EXHIBIT A

State Water Resources Control Board

Division of Water Rights
1001 I Street, 14th Floor ♦ Sacramento, California 95814 ♦ 916.341.5300
P.O. Box 2000 ♦ Sacramento, California 95812-2000
Fax: 916.341.5400 ♦ www.waterrights.ca.gov

Linda S. Adams
Secretary for
Environmental Protection

Arnold Schwarzenegger
Governor

DEC 10 2008

Mr. Eduardo Malacon
Los Angeles Department of Water and Power
111 North Holt Street, Room 1121
Los Angeles, CA 90012

Mr. Dean Messer
California Department of Water Resources
1416 9th Street, Room 620
Sacramento, CA 95814

Dear Messrs. Malacon and Messer:

APPLICATION FOR A 401 WATER QUALITY CERTIFICATION FOR THE CALIFORNIA AQUEDUCT HYDROELECTRIC PROJECT, FEDERAL ENERGY REGULATORY COMMISSION PROJECT NO. 2426, VENTURA AND LOS ANGELES COUNTY

The State Water Resources Control Board (State Water Board) Executive Director has issued a Water Quality Certification pursuant to section 401 of the Clean Water Act for the California Aqueduct Hydroelectric Project, FERC Project No. 2426. A copy of the Water Quality Certification and a copy of the Notice of Determination from the State Water Board, as the responsible agency, are enclosed for your records.

If you have any questions, please contact Jennifer Watts at (916) 341-5397 or by email at jwatts@waterboards.ca.gov.

Sincerely,

Camilla Williams

Camilla Williams, Chief
Water Quality Certification Unit

cc: Tracy Egoscue, Executive Officer
Los Angeles Regional Water Quality
Control Board
320 West Fourth Street, Suite 200
Los Angeles, CA 90013

Alexis Strauss, Director
Water Division
US Environmental Protection Agency,
Region 9
75 Hawthorne Street
San Francisco, CA 94105

Honorable Kimberly D. Bose
Office of the Secretary
Federal Energy Regulatory Commission
888 First Street, N.E.
Washington, D.C. 20426

Dr. Creed Clayton
U.S. Fish and Wildlife Service
2493 Portola Road, Suite B
Ventura, CA 93003

STATE OF CALIFORNIA
STATE WATER RESOURCES CONTROL BOARD

In the Matter of Water Quality Certification for the

RE-OPERATION OF PYRAMID DAM FOR THE CALIFORNIA AQUEDUCT HYDROELECTRIC PROJECT FEDERAL ENERGY REGULATORY COMMISSION PROJECT NO. 2426

SOURCE: Piru Creek

COUNTY: Los Angeles and Ventura Counties

Introduction

The California Department of Water Resources (DWR) and the City of Los Angeles (collectively Licensee) applied to the Federal Energy Regulatory Commission (FERC) on March 17, 2005 for an amendment to the current FERC license for the reoperation of Pyramid Dam (Project), a part of the California Aqueduct Hydroelectric Project, FERC Project No. 2426. FERC Project No. 2426 includes a number of hydroelectric developments that are situated along the length of the California Aqueduct. The application for the license amendment only addresses operation of Pyramid Dam and associated impacts to the 18 mile reach of Piru Creek between Pyramid Dam and Lake Piru. Lake Piru is a non-Project facility operated by United Water Conservation District. A map of the Project vicinity is shown in Attachment A. DWR utilizes Piru Creek for conveyance of State Water Project (SWP) water to its long term contractors. Between 1996 and 2002, total annual outflow at Pyramid Lake ranged between approximately 10,000 – 70,000 acre-feet of water.

Amendments to the FERC license requested in Licensee's application include the modification of minimum flow requirements for Piru Creek below Pyramid Dam required under Article 52 and Exhibit S of the current FERC license, which require Licensee to establish and maintain a year-round trout fishery. DWR requested the license amendment to avoid incidental take of the arroyo toad (*Bufo californicus*), a species listed by the United States Fish and Wildlife Service (FWS) as endangered under the Endangered Species Act. Prior to submittal of the application for a license amendment, Licensee submitted a request to FERC for a temporary waiver from the minimum flow releases under FERC license Article 52 on February 10, 2005. FERC approved the temporary waiver on April 12, 2005. Consequently, DWR has already begun operating Pyramid Dam flow releases to simulate natural flow conditions using the same operating guidelines that will be implemented under the requested license amendment.

The DWR water right at Pyramid Dam and Lake Piru is authorized under Water Right Permit 18709 (Application 25988) issued by the State Water Resources Control Board (State Water Board) and documentation is recorded with the Division of Water Rights (Division). Water Right Permit 18709 is for year round storage of 55,000 acre-feet of water collected from Piru Creek. The beneficial uses of water identified in Permit 18709 are irrigation; domestic; municipal; industrial; water quality; recreational; fish and wildlife preservation and enhancement; and incidental power generation. Lake Piru is also designated as a point of rediversion under various permits and licenses held by DWR that authorize water to be conveyed through the California Aqueduct for distribution at various facilities.

Before FERC can issue a license amendment for the Project, Licensee must obtain water quality certification under section 401 of the Clean Water Act from the State Water Board. (33 U.S.C. § 1341.) The State Water Board must certify that the Project will comply with the applicable provisions of the Clean Water Act, including water quality standards set forth in the Water Quality Control Plan for the California Regional Water Quality Control Board, Los Angeles Region (Basin Plan). The State Water Board must analyze the overall effect of the Project license amendment on water quality and include conditions in the certification, if necessary, to adequately protect the designated beneficial uses identified in the Basin Plan.

Water Quality Certification Conditions

Operational Guidelines to Simulate Natural Hydrology

Article 52 of the current FERC license dictates a continuous minimum stream flow release below Pyramid Dam of 5 cubic feet per second (cfs) from November 16 through April 30, and 10 cfs from May 1 through November 15. This article also includes a requirement for release of additional flow up to 25 cfs from Pyramid Dam into Piru Creek, depending upon the predicted maximum air temperature in the Project area. Additional requirements related to the maintenance of stream flow for the purpose of maintaining a year-round trout fishery are contained in Exhibit S of the current license.

In 2003, FWS expressed concern about higher than natural perennial stream flows in Piru Creek and their impacts to the endangered arroyo toad population that is known to inhabit middle Piru Creek, which is the reach between Lake Piru and Pyramid Dam. These concerns included the effects of increased summer stream flows on non-native species that prey on the toads, such as bullfrogs and crayfish. Additionally, the natural scouring events that are necessary to maintain arroyo toad habitat and that would normally occur during winter storm events are prevented due to the flow management practices under the current license conditions. In communicating their concerns about impacts to arroyo toads, the FWS provided recommendations for managing water releases in Piru Creek that are compatible with survival and recovery of the arroyo toad. These recommendations have been incorporated into the operational scheme proposed by DWR in its license amendment application to FERC.

DWR will operate Pyramid Dam to reflect natural flow conditions by releasing flows from Pyramid Lake to middle Piru Creek at a rate up to approximately 18,000 cfs, which is the maximum volume of water that can be safely released from Pyramid Dam. Inflow to Pyramid Lake will be measured at existing gauging stations that are located above Pyramid Lake on upper Piru Creek and Cañada de los Alamos. A multiplier will be used to account for portions of the Pyramid Lake watershed that are not tributary to either upper Piru Creek or Cañada de los Alamos. Due to operational constraints, the stream release into middle Piru Creek at Pyramid Dam will typically lag measured inflow by approximately one day. Implementation of the proposed project will result in greater volumes of water passing through middle Piru Creek during the rainy season (typically November through April). During the dry season (May through October), flows in middle Piru Creek will gradually diminish in response to decreasing surface water inflow to Pyramid Lake. On rare occasions during dry years, inflow to Pyramid Lake may be reduced to zero.

Radial Gate Testing

Sudden increases or decreases in stream flows can be disruptive to aquatic organisms, especially when they occur during critical life history stages. For this reason, short-term increases in flow to middle Piru Creek associated with testing of the radial gates, stream release valves, or other requirements to test equipment at Pyramid Dam are prohibited between March 15 and June 15 and will be avoided to the extent possible between June 16 and July 31. Scheduled tests that require releases that last longer than 15 minutes will require prior notification to the FWS. This allows the radial gates at Pyramid Dam to be exercised, and provides for testing equipment, as mandated by FERC or other agencies, that would otherwise increase flows by up to 50 cfs for short periods of time.

Monitoring Requirements

Monitoring for federally listed threatened and endangered species and for California species of special concern within the Project area is included to better understand how implementation of the Project affects these species and will allow for collection of information about their status in middle Piru Creek. DWR will develop a monitoring plan that includes annual breeding surveys for federally listed arroyo toads and that may also include surveys for California red-legged frogs, and for two California species of special concern: Southwestern pond turtles and Two-striped garter snakes. The monitoring plan may also need to include surveys for exotic species known to occur in middle Piru Creek, such as bullfrogs and crayfish, which are known to prey upon arroyo toads.

WATER QUALITY CERTIFICATION FOR FEDERAL PERMIT OR LICENSE

BY THE EXECUTIVE DIRECTOR:

1. The federal Clean Water Act (33 U.S.C. §§ 1251 *et seq.*) was enacted "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." (33 U.S.C. § 1251(a).) Section 401 of the Clean Water Act (33 U.S.C. §1341) requires every applicant for a federal license or permit which may result in a discharge into navigable waters to provide the licensing or permitting federal agency with certification that the project will be in compliance with specified provisions of the Clean Water Act, including water quality standards and implementation plans promulgated pursuant to section 303 of the Clean Water Act (33 U.S.C. § 1313). Section 401 of the Clean Water Act directs the agency responsible for certification to prescribe effluent limitations and other limitations necessary to ensure compliance with the Clean Water Act and with any other appropriate requirement of state law. Section 401 further provides that state certification conditions shall become conditions of any federal license or permit for the project.

2. The State Water Board is the State agency responsible for certification in California. (Wat. Code, § 13160.) The State Water Board has delegated this function to the Executive Director by regulation. (Cal. Code Regs., tit. 23, § 3838, subd. (a).)

3. The California Regional Water Quality Control Boards have adopted, and the State Water Board and the US Environmental Protection Agency have approved, water quality control plans (Basin Plans) for each watershed basin in the State. The Basin Plans designate the beneficial uses of waters within each watershed basin and water quality objectives designed to protect those uses. Section 303 of the Clean Water Act requires the states to develop and adopt water quality standards. (33 U.S.C. § 1313.) The beneficial uses together with the water quality objectives that are contained in the basin plans constitute state water quality standards under section 303. The State Water Board has also considered the existing water quality conditions and Project related controllable factors.

4. The Los Angeles Regional Water Quality Control Board (Los Angeles Board), has adopted the Basin Plan for the Coastal Watersheds of Los Angeles and Ventura Counties, which identifies industrial service and process supply; agricultural supply; groundwater recharge; freshwater replenishment; water contact recreation; non-contact recreation; warm freshwater habitat; cold freshwater habitat; wildlife habitat; rare, threatened or endangered species habitat; spawning, reproduction, and/or early development habitat; and wetland habitat as existing beneficial uses for Piru Creek between Pyramid Lake and Lake Piru. Additionally, municipal and domestic supply is identified as a potential beneficial use.

5. On June 12, 2008, FERC issued the final environmental assessment (Final EA) for the Project, pursuant to the requirements of the National Environmental Policy Act. The Final EA presents an evaluation of the Project, addresses potential

environmental impacts, and includes responses to comments received on the draft environmental assessment. The Final EA also includes a Finding of No Significant Impact (FONSI).

6. DWR is the lead agency for the Project for purposes of the California Environmental Quality Act (CEQA). (Pub. Resources Code, §§ 21000 *et seq.*) DWR released a Notice of Preparation of a draft environmental impact report (EIR) on May 19, 2004 and held a public scoping meeting on June 17, 2004 in Santa Clarita. DWR subsequently released a draft EIR entitled *The Simulation of Natural Flows in Middle Piru Creek* in November 2004 (State Clearinghouse No. 2004051123) and held a public comment meeting in December 2004. A Final EIR was released in January 2005. The Final EIR was certified by the Director of DWR and a Notice of Determination was filed with the State Office of Planning and Research on February 15, 2005. DWR incorporated conditions into the Project designed to protect the environment.

7. The State Water Board, as a responsible agency under CEQA, has reviewed and considered the documents produced by DWR to support the environmental review required for the issuance of the Section 401 Water Quality Certification. Although the State Water Board was not identified as a responsible agency at the time that DWR circulated the draft and the final EIR, subsequent review of the documents produced by DWR indicate that the State Water Board would not have requested significant changes had it been consulted as a responsible agency. The State Water Board will file a Notice of Determination within five days from the issuance of this certification.

ACCORDINGLY, BASED ON ITS INDEPENDENT REVIEW OF THE RECORD, THE STATE WATER BOARD CERTIFIES THAT THE OPERATION OF THE CALIFORNIA AQUEDUCT HYDROELECTRIC PROJECT BY THE CALIFORNIA DEPARTMENT OF WATER RESOURCES AND THE CITYOF LOS ANGELES UNDER AN AMENDED LICENSE ISSUED BY FERC will comply with sections 301, 302, 303, 306 and 307 of the Clean Water Act, and with applicable provisions of state law, provided that the California Department of Water Resources complies with the following terms and conditions:

1. Pyramid Dam Stream Flow Conditions

Stream releases from Pyramid Dam into Piru Creek shall match natural inflow into Pyramid Lake to the extent operationally feasible and consistent with safety requirements, as described in the following guidelines:

A. Natural inflow to Pyramid Lake will be released into middle Piru Creek at a rate up to approximately 18,000 cfs, which is the maximum safe designed release from Pyramid Dam.

B. Storm releases into middle Piru Creek may be held back at less than the maximum safe designed release of 18,000 cfs if higher releases are deemed a threat to life, safety, or property at Pyramid Dam or downstream of the dam.

C. DWR may elect to appropriate inflow to Pyramid Lake above the safe release flows under the provisions of its existing water rights.

D. Up to 3,150 acre feet of State Water Project water may be delivered to United Water Conservation District via middle Piru Creek between November 1 and the end of February of each water year. During this period, water deliveries may be made over a period of a few days, ramping flows up and down to simulate the hydrograph of a typical storm event, or they may be released more gradually over a longer period.

E. Radial Gate Testing

Releases into middle Piru Creek may be increased for short periods of time to exercise the Pyramid Dam radial gate and stream release valves, to test emergency power sources, to conduct tests mandated by FERC, or to meet short-term operational or maintenance requirements. No such testing will be scheduled between March 15 and June 15. Testing will also be avoided to the extent possible between June 16 and July 31. When testing is conducted, flows shall not increase by more than 50 cfs above current base flows and release events shall not last longer than 15 minutes. Scheduled tests that require larger releases or last longer than 15 minutes require prior notification to the FWS. Unscheduled releases due to equipment failure or emergency situations must be reported to the FWS no later than three business days after the event.

F. All flow requirements of this certification are subject to temporary modification if required by equipment malfunction, emergency conditions or law enforcement activity, or critical electric system emergency beyond the control of the Licensee. The Licensee shall provide advance notification to the FWS prior to any temporary modification, when possible. If advance notification is not possible because an event is unforeseeable, Licensee shall notify the FWS no later than 48 hours from the time that any temporary modification has occurred.

2. Arroyo Toad and Sensitive Species Monitoring Condition

Within one year of issuance of the license amendment, DWR shall file with FERC a plan approved by the Deputy Director for Water Rights for annual breeding surveys of the arroyo toad in middle Piru Creek. Monitoring shall occur, at a minimum, in the lower portion of middle Piru Creek between Lake Piru and Ruby Canyon (a distance of approximately 2 to 3 miles) and shall be conducted by a qualified biologist with experience in identifying arroyo toad larvae and tadpoles. An annual monitoring report shall be submitted to the Deputy Director by October 1 of each year that includes the results of the breeding surveys as well as flow data to document daily releases at Pyramid Dam. If three years of monitoring indicate that the arroyo toad population has shown improvement under the flow modifications identified in this certification, DWR, upon consultation with the State Water Board and FWS, may modify the monitoring frequency required to demonstrate the presence of arroyo toads.

3. This certification is contingent on compliance with all applicable requirements of the Los Angeles Board Basin Plan, except as may be modified by the specific conditions in this certification.

4. Notwithstanding any more specific conditions in this certification, the Project shall be operated in a manner consistent with all water quality standards and implementation plans adopted or approved pursuant to the Porter-Cologne Water Quality Control Act or section 303 of the Clean Water Act. The Licensee shall take all reasonable measures to protect the beneficial uses of water in Piru Creek.

5. Licensee must submit any change to the California Aqueduct Hydroelectric Project that affects the operation of Pyramid Dam that would have a significant or material effect on the findings, conclusions, or conditions of this certification to the Deputy Director for prior review and written approval.

6. DWR shall provide State Water Board staff access to Project sites to document compliance with this certification.

7. The authorization to operate the Project pursuant to this certification is conditioned upon payment of all applicable fees for review and processing of the application for water quality certification and administering the State's water quality certification program, including but not limited to: timely payment of any annual fees or similar charges that may be imposed by future statutes or regulations for the State's reasonable costs of a program to monitor and oversee compliance with conditions of water quality certification.

8. This certification is not intended and shall not be construed to apply to issuance of any FERC license or FERC license amendment other than the FERC license amendment specifically identified in the Licensee's application for certification.

9. This certification does not authorize any act which results in the taking of a threatened or endangered species or any act which is now prohibited, or becomes prohibited in the future, under either the California Endangered Species Act (Fish & G. Code §§ 2050 *et seq.*) or the federal Endangered Species Act (16 U.S.C. §§ 1531 *et seq.*). If a take will result from any act authorized under this certification or water rights held by the Licensee, the Licensee shall obtain authorization for the take prior to any construction or operation of the Project. The Licensee shall be

responsible for meeting all requirements of the applicable Endangered Species Act for the Project authorized under this certification.

10. In the event of any violation or threatened violation of the conditions of this certification, the violation or threatened violation shall be subject to any remedies, penalties, process or sanctions provided for under applicable state or federal law. For the purposes of section 401(d) of the Clean Water Act, the applicability of any state law authorizing remedies, penalties, process or sanctions for the violation or threatened violation constitutes a limitation necessary to assure compliance with the water quality standards and other pertinent requirements incorporated into this certification.

11. In response to a suspected violation of any condition of this certification, the State Water Board may require the holder of any federal permit or license subject to this certification to furnish, under penalty of perjury, any technical or monitoring reports the State Water Board deems appropriate, provided that the burden, including costs, of the reports shall bear a reasonable relationship to the need for the reports and the benefits to be obtained from the reports.

12. In response to any violation of the conditions of this certification, the State Water Board may add to or modify the conditions of this certification as appropriate to ensure compliance.

13. DWR must submit any change to the Project operation that would have a significant or material effect on the findings, conclusions, or conditions of this certification, to the Deputy Director for prior and written approval.

14. This certification is subject to modification upon administrative or judicial review, including review and amendment pursuant to Water Code section 13330 and California Code of Regulations, title 23, division 3, chapter 28, article 6 (commencing with § 3867).

15. The State Water Board reserves authority to modify this certification if monitoring results indicate that continued operation of the Project will violate water quality objectives or impair the beneficial uses of Piru Creek.

16. The State Water Board may add to or modify the conditions of this certification, as appropriate, to implement any new or revised water quality standards and implementation plans adopted or approved pursuant to the Porter-Cologne Water Quality Control Act or section 303 of the Clean Water Act.

17. The State Water Board may add to or modify the conditions of this certification as appropriate to coordinate the operations of this Project and other hydrologically connected water development projects, where coordination of operations is reasonably necessary to achieve water quality standards or protect beneficial uses of water.

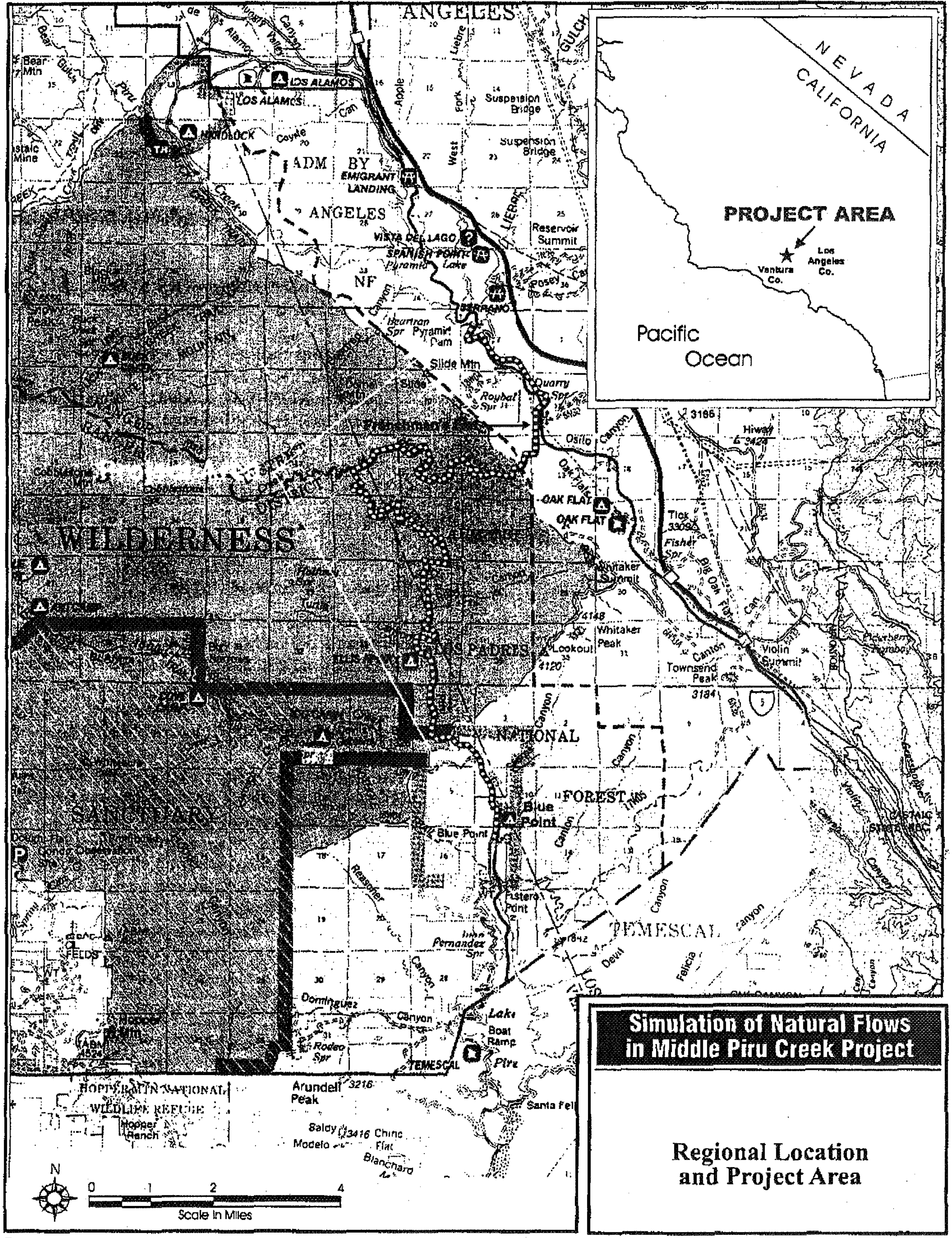
18. The State Water Board shall provide notice and an opportunity for hearing in exercising its authority under conditions 15, 16, and 17 above.

Dorothy Rice

Dorothy Rice
Executive Director

Date: DEC - 9 2008

Attachment A



Simulation of Natural Flows in Middle Piru Creek Project

Regional Location and Project Area

EXHIBIT B

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Southwest Region
501 West Ocean Boulevard, Suite 4200
Long Beach, California 90802- 4213

APR 27 2007

Kimberly D. Bose, Secretary
Federal Energy Regulatory Commission
888 First Street, NE
Washington, D.C. 20426

Re: Motion to Intervene, and Comments on the Draft Environmental Assessment, for the California Aqueduct Project, FERC Project No. 2426-197

Dear Secretary Bose:

Enclosed for filing in the above-referenced proceeding is NOAA's National Marine Fisheries Service's (NMFS) motion to intervene, and comments on the draft environmental assessment, together with a certificate of service for the subject project.

If you have any questions regarding this matter, please contact Anthony Spina at 562-980-4045.

Sincerely,

Mark Helvey

for Rodney R. McInnis
Regional Administrator

Enclosures

cc: Service List

UNITED STATES OF AMERICA
FEDERAL ENERGY REGULATORY COMMISSION

California Department of Water Resources) and the City of Los Angeles) California Aqueduct Project)	FERC Project No. 2426-197

NATIONAL MARINE FISHERIES SERVICE'S COMMENTS ON THE DRAFT ENVIRONMENTAL ASSESSMENT

Overview

NOAA's National Marine Fisheries Service (NMFS) reviewed the Federal Energy Regulatory Commission's (Commission) draft Environmental Assessment (EA) for amendment of the license for the California Aqueduct Project (P-2426-197). Although no species under NMFS' jurisdiction currently exists within the action area (i.e., the middle reach of Piru Creek and adjoining tributaries between Pyramid Lake and Lake Piru), the project reach is within historical spawning and rearing habitat for the Southern California Distinct Population Segment (DPS) of endangered steelhead (*Oncorhynchus mykiss*) and is expected to have a role in the recovery of endangered steelhead. In this context, NMFS offers the following information and comments on the draft EA. These comments supplement and extend the comments NMFS has provided in a previous letter (NMFS 2005[1]) regarding the subject project.

Recovery of endangered steelhead

The draft EA does not provide information that would allow an understanding of how the proposed action would affect the biological, physical, and physicochemical capability of the project reach for future recovery of steelhead. Such information is important, in part, because the Santa Clara River watershed (including the Piru Creek sub-basin) is expected to have a critical role in the recovery of the Southern California DPS of endangered steelhead[2]. The reasons why the Santa Clara River watershed is expected to support recovery of endangered steelhead include the following:

- The Santa Clara River steelhead population has been identified as a "core" population essential for the successful recovery of endangered steelhead due, in part, to the watershed's large size, spawning and rearing habitat quality, relatively reliable winter

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[1] Letter of R. McInnis, NMFS Southwest Region, Long Beach, California, to E. Begley, Department of Water Resources, Sacramento, California, January 11, 2005.

[2] The draft EA states on page 35 that southern California steelhead were listed as endangered on March 19, 1998. As a matter of clarification, this species was listed as endangered on August 18, 1997 (National Marine Fisheries Service 1997. Federal Register 62: 43497-43954).

river discharge, and greater potential for being independently viable (Boughton et al. 2006[3]);

- With regard to Piru Creek, over 38 miles of aquatic habitat exist in the middle reach of the creek, as well as in Fish Creek and Agua Blanca Creek, tributaries to Piru Creek within the project reach;
- Surveys of Piru Creek and tributaries upstream of Santa Felicia Dam reveal that instream habitat is suitable for spawning and rearing of *O. mykiss*. At the time of the surveys, age-0 fish and age-1 and older age groups were common (Moore 1980a[4]). Oversummering habitat for steelhead is available upstream of Santa Felicia Dam, which is a significant ecological finding because oversummering habitat is viewed as the most geographically restricted habitat type for steelhead in southern California (Boughton and Goslin 2006[5]). While elevated temperatures have been occasionally noted in selected creek reaches upstream of the dam, these temperatures are not considered to be problematic for the species (Moore 1980a) and are within the range of water temperatures that juvenile steelhead accept and remain active and forage throughout the day in southern California streams (Spina 2006[6]); and,
- Steelhead historically accessed habitat that now lies upstream of Santa Felicia Dam because evidence indicates contemporary wild rainbow trout upstream of Santa Felicia Dam are descendents of steelhead. Recent genetic investigations by NMFS staff of *O. mykiss* populations, including the population upstream and downstream of Santa Felicia Dam, have concluded that "no substantial genetic differentiation was found between trout populations above and below dams in the 5 river basins studied, which indicates that populations of trout breeding in streams tributary to the dam reservoirs are recently derived from a common ancestral population with trout populations breeding below the dams. This suggests that breeding populations in these upstream tributaries are likely dominated by trout descended from steelhead isolated above the dams following their construction" (Girman and Garza 2006[7]). That steelhead spawned and reared in tributaries within the Santa Clara River watershed, including reaches of Piru Creek that

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[3] Boughton, D. A., P. B. Adams, E. Anderson, C. Fusaro, E. Keller, E. Kelley, L. Lentsch, J. Nielsen, K. Perry, H. Regan, J. Smith, C. Swift, L. Thompson, and F. Watson. 2006. Steelhead of the south-central/southern California coast: population characterization for recovery planning. NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-394.

[4] Moore, M. R. 1980a. Stream surveys, Ojai Ranger District, Los Padres National Forest, Ventura County, California, April 25, 1980.

[5] Boughton, D. A., and M. Goslin. 2006. Potential steelhead over-summering habitat in the south-central/southern California coast recovery domain: maps based on the envelope method. NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-391.

[6] Spina, A. P. 2006. Thermal ecology of juvenile steelhead in a warm-water environment. Environmental Biology of Fishes DOI 10.1007/s10641-006-9103-7.

[7] Girman, D., and J. C. Garza. 2006. Population structure and ancestry of *O. mykiss* populations in South-Central California based on genetic analysis of microsatellite data. Final report of the National Marine Fisheries Service, Southwest Fisheries Science Center, Santa Cruz, California, for the California Department of Fish and Game Project No. P0350021 and Pacific States Marine Fisheries, Contract No. AWIP-S-1.

currently lie upstream of Santa Felicia Dam, is further corroborated by others (Moore 1980b[8]; Titus 2005[9]).

Consequently, NMFS recommends the Commission revise the draft EA to assess the effects of the proposed action on the capability of the project reach to contribute to recovery of endangered steelhead. Aspects to consider in the impact analysis include the effects of planting of domestic trout, effects of delivering water to Lake Piru outside natural runoff events, and effects on the migratory ecology and behavior of *O. mykiss* in the project reach.

Clarification on the relationship between operation of Pyramid Dam and Santa Felicia Dam

The revised environmental document should include a clear and complete description of the relationship between water-management operations at Pyramid Dam and at Santa Felicia Dam. NMFS' current understanding is that Santa Felicia Dam supplies water *at levels* that would not otherwise be achieved if not for Pyramid Dam. For instance, surface water that cannot be captured and stored in Lake Piru (e.g., in the case of high-flow events exceeding storage, causing spills) can be temporarily stored in Pyramid Lake, for later release to Lake Piru. This is supported in the draft EA, which states on page 15 that "[t]he licensees also have the right to store up to 55,000 acre-feet of local runoff in Pyramid Lake when Santa Felicia dam is spilling…" Additionally, "Pyramid Lake supplies the majority of flow to middle Piru Creek and subsequently the majority of inflow to Lake Piru during the dry season…" (page 62 of the Commission's January 2007 EA for the Santa Felicia Hydroelectric Project). Although the proposed action is intended to achieve some level of inflow-outflow release scenario, water deliveries to Lake Piru are expected to continue, according to the draft EA, so this apparent issue regarding the relationship between the two reservoirs remains. Thus, operation of Santa Felicia Dam may be viewed as an "interrelated action" (sensu 50 CFR § 402.02).

Knowing whether operation of Santa Felicia Dam is an interrelated action has important ramifications. According to rules and regulations governing interagency cooperation (50 CFR 402), when contemplating federal actions, agencies are required to consider "effects of the action," which is defined as "the direct and indirect effects of an action on the species or critical habitat, together with the effects of other activities that are interrelated or interdependent with that action…" (50 CFR § 402.02). Therefore, if operation of Santa Felicia Dam is an interrelated action, the effects of Santa Felicia Dam (which are viewed as harmful to endangered steelhead[10]) should be formally considered and the Commission should consult with NMFS on the proposed action, in accordance with section 7 of the U. S. Endangered Species Act (ESA). However,

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[8] Moore, M. R. 1980b. An assessment of the impacts of the proposed improvements to the Vern Freeman diversion on anadromous fishes of the Santa Clara River System, Ventura County, California. Prepared for the Ventura County Environmental Resources Agency, Ventura.

[9] Titus, R. G., D. C. Erman, and W. M. Snider. 2006. History and status of steelhead in California coastal drainages south of San Francisco Bay. Unpublished manuscript.

[10] National Marine Fisheries Service. 2006b. Comments on the environmental assessment for the Santa Felicia Hydroelectric Project, FERC Project No. 2153-012. Letter of R. McInnis, NMFS, Southwest Region, Long Beach, California, to M. Salas, Federal Energy Regulatory Commission, Washington, D.C., January 12, 2006.

whether the operation of Santa Felicia Dam represents an interrelated action is not entirely clear at this time, owing to the inadequacy of the draft EA in this regard.

Migration of steelhead in Piru Creek downstream of Santa Felicia Dam

The draft EA states on page 38 that "several *potential* [emphasis added] obstacles prevent the migration of steelhead on Piru Creek..." NMFS has inspected some of the structures identified in the draft EA (the Piru diversion, and the box culvert near RM 4.5), and believes the subject structures may block passage of steelhead *only during certain, but not all*, discharge conditions. NMFS' experience with improving passage conditions for steelhead at unnatural structures throughout southern and south-central California indicates the passage conditions at the subject structures could be enhanced. Although neither structure is currently in compliance with the ESA, through collaboration with the entities that are responsible for the subject structures, NMFS expects the structures will be modified for the purpose of improving passage conditions for endangered steelhead. The foregoing points should be clarified in the revised environmental document.

Water delivery to Lake Piru and testing of radial gates

To minimize adverse effects of the water deliveries on the migratory behavior and ecology of *O. mykiss*, NMFS recommends that the pattern and relative magnitude of in-channel water deliveries correspond with pattern and magnitude of natural runoff events. Presumably, such an operational scheme would require (1) a reliable ability to instantaneously identify periods when principal tributaries to the middle reach of Piru Creek (e.g., Agua Blanca Creek and Fish Creek) are responding to rainfall events, and (2) the ready capability to facilitate water deliveries from Pyramid Lake according to the pattern and magnitude of flows observed in adjoining tributaries. While delivering water strictly according to natural runoff events may be presumed to support migration of *O. mykiss* to and from spawning and rearing habitats in the project reach, including adjoining tributaries and Lake Piru, validation should be required. A reasonable first step for validating the presumed benefits should include acquiring basic science-based information on the migratory behavior and ecology of *O. mykiss* in the project reach. Accordingly, the Commission should require the licensee to implement an agency-approved (i.e., California Department of Fish and Game, and NMFS) study for the purpose of acquiring data on the migratory behavior and ecology of *O. mykiss* in the project reach. To this end, the study should produce information that would answer the following questions:

- Do *O. mykiss* individuals migrate upstream out of Piru Lake and remain in the middle reach or continue into adjoining tributaries?
- Does the upstream migration correspond to periods of elevated discharge in the middle reach and tributaries?
- What are the characteristics of individual *O. mykiss* that are migrating out of Lake Piru (i.e., are the individuals adults or juveniles, domestic or wild strains)?
- Are the upstream migrants spawning, if so where (e.g., in the middle reach or adjoining tributaries)?

- Are individual *O. mykiss* that are produced from nests of upstream migrants returning to Lake Piru or remaining in the middle reach or tributaries?
- What are the characteristics of individual *O. mykiss* that are returning to Lake Piru (i.e., are the individuals adults or juveniles, showing evidence of smoltification)?
- Does the downstream migration correspond to periods of elevated discharge in the middle reach and tributaries?

In addition to the foregoing fishery study, and as part of the proposed action, the Commission should require the licensee to implement an agency-approved plan to monitoring movement of *O. mykiss* over time and space in the project reach to ensure the selected operating scheme, once fully defined[11] and implemented, is in fact compatible with the migratory ecology and behavior of *O. mykiss*, as determined from the fishery study.

The testing of the radial gates could be of concern, particularly if testing (i.e., water releases) were performed during or shortly after spawning of *O. mykiss*, as is suggested in the draft EA. Increases in water releases have the potential of disrupting fish spawning behavior, causing females to locate nests in areas that would be dewatered following the cessation of the test flows, scour nests and incubating embryos, and displace young fish. Therefore, NMFS recommends that testing of the radial gates be confined to late summer or early fall, when the likelihood of adverse effects on spawning and early life stages is reduced. The magnitude and rate-of-change of the water releases are also of concern, based on the reported effects of rapidly changing discharge causing fish stranding. Hence, the manner of testing should include a specific provision to reduce the likelihood of stranding fish. As part of the proposed action, the Commission should require the licensee to implement an agency-approved (NMFS and California Department of Fish and Game) monitoring plan to validate the effectiveness of the specific provision for minimizing adverse effects of the testing on the fishery resource. Note that some of the data acquired from the assessment of fish migration would be ideally suited to inform some of the uncertainties related to the potential effects of the radial-gate testing on *O. mykiss* nests and early life stages.

Inconsistency of conclusions in the draft environmental assessment

The draft EA is inconsistent when disclosing effects of the proposed action on endangered steelhead. For instance, the draft EA states (on page 8) that "...the proposed project would have no effect on southern California steelhead or its designated critical habitat." In contrast, the draft EA concludes (on page 67) that "[t]he Proposed Action [sic] would improve habitat for steelhead..." and includes recommendations to prevent possible genetic introgression of wild steelhead and domestic trout until biologists from NMFS and the California Department of Fish and Game make a definitive determination regarding future trout-planting practices. Therefore, the draft EA does, in fact, provide information indicating the proposed action would have an effect on endangered steelhead, contrary to what is stated on page 8.

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[11] Note that the draft EA (e.g., on page 50, bottom) suggests the possibility that water deliveries might not correspond with natural runoff events.

Will the proposed action truly effect a water-release schedule that matches the natural flow regime?

For a variety of reasons, the draft EA inspires little confidence that water releases from Pyramid Dam would in fact be consistent with the natural pattern and magnitude of inflow to Pyramid Lake and to the middle reach of Piru Creek from the tributaries. The draft EA (e.g., page 50, bottom) suggests the possibility that water deliveries might not correspond with natural runoff events, which is not surprising because water releases are predicated on a variety of criteria (pages 4 and 5 of the draft EA), including some that may inhibit the release of water according to the natural pattern and magnitude of inflows. Additionally, other than a general statement regarding the Department of Water Resource's model for natural inflow into Pyramid Lake, the draft EA does not appear to provide a clear understanding of the mechanistic process the project proponent would undertake to determine when and how much water to release. The environmental document should fully disclose this process. To develop an expectation of the hydrograph that would result from the proposed action, the environmental document should include simulations (based on historical hydrology data) that predict with some known level of certainty the pattern and magnitude of water releases for a range of water-year types (i.e., wet, normal, dry).

Another reason why water releases may not match the natural pattern and magnitude of runoff events involves the flow-measurement devices that are intended for use under the proposed action. As stated in the draft EA, the flow-measurement devices lack the capability to provide real-time data (including the timing and magnitude of instantaneous discharges). Using real-time data is a preferred means of obtaining a timely and accurate understanding of inflow characteristics for the purpose of guiding the timing, frequency, magnitude, duration, and rate-of-change of water releases from Pyramid Dam. Water releases made in the absence of such information pose a risk of not matching the natural pattern and magnitude of inflows. Water releases that do not match the natural flow regime are expected to increase the potential that the anticipated ecological benefits of the proposed action to native water-dependent organisms would not be fully realized. Given the reported benefits of the natural flow regime (including innate characteristics such as the timing and magnitude of peak flows) to aquatic organisms[12], and the important fact that the intention of the proposed action is to benefit native species, all feasible modifications should be made to the proposed action (including installing flow-measurement devices that will provide real-time data) to ensure water releases would correspond with the natural pattern and magnitude of inflows, and not simply the 24-hour average discharge.

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[12] Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr. K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The natural flow regime: a paradigm for river conservation and restoration. Bioscience 47: 769-784.

Description of the environmental baseline

Generally, the descriptions of the affected environment reported in the draft EA for the various environmental variables are not appropriate for assessing impacts and effects of the proposed action and alternatives, and performing the assessment of cumulative effects. The descriptions of the affected environment in many instances ignores the fact that while anthropogenic activities (e.g., construction and operation of dams and surface-water diversions, land-use activities, conversion of wildlands, groundwater pumping) have caused impacts and effects that have contributed to the current characteristics and condition of the environmental baseline, the impacts and effects of such activities will extend into the future. For instance, Pyramid Dam will continue to block organisms from migrating upstream to historical spawning and rearing habitats, and represents an obstruction in a wildlife migration corridor. Therefore, when considering the impacts and effects of the proposed action and cumulative effects, the impacts and effects due to the proposed action (for example) must be added to the impacts and effects of anthropogenic activities that are expected to extend into the future, including effects of the dam(s).

EXHIBIT C

Population structure and ancestry of *O. mykiss* populations in South-Central California based on genetic analysis of microsatellite data

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Introduction

Steelhead (*Oncorhynchus mykiss*) populations in California south of Monterey Bay are divided into two Distinct Population Segments (DPS), formerly Evolutionarily Significant Units (ESUs). In the South Central California Coast (SCCC) DPS, which extends south from the Pajaro River in Monterey Bay to just north of the Santa Maria River in San Luis Obispo County, steelhead were listed as Threatened under the US Endangered Species Act (ESA) in 1997 (National Oceanic and Atmospheric Administration, 2004). At the same time, steelhead in the Southern California (SC) DPS were ESA listed as Endangered. At the time of ESA listing, this group included fish in coastal drainages from the Santa Maria river to Malibu Creek, but in 2002 it was extended to the Mexican border in San Diego County. A primary limiting factor for steelhead populations in southern California is access to freshwater habitat due to dams and water diversions, which are common in the region. Most of these barriers lack fish passage structures that prevent upstream migration. When fish from the species *O. mykiss* are currently found above such barriers they are considered to be resident rainbow trout, regardless of ancestry, and are not afforded protection under the ESA.

The recovery planning process for steelhead in these two southernmost DPSs is currently underway, yet several important questions regarding population structure of coastal trout in southern California remain. To provide insight into questions of population structure in this geographic area, we have performed genetic analysis of samples from 7 basins in the two DPSs using microsatellite DNA, highly variable genetic markers that can be used to trace ancestry and evaluate even small genetic distinction among populations. Microsatellites, also known as simple tandem repeat loci, have been used in numerous studies of salmonids and have proven to be a valuable tool for elucidating genetic structure (Carlsson & Nilsson, 2001; Castric et al. 2001; Spidle et al. 2001; Wenberg & Bentzen, 2001; Docker and Heath, 2003; Olsen et al. 2003; Deiner, 2004; Garza et al. 2004; Poissant et al. 2005; Crispo et al. 2006).

Previous genetic work on genetic structure of steelhead in this region has relied primarily on mitochondrial DNA (e.g. Berg and Gall 1988; Nielsen et al. 1997), which is a single gene that is often not reflective of population history or true relationships (Chan and Levin 2005) or small numbers of microsatellite loci and inadequate population sampling, which can also lead to inaccurate inference regarding population structure, particularly on a relatively small geographic scale. However, recent work on *O. mykiss* in northern California using a large number of microsatellite loci has demonstrated that genetic structure can be easily identified with such data both at larger scales (Aguilar and Garza, 2006; Garza et al. in review) and at relatively fine ones (Deiner, 2004; Deiner et al. in press; Pearse et al. in press). For example, *O. mykiss* populations in the Russian River separated by waterfalls were highly genetically distinct, whereas those found above and below the two major dams (Warm Springs and Coyote) were found to show low levels of genetic distinction (Deiner et al. in press). In the Klamath River, genetic relationships of trout populations above barriers with those below barriers do not vary with geographic distance, whereas genetic relationships between populations below barriers do (Pearse et al. in press), a pattern referred to as isolation by distance.

In this study, we employ a collection of microsatellite loci to examine the genetic structure of *O. mykiss* in the two southernmost DPSs in California, with a focus on relationships between populations above and below dams. We analyze samples collected in a systematic effort in 2003 from 5 watersheds: the Salinas, Arroyo Grande, Santa Ynez, Ventura and Santa Clara Rivers. We also analyze samples collected opportunistically and in small numbers from the southernmost extent of the range. These include samples from Malibu and Topanga Creeks in Los Angeles County, the Santa Ana and Arroyo Trabuco basins in Orange County, and San Mateo Creek and the Sweetwater River in San Diego County. We also analyze samples of the *O. mykiss* strains raised at Fillmore Hatchery on the Santa Clara River and used in stocking of trout in reservoirs throughout the southern part of the state. In some analyses, we use data from a previous study of *O. mykiss* in the northern part of the state (Garza et al. 2004) to provide a comparative phylogeographic framework.

We then use the results of the genetic analyses to address several aspects of the population structure of *O. mykiss* in this region that may be helpful in the management of this species. First, we evaluate recent ancestry of *O. mykiss* populations in streams above dams in multiple basins to determine if they appear to have been derived from a coastal steelhead lineage or from planted hatchery trout derived from out-of-basin broodstock. This analysis also evaluates whether there is evidence of strong Fillmore Hatchery influence in the current genetic composition of naturally spawned populations in these streams. Second, we evaluate whether population genetic structure in the region is consistent with the delineation of the two DPSs south of Monterey Bay. That is, do the sampled populations form distinct genetic lineages that reflect different demographic and evolutionary trajectories. Finally, we evaluate patterns of genetic differentiation and genetic diversity between sites to provide insight into the levels of recent gene flow and demographic history.

Methods

Sampling Sites

Juvenile *O. mykiss* samples from 20 sites in southern California representing five major drainages from Monterey Bay south to Ventura County were sampled non-lethally by biologists from the National Marine Fisheries Service Santa Cruz Laboratory and the University of California Santa Cruz using a backpack electrofisher and the protocol described in Garza et al. (in review) to stratify sampling within the stream and minimize collection of tissue from siblings (Table 1, Figure 1). Drainages were selected to provide spatial coverage across the current range of steelhead in southern California. Sampling specifically targeted watersheds with large impassible dams, which effectively stop upstream migration into the reservoir from populations downstream of the dam. The reservoirs created by dams in this region have been stocked with trout from the Fillmore Hatchery, located on the Santa Clara River in Ventura County. Since population genetic structure may be influenced by hatchery *O. mykiss* plantings, we also collected and analyzed samples from all distinct Fillmore Hatchery strains.

South-Central California

Two river systems were sampled in the SCCC Steelhead DPS (Figure 1). On the Salinas River, "above-barrier" samples were acquired from Nacimiento Creek, above Nacimiento Dam (1957), and San Antonio Creek, above San Antonio Dam (1965). Tassajara Creek in the Arroyo Seco River drainage and Tassajera Creek in the extreme upper Salinas drainage, were sampled as the below-barrier populations for comparison.

Fish were also sampled from three sites in the Arroyo Grande River drainage, two sites below Lopez Dam, constructed in 1954 by the Army Corp of Engineers for flood control, and one site above. The Lopez Canyon site is located upstream of the county recreation area, above the dam, and the Los Berros Creek and lower mainstem Arroyo Grande sites are below any major barriers to anadromous migration. However, successful passage through the lower river is highly dependent on the timing and magnitude of water releases from the dam and downstream diversions, as the lower section of the river is often dewatered for part of the year.

Southern California

Three drainages were sampled in the SC steelhead DPS, with collections occurring sites in the Santa Ynez, Ventura, and Santa Clara River drainages (Table 1, Figure 1). On the Santa Ynez River, Santa Cruz Creek flows into Lake Cachuma upstream of Bradbury Dam (constructed in 1953), and the North Fork Juncal Creek site lies further upstream above both Gibraltar and Juncal Dams. Hilton Creek is the last tributary below Bradbury Dam, which is the first barrier to upstream migration on the Santa Ynez River, and Salsipuedes Creek is a large tributary in the lower Santa Ynez River. Additional sites were sampled, however, the sample size collected at each site was too small to provide accurate population genetic inference. These sites include: Devil's Canyon (N=3), Indian Creek (N=2) and the mainstem Santa Ynez (N=12) between the Gibraltar and Bradbury Dams. They are analyzed only using model-based assignment clustering techniques described below.

Four sites were sampled on the Ventura River, with two "above-barrier" sites upstream of Matilija Dam (constructed in 1947) and two sites below the dam. The first site is on the mainstem of Matilija Creek just above the reservoir andthe second site is on the Upper North Fork of Matilija Creek. The two below barrier sites are on the North Fork Matilija Creek, which is distinct from the Upper North Fork and has its confluence just downstream of the Matilija Dam, and Bear Creek which is a tributary to the North Fork of Matilija Creek.

The Santa Clara River is the largest drainage in the SC DPS that is consistently occupied by steelhead and also the furthest south of the systematically sampled basins. Three locations were sampled in the Piru Creek drainage upstream of Santa Felicia Dam (constructed in 1954): Lockwood Creek, Piru Creek at Gold Hill, and Piru Creek at Frenchman's Flat. The first two sites are also above Pyramid Dam and its associated reservoir. The two below-barrier sites are Santa PaulaCreek and Lion Canyon in the Sespe Creek drainage. An additional site (Blue Point on Piru Creek, N=12) was sampled in this basin but contained too few samples for population analysis. It is analyzed only using model-based assignment clustering techniques described below.

There are numerous drainages further south than the Santa Clara River in the geographic range of the SC steelhead DPS, including some very large ones (e.g. Los Angeles, San Gabriel Rivers), and a number of smaller drainages in the Santa Monica and Santa Ana Mountains. However, most are very heavily impacted by anthropogenic activity and/or without ocean access for most of the year. Because of this, the Santa Clara River is the southernmost relatively large drainage with substantial anadromous fish habitat and population samples could not be collected from further south. However, tissues were obtained and analyzed from smaller numbers of individuals from several drainages in Los Angeles, Orange and San Diego counties collected over a number of years, including Malibu Creek (N=2), Topanga Creek (N=18), the San Gabriel River (N=1), the Santa Ana River (N=13), San Juan Creek (N=1), San Mateo Creek (N=1) and the Sweetwater River (N=7). However, one of the Topanga Creek fish was not used due to poor sample quality.

DNA Collection and Extraction

The non-lethally collected tissue samples consisted of small caudal fin clips (2-5mm^2) that were placed on blotter paper, inserted into coin envelopes and dried thoroughly in a dessicator. DNA was extracted using the Qiagen Dneasy Tissue Kit, following the manufacturer's recommended protocol for animal tissues and using a BioRobot 3000 (Qiagen Inc.). Approximately 2mm^2 of tissue was digested in 180μL of Qiagen buffer ATL and 20μL proteinase K and kept overnight in a shaking incubator at 55°C. The DNA was then bound to the Dneasy silica-gel membrane with the addition of 200μL Qiagen buffer AL and 200μL of ethanol, washed with 500μL each of Qiagen buffer AW1 and AW2, and finally eluted in 200μL buffer AE (Qiagen, 2000). Extracted DNA was kept frozen at 20°C until it was diluted (20:1 with autoclaved, distilled water) and distributed to 96 well plates for microsatellite amplification via polymerase chain reaction (PCR).

Genotyping

Individuals were successfully genotyped at 18 microsatellite loci in all population samples collected and were therefore used in the population genetic analyses (Table 2). PCR reactions were carried out in 15μL aliquots containing 4μL purified and diluted template DNA, 6.35μL H_2O, 1.5μL ABI 10X II PCR buffer, 0.9μL $MgCl_2$, 1.2μL dNTPs, 0.05μL ABI Amplitaq DNA polymerase, and 1μL fluorescent-labeled oligonucleotide primers (Integrated DNA Technologies, Inc.). Promega, Inc. reagents were used for Omy1011 reactions due to more consistent and reliable amplification. Multiple thermal cycler (MJ Research PTC 225) routines were employed to maximize PCR product. The typical profile consisted of a two minute pre-denaturation at 95 C, then two amplification stages: (a) 10 cycles of denaturation at 95 C for 15s, annealing at 53 C for 15s, and extension at 72C for 45s; (b) 25 cycles at 89 C for 15s, 55 C for 15s, and 72C for 45s. The routine concluded with a final extension phase of 72 C for 5 minutes and indefinite hold at 4C. PCR products were pooled to equalize peak heights and take advantage of multiple label colors and two non-overlapping ends of the measurable size range (50bp-500bp) within each lane. A mix of Formamide, loading dye and internal size standard was added to the pooled PCR product, denatured at 95 C for 3 minutes and immediately transferred to ice. The samples were electrophoresed with either an ABI Prism 377 DNA sequencer or an ABI 3100 genetic analyzer. Gel imaging, lane tracking and allele size for loci run with the ABI 377 were scored with GENESCAN version 3.1.2 and GENOTYPER version 2.1 software (Applied Biosystems). Loci analyzed with the ABI 3100 were analyzed with Gene Mapper version 4.0 software. At least two people performed all size scoring independently, discrepancies were identified and, if a resolution was not reached, the sample was rerun. If a discrepancy persisted through the second analysis, the fish was not scored at that locus. A representative fraction were re-genotyped as a control for data quality.

Data Analysis

Expected heterozygosity (Nei 1987), observed heterozygosity and number of alleles were calculated for each sample population. In order to compensate for variation in sample sizes, genetic diversity was also assessed using allelic richness as estimated with the rarefaction method in FSTAT version 2.9.3.2 (Goudet 2001). FSTAT was also used to calculate the population inbreeding coefficient (F_{IS}), with the probability of significance determined by 10,000 permutations. Deviations from Hardy-Weinberg equilibrium were examined utilizing the Markov Chain Monte Carlo (MCMC) approximation of an exact test (U test) implemented in the GENEPOP program version 3.4 (Raymond and Rousset 1995). The alternative hypotheses (H1) of heterozygote deficiency and heterozygote excess were both tested with Markov chain parameters of 10000 (dememorization), 1000 (batches) and 1000 (iterations per batch). Linkage (gametic phase) disequilibrium was also evaluated to ensure segregation independence of the 18 microsatellite loci in each of the sample populations and using the same type of MCMC approximation of an exact tests as implemented in GENEPOP. Markov chain parameters were the same as those used for the heterozygosity exact tests.

The mean ratio M, the number of alleles/(range in allele size + 1), was also calculated to test for recent reductions in effective population size and significance was evaluated with 10,000 simulated datasets from populations at equilibrium using the program M_P_Val (see Garza and Williamson 2001). During a population decline, alleles are lost and gaps appear in the allele frequency distributions. As a result, the number of alleles decreases more rapidly than the range in allele size and M decreases (Garza and Williamson 2001). When 18 variable loci are assayed and conservative assumptions about the mutation process are made, a value of $M < 0.71$ indicates that the population under study has experienced a recent reduction in effective population size.

Genetic differentiation between sample populations was examined with several methods. Using the test for genic differentiation in GENEPOP version 3.4, a Fisher's exact test was employed to calculate the probability of the null hypothesis (H_O) that allele frequencies were identical across populations (Raymond & Rousset 2001). Pairwise differentiation between all pairs of populations was also quantified using F_{ST}, as estimated by Weir and Cockerham's (1984) Θ estimator, and significance (> 0) assessed by the permutation algorithm in ARLEQUIN (Excoffier et al. 2005) with 10,000 replicates.

The distribution of molecular variation was assessed to identify informative groupings of sample populations using the Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) option in ARLEQUIN version 3.0. Molecular variance was partitioned into components of among groups (F_{CT}), among populations within groups (F_{ST}) and within sample populations (F_{SC}). Statistical significance of variance components was assessed with 10,000 permutations and p-values defined as the percent of random values greater than the observed value. All drainages with multiple above and below barrier populations were included in the analysis (N=4). Within-drainage comparisons were made between above and below barrier groups.

Individual-based assignment tests were used to further evaluate the degree of recent gene flow between sample populations of trout. Fish were assigned to their most likely population of origin, utilizing the Bayesian algorithm of Rannala and Mountain (1997) as implemented in GeneClass version 2.0.g (Piry 2004). Although application of assignment tests can be used to detect first generation migrants (Rannala and Mountain 1997), misassignment, or assignment of an individual to a population other than that of its sampled location, should not be interpreted as migration with juvenile fish, but as a signal of recent ancestry. Since such long-distance migration events are infrequent, patterns of misassigned fish highlight similarities in genetic composition (allele frequencies) between sample populations/locations. Misassignments may also occur randomly if an individual expresses a genotype composed of alleles that are common to many groups. This type of analysis was the only one possible with the smaller samples of fish from the southernmost basins described above, as well as the samples from sites in systematically sampled basins that were not of sufficient size for population level analyses. In these analyses, only the larger population samples and the Fillmore Hatchery strains were used as reference populations for potential assignment. The other type of analysis applied to all of the samples is the Bayesian model-based individual clustering method implemented in the program structure (Pritchard et al. 2000). In this analysis, a prior hypothesis about the number of genetic "populations" is used to partition the dataset into clusters and then fractionally assign the ancestry of each individual fish to each of the clusters without regard to geographic location of origin.

Phylogeographic trees were constructed using matrices of Cavalli-Sforza & Edwards' (1967) chord distance (D_{CE}), using the software package PHYLIP version 3.5c (Felsenstein 1993). The neighbor-joining algorithm was used to determine tree topology and a consensus tree was assembled from 1,000 bootstraps of the distance matrix with the PHYLIP CONSENSE component. Internal branch lengths on the consensus tree are scaled by the number of times that relationship was found in the neighbor-joining trees constructed with the bootstrap samples, which is a measure of confidence in that branch. These analyses were also carried out after combining the population samples described here with the 60 population samples analyzed by Garza et al. (in review), but only with the 15 loci where the data could be easily combined, due to differences in original data collection methods.

Results

The results described here generally only include the sites with adequate sample sizes for population genetic analyses (see above and Table 1). These results describe the population genetic structure found among watersheds as well as across barriers within basins. Combination of the new data with those from 60 additional population samples previously described (Garza et al. in review) provides broader geographic context for interpretation of genetic relationships. The phylogeographic results also bear upon the issue of introgression and/or hybridization of planted hatchery trout with native trout in the basins under study. In addition, the results provide a comparison of levels of genetic diversity among the sites sampled for this study.

Genetic Structure

The genetic structure of *O. mykiss* populations in the SCCC and SC DPSs is represented in an unrooted, neighbor-joining dendrogram with branch lengths scaled by chord distances (Figure 2a). The pattern of population clustering (topology) of the tree has several salient features. First, population samples from the Ventura, Santa Clara and Salinas Rivers, both those sampled above and below barriers, form monophyletic lineages on the tree, whereas the population samples from the Santa Ynez and Arroyo Grande Rivers are interspersed with one another and in a central position in the tree. The Fillmore Hatchery strains all clustered together, and are separated by a long internal branch from all of the naturally spawned population.

The bootstrap consensus tree (Figure 2b) had very similar topology to the chord distance/neighbor joining tree, clustering the populations from the Salinas, Ventura and Santa Clara Rivers, as well as the hatchery populations, with moderate bootstrap support for monophyletic lineages, and interspersion and sparse bootstrap support for monophyletic lineages of the Santa Ynez and Arroyo Grande populations. In addition, very high bootstrap support (>80%) was observed for clusters of populations within some tributaries of the Salinas, Ventura and Santa Clara drainages. For example, the two sample locations below Matilija Dam on the Ventura River always clustered together, as did the two samples above both Pyramid and Santa Felicia Dams. It is important to point out that, although these groups were most closely related in our study, the next most similar population samples were those on the other side of the dam. In addition, samples from the late and early components of the Mt. Whitney trout strain from Fillmore Hatchery always clustered together, as did samples from two consecutive year-classes of the Hot Creek strain. The lack of interspersion of the hatchery strains with the wild populations in the trees and their separation by long internal branches with high bootstrap support indicates a general lack of contribution of fish planted from Fillmore Hatchery to reproduction in trout population in streams above or below the dam reservoirs.

Evaluation of the phylogeographic trees constructed with the combined dataset, which has dense coverage of coastal steelhead populations all the way to the Oregon border, provided geographic context for the analysis of population samples in the SCCC and SC steelhead DPSs. The chord distance/neighbor joining tree and the bootstrap consensus tree are presented in Figure 3. Several features of the trees stand out. First, all of the southern steelhead population samples described here cluster with all of the other populations from south of San Francisco Bay. These populations are separated from all of those north of San Francisco Bay (inclusive) by a relatively long internal branch. Second, there is no strong signal of geographically-based reductions in gene flow in the southern populations above the level of the basin. That is, there are not internal branches that separate populations into groups that correspond to the three currently recognized DPSs in this region. This is consistent with the results of Garza et al. (in review), who found a similar lack of concordance with genetic structure and steelhead ESU/DPS boundaries in other parts of California. Another pattern evident in the combined phylogeographic trees that is concordant with the earlier work is the general lack of strict concordance between geographic and genetic population structure at small spatial scales, and the overlapping genetic distances of population samples from the same basin with those from geographically proximate basins.

Exact tests identified significant differences in allele frequencies between all pairs of sample sites, although only marginally so for two pairs. Similarly, pairwise F_{ST}, the proportion of genetic variation that separates the population samples, was significantly ($p<0.001$) different from zero for all but two comparisons following correction for multiple comparisons (Appendix 1), the Lockwood Creek and Piru Creek at Gold Hill sampling sites ($F_{ST}=0.004$, $p=0.002$), above both dams on the Piru Creek tributary of the Santa Clara River, and the 2002 and 2003 year classes of the Hot Creek Virginia strain

(F_{ST}=0.009, p=0.008) from the Fillmore Hatchery. Thus, sites from Lockwood Creek and Piru Creek at Gold Hill were combined for further analyses of genetic structure (e.g. AMOVA) among geographic sites. Overall, the mean value of F_{ST} was 0.124, indicating that approximately 12% of all of the genetic variation in the dataset was partitioned between population samples. Mean F_{ST} for within-basin comparisons was 0.086 while the mean value for between-basin comparisons was 0.108. A two-tailed t-test found the distribution of between-basin comparisons to be significantly higher ($p<0.001$) than the distribution of within-basin comparisons.

AMOVA analysis indicated that within-population variation was the dominant component of molecular variance for all population groupings evaluated in this study (Table 3a, b). The molecular variance was generally greater among populations within groups than between groups, indicating substantial differentiation between sample sites and a generally lack of elevated differentiation between pairs of populations above and below dams. Differences between basins accounted for 3.42% of the overall variation, when only below-barrier populations were considered and 2.5% when all populations were considered (Table 3a). When groupings that separated populations to the north and south of a particular geographic point were considered (Table 3, Groupings 4-7), the Ventura River break (separating the Ventura and rivers to the north from the Santa Clara River) yielded the largest genetic differentiation between groups of basins. Grouping the sites according to the current DPS designation (the Arroyo Grande break) yielded a level of genetic variation between groups that was lower than that for divisions between most other groups of basins in the study area. However, none of the geographic groupings of population samples from different basins yielded results that explained more than about

2% of the total genetic variation in the study, indicating that between-basin geographic and genetic population structure are not strictly concordant, a result consistent with that found in the analysis of phylogeographic trees.

Evaluating the structure of molecular variation within each drainage separately (Table 3, Groupings 8-12), differences between above and below barrier groups were not significantly different from zero for the Salinas, Arroyo Grande, and Santa Ynez Rivers. In contrast, differentiation between above-barrier and below-barrier sites in the Ventura and Santa Clara River basins were significantly different from zero. However, only the Ventura River showed a greater proportion of variance between groups than within groups, suggesting a larger difference between above and below barrier populations. Even so, the proportion of molecular variation partitioned above and below Matilija Dam is still only ~6% and this is partially due to the great similarity between the two above-barrier populations with each other and the two below-barrier populations with each other (Figure 2, Appendix 1).

Assignment tests readily distinguished individuals sampled from various river locations throughout southern California. Overall, fish were assigned to the location from which they were sampled with an accuracy of 93% and to the basin of origin with 98.9% accuracy (Table 4). Only 105 fish of 1505 were misassigned to a population location other than the one where they were sampled and, of those, only 16 were assigned to a location outside of their sample drainage. The largest number of reciprocal misassignments was between the two sites on upper Piru Creek above both dams, Gold Hill and Lockwood Creek. One-third of fish at these sites were misassigned which is only marginally better than the one half expected with random assignment. This is consistent with the non-significant differentiation (F_{ST}) value between them, indicating that they are not separate populations. When these samples are combined, the total assignment accuracy to population climbed to 96.5%. Only two fish from rivers assigned to the various hatchery strains, one each from the Salinas and Arroyo Grande (Table 4).

The individual assignment tests were also performed with the fish collected from locations where insufficient numbers were present for full population genetic analyses using the 5 basin population samples and the Fillmore Hatchery strains as potential populations of origin. The results of these assignment tests are presented in Table 5. A larger proportion of fish from these southern basins assigned to Fillmore Hatchery Strains then from basins further north (29.3% [12 of 41] in the south vs. 0.1% [2 of 1505] in the northern 5 basins). These values are not strictly comparable, because the population of origin was not available for assignment with the small samples. However, the second most likely population for assignment for the fish in the northern 5 basins included a Fillmore Hatchery stock as second choice less than 1% (11 of 1505) of the time. The one fish from San Juan Creek (Arroyo Trabuco) was assigned to hatchery stocks, as were almost half of the fish from Topanga Creek (8 of 17) and the Sweetwater River (3 of 7).

Genetic Diversity

Expected heterozygosity ranged from 0.582 to 0.707 with a mean value of 0.634 over the 20 sample populations evaluated (Table 1). After adjusting the mean number of alleles per population for the smallest sample size (N=23), mean allelic richness was 5.6 over all samples. Both the number of alleles observed (9.7) and allelic richness (6.7) was highest in the Lopez Canyon population from Arroyo Grande, whereas the Bear Creek population from the Ventura River had both the lowest observed number of alleles (4.9) and allelic richness (4.6). An analysis of the number of alleles present in the combined data set of northern and southern California population samples found a significant pattern of reduction in diversity in the southern populations (data not shown). The 60 populations surveyed in the two populations included 35 from south of the Golden Gate and 45 from further north in California. However, only 10 of the 35 lowest diversity values in the combined dataset were observed in populations from north of the Golden Gate. This is consistent with the pattern observed by Garza et al. (in review) of a strong correlation between latitude and allelic diversity. It is worth noting that the Fillmore Hatchery strains, when included in this analysis, all had allelic diversity values that were among the very lowest observed. Values for the M-Ratio were all significant, and ranged from 0.505 to 0.667 and averaged 0.582 over all sample populations, indicating widespread effective population size reductions.

Values of F_{IS} were found to be significantly greater than zero ($p<0.01$) in five of the sample populations, suggesting potential inbreeding, sampling of two distinct populations or null alleles (Table 1). However, statistical evaluation of heterozygote excess and deficiency yielded only 26 of 453 significant tests ($p<0.001$), which appeared randomly distributed across loci and populations. Similarly, tests for linkage disequilibrium revealed that over all populations, only 11 loci pairs (out of 153) showed significant disequilibrium ($p<0.001$). Overall, deviations from equilibrium were similar to that expected by chance alone and were not expected to impact the other analyses.

Discussion

Genetic analyses of microsatellite data presented here successfully address questions regarding population genetic structure in the South Central California Coast and Southern California steelhead DPSs. The data and analyses allow the evaluation of specific hypotheses regarding the impact of dams on the genetic structure of steelhead, the effects of large-scale stocking of rainbow trout in the reservoirs above these dams, and the concordance of genetic population structure with existing DPS boundaries.

Specifically, no substantial genetic differentiation was found between trout populations above and below dams in the 5 river basins studied, which indicates that populations of trout breeding in streams tributary to the dam reservoirs are recently derived from a common ancestral population with trout populations breeding below the dams. This suggests that breeding populations in these upstream tributaries are likely dominated by trout descended from steelhead isolated above the dams following their construction.

Phylogeographic and AMOVA analyses also failed to find a signal of reduced gene flow at any point on the coast, including between the Santa Ynez and Arroyo Grande Rivers, the location of the current boundary between the two steelhead DPSs in this region. This indicates that there is no significant genetic differentiation separating populations to the north or south of any point in the geographic region of study here and indicates that there is no genetic basis for delineation of these two distinct population segments. Several different types of analysis indicated that the primary level of structure for steelhead in the southern part of California is the local population, followed by the river basin, then the region. Evaluation of the results of phylogeographic analyses of the current dataset with that of Garza et al. (in review) indicates that there is no evidence for any geographically defined "breaks" in gene flow, that result in genetically distinct lineages including multiple river basins. To the extent that a DPS is intended to represent a unique genetic lineage within a species, the current delineations south of the Golden Gate are not consistent with population genetic structure.

Finally, several analyses indicate that trout populations breeding in streams above dam reservoirs and those breeding below dams have not been heavily introgressed by planted trout from Fillmore Hatchery or any other hatchery stock that is derived from out of basin broodstock. This does not mean that there has been no influence of hatchery plants on any of the populations surveyed here, or that their will not be in the future, but only that the vast majority of fish sampled for this study are not directly descended from hatchery raised fish or their recent progeny. However, use of individual-based assignment analyses to identify the recent ancestry of small numbers of fish from basins in Los Angeles, Orange and San Diego Counties found a substantial signal of hatchery ancestry, particularly in Topanga Creek.

Each of these areas of inference is described in more detail below.

Overall Genetic Structure

Analysis of population genetic structure found evidence forhierarchical structure similar to that found in steelhead populations further to the north (Garza et al. in review). The majority of genetic variation was at the level of individual local population with multiple analyses. Tests of genetic differentiation were significant for nearly every location sampled and the differentiated populations were represented by relatively long terminal branches on the phylogeographic trees. In the AMOVA analyses approximately 90% of the molecular variance was partitioned among individual populations in almost all analytical frameworks evaluated. These results are also consistent with the very high assignment accuracy (>95%) to differentiated populations and the almost perfect assignment accuracy to basin of origin. This last result indicates that these data are useful as a reference baseline for genetic stock identification techniques to determine basin and tributary of origin for individual trout in management or forensic applications. In contrast, the high genetic similarity of the Gold Hill and Lockwood populations from upper Piru Creek (Santa Clara River), which were also the most spatially proximate samples not separated by a dam, help delineate the lower geographic limit at which population structure might be observed.

Analysis of population structure at a higher spatial scale found variable results. Certain populations from within a basin always clustered together with high bootstrap support, reflecting high levels of recent gene flow. For example, the three locations from above dams on Piru Creek always formed a well-supported cluster, as do the two populations above Matilija Dam on the Ventura River. In addition, the two Ventura River populations from below the dam form a well supported cluster, as do the Salinas River populations from above San Antonio Dam and below it, but relatively far downstream, in Tassajara Creek in the Arroyo Seco drainage. All population samples, both above and below dams, from the Salinas, Ventura, and Santa Clara Rivers formed basin-specific lineages in some of the phylogeographic trees, although they were generally not supported by high bootstrap values. In contrast, the Santa Ynez and Arroyo Grande River population samples were interspersed and found basally in the trees, with populations separated by short internal branches. An alternative, Bayesian model-based clustering method that uses no prior information about geographic origin of the samples found that, with an hypothesis of three genetic groups present in the 20 population samples and the hatchery strains, the hatchery strains formed one group, the Santa Clara River populations formed another and all of the other population samples formed another (Figure 4). This result is consistent with the AMOVA, which found the highest proportion of variance partitioned between regions when the framework separated the Santa Clara River from all others. These results together suggest that the Santa Clara River trout populations are the most distinct of the 5 basins studied here. This may be a consequence of greater influence of hatchery introgression on these populations, as they consistently cluster with Fillmore Hatchery strains on the trees and the hatchery is located on the Santa Clara River.

The more general finding of lack of strict concordance of geographic and genetic clustering for populations from geographically proximate basins is consistent with the pattern found by Garza et al. (in review) for 60 populations of steelhead from the Oregon border to Morro Bay and is indicative of relatively high levels of gene flow (straying and subsequent reproduction) between basins separated by small coastline distances. It is also important to note that construction of such trees requires simultaneous estimation of many population relationships and it is expected that some of them will not be properly resolved with only 18 loci and closely related populations, so particular emphases in interpretation of the results should not focus on any particular population relationship, as estimated from either the trees or F_{ST} values.

Evaluation of Distinction of SCCC and SC DPSs

The current analyses do not provide evidence for a significant genetic distinction between steelhead in the two southern California DPSs. In the AMOVA, the proportion of molecular genetic variance partitioned between populations in the two DPSs was only 1.61% of the total variation and was only marginally significantly different from zero (Table 3, Grouping 5). The grouping that separated the Santa Clara drainages from all others had the highest proportion of genetic variation partitioned of any of the possible groupings of drainages to the north and south of any geographic point (Table 3), but it still explained a very small proportion of the total molecular variation. The phylogeographic trees also failed to yield branches that separated populations from the two DPSs into distinct genetic lineages. These analyses demonstrate that there are not substantial differences in the evolutionary histories of populations in the SCCC and the SC DPSs.Such methods are, indeed, sufficiently powerful to detect structure above the level of a river basin that is reflective of distinct evolutionarily history, similar to that assumed for an ESU, in steelhead in northern California with the approaches used (Garza et al. in review). Nevertheless, further analyses with population samples from additional year-classes might be helpful in confirming this result.

Evaluation of Distinction Between Above and Below Barrier Sites

Examination of the phylogeographic trees indicates that trout above and below dams in the same basin are generally closely related and in many cases the most genetically similar populations in our study. However, the magnitude of differentiation between above and below barrier populations was variable in the five basins examined

(Table 3, Groups 8-12). While all pairs of population samples were significantly differentiated, the AMOVA results found that a non-significant proportion of the genetic variation was due to differences between above- and below-dam populations in the Salinas, Arroyo Grande, and Santa Ynez basins. This indicates recent common ancestry for these populations and/or contemporary gene flow (through downstream migration or transplantation in either direction) across the dams. The genetic similarity of these populations indicates that there has not been substantial divergence of trout populations breeding in streams above dam reservoirs since they were isolated by construction of the dams decades ago.

In the Santa Clara and Ventura drainages, the proportion of genetic variation explained by the presence of dams was significantly different than zero and average F_{ST}= 0.109 for sites above and below the dams in the Santa Clara drainage (when the Lockwood and Gold Hill samples are combined) and 0.100 for the Ventura drainage (Appendix 1). Although differentiation within groups still explained a greater percentage of the overall variation, pairwise F_{ST} values were generally lower between above-barrier sites (average F_{ST}= 0.069) and below-barrier sites (average F_{ST}= 0.059) then for comparisons of an above-barrier and a below-barrier site (see above).

For comparison, Deiner et al. (in press), using the same microsatellites, found comparisons of trout populations above Coyote and Warm Springs dams in the Russian River with populations below to be very similar (average F_{ST}= 0.057) to both that observed between populations below barriers in the 5 basins studied here and to differentiation observed between eight sites below barriers in the Russian River. In contrast, they also found differentiation between *O. mykiss* populations above and below natural barriers (e.g. waterfalls) to be considerably higher (average $F_{ST} = 0.158$) than those found across the dams in the Santa Clara and Ventura drainages (average $F_{ST} = 0.105$). However, pairwise F_{ST} is highly correlated with genetic diversity and, therefore, population size in California steelhead (e.g. Garza et al. in review). Many of the population sizes in areas above waterfalls in the Russian River basin are highly constrained by available habitat area, whereas population sizes above dams in the Ventura and Santa Clara Rivers appear to be less constrained, based on genetic diversity, so it is hard to conclude anything from this comparison.

Impact of Stocking of Study Basins with Trout from Fillmore Hatchery

The results of this study indicate that trout raised at Fillmore Hatchery and planted extensively in dam reservoirs in the study basins have not made a substantial contribution to reproduction in the populations of *O. mykiss* studied here. There is no evidence of widespread admixture or introgression of hatchery trout into breeding populations of naturally spawning trout either above or below the dams. Individual-based assignment tests identified only two fish sampled from wild populations as belonging to hatchery lineages and tests for population or genic differentiation were highly significant in all comparisons of hatchery and wild population/strain samples. In addition, phylogeographic tree analysis and model-based clustering (Figure 2, 3 and 4) clearly identified the Fillmore hatchery strains as highly divergent from the wild *O. mykiss* populations sampled. It is worth noting that this does not mean that there has been no introgression of hatchery fish into populations of native trout in these basins. Small numbers of hatchery fish may achieve reproductive success in some local populations and/or in some years, including those studied here. Moreover, if hatchery strains much more genetically similar to the native populations in this area were raised in a hatchery and released in the study area at some point in the past, then it is possible that some of these populations have hatchery ancestry. For example, it is known that steelhead from various other rivers tributary to Monterey Bay have been raised at the Kingfisher Flat (Big Creek) Hatchery on Scott Creek in Santa Cruz County and released in the Arroyo Seco (including Tassajara Creek) drainage of the Salinas River (Dave Strieg, Monterey Bay Salmon and Trout Project, personal communication). However, they do not maintain a hatchery strain and it seems unlikely that there has been substantial activity of this nature further south, as it is clear that there is little genetic influence of the hatchery strains that are currently commonly raised in California.

A previous study of trout in the Santa Ynez drainage suggested that there was significant introgression of native fish with hatchery fish in the upper basin (Greenwald and Campton 2005). However, this is likely an artifact of the weak power associated with using a single mitochondrial locus. Overall, fish sampled from these sites in southern California appear to share little ancestry with the hatchery strains included in this study. This may be a consequence of simple differences in timing of reproductive maturity or behavior of the two types of fish, which may in turn be a result of either domestication selection or ancestral differences in these traits.

Analyses of the small numbers of fish collected south of the Santa Clara River did, however, reveal a substantial signal of hatchery ancestry. The Topanga Creek fish sampled were a mixture of fish with either predominately hatchery or native steelhead genotypes, as well as some fish that appear intermediate (Figure 4b). Likewise, the fish from the Sweetwater River appear to be primarily of hatchery origin, although individual assignments (Table 5) do suggest that there is some native steelhead ancestry. Finally, the fish from San Juan Creek (Arroyo Trabuco) is of clear hatchery ancestry, whereas those from Malibu, San Gabriel and San Mateo Creeks are clearly not (Figure 4b, Table 5).

The contrasting results for the Sweetwater River fish with the two assignment methods are most likely a consequence of the way that the two analyses are conducted. In the probabilistic method (Table 5), each of the individual population samples is a potential source and the allele frequencies in each are estimated from only those individuals in the population, whereas in the other, semi-Bayesian method (Figure 4) the data are partitioned so as to assign ancestry of each individual fish to one of the three primary clusters, which are constructed without geographic information and characterized by the allele frequencies from all of the constituent fish. In this latter analysis, there are only three choices, the cluster comprising only the Fillmore strains, the one dominated by the Santa Clara River and the one that includes most individuals in all of the other rivers surveyed. When there are only these three choices, then the most similar cluster for all of the Sweetwater fish is the one dominated by Fillmore Hatchery strains. When all of the individual population samples are potential sources for these fish, then four of the Sweetwater fish appear more similar to a coastal steelhead population. However, both analyses force each fish to assign to one of the provided source populations, even when the actual source is not present. It is possible that reason for this discrepancy between the two methods in the primary affinity for four of the seven Sweetwater River fish is because the actual source, or a genetically similar proxy population, is not available for assignment. This unrepresented source population would almost definitely have to be from an isolated rainbow trout lineage, as the coastal steelhead lineages used in the analyses encompass the full range of genetic variation likely to be present in any steelhead-derived fish in Southern California. This hypothesis is supported by the semi-Bayesian assignment analysis that uses as a potential source population all of the hatchery rainbow trout strains, thereby encompassing a broader sample of the genetic variation present in California rainbow trout, and finds all of the Sweetwater fish to be more similar to the hatchery rainbow trout than either of the two Southern California steelhead lineages. Analysis of more samples from California rainbow trout populations, as well as evaluation of additional genetic haplotype markers currently under development by the authors, should provide greater resolution of this issue in the future.

Genetic Diversity

Genetic diversity was variable between sample sites, with heterozygosity varying by as much as 20% between the Ventura-Bear and Arroyo Grande- Lopez Canyon sites, and allelic richness by almost 50% between the same sites. The more variable sites had levels of genetic diversity similar to those found in steelhead populations in the northern part of coastal California (Garza et al. in review, Deiner et al. in press). However, the majority of the population samples examined here have levels of diversity that are among the lowest observed in California steelhead populations, falling in the lower part of the distribution of allelic diversity for these 18 microsatellite loci in the 60 population samples from the two studies. Similarly, estimates of the M ratio, which uses a comparison of two measures of genetic diversity that decline at different rates following a reduction in population size, suggest widespread, recent decreases in effective population size, and consequent loss of genetic diversity in the populations examined here, although it is not clear of what magnitude. It is also worth noting that the hatchery stocks have among the lowest levels of genetic variation observed in this study or that of Garza et al. (in review), so the prospect of inbreeding, and consequent inbreeding depression, in these hatchery strains and any populations established from them are of concern. Moreover, although the populations studied here appear to have experienced little introgression with these hatchery strains, changes in environmental conditions or stocking practices in the future could result in such admixture and the consequent reduction in effective population size that would occur (Ryman and Laikre 1991) would be of concern and possibly complicate efforts to retain and recover viable populations.

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Figure 1: Map showing the approximate locations of the samples systematically collected for this study. The 5 basins sampled are highlighted in color.

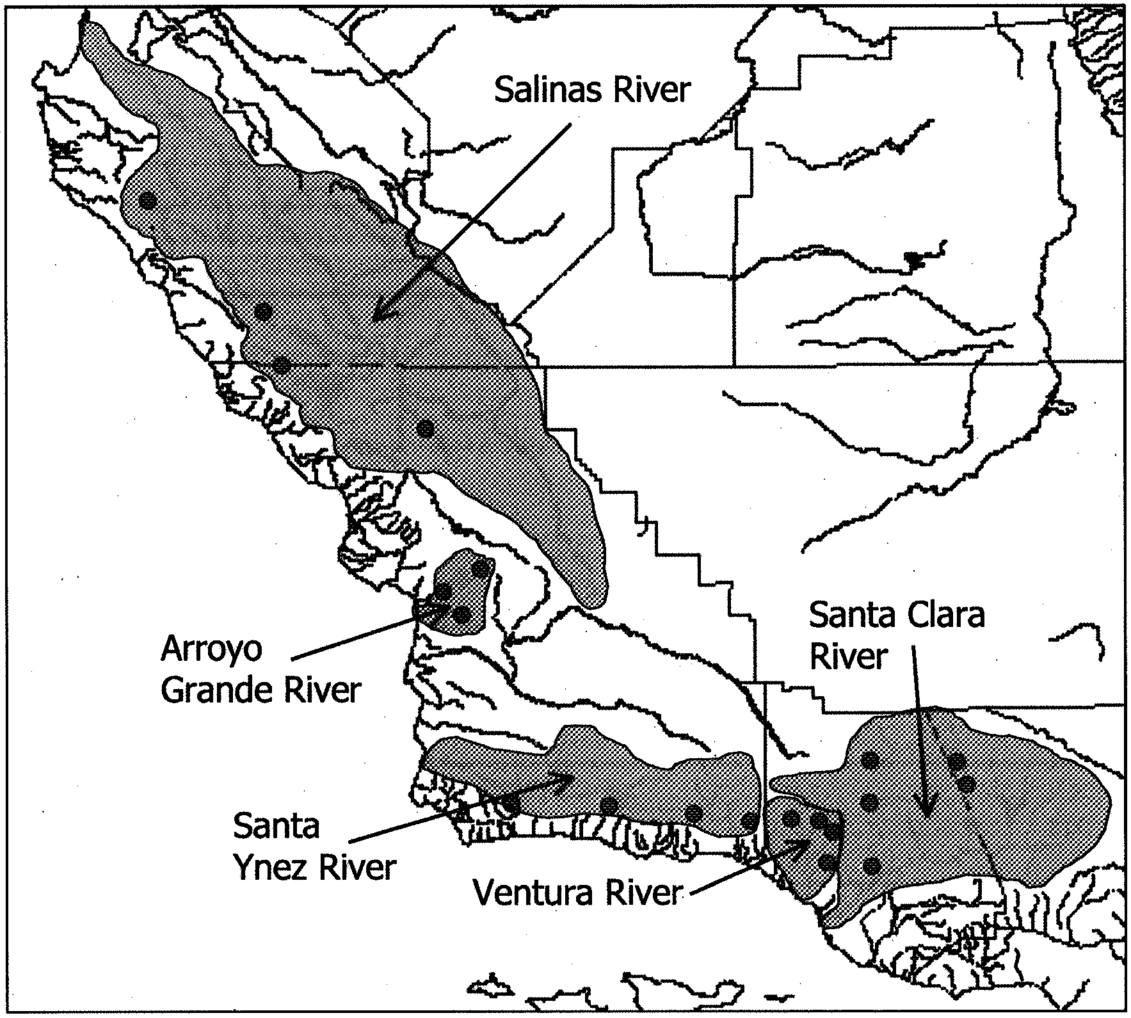

Figure 2. a) A neighbor-joining tree depicting relationships between populations samples constructed using chord genetic (Cavalli-Sforza and Edwards) distances. Branch lengths are proportional to genetic distance.

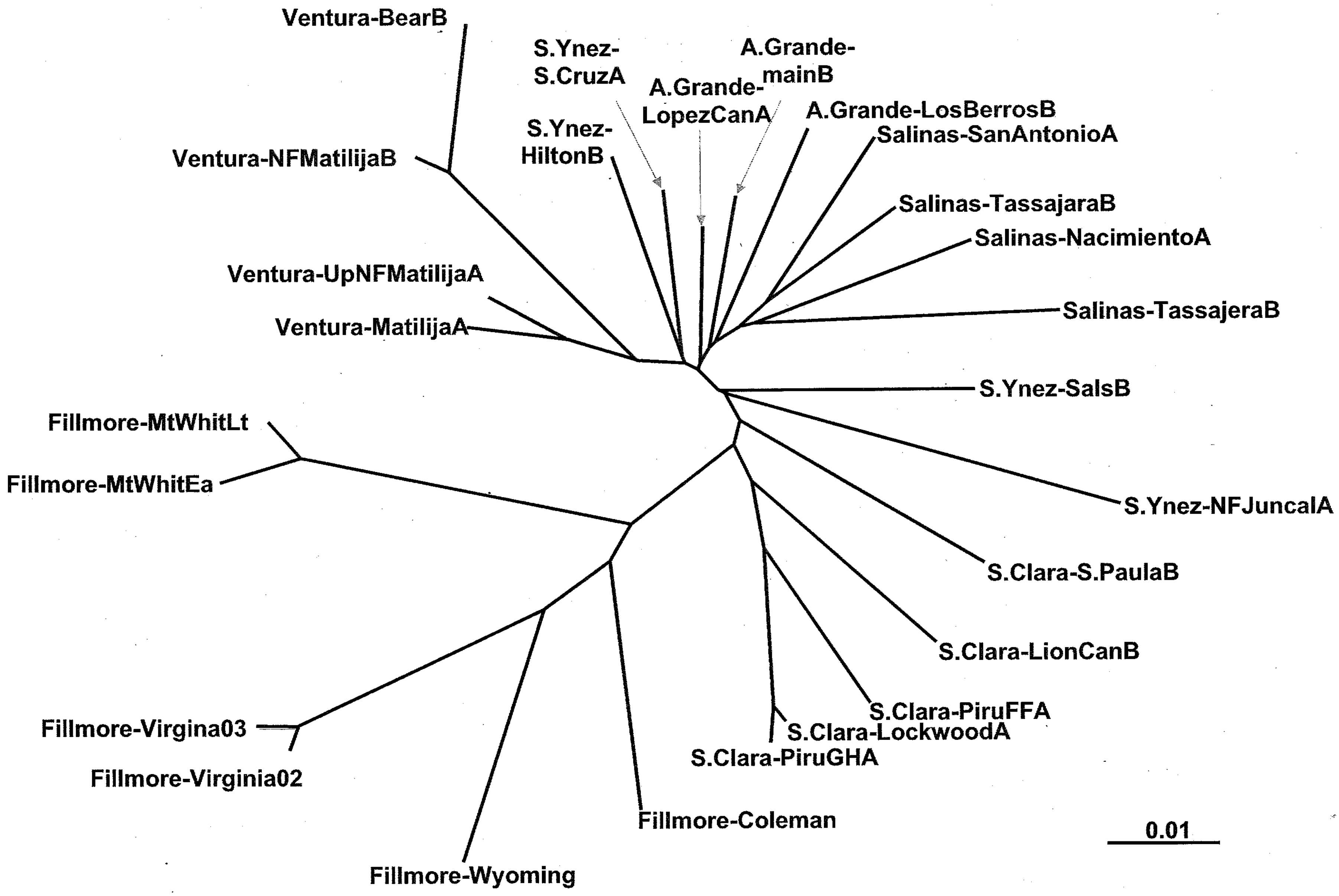

Figure 2. b) Majority rule consensus of neighbor-joining trees constructed with chord distances calculated from 1000 bootstrap datasets. Numbers on internal branches are the percentage of bootstrap replicates in which that grouping was found.

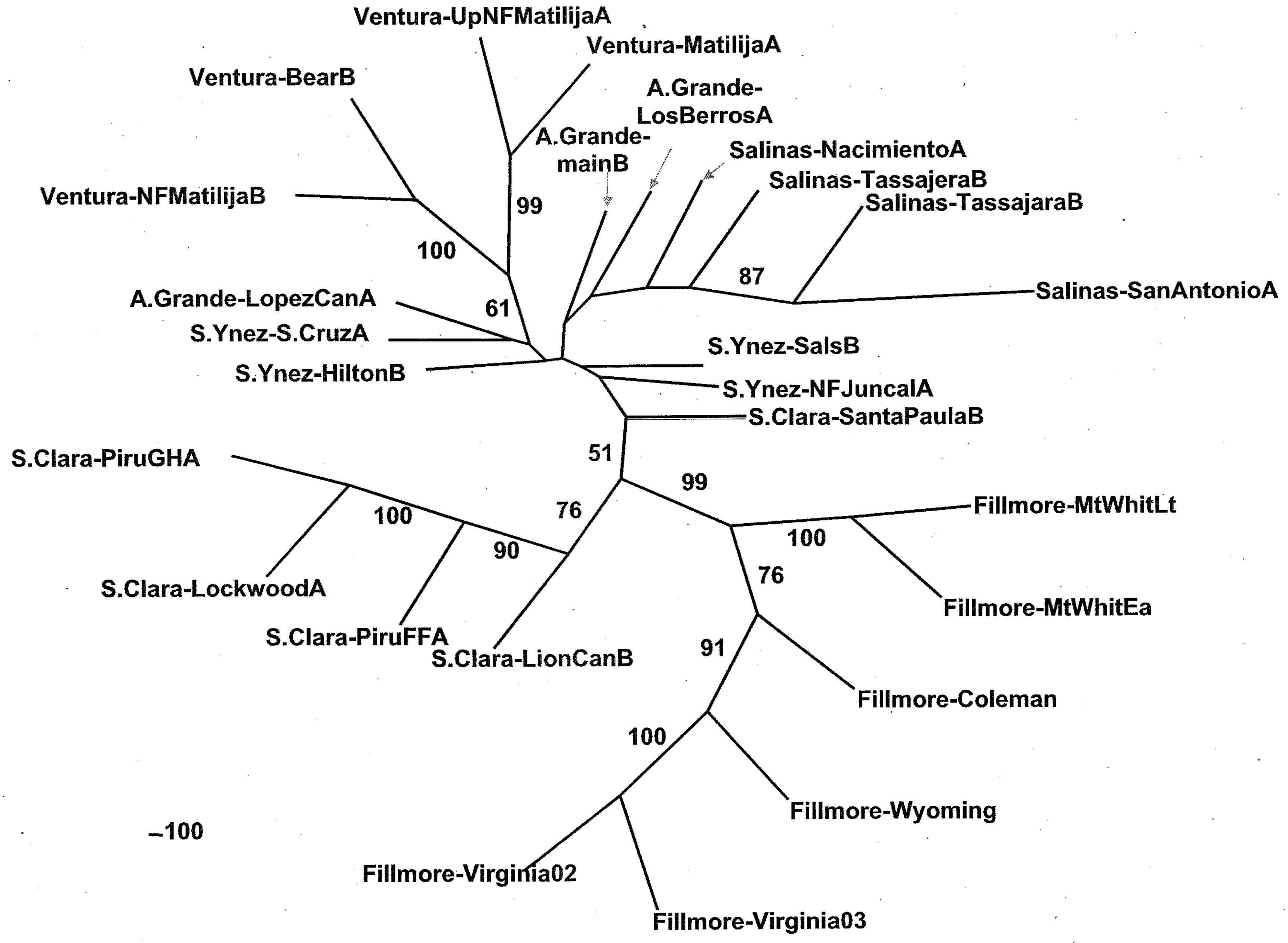

Figure 3. a) Neighbor-joining tree constructed with chord distances calculated from combined northern and southern California steelhead population data from 15 microsatellite loci. Branch lengths are proportional to genetic distance.

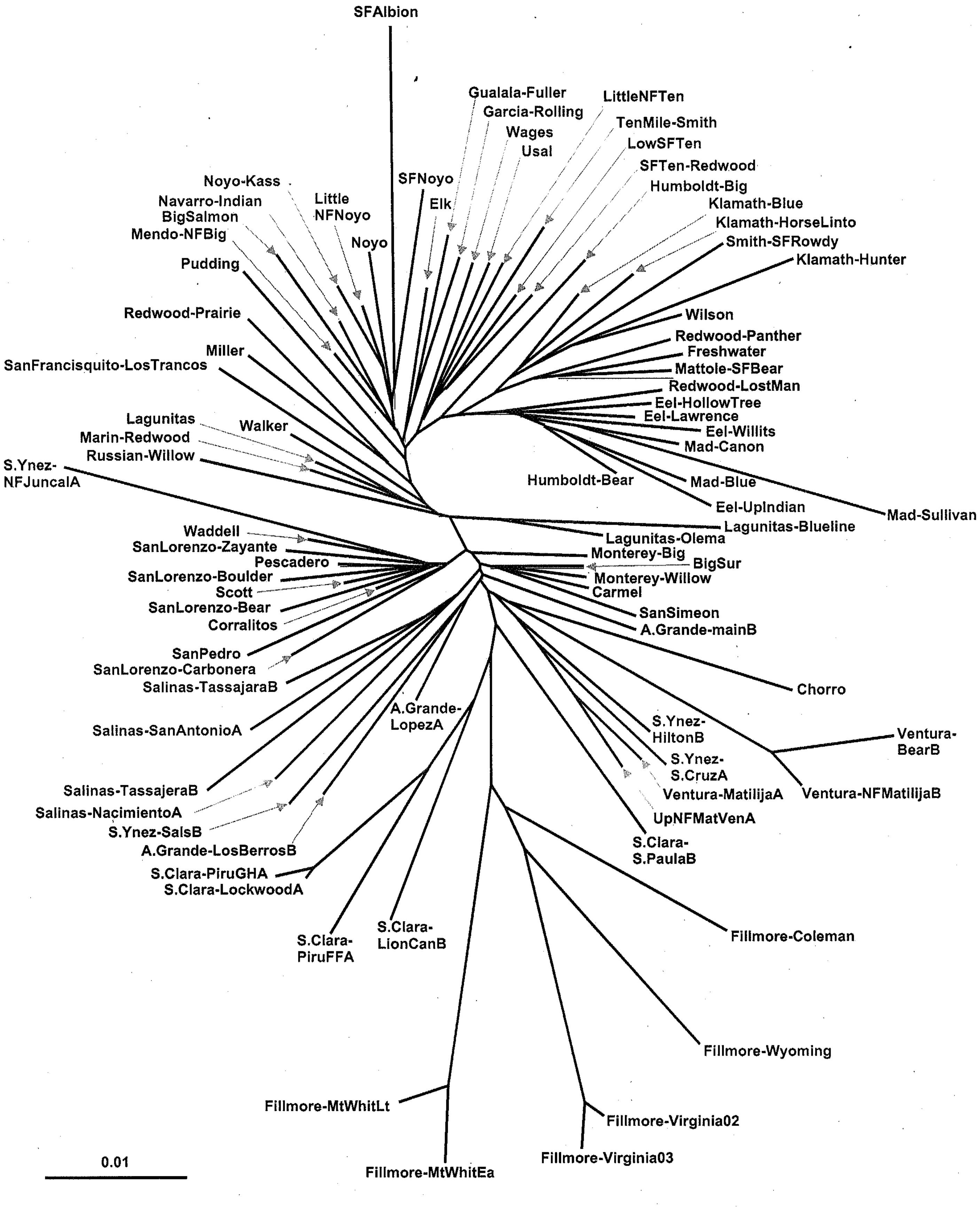

Figure 3 (cont.). b) Majority rule consensus of neighbor-joining trees constructed with chord distances from 100 bootstrap samples of the combined northern and southern California steelhead populations dataset of 15 microsatellite loci. Numbers on internal branches are the percentage of bootstrap replicates in which grouping was found.

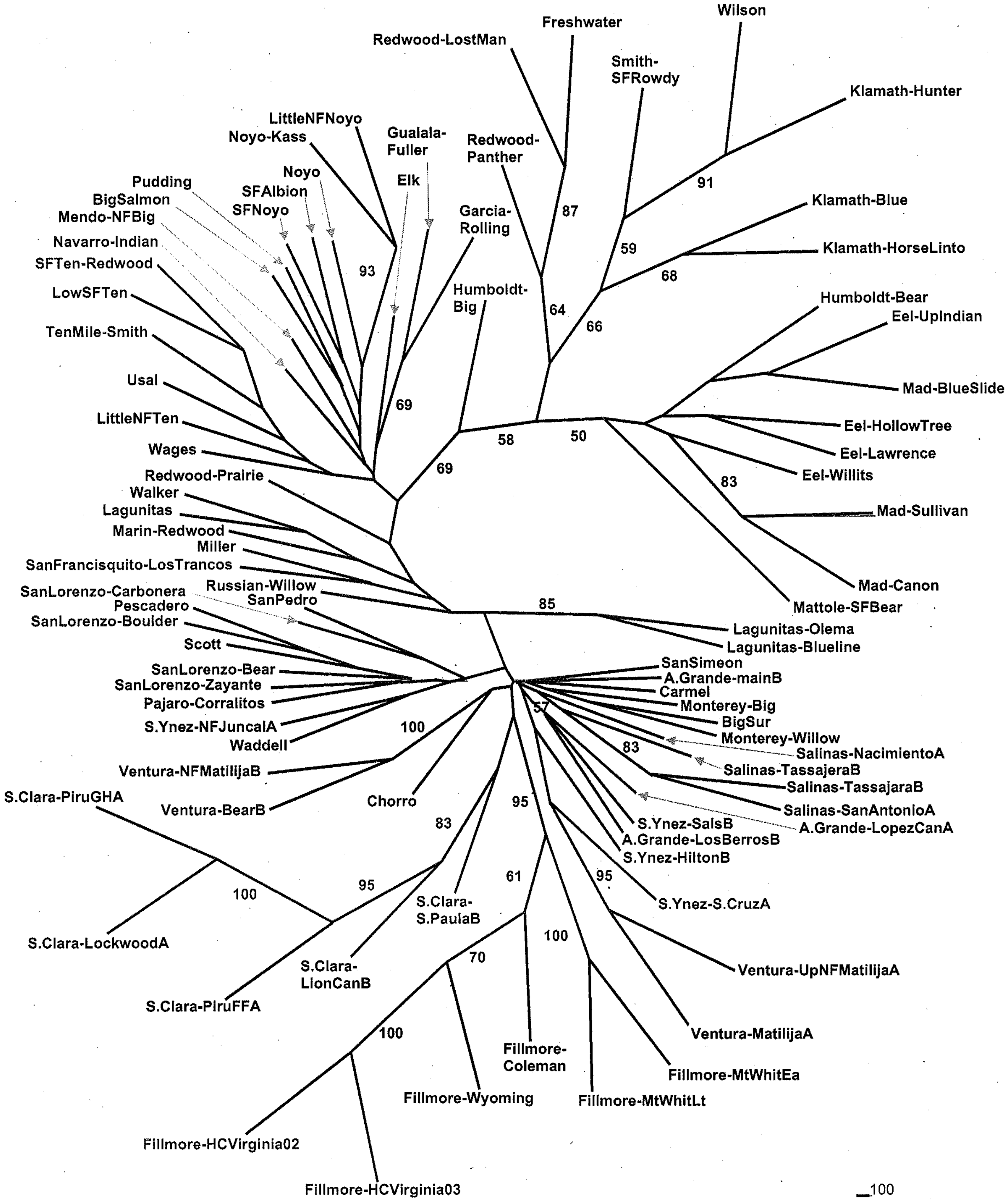

Figure 4. a) Summary of results for individual ancestry assignment using the model-based clustering method implemented in the software package *structure*. Green, blue and red represent the fractional ancestry of each fish as it is assigned to each of the 3 clusters. Groups 1-4, Salinas River; 5-8, Arroyo Grande; 9-15 Santa Ynez; 16-21, Ventura; 22-27, Santa Clara; 28-33, Fillmore Hatchery; 34-South of Santa Clara.

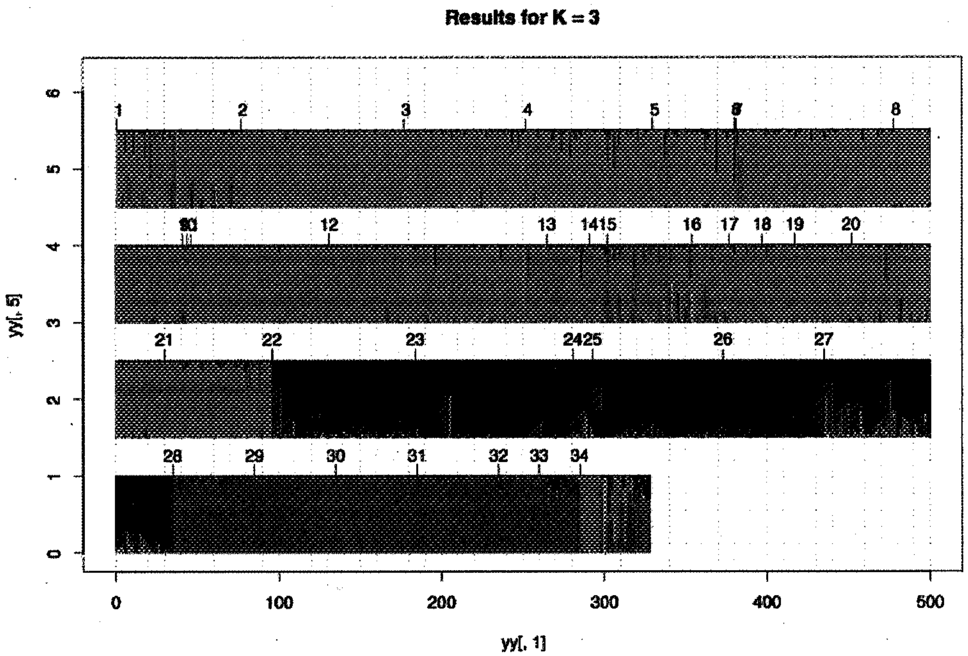

Figure 4. b) Expanded view of ancestry results for fish south of Santa Clara River.

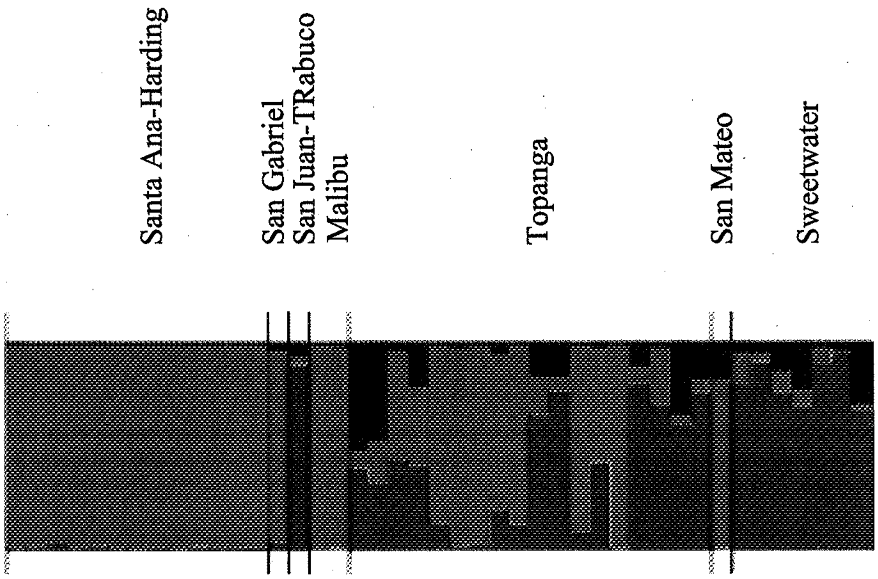

Table 1: Descriptive statistics of 20 populations of O. mykiss from 5 basins and 6 samples from Fillmore Hatchery strains. N=Sample size, He=Expected heterozygosity, Ho=Observed heterozygosity, A=Observed no. of alleles, R=Allelic richness

Drainage	**Population**	**Barrier**	**N**	**He**	**Ho**	**A**	**R**	**Fis**	**M-Ratio**
Salinas	Nacimiento	A	76	0.649	0.660	8.5	6.1	-0.018	0.558
	SanAntonio	A	100	0.637	0.596	7.9	5.8	**0.065**	0.548
	Tassajara	B	75	0.649	0.638	8.8	6.0	0.017	0.611
	Tassajera	B	78	0.632	0.610	7.3	5.6	0.035	0.565
Arroyo Grande	ArrGrande	B	51	0.673	0.662	8.5	6.6	0.017	0.599
	LopezCan	A	97	0.704	0.728	9.7	6.7	-0.034	0.653
	LosBerros	B	63	0.667	0.671	7.2	5.9	-0.006	0.585
Santa Ynez	Hilton	B	52	0.626	0.589	7.3	5.7	**0.060**	0.528
	NFJuncal	A	85	0.585	0.611	6.2	4.8	-0.045	0.548
	Salsipuedes	B	134	0.615	0.588	7.6	5.3	**0.044**	0.505
	SantaCruz	A	26	0.652	0.660	6.6	5.9	-0.012	0.533
Ventura	Bear	B	23	0.617	0.599	4.9	4.6	0.030	0.513
	Matilija	A	75	0.642	0.606	7.6	5.6	**0.057**	0.592
	NFMatilija	B	78	0.631	0.623	6.2	5.0	0.013	0.605
	UpNFMatilija	A	66	0.651	0.667	7.6	5.8	-0.024	0.591
Santa Clara	LionCan	B	88	0.600	0.606	8.3	6.0	-0.010	0.667
	Lockwood	A	97	0.586	0.592	7.1	5.3	-0.009	0.552
	PiruFF	A	80	0.606	0.616	6.8	5.1	-0.018	0.578
	PiruGH	A	62	0.582	0.579	6.9	5.3	0.006	0.612
	SantaPaula	B	100	0.707	0.691	8.2	6.4	0.023	0.544
Fillmore Hatchery	Coleman	n/a	50	0.637	0.643	7.3	5.8	-0.010	0.629
	HCVirginiaA	n/a	50	0.647	0.612	6.5	5.2	**0.054**	0.631
	HCVirginiaB	n/a	50	0.655	0.651	5.5	4.9	0.006	0.624
	HCWyoming	n/a	50	0.666	0.673	6.7	5.5	-0.011	0.570
	MtWhitEa	n/a	25	0.597	0.629	6.1	5.3	-0.055	0.614
	MtWhitLt	n/a	25	0.601	0.613	5.8	5.1	-0.020	0.617
		Mean	66	0.634	0.634	7.1	5.5	0.001	0.582
		SD	29	0.033	0.038	1.2	0.6	0.042	0.042
		Var	812.62	0.001	0.001	1.6	0.4	0.002	0.002

A= above barrier, B = below barrier

Table 2. The microsatellite loci studied with the species in which they were originally described and the original reference.

Locus	Species	Reference
Omy 27	*O. mykiss*	McConnell et al. 1995
Omy 77	*O. mykiss*	Morris et al. 1996
Omy 1011	*O. mykiss*	Morris et al. 1996
One 11b	*O. nerka*	Scribner et al. 1996
One 13b	*O. nerka*	Scribner et al. 1996
Ots 1b	*O. tshawytscha*	Banks et al. 1999
Ots G3	*O. tshawytscha*	Williamson et al. 2001
Ots G43	*O. tshawytscha*	Williamson et al. 2001
Ots G85	*O. tshawytscha*	Williamson et al. 2001
Ots 103	*O. tshawytscha*	Small et al. 1998
Ots G243	*O. tshawytscha*	Williamson et al. 2001
Ots 249b	*O. tshawytscha*	Williamson et al. 2001
Ots 253b	*O. tshawytscha*	Williamson et al. 2001
Ots 401	*O. tshawytscha*	Williamson et al. 2001
Ots 409	*O. tshawytscha*	Williamson et al. 2001
Oki 23	*O. kisutch*	Smith et al. 1998
Ssa 85	*Salmo salar*	O'Reilly et al. 1996
Ssa 289	*Salmo salar*	McConnell et al. 1995

Table 3. (a Analysis of molecular variance results with various hierarchical groupings of sites above and below dams. Details of groupings are shown in Table 3b. Groupings 8-12 consist of pooled above-barrier sites vs. pooled below-barrier sites for each drainage.

			Among Groups			**Among Populations within Groups**			**Within populations**		
Grouping	**Description**	**Nb**	**Var**	**%**	**F_{CT}**	**Var**	**%**	**F_{ST}**	**Var**	**%**	**F_{SC}**
1	Interdrainage - below only	5	0.217	3.42	0.034	0.474	7.46	0.077	5.663	89.11	0.109
2	Interdrainage - all pops	5	0.158	2.50	0.025	0.535	8.46	0.087	5.635	89.04	0.110
3	All above - all below	2	0.019	0.31	0.003*	0.660	10.45	0.105	5.630	89.24	0.108
4	Salinas River Break	2	0.098	1.53	0.015*	0.632	9.88	0.100	5.663	88.58	0.114
5	Arroyo Grande break	2	0.103	1.61	0.016	0.615	9.63	0.098	5.663	88.76	0.112
6	Santa Ynez break	2	0.106	1.66	0.017	0.611	9.58	0.097	5.663	88.76	0.112
7	Ventura River break	2	0.136	2.13	0.021	0.610	9.51	0.097	5.663	88.36	0.116
8	Salinas -above, below	2	-0.043	-0.70	-0.007*	0.498	8.06	0.080	5.725	92.64	0.074
9	Arroyo Grande-above, below	2	0.030	0.46	0.005*	0.309	4.80	0.048	6.090	94.74	0.053
10	Santa Ynez - above, below	2	0.032	0.52	0.005*	0.712	11.76	0.118	5.310	87.72	0.123
11	Ventura - above, below	2	0.389	6.16	0.062	0.206	3.26	0.035	5.719	90.57	0.094
12	Santa Clara - above, below	2	0.258	4.16	0.042	0.419	6.75	0.075	5.524	89.08	0.109

* non-significant genetic differences among groupings

Table 3. (b The groups of population samples included in each of the AMOVA hierarchical partitions in Table 3a. Number next to each site indicates which group in Table 3a. Number of groups for each test is also indicated.

1. Interdrainage - below only	2. Interdrainage - all pops	3. All above - all below	4. Salinas/Arroyo Grande break	5. Arroyo/Santa Ynez break	6. Santa Ynez/ Ventura break	7. Ventura/ S.Clara break
# Groups = 5	# Groups = 5	# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2
1 SLTassajaraB	1 SLTassajaraB	1 SLNacimientoA	1 SLTassajaraB	1 SLTassajaraB	1 SLTassajaraB	1 SLTassajaraB
1 SLTassajeraB	1 SLTassajeraB	1 SLSanAntonioA	1 SLTassajeraB	1 SLTassajeraB	1 SLTassajeraB	1 SLTassajeraB
2 AGArrGrandeB	1 SLNacimientoA	1 AGLopezCanA	2 AGArrGrandeB	1 AGArrGrandeB	1 AGArrGrandeB	1 AGArrGrandeB
2 AGLosBerrosB	1 SLSanAntonioA	1 SYNFJuncalA	2 AGLosBerrosB	1 AGLosBerrosB	1 AGLosBerrosB	1 AGLosBerrosB
3 SYHiltonB	2 AGArrGrandeB	1 SYSantaCruzA	2 SYHiltonB	2 SYHiltonB	1 SYHiltonB	1 SYHiltonB
3 SYSalsB	2 AGLosBerrosB	1 VTMatilijaA	2 SYSalsB	2 SYSalsB	1 SYSalsB	1 SYSalsB
4 VTNFMatilijaB	2 AGLopezCanA	1 VTUNFMatilijaA	2 VTNFMatilijaB	2 VTNFMatilijaB	2 VTNFMatilijaB	1 VTNFMatilijaB
4 VTBearB	3 SYHiltonB	1 SCLockwoodA	2 VTBearB	2 VTBearB	2 VTBearB	1 VTBearB
5 SCLionCanB	3 SYSalsB	1 SCPiruFFA	2 SCLionCanB	2 SCLionCanB	2 SCLionCanB	2 SCLionCanB
5 SCSantaPaulaB	3 SYNFJuncalA	1 SCPiruGHA	2 SCSantaPaulaB	2 SCSantaPaulaB	2 SCSantaPaulaB	2 SCSantaPaulaB
	3 SYSantaCruzA	2 AGArrGrandeB				
	4 VTBearB	2 SLTassajaraB				
	4 VTNFMatilijaB	2 SLTassajeraB				
	4 VTMatilijaA	2 AGLosBerrosB				
	4 VTUpNFMatilijaA	2 SYHilton				
	5 SCLionCanB	2 SYSals				
	5 SCSantaPaulaB	2 VTNFMatilijaB				
	5 SCPiruLockA	2 VTBearB				
	5 SCPiruFFA	2 SCLionCanB				
	5 SCPiruGHA	2 SCSantaPaulaB				

8. Salinas - above, below	9. Arroyo Grande - above, below	10. Santa Ynez - above, below	11. Ventura - above, below	12. Santa Clara - above, below
# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2
1 SLTassajaraB	1 AGArrGrandeB	1 SYHiltonB	1 VTNFMatilijaB	1 SCLionCanB
1 SLTassajeraB	1 AGLosBerrosB	1 SYSalsB	1 VTBearB	1 SCSantaPaulaB
2 SLNacimientoA	2 AGLopezCanA	2 SYNFJuncalA	2 VTMatilijaA	2 SCPiruLockA
2 SLSanAntonioA		2 SYSantaCruzA	2 VTUpNFMatilijaA	2 SCPiruFFA

Table 4. The results of assignment tests are presented with misassignments off of the diagonal. Rows represent distribution of assignments for all fish collected at that site.

	SLNacimientoA	SLSanAntonioA	SLTassajaraB	SLTassajeraB	AGArrGrandeB	AGLopezCanA	AGLosBerrosB	SYHiltonB	SYNFJuncalA	SYSalsB	SYSantaCruzA	VTBearB	VTMatilijaA	VTNFMatilijaB	VTUpNFMatilijaA	SCLionCanB	SCPiruLockA	SCPiruFFA	SCPiruGHA	SCSantaPaulaB	FHColeman	FHVirginia02	FHVirginia03	FHWyoming	FHMtWhitEa	FHMtWhitLt
SLNacimientoA	**75**																							1		
SLSanAntonioA		**99**	1																							
SLTassajaraB		1	**70**	1		2	1																			
SLTassajeraB				**78**																						
AGArrGrandeB					**48**	1					1		1													
AGLopezCanA						**94**					1		1									1				
AGLosBerrosB						1	**62**																			
SYHiltonB						1	1	**45**		1	3					1										
SYNFJuncalA									**85**																	
SYSalsB						2				**132**																
SYSantaCruzA					1						**25**															
VTBearB												**18**		5												
VTMatilijaA											1		**63**		11											
VTNFMatilijaB						1						3	1	**73**												
VTUpNFMatilijaA											1		7		**58**											
SCLionCanB																**88**										
SCPiruLockA																	**69**		28							
SCPiruFFA																		**77**	2							
SCPiruGHA																	25		**37**							
SCSantaPaulaB																				**100**						
FHColeman																					**48**			2		
FHVirginia02																						**29**	20			1
FHVirginia03																						15	**35**			
FHWyoming																								**50**		
FHMtWhitEa																									**16**	9
FHMtWhitLt																									9	**16**

Table 5: Assignments of individual fish from small populations in Los Angeles, Orange and San Diego Counties. The top three choices for assignment as population of origin are displayed, along with the probability of assignment to that population. Fish assigned to Fillmore Hatchery strains are in bold type.

Sample assigned	Assigned to:	Probability	Second choice	Probability	Third Choice	Probability
Malibu	SLTassajaraB	52.758	AGArrGrandeB	45.059	VTNFMatilijaB	2.18
Malibu	AGArrGrandeB	92.454	AGLopezCanA	6.393	SYHiltonB	0.439
Topanga	FHColeman	53.736	AGArrGrandeB	32.124	SCLionCanB	14.129
Topanga	AGLopezCanA	84.858	FHColeman	15.124	SLTassajaraB	0.018
Topanga	FHColeman	77.651	AGLopezCanA	21.9	SCLionCanB	0.232
Topanga	AGLopezCanA	99.187	AGArrGrandeB	0.79	SLSanAntonioA	0.015
Topanga	AGLopezCanA	99.156	AGArrGrandeB	0.732	SYHiltonB	0.098
Topanga	AGLopezCanA	46.963	SLTassajaraB	41.806	AGArrGrandeB	11.008
Topanga	AGLopezCanA	76.743	SYSantaCruzA	11.536	SYHiltonB	6.794
Topanga	AGLopezCanA	94.65	AGArrGrandeB	5.294	SLNacimientoA	0.034
Topanga	FHColeman	100	SCLionCanB	0	SCPiruFFA	0
Topanga	FHColeman	100	SCPiruFFA	0	SCLionCanB	0
Topanga	AGArrGrandeB	99.201	SYHiltonB	0.489	AGLopezCanA	0.302
Topanga	SLNacimientoA	97.002	SYHiltonB	1.229	AGLopezCanA	0.798
Topanga	AGArrGrandeB	90.548	SLNacimientoA	9.449	SLTassajaraB	0.003
Topanga	FHColeman	99.735	SCLionCanB	0.261	FHWyoming	0.004
Topanga	FHColeman	100	AGLopezCanA	0	SLSanAntonioA	0
Topanga	FHColeman	100	SCLionCanB	0	FHWyoming	0
Topanga	FHColeman	99.815	FHWyoming	0.176	AGLopezCanA	0.004
San Gabriel	AGArrGrandeB	99.443	SLTassajaraB	0.491	AGLopezCanA	0.066

Table 5 (cont.)

Sample assigned	Assigned to:	Probability	Second choice	Probability	Third Choice	Probability
Santa Ana-Harding	AGLopezCanA	99.917	SYHiltonB	0.033	SLSanAntonioA	0.027
Santa Ana-Harding	AGLopezCanA	95.85	SYHiltonB	3.106	VTUpNFMatilijaA	0.529
Santa Ana-Harding	AGLopezCanA	99.037	SLSanAntonioA	0.827	SLTassajaraB	0.136
Santa Ana-Harding	AGLopezCanA	90.149	SYHiltonB	9.779	SLSanAntonioA	0.032
Santa Ana-Harding	AGLopezCanA	99.837	SLSanAntonioA	0.114	SLTassajaraB	0.05
Santa Ana-Harding	SYHiltonB	98.447	AGLopezCanA	1.544	SLTassajaraB	0.004
Santa Ana-Harding	AGLopezCanA	98.311	SYHiltonB	1.092	SLTassajaraB	0.59
Santa Ana-Harding	AGLopezCanA	96.493	SYHiltonB	3.388	SLSanAntonioA	0.102
Santa Ana-Harding	AGLopezCanA	99.899	SLTassajaraB	0.075	SYHiltonB	0.021
Santa Ana-Harding	AGLopezCanA	92.828	SYHiltonB	6.732	SLTassajaraB	0.233
Santa Ana-Harding	AGLopezCanA	99.744	SLSanAntonioA	0.25	SYHiltonB	0.004
Santa Ana-Harding	AGLopezCanA	99.959	SLTassajaraB	0.023	SLSanAntonioA	0.01
Santa Ana-Harding	AGLopezCanA	99.394	SLSanAntonioA	0.35	SLTassajaraB	0.241
San Juan-Trabuco	FHVirginia02	72.683	FHVirginia03	22.052	SCSantaPaulaB	5.26
San Mateo	AGLopezCanA	58.792	SLTassajaraB	17.472	SLNacimientoA	12.502
Sweetwater	AGLosBerrosB	93.096	FHMtWhitLt	6.731	FHVirginia02	0.092
Sweetwater	FHMtWhitLt	96.525	FHMtWhitEa	3.432	FHWyoming	0.031
Sweetwater	SLTassajaraB	51.678	AGArrGrandeB	27.378	SLNacimientoA	17.361
Sweetwater	SYnezHilton	98.229	SCLionCanB	1.084	VTBearB	0.672
Sweetwater	FHMtWhitEa	52.908	FHMtWhitLt	47.092	FHColeman	0
Sweetwater	FHColeman	66.218	FHMtWhitEa	23.739	SCLionCanB	4.418
Sweetwater	SCLionCanB	100	FHMtWhitLt	0	FHMtWhitEa	0

Appendix 1. Pairwise F_{st} values are presented for all sites.

	SLSanAntonioA	SLTassajaraB	SLTassajeraB	AGArrGrandeB	AGLopezCanA	AGLosBerrosB	SYHiltonB	SYNFJuncalA	SYSalsB	SYSantaCruzA	VTBearB	VTMatilijaA	VTNFMatilijaB	VTUpNFMatilijaA	SCLionCanB	SCPiruLockA	SCPiruFFA	SCPiruGHA	SCSantaPaulaB	FHColeman	FHVirginia02	FHVirginia03	FHWyoming	FHMtWhitEa	FHMtWhitLt
SLNacimientoA	0.078	0.067	0.087	0.066	0.066	0.074	0.089	0.139	0.118	0.063	0.149	0.102	0.137	0.087	0.117	0.106	0.082	0.106	0.094	0.112	0.161	0.157	0.144	0.173	0.161
SLSanAntonioA		0.054	0.086	0.058	0.055	0.073	0.086	0.133	0.101	0.076	0.144	0.107	0.119	0.077	0.131	0.119	0.123	0.114	0.088	0.117	0.171	0.167	0.149	0.170	0.157
SLTassajaraB			0.082	0.054	0.052	0.070	0.069	0.119	0.094	0.069	0.126	0.103	0.113	0.082	0.127	0.117	0.091	0.119	0.077	0.115	0.156	0.152	0.135	0.171	0.154
SLTassajeraB				0.082	0.082	0.081	0.091	0.153	0.147	0.110	0.166	0.134	0.149	0.111	0.158	0.139	0.119	0.134	0.124	0.137	0.190	0.189	0.152	0.193	0.177
AGArrGrandeB					0.047	0.051	0.065	0.109	0.085	0.045	0.095	0.070	0.076	0.049	0.113	0.090	0.076	0.094	0.070	0.099	0.151	0.146	0.129	0.155	0.138
AGLopezCanA						0.058	0.056	0.106	0.073	0.038	0.107	0.055	0.089	0.045	0.114	0.102	0.099	0.103	0.057	0.103	0.138	0.130	0.121	0.149	0.137
AGLosBerrosB							0.077	0.131	0.091	0.071	0.127	0.096	0.108	0.086	0.131	0.124	0.121	0.124	0.089	0.106	0.148	0.143	0.128	0.168	0.144
SYHiltonB								0.147	0.102	0.063	0.132	0.090	0.108	0.071	0.137	0.131	0.110	0.132	0.090	0.120	0.158	0.156	0.137	0.180	0.167
SYNFJuncalA									0.134	0.145	0.183	0.160	0.169	0.137	0.178	0.168	0.175	0.181	0.118	0.161	0.197	0.203	0.198	0.200	0.195
SYSalsB										0.090	0.155	0.103	0.121	0.105	0.128	0.140	0.138	0.146	0.088	0.126	0.167	0.162	0.164	0.158	0.143
SYSantaCruzA											0.117	0.062	0.100	0.058	0.119	0.129	0.087	0.130	0.066	0.120	0.152	0.144	0.134	0.176	0.162
VTBearB												0.114	0.034	0.105	0.165	0.154	0.156	0.162	0.124	0.154	0.166	0.155	0.171	0.194	0.180
VTMatilijaA													0.093	0.035	0.150	0.123	0.114	0.127	0.094	0.138	0.173	0.160	0.147	0.162	0.156
VTNFMatilijaB														0.086	0.141	0.142	0.144	0.147	0.110	0.137	0.162	0.152	0.160	0.172	0.160
VTUpNFMatilijaA															0.145	0.113	0.104	0.117	0.082	0.115	0.170	0.165	0.148	0.162	0.146
SCLionCanB																0.104	0.109	0.112	0.084	0.139	0.174	0.157	0.162	0.183	0.171
SCPiruLockA																	0.088	**0.004**	0.115	0.121	0.191	0.174	0.173	0.169	0.159
SCPiruFFA																		0.085	0.096	0.124	0.176	0.167	0.166	0.183	0.167
SCPiruGHA																			0.119	0.125	0.194	0.177	0.172	0.186	0.174
SCSantaPaulaB																				0.114	0.131	0.119	0.127	0.142	0.134
FHColeman																					0.118	0.120	0.110	0.126	0.108
FHVirginia02																						**0.009**	0.086	0.154	0.148
FHVirginia03																							0.086	0.161	0.151
FHWyoming																								0.138	0.129
FHMtWhitEa																									0.014

EXHIBIT D

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Alternate Flow Regime to Protect Rare Native Species in Middle Piru Creek (Los Angeles and Ventura Counties, California)

January 5, 2009

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[2]Pacific Coast Conservation Alliance

Alternate Flow Regime to Protect Rare Native Species in Middle Piru Creek (Los Angeles and Ventura Counties, California)

On December 10, 2008, the California State Water Resources Control Board issued a Water Quality Certification for the California Aqueduct Hydroelectric Project, FERC Project No. 2426, certifying that the project protected beneficial uses identified under the Clean Water Act. This project changes the operation of Pyramid Dam so that releases from Pyramid Lake that are discharged down Piru Creek are roughly equivalent in amount and timing of water entering Pyramid Lake from natural sources, with the addition of 3,150 acre-feet of water deliveries during the winter. This report assesses the environmental impacts of the proposed project, especially on rare and endangered species, and proposes an alternative flow regime that would maintain rare and endangered species. It responds both to the Water Quality Certification issued by the Water Resources Control Board and to the final Environmental Assessment ("EA") approved by the Federal Energy Regulatory Commission ("FERC") approved in June 2008.

The proposed project raises an interesting set of questions because it was formulated in response to a request by the U.S. Fish and Wildlife Service ("USFWS") to reinstate a "natural" flow regime to avoid unauthorized take of arroyo toads (Letter from Bridget Fahey, USFWS to Eva Bagley, Department of Water Resources, August 20, 2003). Although the project and analysis of its impacts are discussed in terms of recreating "natural" conditions, middle Piru Creek remains highly modified and influenced by the two reservoirs at either end (Pyramid Lake and Lake Piru). Thus, although the flow regime may be considered more "natural," the system is by no means restored to natural conditions, especially given the water deliveries during the winter. Consequently, assessing whether actions will benefit or adversely affect native species remains difficult. In the sections that follow, we consider the complexity of this situation and the potential impacts of implementing the proposed action over the long term.

Those assessing environmental impacts often rely on the idea of something being "natural" as being synonymous with not having adverse biological impacts. The Department of Water Resources ("DWR") and FERC take shortcuts in their analysis by asserting that the changes in flow regime are more "natural" and therefore do not cause significant adverse impacts. Not only is such analysis flawed, because a return to natural conditions may have adverse impacts on state and federally protected species (e.g., removal of a water source that supports an endangered species), but the proposed new flow regime is not "natural" either. The new flow regime is unnatural in any number of ways, including the temperature of the released water, the lack of sediment suspended in released water, and the non-equivalency of simulating assumed surface flows with natural hydrologic flow in a watershed, including subsurface flows.

In this report we review why the proposed flow regime will have significant adverse impacts on sensitive species and beneficial uses in middle Piru Creek and why it will not provide some of the benefits that have been asserted by project proponents. In particular, we present evidence that the proposed flow regime would not reduce the impact of exotic bullfrogs (*Lithobates catesbeianus* [=*Rana catesbeiana*]) on arroyo toads (*Bufo californicus*). We then present an alternative flow regime that will minimize impacts to native species and propose mitigation measures to offset some of the adverse impacts of the project.

Our proposed flow regime includes the release of water flowing into Pyramid Lake during the winter, which is an element of the proposed project, because these peak flows would change the morphology and vegetation of middle Piru Creek in a manner that would benefit arroyo toads. We differ from the proposed project in the provision of water during the spring and summer, proposing a minimum 15 cfs baseflow from March 15 to August 31, with a gradual decline from September 1 until the winter when releases would be tied to inflows. This proposed regime would provide adequate water for reproduction of arroyo toads, California red-legged frogs (*Rana draytonii*), and native rainbow trout (*Oncorhynchus mykiss*), while the winter storms would rework the vegetation and morphology of the stream to provide appropriate habitat for arroyo toads. This approach also ensures that water is available to mitigate against the drier and hotter climate that is expected in southern California.

1 Elements of the Proposed Project

1.1 Release of Winter Flows in Approximate Quantity and Velocity as Flowing into Pyramid Lake

The proposed project would release flows into Piru Creek equivalent to the inflows into Pyramid Lake as measured upstream and adjusted for inflows from ungauged tributaries. The major difference from previous conditions is that stormwater flowing into Pyramid Lake (which occurs predominantly during the winter) would be released, with a slight delay, into middle Piru Creek. This change would allow peak winter stormflows to reach the maximum operational limit of 18,000 cfs. We identify these events, tied to natural precipitation, as "peak winter flows." Assuming this methodology were effective, it would allow large flows during wet years that would dramatically affect the ecology of the downstream reach. These peak winter flows have been absent during the course of the operation of Pyramid Lake and only returned to Piru Creek under the interim operating agreement. Such peak flows dramatically influence the geomorphology and vegetation of southern California riparian systems. Specifically, they scour and deposit sediments, widen channels, and remove emergent aquatic and riparian vegetation. These flows keep upland plant species from colonizing riparian zones where they would be subjected to submerging, scouring, physical damage, and low soil fertility (Nilsson & Berggren 2000). Lack of peak winter flows leads to maturation of vegetation and overall increased canopy cover and dramatically decreased active flood plain area (Graf 2006; Ligon et al. 1995).

1.1.1 Arroyo Toad

Scouring peak winter flows, as caused by natural storms, are necessary to create and maintain ideal habitat for arroyo toads (Campbell et al. 1996; Madden-Smith et al. 2003; U.S. Fish and Wildlife Service 1999). Large floods remove vegetation that grows under regulated conditions and provide the open breeding habitat and adjacent terraces as well as access to and from those terraces that are necessary for the long-term survival of arroyo toads (Haas 2005).

The arroyo toad survey report from middle Piru Creek during 2005 provides ample evidence that peak winter flows improved the geomorphology of the creek as toad habitat (Sandburg 2006). The floods washed out the incised channel that had developed over years of regulated flows, removed vegetation that had invaded breeding habitat and adjacent refugia, increased stream sinuosity, and established systems of pools and terraces preferred by toads (Sandburg 2006).

Studies from the Santa Margarita River by W. E. Haas provide additional evidence of the beneficial effects of winter scouring floods (Haas 2004, 2005). During the period 2001–2004, Haas monitored arroyo toad populations over 8 km of the Santa Margarita River at the Fallbrook Naval Weapons Station. The hydrograph for this period is shown below (Figure 1). Haas's study area along the Santa Margarita River presents an excellent situation for comparison with Piru Creek due to similarities in hydrology and geomorphology. Although the daily flows are not directly comparable, the geomorphology of Haas's study area and the flow patterns exhibited in this system provide insight for predicting the response of arroyo toads to flow regimes in Piru Creek.

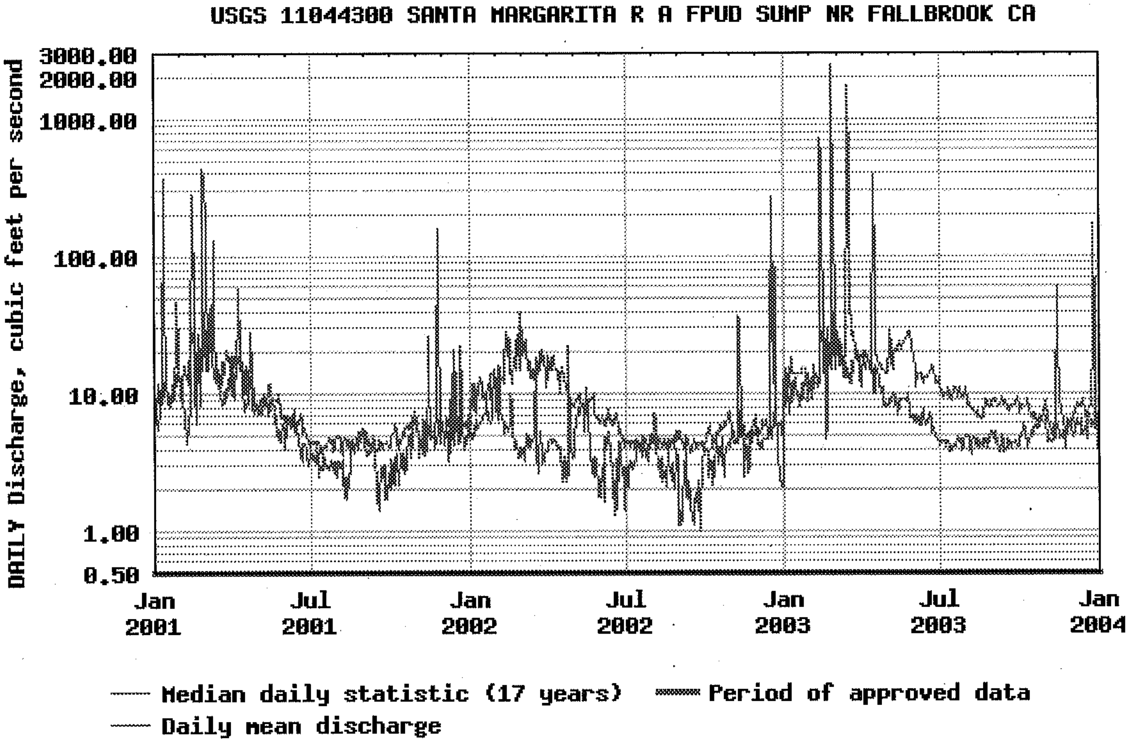

Figure 1. Hydrograph of Santa Margarita River as measured by the USGS (Gauge Station # 11044300). The gauging station is located approximately 1.3 km east of the eastern edge of Haas's Santa Margarita River study site on Detachment Fallbrook.

The results of the study showed the following:

2001: Virtually no breeding occurred along the portion of the Santa Margarita River that most resembles Piru Creek. Little scouring occurred in the period between 1998 and 2002. Hence, in 2001, the river was channelized and densely populated by cattails (*Typha* spp.); where the river broadened, watercress (*Rorippa nasturtium-aquaticum*) formed margins along the river's edge where cattails were absent.

2002: In February of 2002, because a major fire (the Gavilan Fire) affected the eastern half of the study area, access to the river from non-breeding habitat was made possible along the eastern half of the station. This was also the year of the lowest annual rainfall total in San Diego County since recordkeeping was initiated in 1850. Still, some arroyo toad breeding was documented along the eastern half of the study area, facilitated primarily because freshwater marsh vegetation had been burned along and within the river channel and exotic herbaceous layer vegetation had been eliminated from adjacent terraces.

2003: Three major events (in February and March) and a lesser event in April (Figure 2) resulted in scouring and re-formation of the riverbanks, which facilitated arroyo toad breeding along much of the northern edge of the weapons station. Breeding in 2003, an unexceptional rainfall year, was of similar density of number of breeding males to 1998, a year described meteorologically as an El Niño. Breeding activity did not commence in 2003 until very late in April, that is, following the last of three major flow peaks.

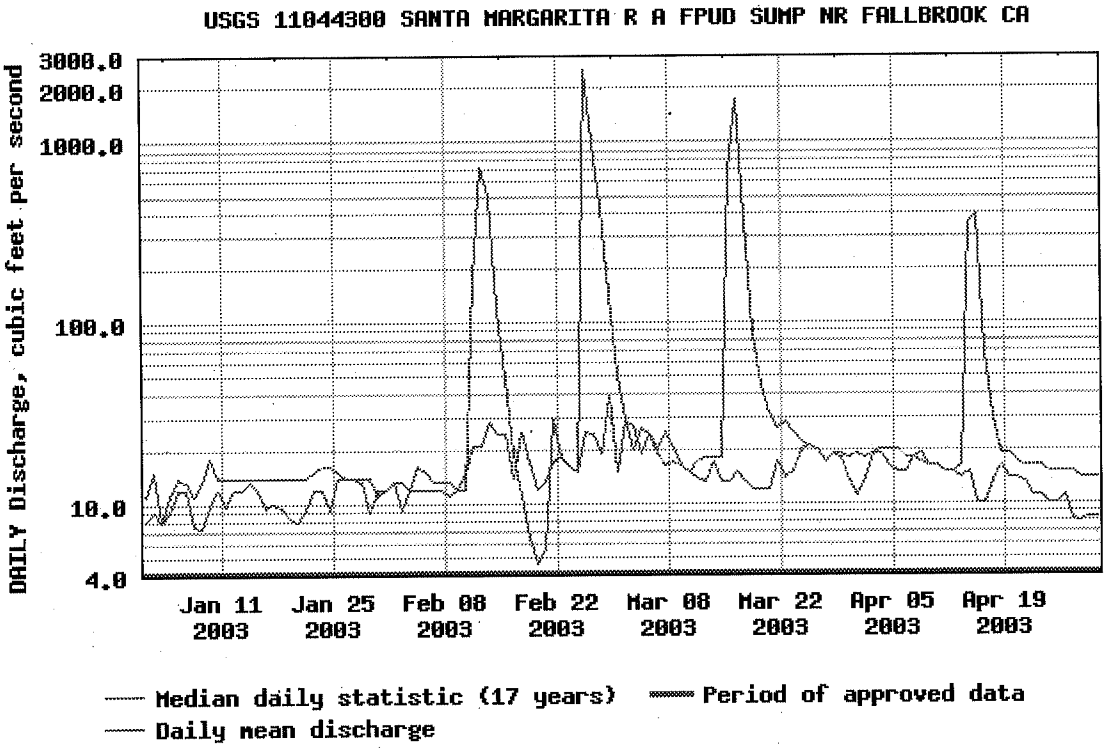

Figure 2. Hydrograph of Santa Margarita River as measured at USGS Gauge Station # 11044300 between 1 January and 30 April 2003.

Given the presence of suitably low flows and suitable/available breeding sites, the following conclusions can be made regarding the arroyo toad along portions of the Santa Margarita River:

- Peak breeding of the arroyo toad is not tied to either low or high annual rainfall totals. Periodicity and timing of rainfall and resultant flows dictate stream character breeding suitability;

- Presence of suitable breeding sites, even when stream conditions are favorable, does not ensure arroyo toad breeding. Access to breeding sites (that is, the absence of vegetation barriers, native and non-native) is required to facilitate arroyo toad breeding;

- Scouring and consequent re-structuring/re-forming river banks (along with in-stream vegetation removal) appear to be the most important factors that facilitate arroyo toad breeding, especially in areas where exotic vegetation has become problematic;

- An incised channel limits (but does not absolutely preclude) arroyo toad breeding substantially.

The benefits of winter floods to arroyo toads depend on the presence of sufficient rocks, cobbles, gravel, and other material to be worked downstream. As described by Madden-Smith et al. (2003), "A balance of scouring flows and sufficient sediment supply is required to maintain arroyo toad breeding habitat." Because Pyramid Lake traps sediments, the creek downstream suffers increased erosion (Baxter 1977; Ligon et al. 1995; Nilsson & Berggren 2000), which destroys arroyo toad habitat (Madden-Smith et al. 2003). The top four miles of Piru Creek have already been degraded as habitat because of this phenomenon. Farther down the creek, tributaries and erosion of upstream reaches provide sufficient sediment to maintain toad habitat. The proposed release of peak winter flows will result in the gradual elimination of habitat even in the middle reaches of the creek through removal of sediment without adequate replenishment.

The proposed increase in peak winter flows has the potential to provide dramatic short-term benefits for arroyo toads by removing dense riparian vegetation and creating a more suitable stream morphology. However, the action also has foreseeable long-term negative effects in the form of increased movement of alluvia and sediments downstream, thereby reducing breeding habitat. This adverse impact is neither fully discussed nor mitigated in the EA, which improperly defers mitigation to future nonbinding discussions to increase sediment supply.

1.1.2 Native Fishes

Peak winter flows are associated with increased reproductive success of native fishes (Baltz & Moyle 1993; Brown & Ford 2002; Brown & Moyle 1997; Moyle & Light 1996a, b). As summarized by Marchetti and Moyle (2001) from a study in northern California, "[C]onditions for native species improved during years with large peak flows in winter and sustained flows in summer." Furthermore, as discussed below, peak winter flows can reduce populations of exotic fishes.

The vegetation changes resulting from peak flows are not entirely beneficial to native fishes. Elimination of streamside vegetation through peak winter flows can reduce refugia for fishes in the summer. Overhanging willow vegetation provides lower temperature conditions for native fishes to escape to in the summer (Marchetti & Moyle 2001), although recent research shows that juvenile steelhead tolerate and forage in conditions warmer than previously presumed (Spina 2007).

1.1.3 Exotic Predators

Many of the exotic species that have invaded and threaten native species in California streams are specialists of lentic (slow-moving) habitats (Power et al. 1996). These include bullfrogs (Hayes & Jennings 1986), large-mouth bass and related species (Moyle 1976; Moyle et al. 1986), and mosquitofish (Meffe 1984; Meffe & Minckley 1987). It is widely asserted that high peak flows will wash out exotic bullfrogs and exotic fishes from California riparian systems (Kats & Ferrer 2003; Madden-Smith et al. 2003; Marchetti & Moyle 2000; Meffe 1984).

The most applicable research to the effects of peak winter flows on bullfrogs was completed to provide guidance for managers seeking to protect red-legged frogs (Doubledee et al. 2003). Because bullfrog larvae, which can (but not always) require two breeding seasons to mature, remain in streams during periods of peak winter flows, they are susceptible to being washed away. Doubledee et al. (2003) constructed a mathematical model to assess the flooding regime that would minimize bullfrog survival and maximize red-legged frog survival, using data from Ventura and Santa Barbara counties, including Piru Creek, to calibrate the model. The results predicted that coexistence between red-legged frogs and bullfrogs would be highest if flooding occurred at least every five years; a scenario similar to what we propose (i.e., scouring releases every 5–7 years) is a necessary management practice to maintain the health of the Lower Piru Creek system.

Notwithstanding the literature stating that winter floods can wash out invasive exotic species, releases from Pyramid Lake can introduce exotic fish downstream. As described by Sweet (1992), "Every water release from Pyramid Dam thus carries the threat of further exotic species being introduced." Moreover, adult bullfrogs are capable of overwintering in a host of refugia and are not tied to aquatic habitats year-round. Bullfrog larvae are hardy and while some may perish during release events, others (even if in small numbers) will persist (Haas unpublished data). It is not the population size that makes the bullfrog an egregious predator but rather its persistence.

1.1.4 Exotic Plants

Sweet (1992) observed that tamarisk (*Tamarix ramosissima*) had been established in Piru Creek and attributed its success to the summer flows. The lack of flooding and stable hydrology downstream of dams causes the densest infestations of tamarisk (Shafroth et al. 2005). On average, however, tamarisk is found in drier conditions than are native riparian species such as willows and cottonwoods (Cooper et al. 2003; Shafroth et al. 2000) and tamarisk is less tolerant to flooding and scouring than are native trees (D'Antonio et al. 1999). Consequently the release of peak winter flows will help to decrease this invasive species. With a decrease in scouring floods, tamarisk, along with other woody vegetation, will increase (Shafroth et al. 2002). Release of scouring winter flows, as proposed in the project, would have a beneficial effect on the riparian vegetation by favoring native trees over exotic tamarisk.

1.1.5 Western Pond Turtle

Reestablishment of winter flows is expected to benefit western pond turtle (*Actinemys marmorata*) because the flows will rework the incised channel to create broader, shallower pools preferred by the turtles (Holland 1994).

1.1.6 Two-striped Garter Snake

By reducing populations of exotic predators, peak winter flows can be expected to benefit two-striped garter snakes (*Thamnophis hammondii*).

1.2 Proposed Elimination of Summer Flows Does Not Protect RARE Beneficial Use

The proposed flow regime does not include any predictable summer releases and consequently differs from the constant 25 cfs flows through the summer that is the baseline condition for analysis. The impact analysis therefore must consider the impacts of this change, regardless of whether it is perceived to be a return to a "natural" condition or not. The elimination of predictable summer water releases will not protect the existing beneficial uses in middle Piru Creek, primarily by adversely modifying habitat for rare species (RARE).

1.2.1 Arroyo Toad

Arroyo toad breeding is low or absent during years with low precipitation (Haas 2001, 2004; Holland et al. 2001; Jennings & Hayes 1994; Sweet 1992). Once female toads emerge from overwintering, they must forage for a period of time to be able to produce eggs. In dry years, breeding habitat may be gone by the time eggs have matured. Elimination of summer base flow in Piru Creek will decrease or eliminate arroyo toad recruitment during dry years.

The agencies promoting this project have asserted that reduction in summer flows to match input to Lake Pyramid will have benefits for arroyo toads by eliminating breeding habitat for the bullfrog, an exotic predator. Although bullfrogs may indeed depredate arroyo toads, their ecologies are sufficiently distinct that it is rare for the two to co-occur in quintessential arroyo toad breeding habitat consisting of low-gradient, shallow, sandy/gravelly streambed. The reduction of summer flows rather may have a far more adverse effect on arroyo toads than on bullfrogs by limiting the length of the arroyo toad breeding season and by concentrating arroyo toad larvae (and subsequently neonates) in deeper pools. These deeper pools, compared with quintessential arroyo toad breeding habitat, are more likely harbor bullfrogs (and bullfrog larvae) and thus make depredation of arroyo toads (or death by other means such as limitation of larval foraging habitat) more likely. This phenomenon has been observed at numerous arroyo toad breeding sites throughout the range of the species including along the Santa Margarita, San Luis Rey, and Sweetwater rivers (Haas unpublished manuscript, 2008).

Rather than eliminating exotic predators of arroyo toads, reduction of summer flows would have the result of creating isolated ponds of water where aquatic species are concentrated. Concentrated food resources result in greater competition between native and exotic species (Kiesecker et al. 2001). Concentration in isolated pools is likely to exacerbate the impacts of interactions with exotic species already existing in the system.

With the previous summer water release of 25 cfs and no winter storms, Piru Creek developed an incised channel and extensive riparian vegetation. These conditions are adverse to arroyo toad breeding success. With the large winter storms that reconfigured the channel to make it broader and shallower and removed the extensive riparian vegetation, the summer flows of 25 cfs were beneficial for arroyo toads (Sandburg 2006, p. 49):

> A 25 cfs flow in the entrenched channels of year 2004 were excessive for arroyo toad breeding, but **this same flow was suitable and productive** in naturally widened 2005 channels [emphasis added].

This is exactly what Haas found along the Santa Margarita River in 1998 (following a winter of El Niño rains) and again in 2003 following serendipitously well-timed rainfall events.

Furthermore, the guarantee of water being released into Piru Creek during dry years is a buffer against climate change. If the climate becomes drier and little or no summer water is released from Pyramid Lake for an extended period, then there is a risk of creating conditions adverse to arroyo toad breeding, with a long-term potential for extirpation if lack of surface water precludes breeding for an extended period or a shortened breeding season and elevated predation pressure in remaining pools causes a downward population trajectory. From a practical perspective it is far better to ensure that such water continues to be available rather than waiting until a future crisis to try to negotiate the release of water from Pyramid Lake after a switch to the proposed flow regime. Under such a scenario, the needs of riparian-dependent species in Piru Creek are not likely to prevail.

1.2.2 Red-legged Frog

The California red-legged frog requires aquatic and riparian habitats. In the vicinity of water, adults favor dense, shrubby or emergent riparian vegetation (protective embankment overhangs may also be favored hiding spots) closely associated with pools and ponds greater than two feet deep, usually with still or slow-moving water. Jennings et al. (1993) suggested that intermittent streams must retain surface water in pools year-round for red-legged frogs to survive. However, California red-legged frogs may aestivate in small mammal burrows, under large rocks, and in moist leaf litter, and may be found several hundred feet from their riparian haunts (Jennings et al. 1993). Where climatic conditions are extreme (e.g., in southern California) well-vegetated terrestrial areas within a riparian corridor near to the streambed may provide important sheltering habitat during periods of drought and for overwintering.

California red-legged frogs breed variably in the period between November and March depending on rainfall regime, temperature, and presence of breeding habitat. Eggs are typically attached to emergent vegetation at or near the surface. Eggs may be dislodged during periods of elevated flows, especially when these occur in pulses. Eggs hatch in one to two weeks (egg development in colder climates may take considerably longer) and neonates emerge approximately 3.5–7 months later. Rate of larval development is tied to several factors, the most important of which is availability of forage, along with water and air temperatures. The northern red-legged frog (*Rana aurora*) has the lowest upper (21°C) and lower (4°C) lethal embryonic temperatures of any North American ranid frog (Licht 1971). Similar data are not available for the California red-legged frog, and although each may be expected to be several degrees higher because of the more southerly distribution, temperature of water releases (especially dependent on whether water is released from the surface or from below the surface) must be considered in developing a programmatic approach to water releases where this species occurs. Also, water temperatures downstream from release sites may experience dramatic increases during the period of larval development if overstory vegetation is reduced (e.g., due to reduced flows) and/or if pools begin to dry prematurely such that water temperatures rise, especially if subjected directly to radiant heat.

Drying of the streambed in and of itself may or may not adversely affect adult red-legged frogs, especially if adequate shade and moisture are available in the vicinity of drying pools. However, depending on many features (e.g., timing of egg deposition, water and air temperatures, level of competition within breeding pools), larvae may require breeding pools as late as June or July (rarely August). Thus, release regimes with late summer reductions in flow may have significant adverse effects on red-legged frog recruitment. Maintenance of emergent vegetation within instream pools is important to egg deposition sites. Thus, periodic, especially regular, drying of the streambed may have significant, adverse impacts on red-legged frogs and is contraindicated for the persistence of this species along Piru Creek. It should be noted that middle Piru Creek is designated critical habitat for the red-legged frog, but not the arroyo toad. In sum, the proposed regime would have a potentially significant impact on the red-legged frog, whereas the alternative regime would support the species.

1.2.3 Native Fishes

In California and other Mediterranean climates, numbers of native fishes are increased with increased flows, while exotic species increase with decreased flows (Marchetti & Moyle 2001; Moyle et al. 1986). Reducing summer flows would degrade habitat for native species, which prefer cooler water, and promote reproduction of exotic species (Marchetti & Moyle 2001). Most exotic fishes spawn in the summer (Moyle 1976). Although native fishes are adapted to surviving multiple years of low flow, they can do so only if there are sufficient refugia (Marchetti & Moyle 2001). Summer flows are beneficial to the native fishes, as described by Marchetti and Moyle (2001) for Putah Creek near Sacramento:

> [H]igher summer flows also favored native fishes by providing longer reaches of cool flowing water where juveniles of the native fishes could find suitable conditions for rearing, while simultaneously reducing the favorability of the habitats for spawning and rearing of alien fishes.

Therefore, the proposed license amendment would have a potentially significant impact on native fishes, including resident rainbow trout, whereas the alternative regime would support them. The resident rainbow trout in middle Piru Creek are not from hatchery stock and genetically cluster endangered southern steelhead elsewhere in the Santa Clara River watershed (Girman & Garza 2006).

1.2.4 Western Pond Turtle

Reduced summer flows would decrease the number of shallow ponds available for basking and foraging by western pond turtles. Reduced flows would furthermore concentrate turtles into fewer areas where chances of adverse impacts from recreation would be expected. This concern was raised by the Department of Fish and Game in its comments on the draft EA.

1.2.5 Exotic Predators

Exotic predators, including American bullfrogs, fishes, and invertebrates, are present in Piru Creek and could be affected by decreased summer flows. Reduction in bullfrogs in particular is promoted as a primary benefit of the flow regime proposed by DWR.

Doubledee et al. (2003) investigated draining ponds as a method to control bullfrogs. This approach is roughly analogous to eliminating summer flows in Piru Creek; along certain sections of the creek no ponds will remain. In a modeling experiment that included a full life history description of bullfrogs, Doubledee et al. (2003) found that draining ponds every two years would reduce bullfrog populations by 50%, but this result did not take emigration into account. They go on to recommend that ponds within 5–10 km of each other should be drained simultaneously to reduce bullfrog populations. If applied to Piru Creek, these results suggest that it would be nearly impossible to eliminate bullfrogs by reducing summer flows. Ponds of water would still remain as refugia in the creek (where competition with native species would be heightened), the artificial reservoirs would serve as perpetual sources of immigrants, and bullfrogs would rapidly return. Incidentally, active control of bullfrogs (shooting) has not been effective in the past (Rosen and Schwalbe 1995). The only successful eradication projects have taken place in isolated ponds that could be entirely drained and all bullfrogs killed (Ficetola et al. 2007).

Although decreased summer flows would limit bullfrog reproduction to some degree, adult bullfrogs easily persist through dry periods and rapidly reproduce when conditions are suitable. In an eight-year study in the San Diegito River system, Haas found that extended periods during which dry conditions occur do not necessarily eliminate adult bullfrogs from local areas. Moreover, in studies of the arroyo toad in Santa Maria Creek, Haas found three live adult bullfrogs and a young blue catfish (*Ictalurus furcatus*) buried > 8 inches deep in relatively moist soils within the creek's riverwash in August, more than a month after surface flows had vanished. The ability of exotic invasive species to persist must not be underestimated. As shown by removal attempts (through shooting), populations rebound within 3–4 months when stressful conditions are removed (Rosen & Schwalbe 1995). Exotic aquatic predators, including invertebrates such as crayfish, rebound quickly when flushed out by winter storm flows (Kats & Ferrer 2003).

Periodic drying as would be experienced in middle Piru Creek under the proposed flow regime would not be sufficient to reduce populations of bullfrogs. Attempts to remove bullfrogs from isolated ponds involve complete drying followed by excavation of sediment to remove adults and larvae (Banks et al. 2000). Even with this effort, bullfrogs were reproducing in the pond two years later (Adams & Pearl 2007). Mathematical modeling of bullfrog dynamics predicts that complete drying would be necessary every other year to allow coexistence with red-legged frog (Doubledee et al. 2003). Such drying would not be desirable in Piru Creek, because it would have adverse impacts on native species (Maret et al. 2006).

Moreover, the data upon which Adams and Pearl (2007) based their findings may not be fully applicable to warm southern California meta-populations of the bullfrog, which may be able to complete their transformation during the first summer in warm climates (Bury & Whelan 1984;

Cohen & Howard 1958) as opposed to many other locations where two seasons are required for their transformation. Indeed, bullfrog larval transformation has been reported at four months in Louisiana (George cited in Willis et al. 1956) and at six months in California (Cohen & Howard 1958). For precisely this reason draining isolated ponds to control bullfrogs must be done rapidly, typically under controlled circumstances, so that larval development and transformation do not occur before water is removed and, importantly, to avoid selection of rapidly developing larvae (Adams & Pearl 2007). It is specious, therefore, to argue that establishing a "natural" flow regime in middle Piru Creek is going to reduce the impacts of bullfrogs by reducing their number with summer drying (see Figure 3).

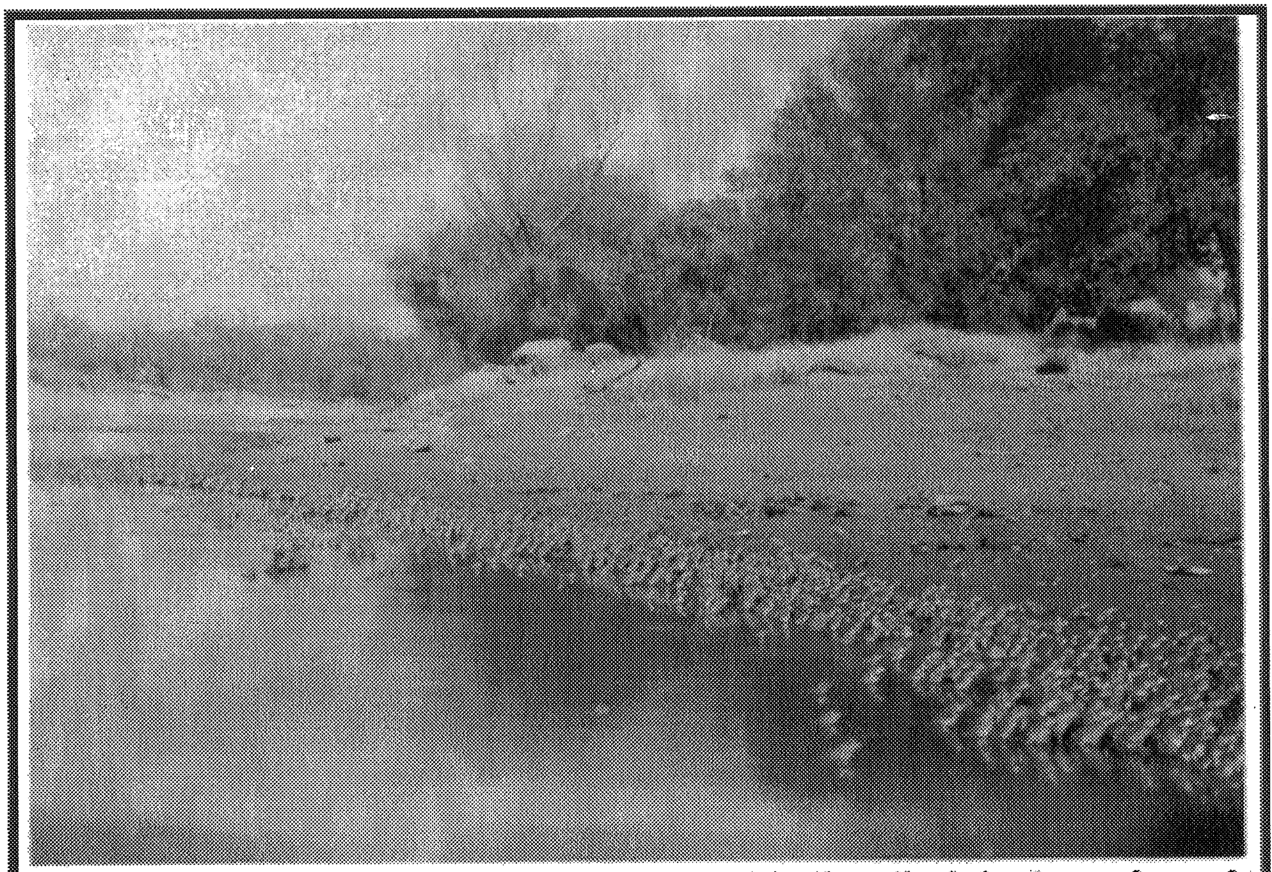

Fig. 1. In 1950 thousands of recently metamorphosed bullfrogs lined the shores of one of the earthen reservoirs at the San Joaquin Experimental Range, California. The eggs had been laid only about seven months earlier. This reservoir went completely dry during the late summer of the previous season.

Figure 3. Photograph and caption reproduced from Cohen and Howard (1958) showing that permanent water is not needed for bullfrog development.

1.2.6 Exotic Plants

Removal of summer flows will not likely reduce extent of tamarisk because plants are already established and can easily reach subsurface water with their taproots. Furthermore, as discussed above, tamarisk cover is documented to increase under lower water conditions in southwestern rivers. Compared with allowing peak winter flows with scouring floods, the evidence does not support use of removal of summer water as a means of tamarisk control (Shafroth et al. 2002; Shafroth et al. 2000; Stromberg et al. 2007a; Stromberg et al. 2007b). To the contrary, summer water releases from reservoirs have been shown to mitigate the effects of natural drought on native seedling mortality when they occur after winter flooding events that are conducive to establishment of native trees (Lytle & Merritt 2004).

1.2.7 Climate Change Scenarios

It is shortsighted to commit to the long-term management of middle Piru Creek by institutionalizing the proposed "flow in/flow out" release schedule for Pyramid Lake. Under foreseeable future climate change scenarios, this region is likely to be warmer and drier (Westerling & Bryant 2008). In an extended drought, the sensitive amphibians currently supported in Piru Creek could face extirpation as a result of inadequate breeding habitat. Because releases of water from Pyramid Lake were originally established as mitigation for destruction of stream habitat, the rights to such flows should be retained to be able to maintain the remaining downstream habitat under future climate emergencies.

2 Proposed Alternative Flow Regime for Piru Creek

The stated purpose of the proposed project is to avoid take of arroyo toad by water releases along Piru Creek. This stated purpose is misguided. The purpose should instead be to devise a flow regime that protects all of the beneficial uses of the creek, including as habitat for rare species other than arroyo toad. The currently proposed flow regime attempts to create a "natural" flow regime, but because of the capping off of peak winter flows at 18,000 cfs (far below the natural flows of 54,000 cfs) and by delivering water in addition to natural flows during the winter, the hydrological conditions in Piru Creek between Lake Pyramid and Lake Piru will not be natural under the proposed project.

The implementation of the flow regime by eliminating all "artificial" summer baseflow will not be effective in reducing populations of exotic bullfrogs or protecting the beneficial uses of middle Piru Creek. Rather, the research demonstrates that reduction of the extent of surface waters without complete drying may exacerbate the adverse impact of introduced predators such as bullfrogs on native fish and amphibian species. Furthermore, the scientific literature and observations on Piru Creek indicate that additional summer water releases benefit sensitive native species. We therefore offer an alternative flow regime.

2.1 Flow Characteristics

2.1.1 Winter Flows

From the period of the first winter storm to May 1, a volume of water equivalent to that which flows into Lake Pyramid will be released from it, within the operational constraints of Pyramid Dam. At a period of at least once every 5–7 years, a release event of significant volume adequate to produce scouring flows must be implemented if such flows do not occur naturally from rainfall events. Evidence from both Piru Creek and the Santa Margarita River suggest that floods of this periodicity are necessary to rework sediments and clear vegetation (Haas 2005; Sandburg

2006). Models predicting coexistence of red-legged frogs with bullfrogs suggest flooding every five years (Doubledee et al. 2003). The three-year period of variance we recommend (years five, six, or seven) offers sufficient leeway to accommodate periods of extended drought-like conditions and/or a natural scouring event in a high rainfall year. Any water deliveries will take place during the winter period (November to February) and be released to emulate the flows of a winter storm in volume and timing. Water deliveries can be used for the scouring flows described above.

2.1.2 Summer Flows

As discussed above, many species depend on sufficient water to complete reproduction. While some native species can withstand droughts, others, such as the native rainbow trout, require water year-round and many native riparian species thrive with additional water at appropriate times. The project proponents describe inflow to Lake Pyramid from 1977–2002 and adjusted for watershed area as peaking at 255 cfs in February and decreasing to an average of 8–9 cfs from August through October.

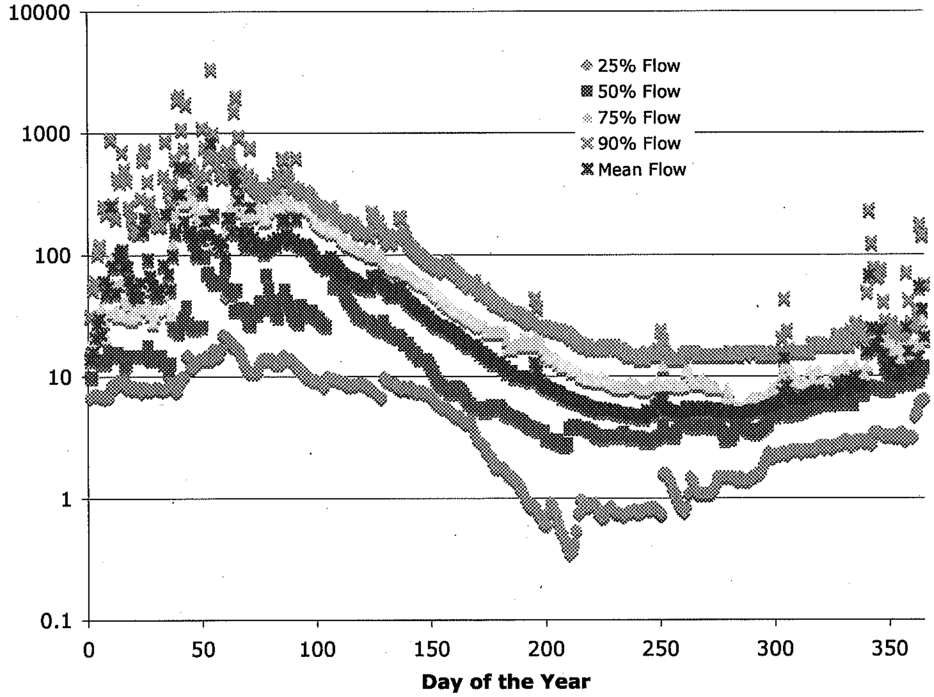

Figure 4. Gauged flow (cfs) in Piru Creek above Pyramid Lake near Bucks Creek (USGS Gauge Station # 11109375) during a 17-year period. Mean flows exceed median (50%) flows because peak flows are so large (not logarithmic scale).

The flow into Lake Pyramid reported in the Environmental Assessment is an extrapolation of the record at Piru Creek near Bucks Creek (Figure 4). This record is for a 198-square mile watershed, whilst Piru Creek directly below Pyramid Lake drains 295 square miles. Adjusting this record upward to account for the larger watershed produces the mean flow numbers reported by the agencies. This method is a back-of-the-envelope extrapolation because it does not account for any groundwater baseflow or spring-fed input that could be provided in the additional 100 square mile watershed above Pyramid Lake.

This record, although only documenting a 17-year history, shows that the outflow of 25 cfs during the summer and fall is within the range of natural variation. This occurs during wet years, and we estimate based on flows from Piru Creek at Bucks Creek (Figure 4) that this flow level has occurred twice in 17 years. During the driest years, stream flow has been recorded at 2 cfs or less. Stream gauges are notoriously inaccurate at low flows, however, so these numbers should be used for analysis only with caution. Such extreme conditions occurred on average once every four years during the 17-year record. As discussed above, this is not a sufficiently long period to effectively reduce the number of adult bullfrogs in middle Piru Creek.

We therefore propose a flow regime that ensures that conditions in middle Piru Creek are always at or above the 75th percentile. This summer flow, combined with the natural winter flows to maintain a desirable stream morphology (see Sandburg 2006), would protect the beneficial uses of middle Piru Creek, including as rare species habitat and warm and cold fish habitat.

Under this scheme, inflow to Lake Pyramid would be released as outflow during the winter season from November to March 15. From March 15 to August 31 water would be released at 15 cfs (or natural inflows, whichever is greater), then decreased by 1 cfs every 2 days between September 1 and September 20 to achieve and maintain a 5 cfs minimum flow from September 20 until the first winter storm. During the winter, inflows would be released as outflows. Flows would be increased gradually to meet the 15 cfs flows in March during years when flows were less than 15 cfs leading up to March 15. In all instances these are minimum flows, to be exceeded if calculated inflows are greater.

These proposed flows are greater than in the average year and would eliminate drought years. Although this amount is less than the 25 cfs maintained since 1995, it should be sufficient to sustain native rainbow trout populations because the population has survived drier conditions than this in the past.

This proposal is based on the judgment that increasing summer flows, as long as there are adequate winter storm events to keep the stream channel sufficiently broad and shallow, will not have significant adverse impacts on arroyo toads. The adverse impacts of bullfrogs and other non-native predators will be reduced by the floods creating a stream morphology with habitat that is more amenable to arroyo toads than to bullfrogs. We also conclude that the impacts of non-native predators would be exacerbated by a flow regime that allowed for partial drying that would concentrate aquatic species into smaller and smaller pools, increasing pressure from predators. The basis for these judgments follows.

Arroyo toads do not require intermittent streams, and are more commonly found around perennial water in the northern part of their range (Cunningham 1962), which includes all areas north of Orange County. Cunningham (1962) found them to be most common along stretches of stream that were perennial, in areas were there are shallow reaches with sandy and gravelly beaches, and few boulders. The species recovery plan also describes habitat as including perennial streams that are flooded on a “fairly regular” basis (U.S. Fish and Wildlife Service 1999).

Perennial flows in Piru Creek therefore cannot be construed as causing take of arroyo toads or constituting an adverse modification of habitat in any manner. To the contrary, they are an improvement to habitat conditions for this species.

The presence of bullfrogs in Piru Creek (and their continued down-washing from Lake Pyramid) complicate the situation, but elimination of summer flows is not an effective solution to control their numbers (Adams & Pearl 2007). Eradication programs will not control bullfrogs at the population level in complex environments (Adams & Pearl 2007). Once established, bullfrogs are "difficult or impossible to directly control or eradicate" (Adams & Pearl 2007), although direct control of problem individuals may be beneficial (see below).

The most promising avenue to allow for coexistence of rare native amphibians with bullfrogs is through management of habitat structure rather than through alteration of hydroperiod (Adams & Pearl 2007). Bullfrogs and arroyo toads have quite different habitat preferences — bullfrogs prefer deep pools with extensive vegetation while arroyo toads prefer shallow pools with little vegetation. Providing habitat diversity, by changing stream morphology with winter storm flows in this instance, should decrease encounters between the two species (Smith 1972). Native amphibians, even red-legged frogs, can coexist with bullfrogs if habitat conditions mitigate for the adverse effects of bullfrogs (Adams 2000).

We also base this proposed regime on extensive research currently underway to examine the effects of water release on arroyo toads and the Coast Range newt (*Taricha torosa*) by Haas in the San Diego River watershed. For purposes of this discussion, the Coast Range newt serves as a surrogate for one or more Piru Creek species, including the red-legged frog or native fishes that require deep drop pools for breeding. These studies show that release rates of 6, 12, and 24 cfs may benefit both species, depending on the nature and timing of the releases. The preliminary conclusions of this research are as follows.

- Changes or fluctuations in release rate may have detrimental effects on the arroyo toad. Depending on the nature of the fluctuation, rapid increase in flow rate may wash out egg masses and larvae whereas even minor variations in stream channel height my subject egg masses and larvae to desiccation (via lowering of the water level) or washout (e.g., if the increase in height results in exposure to increased flow rates that are beyond the species' tolerance).

- Fluctuations in release rate have much less of an adverse effect on the Coast Range newt than on arroyo toad. This suggests that fluctuations in release rate would affect red-legged frog and native fishes less than arroyo toad in Piru Creek.

- The effect of the release on either species is dependent not only on the release rate but also the effect of the release relative to pre-release ambient flows. Thus, a complete analysis of the impacts of a flow regime would include an analysis of the effects of a release rate on different in-stream habitats (e.g., the low-gradient sandy habitats of the arroyo toad and the higher gradient reaches that exhibit deeper, including pooled, water that support red-legged frogs and native fishes) and take into account the effects of a release under various flow baselines. The arroyo toad requires breeding sites with channels, ox bows, or other breeding sites of less than 12-inch depth and flow rates no greater than 0.5 feet/second (15 cm/second).

- Winter releases of significant volumes should thus be timed to be complete several weeks before the onset of arroyo toad breeding.
- Arroyo toads benefit from lower rates of release especially if the release period is extended through the breeding season (e.g., between mid-March and mid-August). This is true even for the southernmost arroyo toad populations despite their ability to aestivate until fall and winter rains commence. A constant release regime of low volume, however, does not benefit the newt to the same extent it does the arroyo toad. Thus, to benefit more than just the arroyo toad, we proposed a higher release volume.

A final important issue must be considered in managing the system for persistence of native amphibians. Bullfrogs are reservoirs and vectors of the fungal pathogen *Batrachochytrium dendrobatidis* (Daszak et al. 2004; Sánchez et al. 2008), which causes a potentially fatal skin infection in many amphibians, including arroyo toads (Pessier et al. 1999) and red-legged frogs (Padgett-Flohr 2008). Reduction in bullfrog numbers is desirable for this reason as well. Future research may describe the mechanism and risk factors for exposure to *B. dendrobatidis* when carried by bullfrogs as a reservoir. Arroyo toads should be at less risk than red-legged frogs because of their habitat preferences, but the status of these populations should be monitored and researchers should take appropriate precautions to avoid spreading the disease (e.g., cleaning boots and equipment with bleach solution).

2.2 Adaptive Management and Mitigation Measures

The final Environmental Assessment and Clean Water Act certification err in failing to provide adequate mitigation measures to offset the predictable adverse impacts of the proposed project and to protect existing beneficial uses. For example, the EA proposes only monitoring as mitigation for impacts of winter flooding on red-legged frogs, but establishes no defined actions to be taken to mitigate adverse impacts of such flooding. Monitoring, without associated triggers for mitigative action, does not constitute effective mitigation. Furthermore, most conservation monitoring lacks sufficiently rigorous statistical design to evaluate hypotheses and is thus can be "a waste of time" (Legg & Nagy 2006). The EA also offers only monitoring as a mitigation for the long-term but predictable removal of sediment from the upper reaches of middle Piru Creek. The EA provides no management plan for exotic predators swept into middle Piru Creek from Pyramid Lake except the assertion that the winter flows and low summer flows will diminish their abundance. Rather than relying on monitoring as the mitigation, adaptive management programs should be put in place to address these predictable and significant adverse impacts on the biological resources of Piru Creek.

2.2.1 Sediment Provision

The arroyo toad habitat values of the first four miles of stream below Pyramid Dam have already been destroyed by clear water releases. Further stream degradation will take place with the larger and more frequent winter water releases. The Water Resources Control Board should require the DWR to commit to a sediment replenishment program as mitigation for this impact. Precedent for replenishment of sediment below dams to restore in-stream habitat is provided in the restoration of salmon habitat on the Trinity River in northern California (U.S. Department of the Interior 2000). Fine sand and gravel (not silt) should be placed below Pyramid Dam in amounts commensurate with winter rainfall and allowed to be incorporated into the morphology of Piru Creek to restore and maintain habitat for native amphibians and fishes. This action will require additional planning and compliance steps to evaluate the quantity, source, and deposition method for the sediments, but the implementation of such a program should be guaranteed by the current project.

2.2.2 Exotic Species Management

Pyramid Lake is a constant source of exotic species that will be distributed downstream by the proposed flow regime. Although complete removal of these species, especially bullfrogs, is not feasible (Adams & Pearl 2007), this impact can be mitigated through an ongoing control plan. Sweet, in an online report, asserts that single bullfrogs at arroyo toad and red-legged frog breeding pools can cause significant harm to a population and removal of those individuals would provide a short-term benefit. Removal of problem individuals would be effective because arroyo toad breeding occurs before bullfrogs begin moving between pools. The management program should selectively remove adult bullfrogs in arroyo toad habitat during breeding season. Because bullfrogs are essentially impossible to eradicate in this complex environment, such targeted lethal control can at least reduce impacts on breeding populations of endangered species.

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January 5, 2009

Via Fax & U.S. Mail

Dorothy Rice, Executive Director
State Water Resources Control Board
P.O. Box 100
Sacramento, CA 95812-0100
Fax: 916 341-5620

Re: Request for Preparation of Staff Record

Dear Director Rice,

This firm represents California Trout and Friends of the River. On behalf of these clients, we intend to petition the State Water Resources Control Board to reconsider the Section 401 Water Quality Certification ("401 certification") for the California Aqueduct Hydroelectric Project, Federal Energy Regulatory Commission Project No. 2426, issued on December 9, 2008. Pursuant to California Code of Regulations, Title 23, Section 3867(d)(9), we hereby request that the Board prepare the staff record, to the extent that such a record exists, for this 401 certification.

Very truly yours,

SHUTE, MIHALY & WEINBERGER LLP

Winter King

cc: Matt Bullock, Staff Counsel, SWRCB
Camilla Williams, Chief, Water Quality Certification Unit

P:\CALTR\MAT 2\Piru Creek\SWRCB Petition for Reconsideration\kbb002(request for record).doc

** Transmit Confirmation Report **

P.1
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TO:

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State Water Resources Control Board

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MESSAGE:

If there are any problems or questions related to this fax, please call our Receptionist at 415/552-7272