4.1.3 Pyramid Reach Fish Populations Study

4.1.3.1 Project Nexus

Continued Project O&M and Project-related recreation activities have the potential to affect fish populations in Pyramid reach (i.e., the 18.4-mile-long section of Piru Creek from Pyramid Dam to the NMWSE of Lake Piru).

4.1.3.2 Existing Information and Need for Additional Information

Existing, relevant, and reasonably available information regarding fish populations in Pyramid reach is provided in Section 4.5 of the Licensees' PAD. As a summary, surveys conducted by CDFW in Pyramid reach in 1987 detected two native species (rainbow trout and prickly sculpin) and five introduced fishes (bluegill, green sunfish, largemouth bass, catfish, and brown trout). CDFW stocked Pyramid reach with rainbow trout and largemouth bass in the 1930s, and with rainbow trout from the1940s to August 2008.

Additional information, which will be provided by this *Pyramid Reach Fish Populations Study*, is needed to determine the presence and locations of the fish community that occur in Pyramid reach that could be affected by the Project.

4.1.3.3 Study Goals and Objectives

The goals of this *Pyramid Reach Fish Populations Study* are to: (1) characterize fish species composition and relative spatial distribution; (2) estimate abundance (i.e., fish per mile in areas feasible for electrofishing) or relative abundance of fish by species; (3) analyze fish population size-structure and age-class structure; and (4) calculate the fish condition factor in Pyramid reach. The objective of this *Pyramid Reach Fish Populations Study* is to fill recognized gaps in existing information on the presence and extent of fishes in Pyramid reach.

4.1.3.4 Study Methods

Study Area

The study area for the *Pyramid Reach Fish Populations Study* includes Pyramid reach as shown in Figure 4.1-7 below.

General Concepts and Procedures

- Personal safety is the most important consideration of each fieldwork team. Fieldwork will only occur in safely accessible areas and under conditions deemed safe by the field crews. Locations within the study area that cannot be accessed in a safe manner (e.g., locations containing dense vegetation or unsafe slopes) and areas inundated when the surveys are performed, will not be surveyed; these areas will be identified in the data summary and an explanation for survey exclusion will be provided.

- The *Pyramid Reach Fish Populations Study* will begin after FERC issues its Study Plan Determination.

- The *Pyramid Reach Fish Populations Study* does not include the development of requirements for the new license, which will be addressed outside the Study.

- The *Pyramid Reach Fish Populations Study* focuses specifically on fish populations within Pyramid reach, but the study area for the *Pyramid Reach Fish Populations Study* is specific to locations that can support that resource.

- If required for the performance of the *Pyramid Reach Fish Populations Study*, the Licensees will make a good faith effort to obtain permission to access private property well in advance of initiating the Study. The Licensees will only enter private property if permission has been provided by the landowner.

- The Licensees will acquire all necessary agency permits and approvals prior to beginning fieldwork for the *Pyramid Reach Fish Populations Study*.

- Field crews may make variances to the *Pyramid Reach Fish Populations Study* in the field to accommodate actual field conditions and unforeseen problems. Any variances in the *Pyramid Reach Fish Populations Study* will be noted in the data resulting from the *Pyramid Reach Fish Populations Study*.

- To prevent the introduction and transmittal of amphibian chytrid fungus and invasive aquatic species (e.g., quagga mussels, zebra mussel, and Asian clams), field crews will be trained on, provided with, and use materials (e.g., Quat) for decontaminating their boots, waders, and other equipment when leaving or traveling between water-based study sites. Field crews will follow DWR's Quagga and Zebra Mussel Rapid Response Plan and CDFW's Aquatic Invasive Species Decontamination Protocol which can be found at the following link: (https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=43333). All boats used during the study will follow cleaning protocols, including inspections before and after use. All decontamination requirements in place at Project reservoirs including those of DWR's *Quagga and Zebra Mussel Rapid Response Plan* for the SWP will be strictly followed (DWR 2010).

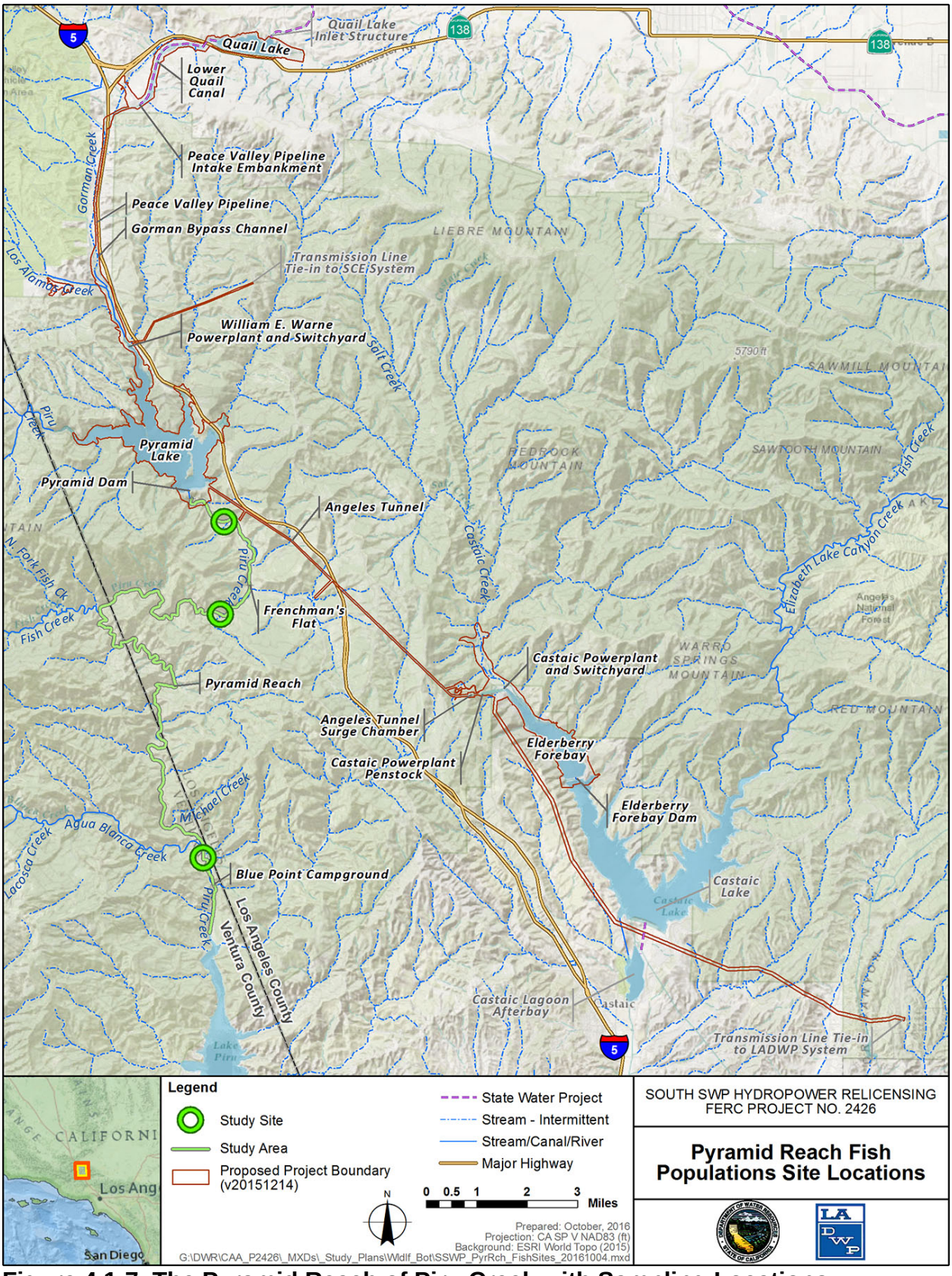

Figure 4.1-7. The Pyramid Reach of Piru Creek with Sampling Locations

Methods

Data collection for the *Pyramid Reach Fish Populations Study* will consist of four steps: (1) classify mesohabitat and channels; (2) conduct eDNA sampling; (3) select sampling sites for fish population sampling; and (4) sample fish population, as described below. Fish sampling will be predicated on the Licensees obtaining necessary federal and State of California permits for sampling. Required permits will include a CDFW scientific collecting permit for streams that do not contain federal ESA-listed species and an ESA section 10(a)(1)(A) authorization from the USFWS for arroyo toad.

Step 1 – Classify Mesohabitat and Channels. Mesohabitat will be classified from the NMWSE of Lake Piru upstream to Pyramid Dam. A three-tiered habitat mapping classification system developed by Hawkins et al. (1993) will be used to assist in the identification of individual habitat units in the field. Level III categories are generally modified/adopted from McCain et al. (1990) and Flosi and Reynolds (1994). Figure 4.1-8 shows the relationship among the three levels. At the broadest level, Level I categorizes habitats as “fast water” and “slow water.” In Level II, fast water and slow water are each subdivided into two categories: turbulent and non-turbulent, and scour pool and dammed pool, respectively. Level III includes the 18 distinct mesohabitat types that will be used to classify habitat for the study. These expand on the Level II classification by separating each habitat type by either gradient, physical structure, or geomorphic process.

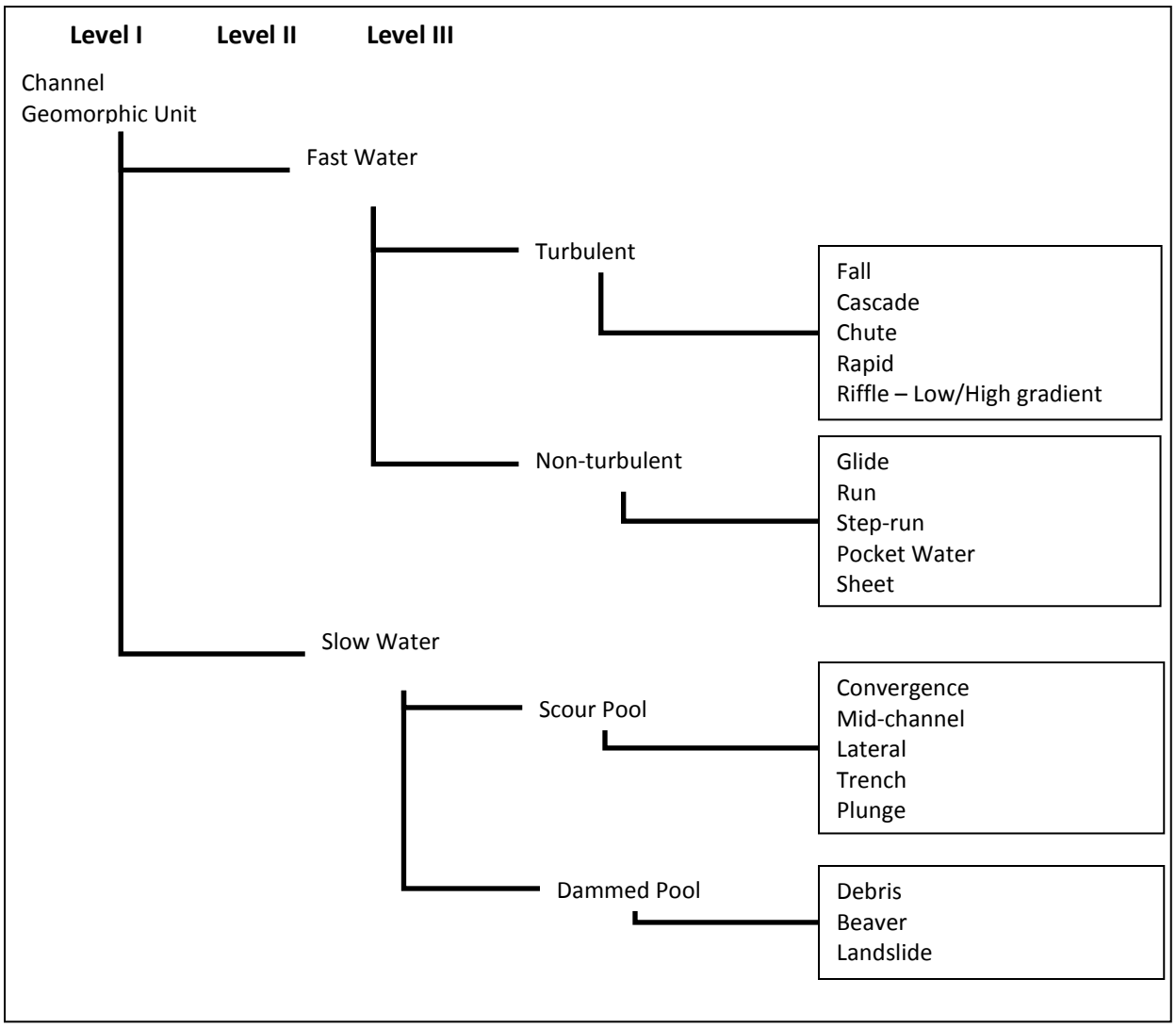

Figure 4.1-8. Key to Habitat Types

Each distinct habitat unit will be numbered consecutively in an upstream direction. Habitat type descriptions are listed in Table 4.1-1 below. Channel and habitat characteristics shown in Figure 4.1-8 and Table 4.1-1 will be assessed in all ground surveys, and the aerial imagery will be used to assess channel and habitat types when streams are clearly visible.

The extent of the ground-based habitat mapping surveys will be determined based on the visibility of the stream from aerial imagery, the length of the sub-reach to be surveyed, and whether the reach is accessible by field crews. Ground-based mapping will be conducted in those stream segments where habitat characteristics are not adequately discernible in the aerial imagery.

Limited ground-based mapping will also be conducted in stream segments that are conducive to mapping using aerial imagery to establish a baseline for mapping the remainder of the reach. Ground-based mapping in streams visible in the aerial imagery will be used to “calibrate the eye” by physically measuring and typing specific habitat units observed in the aerial imagery. Mesohabitat units assessed on the ground will then be “typed” in the remainder of the stream sub-reach using the aerial imagery.

The physical parameters (e.g., bankfull width, pool depth, substrate) measured for each mesohabitat unit during ground-based mapping are expected to be similar for those same mesohabitat units throughout the remainder of the sub-reach. Additional habitat information, such as counting LWD (any un-rooted wood with a minimum length of three feet and minimum diameter or four inches at the large end) in the channel, trout spawning gravel and spawning gravel patch size, and potential fish passage barriers, will be documented during ground based mapping at each fish sampling site.

Table 4.1-1. Habitat Types

I. Fast Water:			Riffles, rapid, shallow stream sections with steep water surface gradient.
	A. Turbulent:		Channel units having swift current, high channel roughness (large substrate), steep gradient, and non-laminar flow and characterized by surface turbulence.
		1.Fall:	Steep vertical drop in water surface elevation. Generally not modelable.
		2.Cascade:	Series of alternating small falls and shallow pools; substrate usually bedrock and boulders. Gradient high (more than 4 percent). Generally not modelable.
		3.Chute:	Narrow, confined channel with rapid, relatively unobstructed flow and bedrock substrate.
		4.Rapid:	Deeper stream section with considerable surface agitation and swift current; large boulder and standing waves often present. Generally not modelable.
		5.Riffles:	Shallow, lower-gradient channel units with moderate current velocity and some partially exposed substrate (usually cobble). • Low gradient – Shallow with swift flowing, turbulent water. Partially exposed substrate dominated by cobble. Gradient moderate (less than 4 percent). • High gradient – Moderately deep with swift flowing, turbulent water. Partially exposed substrate dominated by boulder. Gradient steep (greater than 4 percent). Generally not modelable.
	B. Non-turbulent:		Channel units having low channel roughness, moderate gradient, laminar flow, and lack of surface turbulence.
		1.Sheet:	Shallow water flowing over smooth bedrock.
		2.Run / Glide:	Shallow (glide) to deep (run) water flowing over a variety of different substrates.
		3.Step Run	A sequence of runs separated by short riffle steps. Substrates are usually cobble and boulder dominated.
		4.Pocket Water:	Swift flowing water with large boulder or bedrock obstructions creating eddies, small backwater, or scour holes. Gradient low to moderate.
II. Slow Water:			Pools; slow, deep stream sections with nearly flat water surface gradient.
	A. Scour Pool:		Formed by scouring action of current.
		1.Trench:	Formed by scouring of bedrock.
		2.Mid-channel:	Formed by channel constriction or downstream hydraulic control.
		3.Convergence	Formed where two stream channels meet.
		4.Lateral:	Formed where flow is deflected by a partial channel obstruction (stream bank, rootwad, log, or boulder).
		5.Plunge:	Formed by water dropping vertically over channel obstruction.
	B. Dammed Pool:		Water impounded by channel blockage.
		1.Debris:	Formed by rootwads and logs.
		2.Beaver:	Formed by beaver dam.
		3.Landslide:	Formed by large boulders.
		4.Backwater:	Formed by obstructions along banks (recorded as a comment or note to mapping).
		5.Abandoned Channel:	Formed along main channel, usually associated with gravel bars (not part of the main active channel; recorded as a comment or note to mapping).

Note: Adapted from McCain et al. 1990, and Hawkins et al. 1993.

Step 2 – Conduct eDNA Sampling. The eDNA sampling will be conducted at 1,640-foot intervals using a Garmin GPSMAP 60CSx (or similar) to determine sampling locations, from Pyramid Dam to the NMWSE of Lake Piru. Sampling will be conducted by biologists trained in eDNA collection. Sample collection will occur once during the spring run-off period, at the tail end of the descending limb of the hydrograph. This will limit the dilution effects of high flows and simultaneously maximize the potential for DNA transport in the water column. This is expected to follow a storm event in February or March when it is determined that field crews can safely access the sampling locations in the Pyramid reach. Sampling will be consistent with the protocol described in Bergman et al. (2016). For each sample, a maximum of 2 liters of water will be filtered using sterile tubing and a portable peristaltic pump. No water other than sample blank water will be transported or stored for sampling. Water samples will be filtered through a 0.45 micrometer sterile filter, and stored on ice for transport back to the lab. Samples will be labeled with sampling location, volume of water filtered, and any other information necessary for tracking and chain of custody purposes.

To prevent cross contamination of samples, new filters, tubing, and nitrile gloves will be used for each sample. In addition, after collection each sample filter will be returned to its original packaging and sealed in a secondary container prior to storage in a separate, dedicated transport container. All filters will be kept in the secondary storage container and placed in a -20 degrees Celsius (^{o}C) laboratory freezer until DNA extraction is performed. Any filters that are opened but not used will be considered contaminated and discarded. Field (negative) controls will be taken at the beginning and end of each field day.

eDNA samples will be tested for the presence of DNA from Santa Ana sucker (SSC), arroyo chub (SSC), (FE), and rainbow trout. These fish represent the primary game fish in the reach (i.e., rainbow trout) and the two listed native fishes, although the occurrence of the listed fish species in this reach have only been documented anecdotally. Any incidental sightings of the listed fish species will be noted.

In order to implement surveys that seek to use eDNA to detect species of interest, both a DNA barcode and means to assay for that DNA must exist. DNA barcoding is a technique for identifying species using a short DNA sequence from a standard position in the mitochondrial genome. DNA barcode sequences are very short relative to the entire genome and presently exist for many organisms or can be created reasonably quickly using routine laboratory practices. The Cytochrome B Oxidase subunit 1 mitochondrial region (COI) has emerged as a standard barcode region. Yet, the Cytochrome B (CytB) has proved equally adept at identifying higher animals. The current and most sensitive method to “assay” an eDNA sample for the presence of target DNA (DNA barcode) is quantitative PCR (qPCR). DNA barcodes for species of interest are used to create qPCR primer and probe sets, also referred to as assays.

The species of interest for this Project include: Santa Ana sucker, arroyo chub and rainbow trout. Currently from this list of species an assay exists only for rainbow trout. To detect the DNA from the other species of interest a DNA barcode must be established and qPCR assay must be developed.

Both DNA barcoding and qPCR assay development are standardized published procedures. The following describes the methods for the DNA barcoding and development of qPCR assays for each species of interest.

A minimum of 3 vouchered specimens should be used for DNA barcoding of the mitochondrial genes CytB and COI. In order to capture any genetic diversity of the CytB and COI genes within target species populations, the specimens should include individuals from across the known range.

DNA extractions for each specimen will be amplified by PCR using universal fish primers for CytB and COI. PCR amplification consists of a 15 µl total reaction volume. Each 15 µl reaction is composed of 7.5 µl Promega GoTaq® G2 Hot Start Colorless Master Mix (Promega Corporation), 1 µl 10 nM Forward primer, 10 nM Reverse primer, 3.5 µl ultra-pure nuclease free water, and 2 µl 100ng/ µl normalized DNA. Thermocycling is performed using the Promega Master Mix protocol with an optimized annealing temperature of 55° C and the complete cycle profile of 2 minutes. at 95° C initial denaturation, 40 cycles of 95° C for 30 seconds, 55° C for 30 seconds, 72° C for 1 minute, with a final extension at 72° C for 5 minute. PCR products are separated by electrophoresis in 1 percent agarose (w/v) gel at 90v for 20 minutes. Gels are visualized by BioRad mini trans illuminator (BioRad Laboratories, Inc.). Appropriate bands are excised from the gel using a brand new razor blade for each band and placed into individual sterile micro-centrifuge tubes. DNA is extracted from the agarose gel using QIAquick® Gel Extraction Kit following manufacturer's guidelines. Extracted DNA and primers are submitted to the University of California (UC) Davis DNA sequencing facility for DNA Sanger sequencing. DNA sequence data received from UC Davis DNA sequencing facility are aligned using Geneious alignment software (Geneious, Inc.) and analyzed for a lack of variability across the sequenced regions. Consensus fragments are used as the template for a nucleotide BLAST (Basic Local Alignment Search Tool). Results of the BLAST determine which portion of the CytB and/or COI regions are unique or conserved within species mitochondria yet retain intra species variation sufficient to use as a genetic barcode.

DNA barcode sequences for each species are used as template for qPCR assay design. Commercially available algorithms are used to analyze DNA barcode sequences and generate qPCR primer probe sets or assays. Primer probe sets generated by the algorithm are scored from highest to lowest. The highest scoring primer probe sets are queried for sequence similarity again using a BLAST of the National Center for Biotechnology Information (Nucleotide database as a means of determining in-silico species specificity. Primer and probe sets are then tested for in vitro specificity on the original vouchered specimen and for cross reactivity to any closely related and co-occurring species as well as the assay sensitivity. For all assay design and validation, we take into consideration and explicitly follow any applicable Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines.

DNA from all samples and controls are extracted using PowerWater Sterivex™ DNA Isolation Kit (Mo Bio Laboratories, Inc.) following the manufacturer's recommended

guidelines. A DNA extraction negative control is processed in parallel to ensure sample integrity throughout extraction procedure. The DNA extraction control consists of Sterivex™ filtered the ultrapure water only. DNA extraction controls are processed using the same equipment utilized to extract DNA from all samples. Each sample and all controls are analyzed in triplicate for the presence of the GGS CytB mitochondrial gene using the qPCR primer and probe designed previously. DNA extract from each sample is analyzed in triplicate with each qPCR replicate consisting of a 10 µl reaction volume. Each 10 µl qPCR reaction is composed of 2x Applied Biosystems TaqMan Universal PCR Master Mix, No AmpErase UNG (Thermo Fisher ABI), 500-900 nM initial primer concentration, 2.5-10 uM initial probe concentration, and 4 µl DNA template. Thermocycling is performed using a Bio-Rad CFX 96 Real time System (Bio-ad Laboratories, Inc.) with the following profile: 10 min at 95° C, 40 cycles of 15 second denaturation at 95° C and 1 min extension at 60° C. Six template control (NTC) reactions are run on the plate with the control sample templates consisting of 4 µl of ultrapure water replacing DNA template within reaction volume. Three positive control reactions consisting of 20 ng/µl target species genomic DNA template are also tested in parallel to ensure consistent PCR performance. All PCR master mixes are made inside an ultraviolet (UV) PCR enclosed workstation. A DNA template is added to the master mix outside of the UV PCR workstation on a dedicated PCR set up workbench. All PCR reactions are conducted on instruments located outside of the main lab in a separate portion of the building. Results of the qPCR reactions are analyzed using BioRad CFX manager v3.1 (Bio-Rad Laboratories, Inc.). A sample is considered positive for the presence of target DNA if any one of the three replicates showed logarithmic amplification within 40 cycles.

Step 3 – Select Sampling Sites for Fish Population Sampling. Three representative sample sites will be selected: one in the 2-mile-long section of Pyramid reach between Pyramid Dam and the concrete structure upstream of Frenchman's Flat (stream segment 1); one within a mile downstream of Frenchman's Flat, within the stream segment from the concrete structure upstream of Frenchman's Flat to the confluence of Fish Creek (stream segment 2); and one just upstream of the confluence with Agua Blanca Creek within the stream segment from Fish Creek to the NMWSE of Lake Piru (stream segment 3). The sites will be selected at locations accessible to field crews and will represent the overall habitat ratios found in the reach using the mesohabitat mapping data created for the reach.

Prior to site selection in the field, preliminary sites will be selected using existing aerial imagery and habitat mapping data. Final sampling sites will be selected in consultation with USFS, USFWS, SWRCB, and CDFW. The Licensees will make a good faith effort to schedule the consultation on a day or days convenient to the Licensees and interested relicensing stakeholders, and will provide an email notice at least 30 days in advance of the meeting or site visit.

Sample sites are expected to vary in length, but typically range between 325 and 1,000 feet. Site length will be sufficient to include habitat that represents the ratio of riffle, run, and pool habitat present in the stream segment in which the site is located. Exact site length will be determined in the field by the Licensees.

Step 4 – Sample Fish Population. Multiple-pass depletion sampling (Reynolds 1996 and Temple et al. 2007) using backpack electrofishing equipment will be performed where permitted to capture fish and develop population estimates at the sampled sites for select species. This sampling is expected to occur in the fall (September or October). Upstream and downstream ends of each site will be blocked with fine mesh nets or a fish passage barrier. If required, the nets or passage barrier would span the full width and depth of the stream except where an upstream fish passage barrier obviates the need for head-end blocking or where edge or stream margin habitat is to be sampled. If necessary, salt blocks will be placed in the stream immediately above the electrofishing station to increase conductivity. Salt blocks will be used when fish are observed escaping the direct path of the electric field generated by the electrofishing unit at elevated settings.

Field crews will consist of at least two netters for each shocker. The Licensees will follow Temple, et al. (2007), who recommends one backpack electroshock crew for streams less than 24.6 feet wide and two backpack electrofish crews for streams 24.6 – 49.2 feet wide. In wadeable streams wider than 49.2 feet, the number of electroshocking crews will be expanded as necessary to assure effective and accurate sampling. Electrofishing will be conducted by a qualified professional biologist who is trained in electrofishing techniques, and will be implemented only where permitted by USFWS and CDFW.

Captured fish will be retained in aerated buckets and/or live cars until each pass is completed. Fish will be sedated as required in accordance with generally accepted scientific methodology and regulatory approvals. All fish will be identified to species and counted. Up to 50 individuals of each species will be measured to the nearest millimeter (fork length) and weighed by digital scale to the nearest gram. Effort will be made to measure representative fish species in all size classes, within the subsample of the measured species. The actual number of measured species will be determined through professional judgment based upon the size class homogeneity of the sample (i.e., number of size classes represented). If the Licensees are granted the appropriate scientific collecting permits to collect Santa Ana sucker and arroyo chub, and individuals are found during field sampling, tissue samples will be collected and turned over to CDFW and USFWS for analysis.

Scale samples will be collected on a subsample of larger, less abundant game fish for validating length-age indices. These species will include rainbow trout, brown trout, and largemouth bass if present during the surveys. Captured fish will be released downstream of the sampling area following completion of each electrofishing pass. Effort will be made to ensure sampling activities in the field will minimize potential injury or mortality to aquatic species. Mortalities and fish condition (spinal trauma, bruising) will be noted and recorded prior to release.

General information and habitat/channel metrics will be collected at each sample site. This information will include a distinct site identification marker, number of shockers, date and time, air and water temperature, conductivity, weather conditions, and GPS location of each end of the site. Metrics collected at each mesohabitat unit within the

sample site will include mesohabitat type, estimated average and maximum depth, estimated average wetted and bankfull width, dominant cover type, dominant and subdominant substrate, and sampling effort, in seconds. Habitat data collected will be consistent with that collected in Step 1.

Prior to electrofishing at a site and after installing both upstream and downstream block nets, the Licensees will walk the stream bank to directly look for the presence of known sensitive species, including WPT, arroyo toad, CRLF, or foothill yellow-legged frog (FYLF). If any sensitive species individuals are observed, the Licensees will note the observation and maintain a safe distance so as to not disturb the individual(s). The field lead will then relocate the site a safe distance upstream or downstream to a location that includes similar habitat types as the selected site, and repeat the procedure.

Precautions to guard against the incidental take of arroyo toad will be determined during the application for an ESA 10(a)(1)(A) permit from the USFWS. Restrictions and limitations imposed by this authorization may have a significant impact on the methods used for this *Pyramid Reach Fish Populations Study*. Any such changes will be noted in the final technical memorandum.

Quality Assurance and Quality Control

Field data gathered during this *Pyramid Reach Fish Populations Study* will be collected in a manner that promotes high quality results, and will be subject to appropriate QA/QC for sample collection equipment, procedures, and cross-checking of data. As part of the QA/QC procedures, extreme care will be taken to ensure the data collected is accurate and maintained in a safe environment.

Analysis

Individual Fish Condition Factor

Fish size and weight data will be summarized by species and sample site. Similarly, standard metrics including minimum, maximum, and mean fork length and weight will be reported. Length and weight data will be used to calculate a relative condition factor (Anderson and Gutreuter 1983) and to provide a general indication of the health of individuals, where factors greater than 1 indicate more healthy individuals. Relative condition factors for electrofishing sites will be calculated for length and weight data collected at all quantitative electrofishing sites.

Fish Species Populations and Biomass

Standing stock estimates in terms of fish population numbers and biomass will be calculated by species for each site and analyzed by age class. Electrofishing data will be analyzed using a scientific software package (e.g., Microfish or other similar program). Capture probabilities (the proportion of fish captured on a given electrofishing pass), size statistics, and biomass will be generated for each sample site using fish capture data. Biomass will be calculated based upon total weight measured for each species. Standing stock estimates will be reported as: (1) numbers and weight (grams)

of fish by species per 328 feet (100 meters) of stream; (2) numbers of fish by species per mile; (3) pounds of fish by species per acre of stream surface; and (4) kilograms of fish by species per hectare.

Game fish species population analysis will include size structure based on RSD. To provide an index of size structure for each site, traditional RSD of each species will be calculated. The RSD will be presented on a scale of 0 to 100 (Anderson and Neumann 1996). RSD will be calculated as the proportion of fish sampled greater than 6 inches, such that: RSD = (# of fish >6-inch in sample) / (# of fish in sample) x 100. The 6-inch length was chosen because it is often used as the smallest size where fish are desired by anglers. A high RSD indicates that a greater proportion of the population consists of fish in the size class desirable to anglers. Non-game fish species will be evaluated using length frequency distributions. No RSD calculations will be made on non-game fish species.

Selected fish species will also include an analysis by age class. Existing length-age indices will be used to determine the age class. Length-age indices are relatively accurate for smaller fish; however, confidence intervals reduce with larger fish. Scales collected, as described above, will be analyzed to assist in identifying age class breaks. Analysis of scales will follow methods described in Minard et al. (1997) and Schneider et al. (2000). Regression analysis will be used to analyze the data and, if necessary, adjust the indices.

Fish Community Analysis

The fish community analysis will also include species composition and relative abundance of the fish community (i.e., percent composition). In addition, the diversity of fish species will be assessed. Possible statistical analysis could ***include the Shannon Weaver Diversity Index, a means of characterizing species diversity. The condition of fish communities will also be evaluated at three levels of biological organization: individual level, populat***ion level, and community level. Evaluation of these three levels will be accomplished using electrofishing data, relative condition factors, and any in-field observations. Moyle et al. (1998) and Moyle and Marchetti (1998) provided the following descriptions of fish health at these levels:

Individual Level

Most fish in a healthy stream should: (1) have a robust body; (2) be free of disease, parasites, and lesions; (3) possess reasonable growth rates for the region; and (4) exhibit appropriate behavioral patterns.

Population Level

Fish populations in healthy stream environments: (1) exhibit multiple age classes indicating that reproduction is regularly occurring; (2) achieve a viable population size (i.e., occur in adequate numbers to maintain a self-sustaining population and the long-term persistence of the population); and (3) consist of mostly healthy individuals.

Community Level

Fish communities considered in good health in California: (1) are typically dominated by co-evolved species; (2) have a predictable structure as indicated by limited niche overlap among species and trophic levels; (3) are resilient in recovering from extreme events; (4) consist of a persistent species membership; and (5) are replicated geographically (i.e., can be found in similar habitats within the drainage or in other similar drainages).

Reporting

Pyramid Reach Fish Populations Study results will be included, to the extent completed and ready for inclusion in the Licensees' ISR, USR, DLA, and FLA.

4.1.3.5 Consistency of Methodology with Generally Accepted Scientific Practices

The methods are consistent with the methods used for recent FERC hydroelectric relicensing efforts in California, including the Drum-Spaulding Project (FERC Project No. 2310), the Yuba-Bear Hydroelectric Project (FERC Project No. 2266), and the Yuba River Development Project (FERC Project No. 2246), with the following exception: eDNA is a newly emerging monitoring tool that will augment the ability for surveys to detect rare, cryptic, and elusive species that are unlikely to be found using conventional methods.

4.1.3.6 Schedule

The *Pyramid Reach Fish Populations Study* will begin after FERC issues its Study Plan Determination. The Licensees anticipate the schedule below will be followed to complete the *Pyramid Reach Fish Populations Study*.

Fieldwork Preparation	July 2017 – July 2018
Habitat Mapping	July 2017 – September 2017
Site Selection	May 2018 – June 2018
Fieldwork (eDNA and sampling)	June 2018 – September 2018
Data QA/QC	August 2018 – November 2018
Data Analysis and Reporting	November 2017 – December 2018

4.1.3.7 Level of Effort and Cost

Based on the work effort described above, the Licensees estimate the current cost to complete this *Pyramid Reach Fish Populations Study* is between $136,000 and $181,000.

4.1.3.8 References

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