Upper Tuolumne River Ecosystem Project

2009 Amphibian and Reptile Assessment Study Plan for the Hetch Hetchy Reach

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Prepared for:

The San Francisco Public Utilities Commission

August 25, 2009

About the Upper Tuolumne River Ecosystem Project

In June 2006, the San Francisco Public Utilities Commission (SFPUC) adopted the Water Enterprise Environmental Stewardship Policy and directed the Water Enterprise to integrate this policy into the planning and operation of the SFPUC water system infrastructure, including Hetch Hetchy Project dams and diversions. The policy establishes a management directive to protect and rehabilitate ecosystems affected by water system operations, within the context of meeting water supply, power generation, water quality, and existing minimum instream flow requirements. The policy further directs the nature of SFPUC instream flow releases such that they mimic, to the extent feasible, "…the variation of the seasonal hydrology (e.g., magnitude, timing, duration, and frequency) of their corresponding watersheds in order to sustain the aquatic and riparian ecosystems upon which native fish and wildlife species depend."

Subsequent to adoption of the Environmental Stewardship Policy, the SFPUC initiated the Upper Tuolumne River Ecosystem Project with the goal of conducting a set of long-term, collaborative, science-based investigations designed to (1) characterize historical and current river ecosystem conditions, (2) assess their relationship to Hetch Hetchy Project operations, and (3) provide recommendations for improving ecosystem conditions on a long-term, adaptively managed basis.

The Ecosystem Project will provide data and analyses to (1) support implementation of the Water Enterprise Environmental Stewardship Policy on the Upper Tuolumne River, (2) support ongoing Yosemite National Park Tuolumne Wild and Scenic River planning and management efforts, (3) provide the scientific basis for resolving outstanding issues with the U.S. Department of the Interior related to the 1987 Stipulation under the Raker Act, and (4) implement mitigation and monitoring requirements specified in the Final Programmatic Environmental Impact Report for the Water System Improvement Program (WSIP PEIR).

Primary partners include the SFPUC, Yosemite National Park, Stanislaus National Forest, and the U.S. Fish and Wildlife Service. The study area includes reaches of the Upper Tuolumne River mainstem and major tributaries regulated by the Hetch Hetchy Project, from O'Shaughnessy Dam to Don Pedro Reservoir, Cherry Creek downstream of Cherry Valley Dam, and Eleanor Creek downstream of Eleanor Dam.

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Acknowledgements

Thanks to Don Ashton (U.S. Forest Service and McBain & Trush), Sarah Kupferberg (Questa Engineering), Jeff Maurer (Yosemite National Park), Steve Holdeman (Stanislaus National Forest), and Mike Horvath (SFPUC) for their expertise and input in drafting this study plan.

Preferred citation

McBain & Trush, Inc. and Questa Engineering Corporation 2009. Upper Tuolumne River Ecosystem Project: 2009 Amphibian and Reptile Assessment for the Hetch Hetchy Reach, Draft Study Plan. Prepared for the San Francisco Public Utilities Commission.

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1 Introduction

The decline of aquatic biodiversity in general (Richter et al. 1997), and amphibians in particular is a worldwide phenomenon (Blaustein et al. 2003, Stuart et al. 2004, Mendelson et al. 2006), including the Sierra Nevada of California (Drost and Fellers 1996, Vredenburg et al. 2007). Reptiles are also experiencing dramatic declines worldwide (Gibbons et al. 2000). Various stressors attributed to cause declines in populations downstream of dams include the negative impacts on the timing and magnitude of flow releases from dams (Lind et al. 1996, Kupferberg et al. 2008). Increased understanding of how flow regime affects amphibian and reptile populations is needed to minimize potential adverse impacts of flow management.

For organisms that have evolved with predictable seasonality of runoff, as occurs in snowmelt dominated rivers draining the Sierra Nevada range, flow manipulation can cause fatal mismatches between vulnerable life history stages and altered hydrographs (Lytle and Poff 2004). The first task of the work proposed here is to select appropriate focal species whose life cycle timing, flow requirements, and thermal niches will be representative of the community of amphibians and reptiles occupying the mid-elevational zone of a west slope Sierran river.

Attempts to link flow changes to population responses need to coincide with long-term biological monitoring of an appropriate focal species (Souchon et al. 2008) which can serve as a sentinel for a larger faunal assemblage. It can be a challenge to find a responsive focal species with suitable detectability and generation time. There are many candidates, both vertebrate and invertebrate. For example, mussel larvae (glochidia) are sensitive to the timing of flow fluctuations (Haley et al. 2008, Layzer and Madison 1995), but because adults can live up to 100 years (Bauer 1992) and newly settled cohorts are relatively inconspicuous, there can be long lag times between altered patterns of discharge fluctuation and observable population response (Howard and Cuffey 2006).

At the opposite extreme, benthic macroinvertebrates are good bio-indicators of overall stream health (Rosenberg and Resh 1993), but may not be useful in detecting flow effects because of their natural variability. Abundance of benthic macroinvertebrates can decline precipitously during floods, through catastrophic drift and loss of habitat (Cereghino and Lavandier 1998, Gore 1989, Gore et al. 2001), but can also increase relatively quickly (Brusven and Trihey 1978, Scrimgeour et al. 1988, Wallace 1990). Benthic macroinvertebrate densities also change considerably from year to year for many reasons, and samples require extensive processing.

In contrast, amphibians and reptiles have detectabilities and life spans that may make them suitable as focal organisms to detect the effects of artificial flow fluctuation. For example, the flooding and stranding susceptible egg masses of ranid frogs (which produce discrete clutches and spawn once per year) are conspicuous, and in fact are used as estimators of the number of breeding females (Crouch and Patton 2000, Loman and Andersson 2007, Petranka et al. 2007). In addition, sensitivity to water temperature makes amphibians and reptiles useful as indicators of downstream thermal effects of dams.

Here we propose to examine the hydrologic, hydraulic, and thermal factors that may be limiting to amphibians and reptiles using aquatic habitats in the Hetch Hetchy Reach of the Upper Tuolumne River. This study will collect baseline data on wildlife presence, frequency and diversity, with particular emphasis on the distribution and relative abundance of focal taxa on the portion of the Tuolumne River from O'Shaughnessy Dam downstream to Cherry Creek and nearby unregulated reference reaches on the Merced River, Middle Fork of the Tuolumne River, and/or the Clavey River. After initial baseline surveys, we will transition to a more statistically rigorous sampling method for inference across the study area.

The survey and monitoring aspects of this study will inform other aspects of the Upper Tuolumne River Ecosystem Project, specifically the amphibian, reptile, and benthic macroinvertebrate flow-habitat mapping studies. These data will be instrumental in developing instream flow recommendations in the Hetch Hetchy reach. The amphibian and reptile data collected in 2009 will build on data collected in 2008 to provide baseline data for future monitoring programs and adaptive management.

The proposed 2009 research will be a collaborative effort among the San Francisco Public Utilities Commission, the Stanislaus National Forest, US Fish and Wildlife Service, and Yosemite National Park. The project will be coordinated with a series of investigations that the Yosemite National Park Service personnel are conducting on the downstream impacts of the current flow regime on hydrologic, vegetation, and ecologic conditions, known collectively as the Looking Downstream project. This study will also be augmented with data collected by University of California Berkeley researchers as part of the "Hydropower Effects on Amphibian Species II" project sponsored by the Public Interest Energy Research Group of the California Energy Commission.

2 2009 Study plan

The goals for the 2009 study is to further establish baseline levels of diversity and distribution of amphibians and reptiles at previously sampled locations in the river corridor likely to be occupied, to assess the suitability of physical conditions in habitats used by focal amphibians and reptiles for reproduction, and introduce the use of randomly selected sites to allow extrapolation of results to the entire reach. Emphasis is placed on taxa with pronounced aquatic life stages to determine whether shifts from baseline data represent positive or negative trends, and whether those trends are associated with species-appropriate flow management and consistent with the river ecosystem restoration goals.

2.1 Task 1: Choose focal species

2.1.1 Description

The objective of the task is to reach consensus on which focal species will be used for detailed analyses that extend beyond the general inventory and monitoring aspects of this proposal. A secondary goal would be to approve the general and taxon-specific methods outlined below.

With respect to reptiles, we propose Sierra garter snake, *Thamnophis couchii*, and western pond turtle, *Actinemys* (*Clemmys*) *marmorata*, as focal species. Justifications for these choices include the turtle and garter snake's reliance on river-based prey, and the turtle's status as a CA Species of Special Concern.

With respect to amphibians, we propose foothill yellow-legged frog, *Rana boylii*, and/or Sierra newt, *Taricha torosa sierrae*. Justifications for focusing on the foothill yellow-legged frog include its unique qualities of being exclusively a river-breeding species, its status as a Forest Service Sensitive Species and CA Species of Special Concern, its known sensitivities to altered flow regime, and extensive existing knowledge of its habitat and thermal requirements. However, the rarity of this species in the study reach (one confirmed individual in 2008) introduces uncertainty to the proposed analyses. We must make assumptions about which habitats are likely to be occupied and that the thermal niche of the local genome is similar to populations studied in other locations. Alternatively, the newts are abundant but their thermal biology and flow requirements are less well-known. Newts breed in ponds as well as in lentic (slow or still-water) microhabitats of rivers.

2.1.2 Methods

We propose to discuss the pros and cons of the candidate focal species with SFPUC's partners, specifically the USFS and NPS, after everyone has had time to review this document. Communication will occur among but not necessarily be limited to, Bill Sears, Mike Horvath, Bill Trish, John Bair, Christine Chamfer Don Ashton, Sarah Kupferberg, Jeff Maurer, and Steve Hodgeman.

2.1.3 Schedule

July-August 2009.

2.2 Task 2: Conduct field surveys

2.2.1 Description

To complete the amphibian and reptile inventory, and thus establish baseline data for quantitative comparisons with future surveys, we propose a combination of techniques (Visual Encounter Surveys, VES, Area Constrained Surveys, ACS, and incidental observations). This task is important with respect to adaptive management and assessments of future impacts of flow regime changes.

Baseline data will be collected for three consecutive years (e.g., 2008, 2009, and 2010) using passive sampling techniques for herpetofauna (i.e., reptiles and amphibians). The three-year study duration is the minimum length of time required to show inter-annual variation and variation in detection rates. The first year effort, in 2008, assessed aquatic herpetofauna diversity and distribution. Subsequent surveys proposed for 2009 and 2010 will target those species most likely to respond to changes in the contemporary flow regime and that also have adequate sample sizes for analysis. Pilot testing of hypotheses related to focal species may also occur in 2009, which includes more intensive passive sampling. Information collected will be integrated with the Yosemite National Park Looking Downstream project to develop a comprehensive assessment of existing ecological conditions downstream of O'Shaughnessy Dam.

To increase the inference of the current study to a broader geographic area and promote documentation of ecological trends and patterns in Yosemite National Park overall, additional data collection is proposed at comparable reaches along the Merced River, or possibly above O'Shaughnessy Dam, or comparable elevations in local drainages where focal species are more abundant such as the Clavey River and the Middle Fork Tuolumne River. We believe that identifying an appropriate reference site would allow-comparisons of data. Such comparisons will facilitate our ability to evaluate the salient features of the physical and biotic conditions currently found downstream of O'Shaughnessy Dam. As a hypothetical example, consider the situation that no breeding activity of a focal species is detected in the study reach. Knowledge that breeding had already been initiated in nearby unregulated rivers at similar temperatures would focus our attention on other key variables. The goal is to identify components of a "reference" flow regime that could be used in developing streamflow recommendations.

2.2.2 Study Area

The current study area includes the mainstem of the Tuolumne River between the Cherry Creek confluence on the Stanislaus National Forest and O'Shaughnessy Dam in Yosemite National Park. The Upper Tuolumne River in the study area is approximately 25 kilometers long and ranges in elevation from 660 m to 1105 m (2165' - 3625') (Figure 1). Sampling is proposed within the riparian corridor along the mainstem and any intersecting tributaries up to 1/4-mile. Survey reaches within the study area have been selected by stratified convenience sampling (Hayek 1994), taking into account accessibility (roads and trails), habitat type, and elevation. There are four access points (Figure 1) including 1) the Tuolumne River trail at Early Intake, 2) Mather Pool social trail from Camp Mather, 3) Poopenaut Valley Trail, and 4) O'Shaughnessy Dam Service Road. These four access points divide the study area into five sub-reaches and effectively stratify the study reach by elevation. The Gorge sub-reach is not included in this sampling effort because of difficult access, crew safety, and the extent of immobile bedrock control in the channel. The reaches above and below the Gorge are henceforth referred to as the upper and lower study reaches: Upper = O'Shaughnessy Dam to the downstream end of Poopenaut Valley, and Lower = from Mather Pool to Early Intake. Within each of these survey reaches, transects (subsamples) will be established for surveys.

In addition to proposed surveys along the riparian corridor, off-channel water bodies within 1/4-mile of the mainstem of the river will also be surveyed due to their potential to contribute to amphibian and reptile population dynamics in the study reach.

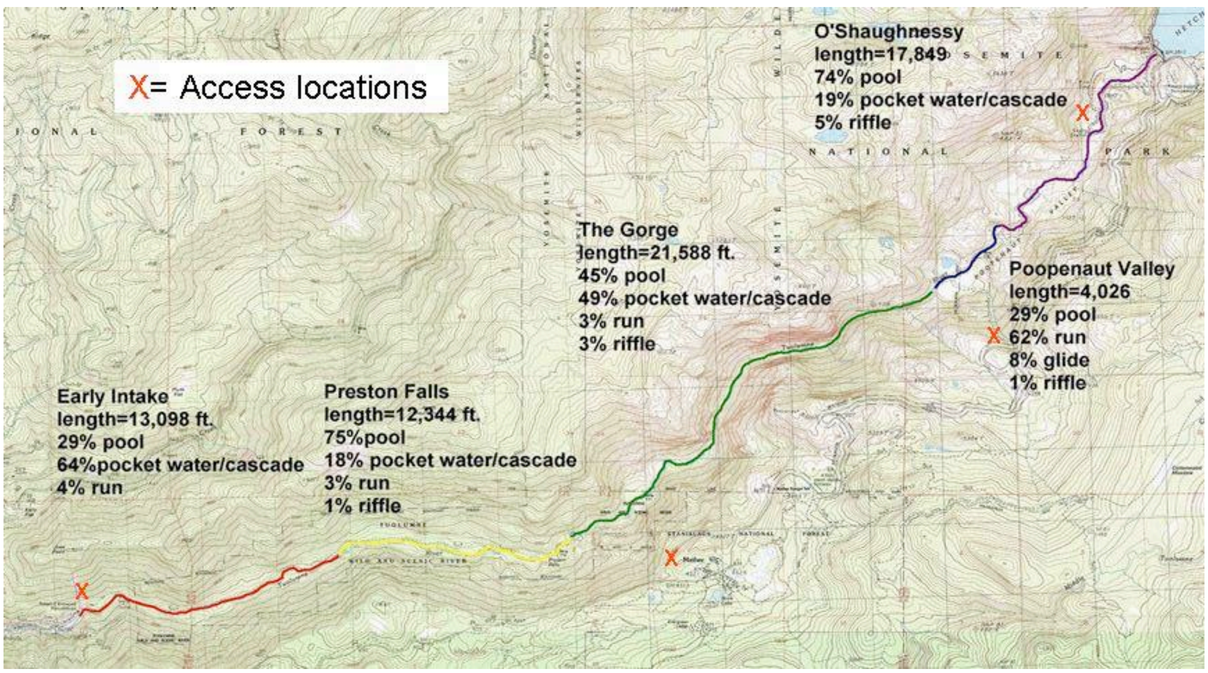

Figure 1. Hetch Hetchy Study Area map showing subreach variation in proportion of habitat types present. Herpetofauna surveys will be conducted in all reaches except for the Gorge reach. For sampling purposes, the Early Intake and Preston Falls subreaches are combined to form the lower sampling reach and the Poopenaut Valley and O'Shaughnessy subreaches are combined to form the upper reach.

2.2.3 Methods

Three to five survey periods per year are proposed between April and late-October. Five surveys are recommended to capture seasonal variation in species life stage, activity level, and detectability within and among species. Initial survey visits in 2008 were designed to confirm habitat distribution and associations to model other reaches, identify potential biological hotspots, and determine focal species for more in-depth investigations in 2009-2010. Follow-up studies on focal species will investigate which species have measurable populations or distributions affected by flow regime. Surveys are generally conducted on foot, except for the assessment of turtle demographics, which is conducted by snorkeling.

We propose a hierarchical sampling strategy during each survey period to detect a wide range of amphibian and reptile species. Three general sampling methods will be used during each field survey and include: passive Visual Encounter Survey, habitat-based Area Constrained Search and Incidental/Opportunistic Encounters.

Visual Encounter Surveys (VES) following standardized protocols (Crump and Scott 1994) will serve as the primary method to detect and quantify the focal species along the river margin. VES are conducted first upon arrival. During VES, observers walk carefully along the transect, (oriented longitudinally along the stream course) visually searching for exposed animals. Observers may scan ahead with binoculars or inspect crevices with a flashlight, but no substrates or cover objects are moved. VES is designed to detect diurnally active animals, and are effective for detecting turtles, foothill yellow-legged frogs, garter snakes, and newts. During their breeding season, toads may also be detected, but outside of breeding season they are primarily nocturnal.

The survey transects established in 2008 and revisited in 2009 will be augmented with randomly selected transects to allow for extrapolation of results reach-wide. The initial site selection was based on expert opinion of unique habitat types and transects were placed to maximize the likelihood of encountering the focal species. Additional VES will be conducted at up to six 100-m transects randomly selected within the downstream reach (Mather Pool to Early Intake) and another six transects will be randomly selected in the upper reach (O'Shaughnessy Dam to the downstream end of Poopenaut Valley). The selection process will be stratified among the habitat types identified within each reach (see Fig. 1 for % composition of riffles, pools, pocket water, etc.) using randomly generated distances and consultation with aerial photographs. With consideration of access and surveyor safety, some randomly generated sites may be eliminated and new sites chosen. Having such a comparison between random sites and highly suitable sites will allow for extrapolation of density estimates from the survey sites to the scale of the entire Hetch Hetchy Reach.

Area-constrained Searches (ACS) are employed to sample specialized or limited habitat types within the survey reach and can occur within or outside of the VES transects area. ACS will focus on areas where preferred habitat occurs and where incidental sightings are clustered. Habitat-based ACS utilize aerial photograph-based maps of study reaches that contain pockets of unique habitat and/or concentrations of individuals (e.g. a newt breeding and oviposition site) that may not be covered in randomly placed units of the VES. ACS involves temporarily moving substrate (rocks, logs) to detect and identify species under cover, then replacing substrate to its original location as closely as possible to minimize habitat disturbance. ACS may include littoral zone searches and dip net swipes (Olsen et al. 1997) conducted in representative lentic habitats in the Poopenaut Valley and side channel sites farther downstream. ACS may also involve snorkeling if the area to be searched is too deep to be effectively searched by foot.

Incidental (or opportunistic) Encounters document reptiles and amphibians encountered en route to sampling sites or animals observed outside of the VES or ACS protocols (e.g. while doing habitat mapping). Encountered herpetofauna will be documented and included in the list of species detected during the field survey, but incidental observations cannot be directly used for estimating density. Incidental encounters can be instrumental for identifying sites where ACS are likely to be fruitful.

The initial field survey in 2008 included a training crew of six people. Up to three teams of two people each will conduct subsequent field surveys each year.

2.2.4 Species Identification

Each animal encountered will be identified to species and life stage (Basey 1976, Zeiner et al. 1988, Jennings and Hayes 1994, Stebbins 2003). Potential amphibian and reptile species that may be encountered are listed in Table 1. When possible, animals will be identified without handling the animals, but in some cases, brief capture (generally less than 2 minutes) may be necessary to confirm identity or photograph diagnostic features.

Table 1. List of potential amphibian and reptile species potentially occurring along the Tuolumne River (O'Shaughnessy Dam to Cherry Creek), Tuolumne County, CA.

Common Name	***Scientific Name***
FROGS & TOADS	
California Toad	*Bufo boreas halophilus*
Pacific Treefrog	*Hyla (Pseudacris) regilla*
Foothill Yellow-legged Frog	*Rana boylii*
Bullfrog*	*Rana catesbeiana*
California Red-legged Frog	*Rana draytonii*
Western Spadefoot	*Scaphiopus hammondi*
SALAMANDERS	
Arboreal salamander	*Aneides lugubris*
Hell Hollow Salamander	*Batrachoseps diabolicus*
Gregarius Slender	*Batrachoseps gregarious*
Sierra Nevada Salamander	*Ensatina eschscholtzii platensis*
Yellow-eyed Salamander	*Ensatina eschscholtzii xanthoptica*
Limestone Salamander	*Hydromantes brunus*
Sierra Newt	*Taricha torosa sierrae*
TURTLES	
Western Pond Turtle	*Emys (Clemmys) marmorata*
SNAKES	
Rubber Boa	*Charina bottae*
Western Yellow-bellied Racer	*Coluber constrictor mormon*
Sharp-tailed Snake	*Contia tenuis*
Northern Pacific Rattlesnake	*Crotalus viridis oreganus*
Ring-necked Snake	*Diadophis punctatus*
Night Snake	*Hypsiglena torquata*
California Kingsnake	*Lampropeltis getula californiae*
Sierra Mountain Kingsnake	*Lampropeltis zonata multicincta*
Chaparrel Whipsnake	*Masticophis lateralis*
Pacific Gopher Snake	*Pituophis catenifer catenifer*
Western Long-nosed Snake	*Rhinocheilus lecontei lecontei*
Sierra Garter Snake	*Thamnophis couchii*
Mountain Garter Snake	*Thamnophis elegans elegans*
Valley Garter Snake	*Thamnophis sirtalis fitchi*
LIZARDS	
Western Whiptail Lizard	*Aspidoscelis tigris*
California Whiptail	*Cnemidophorus (Aspidoscelis) tigris*
Sierra Alligator Lizard	*Elgaria coerulea palmeri*
San Diego Alligator Lizard	*Elgaria multicarinata webbii*
Greater Brown Skink	*Eumeces gilberti gilberti*
Skilton's Skink	*Eumeces skiltonianus skiltonianus*
Coast Horned Lizard	*Phrynosoma coronatum*
Western Sagebrush Lizard	*Sceloporus graciosus gracilis*
Western Fence Lizard	*Sceloporus occidentalis*

The NPS and USFS scientific collecting permits of the biologists involved in the studies will need to be amended to include the handling of the focal species in the chosen locations. Although specimens will not be collected under the proposed data collection methods, uncommon amphibian or reptile species encountered dead may be salvaged for the purposes of voucher or necropsy and retained by the Yosemite Museum.

2.2.5 Data Recording and Analysis

The encounter location for each animal will be marked on an aerial photo and recorded on a handheld GPS unit in UTM coordinates (NAD83). In the case of congregations, such as groups of tadpoles, group size will be counted (for small groups) or estimated (for large groups) and the group's location and count or estimate will be recorded. For each animal observation, habitat type and elevation will be recorded to address species distribution and diversity in the study area.

2.2.6 Schedule

Surveys have been / will be conducted during the following periods:

- End of April 2009 (pre-release, complete)
- Early July 2009 (post-release, complete)
- Early September 2009 (mid-summer)
- October/November 2009 (fall)

2.3 Task 3: Integrate data from the temperature-stage-discharge transects (also referred to as thermal transects) with physical habitat requirements for recruitment of focal amphibians.

2.3.1 Description

To determine whether there would be potentially fatal mismatches between the duration and magnitude of periodic inundation of edgewater habitats and time to hatching of amphibian eggs and development of larvae to metamorphosis (e.g. stranding and scouring events), we will combine the information compiled on topography of thermal transect sites with temperature records from spring and summer 2009. In conjunction with the test flows of 2009, temperature recording data loggers were placed in cross channel arrays at three locations in the lower part of the reach between Preston Falls and Early Intake. Thermistors were located at the channel margins and in deeper water. Specific methods to integrate the temperature data with survey data will depend on whether newts or yellow-legged frogs are used as the focal species. This task is an important step toward developing flow schedule recommendations, and the results should be incorporated into the gaming tool / outcome matrix being developed by Merritt-Smith Consulting. This gaming tool will help integrate output from a variety of ecological data sources and models (e.g., water temperature model, flow-habitat relationships, geomorphic and riparian thresholds) with O'Shaughnessy Dam operations to facilitate rapid assessment of several flow recommendation scenarios.

2.3.2 Methods

For foothill yellow-legged frogs, the integration of thermal, hydrologic, hydraulic, and developmental data will be conducted on a transect-basis at two existing study sites (the Early Intake and Albino Rock study sites, approximately ½ mile and 1 mile upstream of Kirkwood

Powerhouse, respectively). For Western Pond Turtles, the thermal transect is located downstream of Preston Falls at the Fireplace study site. Field work will include tracking water surface stage and lateral water temperatures over a range of flows, then creating stage-to-discharge rating curves for the transect sites. We propose to develop a degree-day model from data collected in a field rearing experiment conducted across a range of thermal conditions (Catenazzi and Kupferberg, unpublished data). Given the temperatures recorded, we will estimate the time to hatching for eggs that might be laid at the thermal transect sites and thus quantify what spring flow step-down schedule could provide sufficient time to hatching. We will also translate calculated unimpaired and contemporary flow fluctuation rates during egg incubation timing to assess the potential impacts of both flow regimes on egg hatching success.

Once tadpoles develop to a stage of swimming competency, the next step in the predictive mapping and transect-based process is to determine whether tadpoles would be able to follow a receding shoreline as discharge declines to summer baseflow. Alternatively, tadpoles could be trapped in isolated side pools apt to dry or swept into areas of swift current if eggs were laid in the early spring prior to snowmelt discharge increasing in late spring. We will examine the panoramic photographs taken in 2008 over a range of flows at the Early Intake and Albino Rock study sites that appear suitable for yellow-legged frog breeding. We will note the changes in the inundated edgewater habitats and identify small topographic features that might act as barriers. We will coordinate with the geomorphic survey crews to measure elevations at a finer scale of resolution if needed. With these data, a combination of a 1-dimensional hydraulic model and Microhabitat Mapping (see Microhabitat Mapping study plan, McBain & Trush 2009) will be used to document water surface elevations and inundation areas on planform maps (2007 orthorectified aerial photographs). The potential stranding or scour consequences from the unimpaired and contemporary flow regimes can then be assessed at these thermal transect sites.

Should Sierra newts be selected as a focal organism, different methods would be employed because there is not the same level of existing knowledge about their thermal requirements and development compared with foothill yellow-legged frogs. We propose to monitor newt developmental and growth rates in relation to temperature by making repeated visits to the thermal transect sites described above where newts are present, capturing larvae with dip nets, taking morphometric data, and then releasing them. Some locations are likely to be more thermally well-mixed than others, based on side pool isolation at summer baseflow. We plan to compare the larval growth rates observed between sites in relation to the compiled temperature data collected under other tasks of the larger project.

To evaluate potential stranding of isolated newt pools, we will make similar use of 2008 panoramic photographs, and coordinate efforts with the geomorphology team to refine the discharge-to-stage-height rating curves at newt breeding sites.

2.3.3 Schedule

Visits can either be in conjunction with VES surveys or at additional dates. For the newt option, visits would occur at approximately 3-4 week intervals during the summer and early fall of 2009. Development of a degree-day model and analysis of temperature data will occur after data loggers have been pulled from the sites.

2.4 Task 4: Assess western pond turtle demographics in pools

2.4.1 Description

This task will be used to assess recruitment status of western pond turtles in the study reach. In 2008, only large adult turtles were observed, suggesting impaired recruitment in the system. In 2009, a few smaller turtles were observed in the Poopenaut Valley terrace ponds, and one in the main channel downstream of Preston Falls. Because this is a long-lived species (60 years or more), impaired recruitment can go unnoticed for many decades before a decline in the adult numbers is observed.

2.4.2 Study Area

Pilot effort (in 2010) will focus on the Preston Falls area ("Fireplace" at 1623+00, locations of one of the three thermal transects), and the Poopenaut Valley terrace ponds.

2.4.3 Methods

Morphometric data will be collected to assess the age structure of the population in the Hetch Hetchy Reach. When western pond turtles *Emys* (*Clemmys*) *marmorata* are captured, we will measure weight, length, age (estimated by annuli counts), and sex (Appendix A1). Snorkel surveys will be conducted in Poopenaut Valley ponds and the Preston Falls reach pool with thermal transects (1623+00). Snorkelers will search along the banks, pools, and under cover objects for turtles (Reese and Welsh 1998). We will also deploy minnow traps to improve the potential for detect hatchlings. Traps (3 per site) will be deployed for six to twelve hours, keeping one end of the trap above water for animals to surface for breath.

2.4.4 Schedule

Visits can either be in conjunction with VES surveys or at additional dates. Two to three days of snorkel surveys should be sufficient for initial assessment in the mainstem. This effort would focus on the lower reach, between Mather Pool and Early Intake. Data collection is proposed for 2010, although an initial effort may be conducted in 2009.

- Late June / early July 2010 (post snow melt peak)
- Late August - September 2010 (mid-late summer)

2.5 Task 5: Assess alternative explanations for foothill yellow-legged frog scarcity within the study reach

2.5.1 Description

This task will consider other potential causes of foothill yellow-legged frog scarcity beyond those directly associated with O'Shaughnessy Dam operation (e.g. presence of chytrid fungus, or long distance to the nearest tributary-based sub-population that may sustain a mainstem meta-population in the Upper Tuolumne River).

2.5.2 Study Area

Limited presence-absence surveys will be conducted on nearby unregulated rivers and streams. These sites can include Merced River below Yosemite Valley, Middle Fork of the Tuolumne River, and/or the Clavey River. Tributary populations could also be sampled (such as Bull Meadow Creek, Jawbone Creek, Corral Creek), to evaluate whether nearby unregulated rivers and streams have similarly scarce populations.

2.5.3 Methods

To determine the presence / absence of chytrid fungus, a sub-sample of amphibians caught during surveys will be swabbed using standard protocols (Appendix A2). Samples will be sent to outside laboratories for analysis. At least two visits will be made to unregulated reference sites.

2.5.4 Schedule

Swabbing can occur during the survey visits opportunistically when animals are captured. Visits to reference sites will occur in:

- Late June / early July 2009 (post snow melt peak)
- Late August - September 2009 (mid-late summer)

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Appendix A1: Turtle Measurement Protocol Synopsis

Measurement of Western Pond Turtles

(*condensed from: Bury, R.B., D. Germano, H.H. Welsh, and D.T. Ashton (Eds.). Manuscript in Review. Western Pond Turtle: Biology, Sampling, Monitoring, Conservation. Handbook for Northwest Fauna, Society for Northwestern Vertebrate Biology.*)

If western pond turtles (*Emmys marmorata*) are observed, and can be hand-captured (if permitted), we will measure them for inclusion in a statewide database being developed. Measurements on captured turtle will include weight, lengths, age (estimated by annuli counts), and gender. Photos will be taken but animals will not be individually marked.

Weight (g) is measured with a spring scales (25, 100, 500 and 1000 gram calibrations) to record the weight to the nearest gram (g). Place a large rubber band around the turtle like a belt and attach the spring scale's clip to the rubber. Clip to the ventral surface so the turtle hangs plastron-up. This minimizes struggling in the first few seconds, allowing for a more accurate measurement. Keep a hand beneath the turtle to catch it if the clip does not hold.

Carapace lengths are measured in straight-line distance across the curved carapace surface. Lengths should be measured to the nearest millimeter (mm) using calipers. Maximum carapace length (CL) is measured using calipers to span the longest distance parallel to the midline, usually from the first or second marginal to the last marginal on one side or another of the midline. This dimension is prone to wear and damage, and thus may skew growth or fitness models. Minimum carapace length (cl) is recorded along the midline of the carapace, from the cleft between marginal at the tail to the cleft between the nuchal and the first marginal anteriorly. These areas are less prone to wear and can provide a more consistent measure.

Plastron lengths may provide a more stable metric for growth and demographic studies, because they are less prone to damage that would affect the measurement. Maximum plastron length (PL) is measured with the calipers flat to the plastron to see which side is longest length (mild asymmetry is common with this dimension). Minimum plastron length (pl) is the midline. Be gentle with the calipers while taking this measurement as the tissue in the midline cleft is sensitive. This measurement may be the most stable length dimension.

Height (HT) is measured as the maximum straight-line distance from the dome of the carapace to the plastron. This helps give a sense of the volume of the animal.

Gender (sex) is be determined by a suite of sexually dimorphic characters. The western pond turtle begins exhibiting sexual dimorphism in morphology and color when turtles are about 100-120 mm in carapace length. These features may be obscure in some turtles and it may be difficult to determine gender, but generally a suite of characters can used to make a determination on sex. Females tend to have a more dome-shaped carapace than the males. Males have a concave or indented plastron allowing them closer access to the female during mating, while the plastron of females is flat or slightly convex providing more space for eggs. The cloaca in females is usually located close to the edge of the carapace or anterior to the edge, while the male's cloaca is located at or posterior to the edge of the carapace. The base of tail of males is generally thicker in diameter; this is where the penis is stored. Eversion of the penis is a positive sign of male gender. Males may have a light-colored maxilla (side of the head below the eye; both sides of the mouth), and light yellow or cream-colored chin, which is usually subtle in young adult males but become more pronounced with time. Males have a more angular snout, whereas the female's is blunt. Even from a distance a mature adult male can identified by the angular snout and light-colored face and chin.

Estimating age of turtles is similar to counting the rings on the cross-section of a tree. There is a significant correlation between the number of rings on turtle scutes and age in years for the western pond turtle. This evidence included recapture of many individuals where one ring was deposited annually. At warmer sites, scute annuli are discernable for 6-10 years, whereas at colder sites, annuli form up to 16 years. After these age periods, turtles no longer lay down discernable rings and age determination is not possible by annuli count (Bury and Germano 1998).

Photographs of the carapace and plastron can be useful in confirming identity of recaptured individuals and can be used to observe changes over time (e.g., growth, injury, healing). Photograph the carapace and plastron. Add a ruler for scale and photograph the plastron again. The ruler should be held on the same plane as the plastron surface so it can used to measure annuli from the photograph. A photograph of the face can help with confirming gender later.

Appendix A2. Chytrid Swab Protocol of the UC Berkeley Briggs et al. NIH Group.

(http://www.amphibiaweb.org/aw/chytrid/swab_instruction07.pdf) Accessed June 12, 2009.

This protocol was developed to allow field biologists to non-destructively sample amphibians in the field for the presence of *Batrachochytrium dendrobatidis*. This document was produced for the Briggs NIH research group based on Boyle et al. (2004).

Supplies:

Swabs - these can be ordered directly or from a distributor:

- Directly: Medical Wire and Equipment: go to http://mwe-usa.com/mwe/mwe.php. The product code is MW113.
- Distributor: Advantage Bundling SP. (catalog number MW113). Advantage Bundling can be reached either by phone, 1-866-Bundling or orders can also be placed through email, sales@advantagebundlingsp.com.

Vials - Screw cap 1.5-ml microcentrifuge tubes, available through Fisher (catalog number 05-669-12). These are standard and can be ordered through other companies, we just give one example. All microcentrifuge tubes used should be sterilized either by autoclaving before use or they can be purchased at a higher price as pre-sterilized (Fisher catalog number 05-669-17).

Marking pen - When possible, use ethanol-proof black markers to label your vials as they tend to persist over time best. Some people prefer to scratch the sample id on the vial because it cannot be washed off or erased by accident.

Procedure:

1. Preferably, capture amphibians by hand. Wear gloves when swabbing animals and change gloves between animals. If you are using a dip net, be aware that *B. dendrobatidis* zoospores could be caught on the net and transferred between individuals, therefore, use different nets whenever possible, or disinfect the net as often as you can (there is no perfect solution to this problem).
2. Swab the underside or ventrum of adult/metamorphs 30 times. Remember you are in effect scraping small amounts of tissue from the skin. Some pressure must be applied, but this does not mean that you must squash the animal. Areas to target are the drink patch, thighs, and webbing between the toes.
3. Air-dry the swab for approximately 5 minutes, avoid direct sunlight if possible (if conditions are too humid to air-dry, then store in 95% EtOH).
4. Break swab ~3 cm from tip and drop into empty screw cap tube. The swab stick should not touch or bump against the top of the vial. Screw the cap on the vial and store in the shade.
5. Samples can be kept a room temperature for a week or maybe longer, but it is best to keep the samples cool and placed as soon as possible in a 4 degree C freezer.