included in the reconnaissance survey for Angels and Mill creeks, respectively. Based on initial review of the study reaches, access, safety, and potential FYLF habitat, the locations of proposed study reaches were determined and are listed in Table 1.

FYLF habitat will be generally characterized as described by Seltenrich and Pool (2002) using updated standard operating procedures and data forms (Appendix A). FYLF habitat will be characterized at each site where breeding or tadpoles are found. FYLF habitat for study reaches will be characterized at sites in the upstream, mid-reach, and lower reach for each reach, regardless of whether FYLF are encountered.

[1] Only study sites that can be accessed safely with the permission of the landowner or occupier will be sampled.

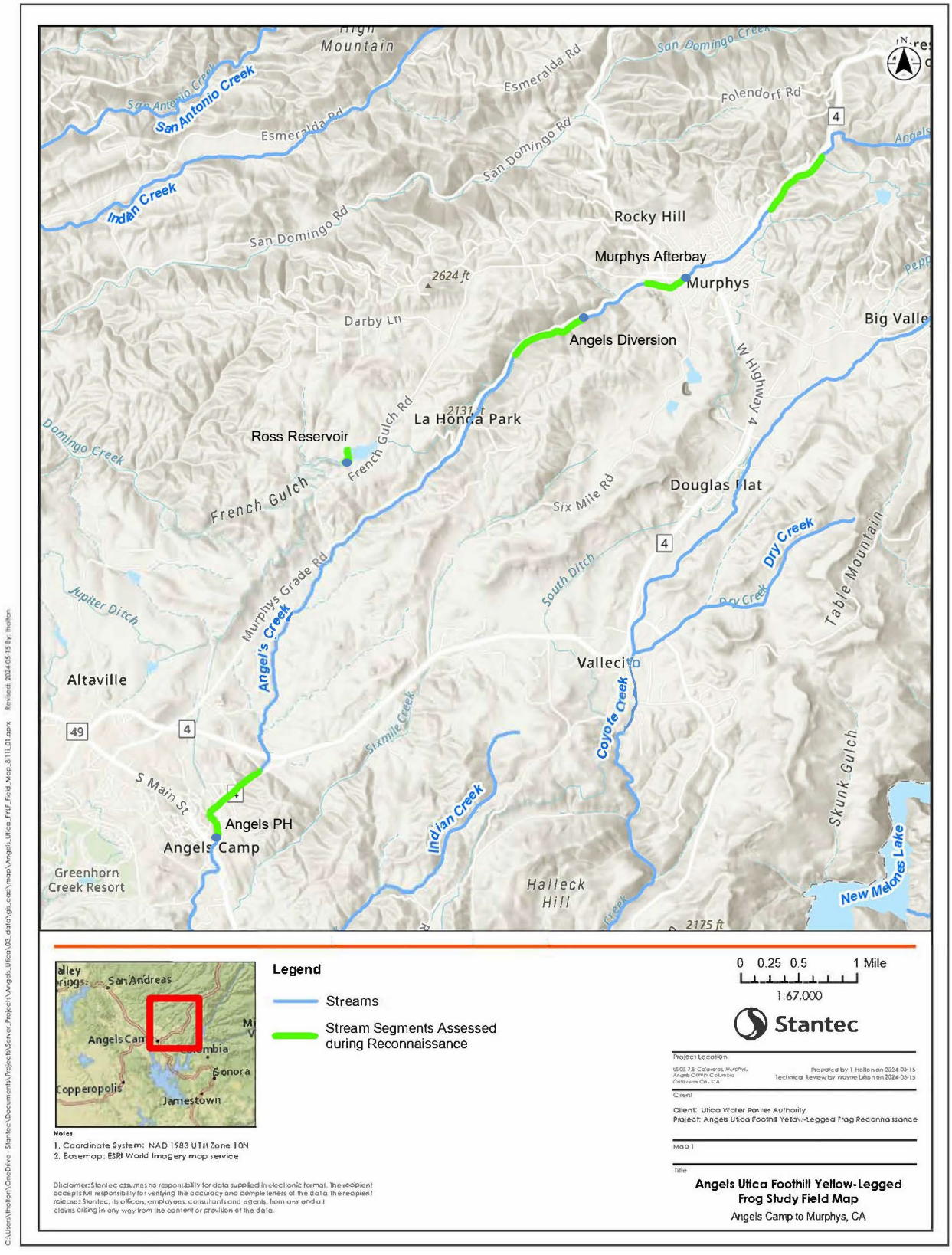

Figure 1. Map of FYLF Sampling Locations on Angels Creek

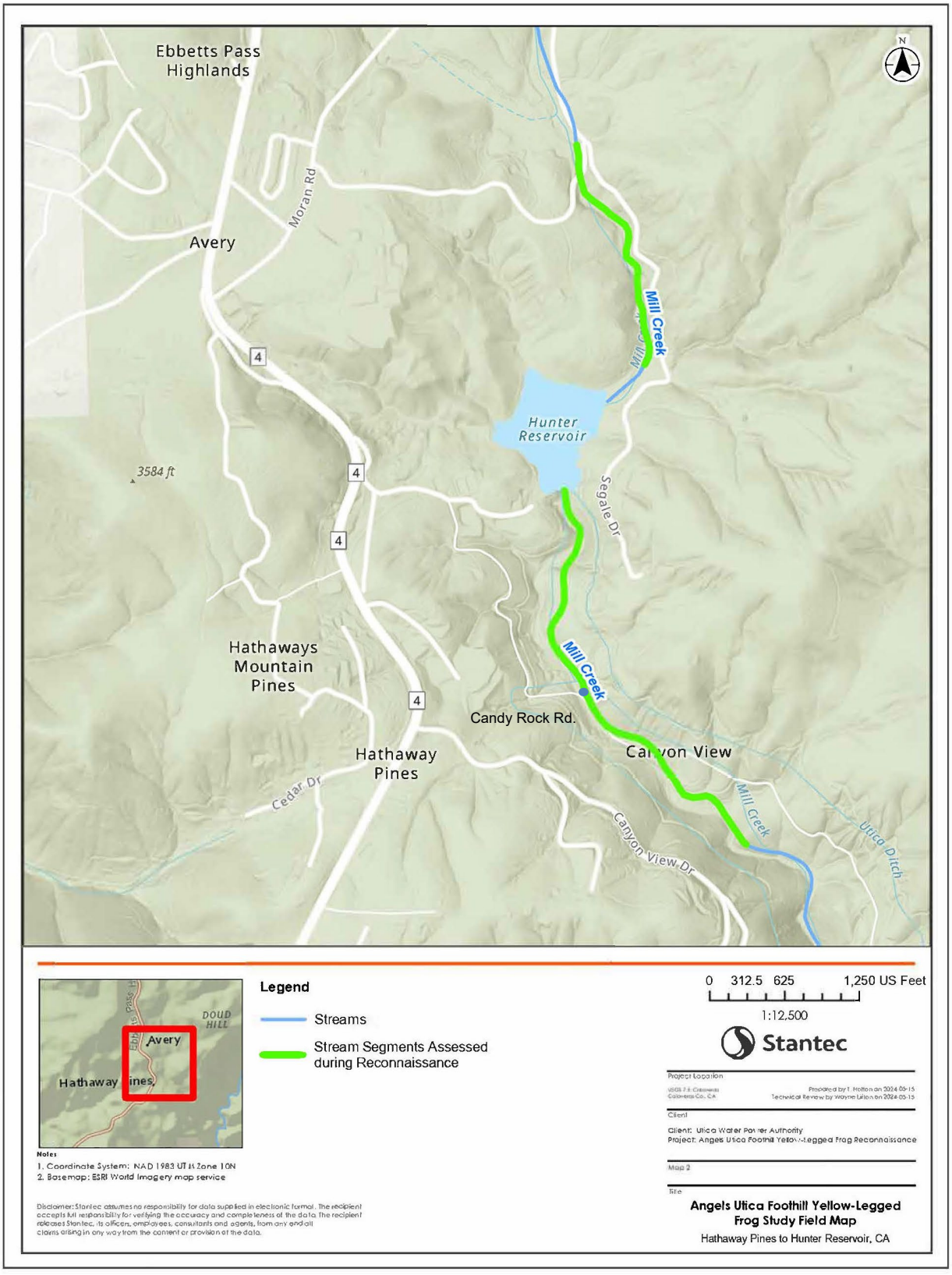

Figure 2. Map of FYLF Sampling Locations on Mill Creek

Table 1. Proposed FYLF Study Reaches

Station ID	Reach Description
Angels Creek	
Upstream of Murphys	Angels Creek upstream of Murphys
Downstream of Murphys	Angels Creek downstream of Murphys Afterbay
Downstream of Angels Diversion	Angels Creek downstream of Angels Diversion
Upstream of Angels Powerhouse	Angels Creek upstream of Angels Powerhouse
Mill Creek	
Mill Creek below Hunter Reservoir	Mill Creek downstream of Hunter Reservoir

7.1.2 Determine the Present Distribution and Abundance of FYLF

A combination of visual encounter survey (VES) and eDNA sampling (discussed further below) will be used to help determine the presence and distribution of FYLF in the stream reaches sampled.

7.1.2.1 Visual Encounter Surveys

At each study reach, FYLF VES and habitat assessment methods will follow standard approaches used in recent FYLF studies (Heyer et al. 1994; Peek et al. 2017; Seltenrich and Pool 2002). Three VESs will be conducted in each reach: two VESs during the spring/early summer for egg masses and tadpoles, respectively, in an attempt to bracket the breeding period and one late-summer/early-fall VES targeting post-metamorphic frogs. A two-person crew will conduct the VES.

7.1.3 Characterize FYLF Beeding and Breeding Success

The breeding survey will be key to determining if FYLF breeding occurs in the stream reaches, and the presence of egg masses provides an indication of initial breeding success. The successful incubation of eggs to tadpoles and eventually to metamorphs provides the next generation of frogs, which is the ultimate measure of reproductive success.

7.1.3.1 Breeding Survey

The FYLF breeding survey will be implemented in the spring following the reconnaissance survey. Water temperatures will be monitored at temperature recorders installed at locations identified in Table 2. The breeding survey will take place as water temperatures warm up and downstream temperatures reach water temperatures associated with breeding. The VES will be scheduled at a time when adult FYLFs are expected to be concentrating to breed and when egg masses may be present. All egg masses (and tadpoles) will be visually evaluated without handling to determine their developmental stage, if possible, using *A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification* (Gosner 1960).

Table 2. Water Temperature Monitoring Locations

Station ID	Station Description	Flow Data Available
Angels Creek		
S-78	Outlet of Murphys Afterbay	Yes*
S-124	Fish release at Angels Diversion	Yes*
Angels Powerhouse	Angels Creek 150 feet upstream of the Angels Powerhouse tailrace	(Gage S-124)
Mill Creek		
S-66	Mill Creek before it enters Hunter Reservoir	Yes
S-128	Fish release out of Hunter Reservoir	Yes*
Candy Rock Road	Mill Creek downstream of Candy Rock Road	(Gage S-128)

* Head of reach is located at gaged flow release

7.1.3.2 Tadpole and Metamorph Surveys

VESs during the early summer for tadpoles will provide a measure of breeding success and an additional opportunity to identify the presence of FYLFs. A late-summer/early-fall VES targeting post-metamorphic frogs will provide an indication of the successful production of juvenile frogs.

Survey teams will consist of a minimum of two biologists. Surveys will be completed walking upstream so individuals seeking cover in the stream are not counted twice. Surveyors will wade or walk the shoreline and shallow-water habitats where possible, scanning ahead for approximately 10 meters (m) at a time, searching for all lifestages (eggs, larvae, juveniles, adults) on both sides of the stream wherever possible. Surveyors will carefully check under bedrock and boulder ledges as well as in deep pockets beneath large cobble in low-velocity areas, as eggs may be located in highly cryptic and well-concealed locations only accessible by touch.

A California Native Species Field Survey Form for all FYLFs observed will be prepared and submitted to the CNDDB. All surveyors will adhere to accepted decontamination guidelines to minimize the potential for transmitting diseases/pathogens (e.g., U.S. Fish and Wildlife Service 2005).

8.0 NORTHWESTERN POND TURTLE

8.1 METHODS

8.1.1 Document the Presence of Northwestern Pond Turtle

- The stream reaches to be surveyed for FYLFs also will be assessed for WPTs. In particular, the biologist team will visually inspect pools and backwaters for WPTs in each stream reach during FYLF surveys.

- Stevens (2007) reported the presence of WPT below Ross Reservoir. This area will be surveyed.

- Focused surveys for WPTs will be conducted around Project forebays and reservoirs. VESs will be conducted by foot, with surveyors stopping frequently to examine logs or other basking areas for WPTs using binoculars.

8.1.2 Determine the Present Distribution and Abundance of WPTs

- Methods of surveying for this species in lentic and lotic habitats will generally follow standard visual survey protocols outlined by U.S. Geological Survey (USGS 2006).

- Other native and non-native aquatic species will be documented (e.g., bullfrogs, beavers, snakes, crayfish, and otters).

- Any non-native invasive species identified will be documented.

- California Native Species Field Survey Form will be prepared for all WPTs recorded and submitted to the CNDDB.

9.0 EDNA SAMPLING

eDNA sampling and analysis will be used to support the determination of the presence and relative distributions of FYLFs and WPTs, as well as the potential detection of CRLFs.

- Sampling for eDNA will take place in each study reach during the FYLF breeding survey. During the breeding survey, there is the potential for both adult FYLF and egg masses to be present in the reaches, increasing the potential for detection if FYLFs are present.

- eDNA sampling will take place at the upper portion of the reach, mid-reach, and the lower portion of the reach (3 locations in each reach) (Table 1).

- The sampling approach will generally follow that described by Bergman et al. (2016); the detailed methodology is provided in Appendix B.

- eDNA will be analyzed for FYLF, WPT, and CRLF.

10.0 INCIDENTAL OBSERVATIONS OF OTHER SPECIES

During VESs, the presence of bullfrogs, other amphibians, or reptiles will be noted and reported if these species are detected. In addition, surveyors will take note of non-native invasive species if encountered.

11.0 CONSISTENCY WITH GENERALLY ACCEPTED SCIENTIFIC PRACTICE

The study methods are consistent with published and unpublished scientific methods and practices currently in use in California. They use modern methods of area- and time-constrained surveys to generate acceptable descriptive statistics of relative species abundance (Heyer et al. 1994). Lotic surveys for FYLF follow standard techniques adapted from Heyer et al. (1994), Peek et al. (2017), and Seltenrich and Pool (2002). WPT surveys will follow visual survey protocols outlined by USGS (2006). Sampling and analyses for eDNA will generally follow the methods described by Bergman et al. (2016).

12.0 PRODUCTS

The following products will be developed and distributed in accordance with the schedule shown below.

- All data and analyses will be provided, as appropriate, to interested parties in a Microsoft Excel spreadsheet (electronic format).
- The study methods and results will be documented in a Technical Study Memorandum (TSM). The TSM will include summary tables and maps, as appropriate.
- Draft TSM will be distributed for review-and-comment.
- Comments on the Draft TSM will be addressed, as appropriate, in a Final TSM.

13.0 SCHEDULE

Task	2024 Schedule
Phase 1	
Desktop Assessment	May 3
Field Reconnaissance	May 6–7
Phase 2	
Initial VES FYLF Breeding/Egg Mass Survey	Week of May 13
Agency Site Visit	May 23
Phase 3	
Review Draft Study Plan	~July 12
Plan Review Period	~July 12 to July 22
Finalize Study Plan	~July 22-29
Phase 4	
Tadpole Surveys	July 1–3
Metamorph Surveys	September
Technical Study Memo	December

14.0 REFERENCES CITED

Bergman, P.S., G. Schumer, S. Blankenship, and E. Campbell. 2016. Detection of adult green sturgeon using environmental DNA analysis. *PLOS ONE* 11(4): e0153500.

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Peek, R.A., S.M. Yarnell, and A.J. Lind. 2017. Visual Encounter Survey Protocol for *rana boylii* in Lotic Environments.

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Stevens, D.W. 2007. California Red-legged Frog Habitat Assessment, Utica Power Authority Hydroelectric System Angels Creek, French Gulch Creek, Mill Creek, Eastern Calaveras County, California.

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Utica Water Power Authority (UWPA). 2023a. Utica Project, FERC Project No. 2019; Draft Application for Small Conduit Exemption and License Surrender for Hydroelectric Facility Exhibit E – Environmental Report, December 2023.

UWPA. 2023b. Angels Project, FERC Project No. 2699; Draft Application for Small Conduit Exemption and License Surrender for Hydroelectric Facility Exhibit E – Environmental Report December 2023.

U.S. Fish and Wildlife Service. 2005. Revised Guidance on Site Assessments and Field Surveys for the California Red-legged Frog. Appendix B: Recommended Equipment Decontamination Procedures. August.

U.S. Geological Survey. 2006. Western Pond Turtle (*Emys marmorata*) Visual Survey Protocol for the South Coast Ecoregion. U.S. Geological Survey Protocol. San Diego, California.

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Appendix A

Foothill Yellow-Legged Frog Standard Operating Procedures and Data Sheets for River and Creek Habitat Assessments

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Foothill Yellow-Legged Frog
Standard Operating Procedures and Data Sheets for River and Creek Habitat Assessments

Revised and Updated for Environmental License Compliance Studies Spring Gap-Stanislaus Project (FERC No. 2130)

The following standard operating procedures (SOPs) describe the parameters and data collection methods for completing river and creek habitat assessments for foothill yellow-legged frogs (*Rana boylii*) (FYLF). Site habitat assessments should be conducted at all sites with suitable habitat for FYLFs, regardless of the visual encounter survey (VES) results.

General Habitat Parameters and Assessment Methods

1. All measurements should be recorded in metric units unless otherwise indicated.

2. For consistency, habitat assessments will be started and completed by the same crew members.

3. Where practicable, habitat assessments will start at the bottom of the site and work upstream.

4. If subsites were designated during the VES, a separate site habitat assessment data sheet should be completed for each subsite.

5. All observations and comments pertaining to amphibian habitat will be recorded on the site habitat assessment data sheet. A field notebook should be used to record additional observations and comments on amphibian habitat not included in the data sheet.

6. Right bank and left bank will be designated facing in a downstream direction.

7. Time entries should be recorded in military format (e.g., 4:00 PM is 1600).

8. Distance and length measurements should be taken with a hip chain, metric tape, or range finder.

9. Velocity measurements should be measured with a Marsh-McBurney (or similar) flow meter. Record velocity measurements to the nearest cm/sec.

10. Field data sheets will be QA/QC checked as each sheet is completed. The reviewer's initials and the review date will be recorded at the bottom of the data sheet.

11. Weather conditions: Habitat assessments can generally be conducted on most days throughout the spring, summer, and fall.

12. Photographs of the site and subsites (if applicable) should include all amphibian habitats present, following the procedures outlined in Section 6.2 of the main text. For river sites and subsites, and creek subsites, photo documentation will include pictures from the bottom looking upstream, from the top looking downstream, and from the middle looking both upstream and downstream. If subsites are not designated on a creek, photographs should be representative of the types of habitats present (both macro- and microhabitats) within the survey area. Photographs should be logged on field data sheets and additional notes recorded in the field notebook.

13. If available, copies of aerial photographs should be used to denote site and subsite boundaries, area surveyed, transect locations, and prominent habitat features. If not available, a site drawing should be included on the back of the habitat assessment data sheet. The general location of egg masses, tadpoles or groups of tadpoles, and the general location of frogs should be included on the aerial photograph or site drawing.

14. Site/subsite habitat assessments are conducted on the initial site visit following the VES. On subsequent site visits, a site habitat assessment data sheet should be completed only if there are significant changes in the overall quality of the habitat (e.g., noticeable changes in the extent and/or distribution of amphibian habitat, the amount of vegetation, water depth and velocity).

Upper Portion of Data Sheet

Below are instructions for completing the river and creek site habitat assessment data sheets, beginning with the upper left corner. These two data sheets are similar, and any differences between them are noted. The instructions for each version of the data sheet are included below.

Date – Record the date of the survey in the following format: month / day / year.

Site # – Record the site number designated during the preliminary habitat assessment (e.g., 1, 2).

Subsite # – If applicable, record the subsite number designated during the VES (e.g., 1a, 1b).

River or Creek Name/Location – Record the river or creek name, if available, and describe the location of the site/subsite or use other identifying information (such as a local landmark or relative position). Provide the approximate survey location along the river or creek and include a landmark or other indicator provided on the USGS topographic map. If site/subsite VESs have been conducted, use the same river or creek names and locations on the site habitat assessment data sheet.

USGS Quad – Indicate the name(s) of the USGS 7.5’ topographic map(s) that cover(s) the location of the site/subsite.

Township/Range – As indicated on the USGS topographic map, indicate the township and range that encompasses all or the largest portion of the site/subsite.

Section – Refer to the USGS topographic map for the Section number containing the site/subsite.

¼ Section – Indicate the ¼ section(s) that contain(s) the site/subsite (e.g., NE ¼, SW ¼, etc.).

Elevation – Record the elevation of the site/subsite as determined by a USGS topographic map or by calibrated GPS.

Start UTMs – Record the UTM data of the beginning location for the site/subsite. Record the UTM easting first and UTM northing second (e.g., 704250, 3391520).

Weather – Indicate current weather, including sky conditions and wind conditions, experienced during the VES. Use the following general guidelines:

Sky conditions:

- Overcast: > 60% cloud cover
- Partly overcast: 20% – 60% cloud cover
- Clear: < 20% cloud cover

Wind conditions:

- Inclement: winds > 20 mph
- Fair: winds 10 – 20 mph
- Ideal: calm to winds < 10 mph

Example: Weather that is Overcast/Fair = greater than 60% cloud cover and winds between 10 and 20 mph.

Total Site Length – Record the total length of the site. If subsites have been designated, this measurement should include all subsites, including the areas between subsites (i.e., steep bedrock reaches, sand beaches, areas with dense overhanging vegetation, etc.) that are not considered suitable habitat. For rivers, include both banks if subsites have been designated on both sides of the river. For creeks, both banks are included as part of the total site length.

River or Creek Aspect – Indicate the orientation of the river or creek drainage at the site/subsite as follows: Southerly, Northerly, Westerly, or Easterly. Example: A south flowing river or creek would have a "southerly" aspect.

Discharge – Estimate the river or creek flow in cubic feet per second (cfs). Where available, use stream gage data.

Water Temperature – Water temperatures should be recorded in degrees Celsius (°C). In small lentic habitats (side pools, scour pools, etc.), water temperatures should be representative of the habitat. River and creek water temperatures should be obtained along the shoreline in edgewater areas, and at a location further from shore that is representative of the main stream temperature.

Observers – The initials of both crew members should appear in this space. The initials of the crew member filling out the data sheet should appear in the first space.

Initial Site Visit – Check this box if this is the first site visit during the field season.

Follow-up Visit – Check this box if the site has been visited previously during the field season. The site habitat assessment data sheet only needs to be completed during a return visit if there are significant changes in habitat features.

Amphibian Habitat Types – The river and creek SOPs differ, as follows.

For rivers: Circle the habitat type(s) from the list provided on the data sheet that best characterize(s) the site/subsite. If more than one habitat type is present at a site/subsite, circle all that are applicable (i.e., side channel with a low gradient riffle).

- *Boulder/Sedge Margin* – Boulder and sedge habitat occurs in low relief areas along the margin of the river. It is characterized by exposed and submerged boulders with interspersed sedge clumps, slow-moving water, and small pools that may be isolated or connected to the river.

- *Side Channel* – A side channel is smaller than the main channel, and generally only receives a portion of the streamflow, and may dry up at low flows. Side channels may be separated from the main channel in locations where center islands or low gradient cobble/boulder bars (often with vegetation) occur and are usually relatively close to the main river channel. Side channels may totally or partially dry out at lower summer flows.

- *Main Channel Pool* – Main channel pools typically occur in low gradient sections of rivers and characteristically occupy most of the wetted channel. Main channel pools are generally relatively deep with low velocities and variable substrates.

- *Isolated/Scour Pool* – An isolated or scour pool is typically a pool formed at higher flows, and normally receives surface flow only during higher outflow periods. Isolated pools and scour pools may be located in bedrock areas, river bars, or inside channels that have been disconnected from the main river flow. These types of pools may be maintained by subsurface feed from the river, or seeps/springs along the river channel.

- *Pool Tail-Out/Pool Backwater* – Pool tail-outs occur at the downstream end of main channel pools adjacent to the main outflow. These areas are often shallow with slow moving or still water, and cobble or boulder substrates. Backwater pools

occur along the margins of rivers at the edge of the main flow and are typically characterized by reverse flows. Backwater pools may occur at river bends, at the bottom of main channel pools, below channel obstructions, etc.

- *Low Gradient Riffle* –Low gradient riffles are characterized by moderate velocities (20 – 50 cm/sec) with little or no whitewater, and partially or totally submerged substrates.

- *Run/Glide* – Run habitats occur in low gradient sections of rivers, have swift water velocities but no water turbulence, with cobble and boulder substrates. Glides also occur in low gradient sections of rivers, have swift water velocities and substrates consisting of gravel and cobble. Runs occur over a definite thalweg; glides do not.

- *Lateral Bar or Point Bar* – Lateral bars are located along a relatively straight section of river, and point bars are located on the inside of a river bend. Both lateral and point bars may have side channels at higher flows that often become disconnected from the main river during the summer or early fall possibly forming isolated pools. These bars may also have isolated or scour pools. For these habitat types, visually estimate the overall bar gradient from the river channel margin to the edge of the upland habitat as follows:

  Low: < 10° slope

  Moderate: 10 – 20° slope

  High: > 20° slope

- *Cobble/Boulder Island* – Cobble/boulder islands tend to occur in wider reaches of the river where small substrates (cobble, boulders, and gravel) dominate the channel. Vegetation is typically present to some degree on these islands, which are generally relatively stable habitats.

- *Other* – This category includes any habitat type that is not described above. Provide a description of the area.

For creeks: Circle the habitat type(s) from the list provided on the data sheet, that best characterize(s) the site/subsite. For a habitat type that is not listed on the data sheet, describe it in the space provided.

- *Pool* – Pools typically occur in lower gradient sections of the creek and characteristically occupy most of the wetted channel. Pools may be deep or shallow with low velocities.

- *Cascade/Pool* – Cascade/pool habitat usually occurs in higher gradient sections of creeks and is dominated by a series of small falls or steps with pools. The substrate generally consists of boulders or bedrock, or a combination of the two.

- *Isolated/Scour Pool* – An isolated or scour pool is typically a pool formed at higher flows, and normally receives surface flow only during higher outflow periods. Isolated pools and scour pools may be located in bedrock areas, inside channels that have been disconnected from the main creek flow, or in wide low gradient areas with small substrates. These types of pools may be maintained by subsurface flow from the creek, or seeps and springs along the creek channel.

- *Pool Tail-Out/Pool Backwater* – Pool tail-outs normally occur at the downstream end of main channel pools. These areas are typically shallow, with slow moving water and cobble or boulder substrates. Backwater pools usually occur along the margins of creeks at the edge of the main flow, and often have reverse currents. Backwater pools may occur at creek bends, at the bottom of main channel pools, below channel obstructions, etc.

- *Side Pool* – Side pools are hydraulically isolated from the main creek channel and receive little or no surface flow. This type of pool may be fed by a seep or spring that discharges to the creek

- *Bedrock Pool* – Bedrock pools occur along moderate gradient stream channels consisting primarily of bedrock shelves, with depressions and scoured areas. These pools may be filled at different streamflow levels, and may become dry as summer progresses, or are sustained by normal creek flows or by seeps and springs.

- *Side/Split Channel* – A side channel is smaller than the main channel and generally receives only a portion of the creek flow. Split channels are usually similar in size and receive similar flows. Side or split channels are usually located in wider, lower gradient sections of creeks with cobble and smaller substrates. As the summer progresses and flows decrease, side channels often become isolated from the main creek flow and dry up, unless fed by subsurface flow or a seep or spring.

- *Low Gradient Riffle* – Low gradient riffles are characterized by moderate velocities (20 – 50 cm/sec), with little or no whitewater, and totally or partially submerged substrates.

- *Run* – Runs occur in low gradient sections of the creek. They have little water turbulence, swift velocities, and substrate compositions of gravel, cobble, and boulder.

- *Other* – This category includes any habitat type that is not described above. Provide a description of the area.

Site/Subsite Location – For rivers, record on which bank the site/subsite occurs (bank designations are made facing downstream). Both banks are always included in creek VESs.

Site/Subsite Length and Width – Record the length and average width of the surveyed amphibian habitat (e.g., lateral bar, boulder/sedge margin). The location of the top and bottom of the site/subsite should be marked with flagging (or other marker), and indicated on an aerial photograph, site drawing, or topographic map.

Approximate Area of Site/Subsite – This is an indication of the amount of habitat available, which may be greater than the actual area surveyed. This is determined using the length and width measurements above. The area should also be delineated on an aerial photograph or site drawing. Area should be recorded in square meters.

Habitat Features

% Margin Vegetation – Indicate to the nearest 5 % the length of stream bank with vegetation at the edge of the wetted channel within the site/subsite. Circle or write in all types present and indicate the dominant (Dom) vegetation type(s), by checking one or more boxes on the data sheet. This parameter is specific to vegetation along the stream margin that may be used for cover by juvenile/subadult and adult frogs. For creeks without subsites, estimate the percent margin vegetation within the survey area; if this feature is not consistent within the site, record a range in percent.

% Emergent Vegetation – Indicate to the nearest 5 % the total area of aquatic habitat within the site/subsite with emergent vegetation. Circle or write in all types present and indicate the dominant (Dom) vegetation type(s), by checking one or more boxes on the data sheet. For creeks without subsites, estimate the percent emergent vegetation within the surveyed area; if this feature is not consistent within the site, record a range in percent.

% Submerged Vegetation – Indicate to the nearest 5 % the area of aquatic habitat within the site/subsite that has submerged vegetation. Circle or write in all types present and indicate the dominant (Dom) vegetation type(s), by checking one or more boxes on the data sheet. For creeks without subsites, estimate percent submerged vegetation within the surveyed area; if this feature is not consistent within the site, record a range in percent.

% Cover Aquatic – Indicate to the nearest 5 % the total area of aquatic habitat within the site/subsite that provides cover for all life stages of FYLF. Circle or write in all types present and indicate the dominant (Dom) cover type(s), by checking one or more boxes on the data sheet. For creeks without subsites, estimate the percent aquatic cover within the surveyed area; if this feature is not consistent within the site, record a range in percent.

% Cover Terrestrial –Indicate to the nearest 5 % the total amount of terrestrial habitat between the river margin and upland habitat within the site/subsite that provides cover for amphibians. Circle or write in all types present and indicate the dominant (Dom) cover type(s), by checking one or more boxes on the data sheet. For creeks without subsites, estimate the percent terrestrial habitat between the creek and upland habitat within the surveyed area that provides cover for amphibians; if this feature is not consistent within the site, record a range in percent.

% Overhanging Vegetation – Indicate to the nearest 5 % the length of stream within the site/subsite with overhanging vegetation. Circle or write in all types present and indicate the dominant (Dom) vegetation type(s), by checking one or more boxes on the data sheet. For creeks without subsites, estimate the percent overhanging vegetation within the surveyed area; if this feature is not consistent within the site, record a range in percent. This parameter should only include vegetation that is directly overhanging river or creek habitats within 2 m of the water surface. Upper canopy vegetation should be included under % Riparian Canopy.

% Riparian Canopy – Indicate to the nearest 5 % the amount of riparian canopy within the site/subsite. Circle or write in all types of vegetation that provide shade along the margin of the stream and indicate the dominant (Dom) type(s). For creeks without subsites, estimate the percent riparian canopy within the surveyed area; if this feature is not consistent within the site, record a range in percent. This measurement does not include overhanging vegetation (i.e., within 2 m of the water surface).

Aquatic Substrate – Indicate to the nearest 5 % the aquatic substrate types present in the site/subsite. Size classifications for substrate types follow the modified Wentworth (1922) scale.

Substrate Type	Size Range (mm)
Silt/Clay/Mud	< 0.059
Sand	0.06 – 1
Gravel/Pebble	2 – 63
Cobble	64 - 256
Boulder	> 256
Bedrock	–

Substrate Embeddedness – Circle the category that best represents the embeddedness of the aquatic substrate in the site/subsite. Embeddedness is defined as the degree to which larger particles (large gravel, pebble, cobble, and boulder) are surrounded or covered by fine sediment such as sand, silt, or clay. Embeddedness categories for stream channel materials (Platts et al. 1983) are provided below. These categories identify fine sediment as material less than 2 mm in diameter.

Level of Embeddedness	Percent of Gravel, Pebble, Cobble, and Boulder Surfaces Surrounded by Fine Sediment
Low	< 25%
Moderate	25-50%
High	> 50%

Dominant Substrate Shape – Indicate the dominant shape of the substrates at the site/subsite. Classification of substrate shape includes angular, sub-angular, and rounded. This parameter is relative to other substrate within the drainage.

River or Creek Habitat – Indicate to the nearest 5 % the river or creek habitats within the site/subsite. Descriptions of aquatic habitat features (Rosgen 1996) are provided below and should be used as a general guide when classifying aquatic habitat.

Aquatic Habitat	General Characteristics
Riffle	Little to considerable whitewater, moderate to fast velocities >20 cm/s, substrate of gravel and cobble to cobble and boulder - totally submerged to exposed, shallow to moderately deep, < 4% - 7% slope (low gradient to high gradient riffles)
Run	No water turbulence; swift velocity; substrate of gravel, cobble, and boulder; low gradient; occurs over a definite thalweg
Glide	No water turbulence; low to moderate even velocity; substrate of sand, gravel, and cobble; 0- 1% gradient; occurs over a wide channel lacking a definite thalweg
Pool	Low velocity, usually large and deep occupying much of the channel, substrate variable, little or no gradient
Cascade/Pool	Varying velocities, bedrock and boulder substrates, high gradient, pools separated by short cascades
Step-Pool	Varying velocities, boulder substrate, high gradient, pools separated by short riffles or cascades
Pocket Water	Sections of a swift flowing stream containing numerous boulders or other large obstructions that create eddies or scour holes (pockets) behind the obstructions

River or Creek Gradient – Record the average stream gradient within the site/subsite. Stream gradient should be visually estimated as follows:

- Low: < 2 %
- Moderate: 2 – 4 %
- High: > 4 – 10+ %

River or Creek Gradient Change – Indicate if there is a significant change in gradient (e.g., low= riffle to high = bedrock cascade/pool) upstream of the site or between adjacent subsites. If there are several significant gradient breaks associated with adjacent subsites, describe them sequentially in the Comments section at the bottom of the data sheet. Use the categories provided under River or Creek Gradient above. Circle whether the gradient upstream of the site, or the adjacent upstream subsite, is **higher** or **lower** relative to the site/subsite being evaluated.

Change in River or Creek Habitat – Describe the alteration in aquatic habitat associated with a change in gradient, using the habitat types listed above under River or Creek Habitat (e.g., the lower portion of the site is a slow glide that changes to a high gradient riffle upstream due to an increase in stream gradient).

Rosgen Channel Type – Circle the appropriate channel type for the site/subsite, using the letter designations provided on the data sheet. Descriptions of the channel types (Rosgen 1996) are provided below:

A: *Cascading channels in a steeply sloped, 'V-shaped' drainage with a 4 – 10% gradient.*

B: *Stream types that occur primarily on moderately steep to gently sloped terrain, with the predominant landform as a narrow and moderately sloping valley. "B" streams are moderately entrenched, moderate gradient (2 – 4%), riffle dominated channels, with infrequently spaced pools*

C: *Stream types that are located in narrow to wide alluvial valleys with a well developed, slightly entrenched floodplain. "C" stream types are low gradient (< 2%), meandering, alluvial channels dominated by riffle/pool habitat and point bars.*

D: *Stream types that are located in broad valleys with alluvial and colluvial fans. "D" stream types are low gradient (< 2%), braided channels with longitudinal and transverse bars, and are characteristically wide channels with eroding banks.*

DA: *Stream types that are located in broad, low-gradient valleys with fine alluvium and/or lacustrine soils, multiple channels, with an expansive well-vegetated floodplain and associated wetlands. "DA" stream types are riffle/pool, similar to stream types "C" and "E," with well-vegetated, laterally stable bars, and very stable stream banks.*

E: *Stream types that occur in broad valleys or floodplains with meadows and are composed of alluvial materials. "E" types are low gradient (< 2%), meandering riffle/pool channels with stable well-vegetated banks.*

F: *Stream types that occur in meadows or other low gradient areas composed of unconsolidated materials. "F" types are entrenched meandering riffle/pool channels on low gradients (< 2%) with a high width/depth ratio typically associated with high bank erosion rates (meadow streams).*

G: *Stream types that are entrenched "gully" step-pool channels with low to moderate gradient (2 – 4%), and high bank erosion rates.*

Wetted Channel Width – Measure and record the wetted channel width (that portion of channel with water) in meters, within a representative section of the site/subsite.

Bankfull Width – Record the bankfull width (upper limit of high flows) of the channel in meters within a representative section of the site/subsite. This measurement should be based on indicators such as staining on rocks, and scour and debris lines. Many hydrologically controlled streams do not display good indicators, and bankfull widths may be approximations based upon available features.

Water Turbidity – Visually estimate turbidity and circle **Low**, **Medium**, or **High**. Estimates should be based upon the following general guidelines:

- **Low** – able to see submerged substrate (including small gravels) clearly in 0.6 m (2 ft) of water
- **Medium** – unable to distinguish small gravels in 0.6 m (2 ft) of water
- **High** – unable to readily distinguish large gravels in 0.6 m (2 ft) of water

  Note: turbidity estimates should consider lighting conditions (shading may affect ratings).

Water Color – Circle **Clear** or **Discolored** (e.g., tannin staining).

Bank Gradient – Circle the appropriate gradient and applicable stream bank (L/R). Record the average bank gradient within the site/subsite; if this feature is not relatively consistent within the surveyed area, indicate a range. For both rivers and creeks, use the categories below.

- Low: < 15°
- Moderate: 15 – 40°
- High: > 40°

Active Bank Erosion – Indicate whether active erosion is occurring within or adjacent to the site/subsite.

Inundated River Bar – For river sites/subsites only, indicate whether inundated bars are present, and record the **Approximate Area** (m^2) of the river bar that is inundated at the existing flow level. This area should include all potentially suitable habitats from the water line out to the point where there is a noticeable change either in slope or in substrate characteristics, or both. The inundated portion of the bar represents an extension of the existing shoreline amphibian habitat that would likely remain amphibian habitat at lower flows. **Average Depth** of the inundated area should be recorded to the nearest 5 cm. Estimate the **Velocity Range** (e.g., 0–5 cm/sec) within a representative portion of the inundated area using a flow meter, or if experienced, visually estimate.

Edgewater – For river sites/subsites only, indicate if there are shallow water areas (typically < 45 cm deep) along the margin of the river with calm or slow-moving water and primarily cobble and boulder substrates. Record the **Average Depth** (cm), **Approximate Area** (m^2), and the **Location in Site/Subsite** of the edgewater habitat.

Tributary Nearby – Indicate if there is a tributary confluence at or in close proximity to the site/subsite. Record the tributary **Location** in reference to the site (upstream (**U/S**) or downstream (**D/S**), and left bank (**LB**) or right bank (**RB**) facing downstream), the approximate **Distance** (m) from the site/subsite, and whether it is **Perennial** or **Ephemeral**. Note: if a tributary is not visible from the top or bottom of the site, consult a USGS 7.5' topographic map and estimate the distance from the site/subsite to the nearest tributary.

Upland Habitat Type – Circle the appropriate upland habitat type adjacent to the site/subsite from the list provided or add an alternative habitat type if necessary.

Fish Present – Record fish that are observed within or adjacent to the site/subsite. Indicate if fish are either suspected or known to occur but were not observed. For **Type**, circle or write in the species groups that are present. The following are representative classifications:

- Salmonids – trout and salmon
- Centrarchids – bass and sunfish
- Cyprinids – minnows

Herpetofauna and Life Stage – Circle or write in all that apply. Indicate the approximate number and the life stages present (A = adult, J = juvenile/subadult, T = tadpole, E = egg).

Other Species Observed – Record other species observed at the site/subsite.

Impacts to Amphibian Habitat – Indicate if there are existing activities within or adjacent to the site/subsite that could impact amphibians or their habitat. If possible, assess the potential level of impact (low, moderate, or high).

Photographs – Record the photograph number(s) and description of photo or reference to notes and description recorded in the field notebook.

Comments – Comments will be noted at the bottom of the site habitat assessment data sheet. Comments should include observations of conditions potentially affecting amphibians or their habitat that are not included on the data sheets. Such observations might include: a discharge entering the stream, logging and construction activities, recreation activities, notes on particularly good or particularly poor habitat, and other conditions that stand out or are relatively uncommon.

QA/QC – Record the initials of the person who reviews the data sheet for completeness and the date it is reviewed. The reviewer should not be the person who completes the data sheet. The reviewer should complete the QA/QC review before leaving the site.

References

Platts, W.S., W.F. Megahan, and G.W. Minshall. 1983. Methods for evaluating stream, riparian, and biotic conditions. U.S. Forest Service. In: Bain, M.B., and N.J. Stevenson, editors. 1999.

Aquatic habitat assessment: common methods. American Fisheries Society, Bethesda, Maryland.

Rosgen, D. 1996. Applied river morphology. Wildland Hydrology, Pagosa Springs, CO.

Wentworth, C.K. 1922. A scale of grade and class for elastic sediments. Journal of Geology 30: 377- 392.

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Foothill Yellow-Legged Frog
River Habitat Assessment

Date: mm___dd___yy____ Site #:_____ Subsite #:____ River Name/Location:_____
USGS Quad: _____ Township: _____ Range:_____ Section:_____ ¼ Section:_____ Elevation:_____
Start UTMs: _____ Weather: *Sky*: Overcast Partly Overcast Clear *Wind:* Inclement Fair Ideal
Total Site Length: _____ River Aspect: _____ Discharge (cfs) _____ Water Temp: (edgewater) ____ (main channel) _____
Observers: _____ _____ Initial Site Visit □ Follow-up Site Visit □

AMPHIBIAN HABITAT TYPES:

- Boulder/Sedge Margin
- Side Channel
- Main Channel Pool
- Isolated/Scour Pool
- Pool Tail-Out/Pool Backwater
- Low Gradient Riffle
- Run/Glide
- Lateral Bar or Point Bar Bar gradient: low (<10º) moderate (10–20º) high (>20º)
- Cobble/Boulder Island
- Other: _____

Site/Subsite: Location: right bank left bank Length: _____ Width: _____

Approximate Area (m^2): _____

HABITAT FEATURES:

% Margin Vegetation: ____ Type: forbs grass sedge rush blackberry other: _____
Dom.: □ □ □ □ □ □

% Emergent Vegetation: ____ Type: grass sedge rush pondweed other: _____
Dom.: □ □ □ □ □

% Submerged Vegetation: ____ Type: algae rooted aquatic veg other: _____
Dom.: □ □ □

% Cover Aquatic: _____ Type: rootwad aquatic veg. woody debris gaps between substrate other : _____
Dom.: □ □ □ □ □

% Cover Terrestrial: _____ Type: duff/leaf litter burrows woody debris undercut bank other: _____
Dom.: □ □ □ □ □

% Overhanging Vegetation: ____ Type: willow blackberry alder dogwood other: _____
Dom.: □ □ □ □ □

% Riparian Canopy: _____ Type: willow ash alder maple oak conifer other: _____
Dom.: □ □ □ □ □ □ □

Aquatic Substrate (%): silt/clay _____ sand _____ gravel/pebble _____ cobble _____ boulder _____ bedrock
Substrate Embeddedness: low (<25%) moderate (25-50%) high (>50%)
Dominant Substrate Shape: angular sub-angular rounded
River Habitat (%): riffle _____ run _____ glide _____ pool _____ cascade/pool _____ step-pool _____ pocket water
River Gradient: low (0-2%) moderate (2-4%) high (4-10+%) Wetted Channel Width: ___ Bankfull Width: _____
River Gradient Change: No Yes higher lower Change in River Habitat: _____
Rosgen Channel Type: A B C D DA E F G
Water Turbidity: low moderate high Watercolor: clear discolored (tannins, etc.) Bank Gradient: low (<15°) R / L mod (15-40°) R / L high (>40°) R / L Active Bank Erosion: Yes No Inundated River Bar: present absent Approximate Area (m^2) _ Avg. Depth: _____
Velocity Range: __ Edgewater: Yes No Average Depth: _____ Approximate Area: ___
_____ Location in Site/Subsite: ____ Tributary Nearby:Yes No Location:
U/S D/S LB RB Distance: _____ Perennial Ephemeral

Upland Habitat Type: mixed conifer foothill hardwood/conifer foothill hardwood scrub/shrub other:_____
Fish Present: Yes No Type: salmonid centrarchid cyprinid other: _____
Herpetofauna & Life Stage (A J T E) tree frog ___ bullfrog _____ western pond turtle ____ garter snake ____ other_____
Other Species Observed: _____
Impacts to Amphibian Habitat (circle): grazing recreation industrial other: _____ low mod high

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QA/QC (initials): _____ Date: _____

Foothill Yellow-Legged Frog
River Habitat Assessment

PHOTOGRAPHS: _____

COMMENTS: _____

Foothill Yellow-Legged Frog
River Habitat Assessment

Subsite #: _____ Creek Name/Location: _____ USGS Quad: _____
Township: _____ Range: Section:_____¼ Section:___ Elevation: ___ Start UTMs: ___ Weather:
Sky: *Overcast Partly Overcast Clear* *Wind: Inclement Fair Ideal*
Total Site Length: _____ Creek Aspect: _____ Discharge (cfs) _____ Water Temp: (edgewater) ____ (main channel) _____
Observers: _____ _____ Initial Site Visit □ Follow-up Site Visit □

AMPHIBIAN HABITAT TYPES

- Pool
- Cascade/Pool
- Isolated/Scour Pool
- Pool Tail-Out/Pool Backwater
- Side Pool
- Bedrock Pool
- Side/Split Channel
- Low Gradient Riffle
- Run
- Other _____

Site/Subsite: Length: _____ Width: _____ Approximate Area (m^2): _____

HABITAT FEATURES

% Margin Vegetation: ____ Type: forbs grass sedge rush blackberry other: _____
Dom.: □ □ □ □ □ □
% Emergent Vegetation: ____ Type: grass sedge rush pondweed other: _____
Dom.: □ □ □ □ □
% Submerged Vegetation: ____ Type: algae rooted aquatic veg other: _____
Dom.: □ □ □
% Cover Aquatic: _____ Type: rootwad aquatic veg. woody debris gaps between substrate other: ____
Dom.: □ □ □ □ □
% Cover Terrestrial: ____ Type: duff/leaf litter burrows woody debris undercut bank other: _____
Dom.: □ □ □ □ □
% Overhanging Vegetation: ____ Type: willow blackberry alder dogwood other: _____
Dom.: □ □ □ □ □
% Riparian Canopy: _____ Type: willow ash alder maple oak conifer other: _____
Dom.: □ □ □ □ □ □ □

Aquatic Substrate (%): silt/clay ____ sand ____ gravel/pebble ____ cobble ____ boulder ____ bedrock ____
Substrate Embeddedness: low (< 25%) moderate (25-50%) high (> 50%)
Dominant Substrate Shape: angular sub-angular rounded
Creek Habitat: riffle: ____ run: ____ glide: ____ pool: ____ cascade/pool: __ step-pool: ___ pocket water: ___
Creek Gradient: low (0-2%) moderate (2-4%) high (4-10+%)
Creek Gradient Change: No Yes higher lower Change in Creek Habitat: _____
Rosgen Channel Type: A B C D DA E F G
Wetted Channel Width: _____ Bankfull Width: _____
Water Turbidity: low moderate high Watercolor: clear discolored (tannins, etc.)
Bank Gradient: low (<15°) R / L mod (15-40°) R / L high (>40°) R / L Active Bank Erosion: Yes No
Tributary Nearby: Yes No Location: U/S D/S LB RB Distance: _____ Perennial Ephemeral

Upland Habitat Type: mixed conifer foothill hardwood/conifer foothill hardwood scrub/shrub other: _____
Fish Present: Yes No Type: salmonid centrarchid cyprinid other: _____
Herpetofauna & Life Stage (A J T E) tree frog ____ bullfrog _____ w. pond turtle _____ garter snake ____ other: _____
Other Species Observed: _____
Impacts to Amphibian Habitat: grazing recreation industrial other: _____ low mod high

PHOTOGRAPHS: _____
COMMENTS: _____

QA/QC (initials): _____ Date: _____

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APPENDIX B

eDNA Methods

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1 Field Methodology

1.1 Materials

A materials list for sampling is as follows:

- Cordless drill (brushless) to power the pump driver
- Pump driver bit (0.5-inch spade)
- Backup drill battery and battery charger
- Cole Parmer Peristaltic Pump Masterflex® Easy Loader II Model#77200-52
- U.S. Environmental Protection Agency (USEPA)–approved Millipore Sterivex™
- 0.45 micrometer (µm) sterile filter units (USEPA# 90260-ITA-001)
- Masterflex® spooled peroxide-cured silicon tubing, L/S 15
- Tube adaptor (Cole Parmer 30800-22)
- Inlet caps (Qiagen Mat. No. 1104193)
- Outlet caps (Qiagen Mat. No. 1104194)
- Graduated beaker
- Nitrile gloves (non-powdered)
- Ziploc® bags
- Permanent markers
- Backpack and chestpack
- Global positioning system (GPS) unit with extra batteries
- Cooler with blue ice pack
- Ice chest with blue ice packs
- Garbage bags

1.2 Contamination Prevention Guidelines

The field team followed the following contamination prevention guidelines.

1.2.1 Guidelines

- The Genidaqs protocol is crafted to reduce the possibility of contamination, primarily through the use of a filter with filtration material shielded within an external housing. Therefore, the filtration material is never handled by the user. Irrespective of filter, following the procedure ensures the best chances of success.

- Prior to project initiation, Genidaqs recommends that field personnel be trained on the collection protocols, which in part consists of field personnel verifying that they can reliably generate both negative and positive field collections.

- The Genidaqs protocol uses pre-packaged sterile material. Material should be transported prior to field use in “clean” containers.

- Ideally, there should be a “clean” person handling the filter after water collection who bags and tags. If a single user is in the field, change sterile gloves at each site.

- Consumables are considered single use. Following use, filter packaging, silicon tubing, gloves, etc. are immediately placed in “dirty” storage.

- When sampling a flowing water system, conduct collections moving upstream.

- Take field negative controls.

1.3 General Protocol for Sterilizing Instruments and Equipment for Analyzing Genetic Samples

- Materials needed:
  - 20% household bleach solution (mixed with tap water)
  - Deionized (DI) water in wash bottle
  - 70% ethanol (ETOH) in wash bottle
- Kimwipes or paper towels
- Rinse all tissue and body fluids off of instruments, vials, petri dishes, and any other item being exposed to sample tissue using tap water.
- Submerge items in 20% bleach solution for at least 1 minute. Be sure to completely expose all surfaces to bleach, including opening and closing scissors while submerged.
- Remove items from bleach and rinse with DI water. Make sure to remove all bleach.
- Rinse all items with 70 to 100% ETOH.
- Thoroughly dry items with a fresh Kimwipe or paper towel, or air dry on clean paper towels.

1.4 Genidaqs Sample Collection Protocols[2]

1.4.1 Water Collection and Filtration

- Water samples may be collected from either the bank margin, or, where this is infeasible due to dense vegetation or steep topography, by vessel at the channel center.
- For each sampling event, water is filtered directly from the water body at an approximate depth of 2 to 6 inches below the surface. We have found a fishing pole or telescoping pole useful for holding tubing under water and away from the user.

[2] Bergman, P.S., G. Schumer, S. Blankenship, and E. Campbell. 2016. Detection of adult green sturgeon using environmental DNA analysis. PLOS ONE 11(4): e0153500.

- Sterile Masterflex® spooled peroxide-cured silicon tubing, L/S 15 with an internal diameter of 4.8 millimeters (mm) is used in association with a portable Masterflex® L/S Easy-Load II Model #77200-52 peristaltic pump powered by a cordless hand drill. The tubing is clamped within the pump, so the pump never comes into contact with the water. Peristaltic action draws water from the source.

- Water samples are filtered through a Millipore Sterivex™-GP 0.45 µm sterile filter unit. Partially tear open filter packaging to expose filter and connect to tube adapter. The filter only connects to the tube adapter in one way, ensuring proper connection. Note that water is expelled out of the filter and is not sucked into the filter, which is a reverse orientation to that of grab samples using disc filter apparatus.

Pro tip: If the tube adapter is not used (or forgotten), the tube itself can be press fit over the filter inlet, and the process will work. Note: if the filter is installed backward, water will simply flow through the filter assembly and not come into contact with the filter material (i.e., no organic material will be captured).

- All water filtration occurs directly at each site. No water is transported or stored during sampling nor is any water transported between sampling sites.

- Sample filtrate is captured and measured in a graduated beaker to verify the volume filtered for each sample. Filtration ceases when either the target volume is reached or the filter clogs. Reverse the pump to drain remaining water from inside the filter assembly.

- Try to filter 1 liter of water per filter, if possible.

Note: It is very important to remove water from inside the filter. If pumping is ineffective, the filter can be manually shaken to remove water.

- In water with high levels of particulates, sediment may accumulate inside the filter assembly. This is fine as long as residual water is removed from the filter assembly.

- Filtrate is poured out after completion of sampling at each site.

- After filtration, the inlet and outlet of filters are capped at each end, labeled with location ID and volume filtered, placed into a sterile secondary container (Ziploc®), sealed, and immediately placed in a cooler. The filter is considered self-contained at this point.

- Tubing and gloves are immediately disposed of after each use into a sealed trash bag.

- All filters are kept in a cooler for the duration of the sampling day, after which they are transferred to a −20-degree Celsius (°C) laboratory freezer. The filters are stored within individually sealed secondary containers until DNA extraction.

Pro tip: Filters can be wiped with bleach to sterilize the outside of each filter prior to storage and placed into a new secondary container labeled appropriately for the project. This bleach application step breaks the connection between field and lab environments.

- To ensure that field equipment is free of contamination, DNA field controls are taken for each sampling day. Each field control consists of Sterivex™ filtered water processed in the same fashion as the field samples. The field controls are processed for the presence of target species DNA in parallel with all samples.

Pump Apparatus

Filter Apparatus

Pump Apparatus with Silicone Tubing