



# CALFED SCIENCE FELLOWS PROGRAM



In cooperation with the  
California Sea Grant College Program

## FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE  Postdoctoral Researcher  Ph.D. Graduate Student

PROJECT NUMBER \_\_\_\_\_

PROJECT TITLE Linking freshwater sources of California Chinook salmon to their ocean distributions using physical and natural tags of origin

### FINANCIAL SUMMARY

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Will animal subjects be used?  Yes  No

APPROVAL DATE: \_\_\_\_\_ PROTOCOL #: \_\_\_\_\_ PENDING: \_\_\_\_\_

Does this application involve any recombinant DNA technology or research?  Yes  No

## Linking freshwater sources of California Chinook salmon to their ocean distributions using physical and natural tags of origin

### INTRODUCTION:

Processes occurring in freshwater, estuarine, and marine habitats strongly influence the growth, survival and reproductive success of salmonids. To understand population dynamics for commercially important species, there has been a conceptual shift to ecosystem-based frameworks which aim to incorporate factors important to the survival of individuals over their entire life histories. For salmonids, the extent to which factors occurring in freshwater (i.e., habitat alterations) versus marine environments (i.e., oceanographic conditions/ fishing mortality) affect the persistence of salmon populations is currently debated in the field of fish ecology and has profound implications for conservation and fisheries management. Nonetheless, implementing an ecosystem model has been hindered by the inability to track the source identity of individuals between the freshwater and marine environments.

The extent to which groups of salmon in the ocean consist of separate or mixed populations and the spatial scales that segregation may occur is critical to understanding factors influencing the demography of populations (Pacific Fisheries Management Council (PFMC) 2006). In particular, freshwater populations of Chinook salmon (*Oncorhynchus kisutch*) vary in their extinction risks under the U.S. Endangered Species Act (ESA). In California and Oregon, the abundance of the Upper Klamath ESU is frequently the limiting factor in determining the areas of fishing opportunities and the duration of the Chinook salmon seasons off the north-central California coast, which has large socio-economic impacts (PFMC 2006). Chinook salmon from the California Central Valley make significant contributions to fisheries along the west coast of North America largely due to hatchery supplementation of the fall-run (Barnett-Johnson et al. 2007). However, the low numbers of Central Valley fall-run Chinook salmon returning to spawn in 2007 has required the closure of the 2008 commercial and recreational salmon fishing season off the California and Oregon coasts (PFMC 2008a; PFMC 2008b). Thus, the ability to identify the natal origins of individuals, their abundance and distribution is key to minimizing mortality on protected stocks and target healthy populations in the ocean for harvest.

What we know about migratory patterns and large-scale ocean distributions of salmonids has come from recoveries of artificial tags (coded wire tags; CWTs) on hatchery fish (French et al. 1976; Neave et al. 1964; Neave et al. 1976; Groot and Margolis 1991). Recovery patterns for tagged coho salmon (*Oncorhynchus kisutch*) show distinct region-specific distributions of natal populations, revealing life-history diversity and broad-scale spatial structure in the marine environment (Weitkamp and Neely 2002). Historical coded wire-tag recoveries represent the best time-series available to explore spatial distributions of California Central Valley specific stocks caught in the coastal fishery. Yet, information derived from this approach has been underutilized for Central Valley California stocks due to the small and variable numbers of tagged hatchery fish (<10%) and even fewer naturally produced (i.e. wild) fish (CDFG 2001). While broad-scale distributions for particular sources can be obtained by CWT recoveries, these studies rarely provide sufficient recoveries on smaller spatial scales to quantify segregation of related individuals from the same Evolutionarily Significant Units (ESUs; sensu Waples 1991) or natal populations (individual river or hatchery) within ESUs. However, McKinnell et al. 1997 developed a statistical approach to use marine recoveries of individual release groups and determined that steelhead from the same release groups were traveling in a coordinated manner up to three years after entering the ocean together.

Integrating historical coded wire-tag recoveries with natural tags (i.e., genetic and otolith chemistry/microstructure) is a promising approach to actualizing ecosystem models, understanding the spatial ecology of salmon in the ocean, and monitoring restoration efforts in freshwater habitats. In the past decade there has been a rapid increase in the development of genetic and otolith microchemistry/microstructure techniques as natural tags to understand migratory ecology and spatial structure in fishes because all individuals potentially carry a tag identifying their origin (O'Connell and Wright 1997; Campana and Thorrold 2001; Gillanders 2002; Brown 2006). Molecular techniques can detect restricted gene flow and the resultant genetic divergence among groups over evolutionary time scales, whereas chemical signatures in otoliths (fish ear bones) reflect only those environmental differences experienced by individuals in their lifetime (i.e., ecological time scales). On broad spatial scales of ESUs, Chinook salmon are genetically distinguishable and can be assigned to ESU of origin in mixed-stock fisheries analyses (Moran et al. 2006; Barnett-Johnson 2007). However, molecular techniques cannot identify the specific natal river or hatchery of origin within an ESU with enough power for accurate application to mixed-stock fisheries analyses (Banks et al. 2000; Barnett-Johnson 2007).

Understanding the contribution of individual rivers and hatcheries from the California Central Valley fall-run to the ocean fishery is fundamental to understanding sources that contribute to the persistence of this ESU and thus for freshwater restoration efforts. Currently, no traditional tagging methods (e.g., physical or genetic) are sufficient to identify natal sources for Chinook salmon from the Central Valley (Banks et al. 2000). The chemical composition and daily growth patterns of otoliths have been used as environmental tags to track the origin and movement of individuals through different habitats and have been successfully applied in the California Central Valley (Ingram and Weber 1999; Barnett-Johnson et al. 2007; Barnett-Johnson et al. 2008). Otoliths are formed during the embryonic stage and grow by the daily deposition of calcium carbonate into a protein matrix. Elements (e.g., Sr isotopes) from the surrounding water substitute for calcium and become permanently incorporated into otoliths, thus reflecting the chemical composition of the natal environment experienced by a juvenile salmon (Kennedy et al. 1997, Ingram and Weber 1999, Barnett-Johnson et al. 2008).

## **RESEARCH OBJECTIVES:**

Integrating historical coded wire-tag recoveries with natural tags (i.e., genetic and otolith chemistry/microstructure) is a promising approach to actualizing ecosystem models, understanding the spatial ecology of salmon in the ocean, and monitoring restoration efforts in freshwater habitats. We propose to quantify the contribution of natal sources and the ocean distributions of fall-run Chinook salmon from the California Central Valley by analyzing the historical coded-wire tag database, and through the use of molecular and otolith-based tags of origin. Integrating these tags provides information about the identity of salmon at the scale of Evolutionarily Significant Units (ESUs; molecular tags), hatchery or wild production sources (otolith microstructure and sulfur isotopes), and individual rivers (Sr isotopes) and hatcheries (Sr isotopes/ coded-wire tags; see conceptual diagram, Fig. 1). The proposed research will accomplish two main objectives:

**Objective 1:** Identify ocean distribution patterns and the extent of population specific schooling for California fall-run Chinook salmon through analyses of historical coded-wire tag data

**Objective 2:** Conduct mixed-stock analysis to determine the extent of hatchery supplementation and contributions of individual natal sources using molecular and otolith-based tags

## PREVIOUS RESEARCH:

Our previous work demonstrates that molecular and otolith-based tags are successful in tracking the origin of salmon in the coastal ocean at different scales of freshwater organization. Molecular markers identify fish to ESUs. Strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratios, and daily growth bands (otolith microstructure) recorded in otoliths of Chinook salmon from all major natural and hatchery spawning sites in the California Central Valley, can be used to identify river and hatchery of origin of Central Valley Fall ESU adults in the ocean with high accuracy (94%-98%; Fig. 2; Barnett-Johnson et al. 2008). Using this approach, individuals from seven Evolutionarily Significant Units (ESUs) were found in the ocean samples (Fig. 3A). Adults from different ESUs and natal populations were found to mix among regions (Bodega Bay, Bolinas Bay and Monterey Bay) off the central California coast. However, at the smaller spatial scale of aggregations within a region, fish from all four ESUs included in the spatial analyses (Central Valley Fall/ Late Fall, Sacramento River Winter Run, Upper Klamath-Trinity, and S. Oregon & N. California Coastal) showed significant associations with individuals from their ESU of origin three years after entering the ocean (Barnett-Johnson 2007).

We used otolith microstructure and strontium isotopes as natural tags and determined the natal population (river or hatchery) for Central Valley Fall/Late Fall ESU Chinook salmon, which comprised 73% of the catch off the central California coast in 2002 (Fig. 3A). Forty-three percent of the Central Valley fall-run fish were from a single hatchery source- Coleman National Fish Hatchery (Barnett-Johnson 2007; Fig. 3B). In addition, only  $10\% \pm 6\%$  of the fish were produced in the wild (Barnett-Johnson et al. 2007; Fig. 3B). Randomization tests showed population-specific aggregations for fish from Nimbus and Mokelumne River Hatcheries, indicating that there is a degree of permanence in the composition of aggregations perhaps established during their early freshwater phase. Results from the spatial analyses using a combined genetic, otolith microstructure, and isotopic approach revealed new information about the ocean ecology of Chinook salmon and that hatchery supplementation may be playing a larger role than previously thought.

## APPROACH:

**Objective 1:** Identify ocean distribution patterns (regional) and the extent of population specific schooling (small-scale) for California fall-run Chinook salmon through analyses of historical coded-wire tag data

Linking the freshwater origins of salmon to their ocean distributions is critical to understanding the role of oceanographic conditions to the growth, survival and ultimately the reproductive success of individuals. Yet, our understanding of where they go in the ocean is poorly understood (Pearcy 1992). Coded-wire tags (CWTs), 1 mm-long pieces of uniquely encoded wire inserted into the nasal cartilage of juvenile salmon, have been widely used by fisheries agencies since the early 1970s (Weitkamp and Neely 2002). Because of the long time series and the large amount of information it contains, the CWT database is increasingly being used to address questions regarding salmon movements (Norris et al. 2000), homing fidelity (Hard and Heard 1999; Candy and Beachman 2000), and marine survival (e.g., Coronado and Hilborn 1998; Ryding and Skalski 1999; Hobday and Boehlert 2001).

### ***Regional distributions***

To determine the regional ocean distributions of the tagged sources in the California Central Valley, we will use the historical coded-wire tag database. We have conducted preliminary queries of the database and have selected observations of all tag recoveries for fall-run Chinook salmon from California sources which include: Iron Gate Hatchery, Trinity Hatchery, Coleman National Fish Hatchery, Feather River Fish Hatchery, Mokelumne River Hatchery, and Merced River Hatchery. We extracted the accompanying information for each tag recovery: hatchery origin, month and location of ocean recovery, and release year – catch year (age). Our main objective is to determine persistent spatial patterns in ocean distributions for individual sources of Central Valley fall-run fish.

Marine recoveries will be assessed from return years 1975-2005, during which time fishing effort was reasonably constant (PFMC 2005) and comprehensive sampling was conducted by all appropriate agencies. Recoveries will be restricted to the dominant age class of 3 year old fish. In total, 20,961 tag recoveries were retained after these restrictions and will be included in our analyses. These recovery numbers will be expanded for sampling effort because many catches are subsampled for the presence of CWTs and are provided in the PSMFC database (N=427,143). Recovery areas are based on fishing management units used by the California Department of Fish and Game. The most frequent areas of recoveries of Central Valley fall-run fish from north to south include Central Oregon, Southern Oregon, Northern California, Mendocino, San Francisco Bay, and South Monterey Bay. The proportion of recoveries ( $R_{ij}$ ) by hatchery  $j$  in area  $i$  over all years will be calculated as:

$$R_{ij} = \frac{\sum_k r_{ijk}}{\sum_i \sum_k r_{ijk}}$$

Where  $r_{ijk}$  is the number of recoveries from hatchery  $j$  in recovery area  $i$  in year  $k$ . Previous analyses for coho salmon have showed relatively low interannual variation in recovery patterns compared to the number of fish recovered (Weitkamp and Neely 2002). Thus, the formula we are using gives equal weight to all fish regardless of year recovered. Further exploration of interannual and season variation in recovery patterns, differences in other age classes, and other runs will be explored if the number of recoveries allows for a robust assessment. To determine if the locations of CWT recoveries in the ocean were grouped by source origin, we will develop and analyze the 7 x 7 matrix of the percent recovery for the 7 hatcheries across the 7 recovery areas. This analysis will identify whether fish from a particular origin are found disproportionately in ocean recovery regions. These results will be informative for mixed-stock fisheries management. For example, one result may be that Klamath River fish have different and ocean distributions that are persistent across time than fish from Central Valley hatcheries. In addition, this analysis will provide information on whether fish from hatcheries from the same watersheds (e.g., Sacramento/San Joaquin/Klamath) have similar ocean residence distributions or whether they segregate at regional scales.

### ***Small-scale distributions***

To determine whether there is evidence in the coded-wire tag database for population-specific-schooling we will analyze the marine recoveries from fish from the same release groups. We will determine the extent to which fish released from hatcheries at the same time and

location as juveniles are recovered together in the coastal fishery 2-3 years later. We will use the randomization test developed by McKinnell et al. 1997 to determine whether the numbers of recoveries of fish from the same release groups should be expected if individual Chinook salmon within populations traveled in the ocean in an uncoordinated manner.

The randomization test will quantify how unusual the observed number of coincident recoveries would be if a null hypothesis was true. The frequency of the observed number of coincident recoveries will be tested against the null that all Chinook recovered from any release group migrated in the ocean with no more coordination with their recovered release members than with recovered members of other groups. The alternative hypothesis we are testing is that at least some of the recovered individuals from the release groups traveled in a coordinated manner. This will be quantified by developing a null probability distribution to compare the observed number of matches. An initial query of the CWT database by release group showed 25,000 tags recovered from CA hatcheries from 1975-2005. The scope of populations may be expanded depending on whether this finer scale geographic resolution of smaller hatchery production areas creates too few recoveries for adequate inference regarding population-specific-schooling.

**Objective 2:** Conduct mixed-stock analysis to determine the extent of hatchery supplementation and contributions of individual natal sources using otolith-based tags

The low numbers of 2 and 3 year old fall-run Chinook salmon returning to the California Central Valley has resulted in the closure of the commercial and recreational fishery off central CA and southern OR coasts in 2008. It is unclear whether there will be fishing opportunities in 2009, which would continue to limit the availability of otolith samples from the ocean fishery. However, some Chinook salmon were caught as by-catch in CA as part of the whiting fishery. Collaborators at California Department of Fish and Game- Ocean Salmon Project collected spatial information, tissue, scale and otolith samples from these fish and will provide the samples in support of this project. These samples represent a unique opportunity to determine the proportion of hatchery and wild fish and their natal origins in a year with such high marine mortality. Our previous estimate of hatchery supplementation was  $90\% \pm 6\%$  in 2002, which was one of the highest escapement years on record (PFMC 2006). This study will determine the extent to which marine survivorship of hatchery and wild fish changes across years. In addition, these data will provide information about the relative contributions of individual hatcheries and wild sources to the Central Valley Fall ESU and to the ocean fishery, data critical to evaluating fisheries practices and freshwater restoration efforts.

Chinook salmon from the 2008 ocean population will first be identified to ESU of origin using molecular techniques. DNA analysis will be conducted on all samples using a standardized baseline of microsatellite data from 110 populations, which has been established across 9 laboratories as part of a coast wide effort from Alaska to California and assigned to ESU of origin (Moran et al. 2005; Barnett-Johnson 2007). Hatchery and wild origins will be determined using otolith microstructure and sulfur isotopes as per established techniques (Weber et al. 2002; Barnett-Johnson et al. 2007). We will analyze  $^{87}\text{Sr}/^{86}\text{Sr}$  in otoliths from stocks determined to be from the Central Valley ESU (by genetic identification; conceptual diagram in Fig. 1). Adults will be assigned to specific river or hatchery-of-origin based on the juvenile isotopic baseline previously developed (Barnett-Johnson et al. 2008; Fig. 2).

### ***Hatchery vs. wild***

The inability to distinguish hatchery and wild salmon from the California Central Valley has inhibited the detection of declines or recoveries for wild populations. As a response to this need, new innovative otolith-based tags have been developed to identify hatchery and naturally produced (e.g., wild) salmon in marine and freshwater environments. Weber and colleagues (2002) developed a method based on sulfur isotopic signatures recorded in otoliths. Barnett-Johnson and colleagues (2007) have developed methods based on otolith growth patterns. Both techniques offer promising benefits and have potential limitations that need further consideration before any individual technique is adopted for large-scale application. Conveniently, otolith preparation methods for both techniques can be performed on the same otolith saving considerable time in applying both techniques. By taking an integrated approach, this study will not only provide the answer to an important question, but it will also provide a sound foundation from which to make future decisions about what approach to take for future studies.

Otolith microstructure patterns are known to be influenced by environmental factors such as temperature, amount of food, and feeding frequency (Campana and Neilson 1985; Nielson and Geen 1982). Differences in food abundance in hatcheries and rivers can differentially affect growth rates, resulting in different widths and variation of daily increments in otoliths observed in the two rearing types. Chinook salmon from the California Central Valley are liberally fed on schedules resulting in faster growth and wider more uniform daily increments (Barnett-Johnson et al. 2007). Wild Chinook salmon can experience greater variation in food supply and environmental factors contributing to narrow and irregular daily increments (Fig. 4A; Barnett-Johnson et al. 2007). We have previously shown that otoliths of wild salmon contained a distinct exogenous feeding check likely reflecting an abrupt transition in food resources from maternal yolk not experienced by fish reared in hatcheries. These differences in microstructure provide a quantitative baseline that can distinguish individuals reared in hatcheries or in the wild between years (1999 & 2002), life-history stages (juveniles & adults), geographic regions (California, British Columbia and Alaska) and classified fish with ~91% accuracy (Barnett-Johnson et al. 2007). The microstructure tag is less costly and more efficient than sulfur isotopes and therefore has the potential to be applied to large sample sizes. However, this technique has a misclassification rate of ~10% which may introduce significant error when the abundance of one type is very low.

A complementary approach to otolith microstructure is to use sulfur isotopes in otoliths to distinguish hatchery from wild Chinook salmon. This method is based on the predictable and consistent differences in sulfur isotopic composition ( $\delta^{34}\text{S}$ ) between the natural river diet of juvenile salmon (~2‰) and hatchery feeds for salmon (~15‰; Fig. 4B; Weber et al. 2002). Hatchery feeds for salmon have significant proportions of marine fish meal which is recorded in the protein of the otolith. The difference in sulfur isotopic composition between the hatchery and natural diet of juvenile salmon can be extracted from the otolith using an ion probe (Weber et al. 2002).

The rearing origins of Chinook salmon adults in the 2008 ocean sample will be determined by using both otolith microstructure and sulfur isotopes. However, to estimate the composition of the ocean fishery, we used a maximum likelihood estimation procedure (HISEA) based on finite-mixture distributions rather than deriving the composition by directly classifying individuals (Millar 1987, 1990; Koljonen et al. 2005). The ~150 otolith samples available represent an adequate sample size to estimate the contribution of hatchery and wild fish with good confidence limits. For example, based on binomial probabilities, the 95% confidence

interval for an estimate of 3% wild fish is 1%-8%. Since the abundance of wild fish may be small, the sulfur isotope method minimizes classification error to near zero. This significantly decreases the population-level error estimate. The relatively small sample size makes using sulfur isotopes feasible for this question. However, for larger-scale applications, otolith microstructure is the preferable method. By comparing the results of the two methods, we will be better able to evaluate the error structure in the microstructure technique for broad-scale applications.

### ***Individual rivers and hatcheries***

Sr isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratios in otoliths have proven useful in identifying natal freshwater habitats (Kennedy et al. 1997; Ingram and Weber 1999; Hobbs et al. 2005; Barnett-Johnson et al. 2008), tracking small-scale movement patterns (Kennedy et al. 2000), and chronicling timing of migration between marine and freshwater environments (Koch et al. 1992; Bacon et al. 2004; McCulloch et al. 2005). Sr isotope ratios in watersheds and fish otoliths are controlled by the age and composition of rocks comprising individual watersheds. Sr substitutes for Ca and can be recovered from discrete daily growth increments deposited throughout the life of a fish. Rivers providing salmon rearing habitat in the California Central Valley system drain areas of contrasting lithologies and are successful at identifying individuals to individual rivers and hatcheries of origin (94%-98%; Barnett-Johnson et al. 2008).

$^{87}\text{Sr}/^{86}\text{Sr}$  in the natal portion of the adult otoliths will be measured on a laser ablation inductively coupled plasma mass spectrometer (LA-MC-ICPMS) house at the University of California Santa Cruz Keck Isotope facility as per established techniques (Barnett-Johnson et al. 2005). Adults will be assigned to specific river or hatchery-of-origin based on the juvenile isotopic baseline previously developed (Barnett-Johnson et al. 2008; Fig. 2). However, to estimate the composition of the ocean fishery, we used a maximum likelihood estimation procedure (HISEA) based on finite-mixture distributions rather than deriving the composition by directly classifying individuals (Millar 1987, 1990; Koljonen et al. 2005).

### **TIMELINE**

	2008/ 2009	2009/ 2010
TASKS AND MILESTONES	SONDJFMAMJJA	SONDJFMAMJJA
<b>Objective 1:</b> Coded wire tag analysis	██████████	
<b>Objective 2:</b> Genetic analysis	██████	
Otolith preparation		██████████
Microstructure analysis		██████
Sulfur analysis		██████
Strontium analysis		██████
<b>Report and manuscript prep</b>		██████

## **BENEFITS AND POTENTIAL USERS**

Processes occurring in freshwater, estuarine, and marine habitats strongly influence the growth, survival and reproductive success of salmonids. Nonetheless, implementing an ecosystem model has been hindered by the inability to track the source identity of individuals between the freshwater and marine environments. Integrating historical coded wire-tag recoveries with natural tags (i.e., genetic and otolith chemistry/microstructure) provides a novel approach to explicitly link the freshwater and marine habitats of salmon to better quantify the role of various ecosystem process on the persistence of salmon populations. In particular, understanding the spatial distributions of salmon populations in the ocean provides the necessary information to link important oceanographic factors to the realized population sizes in freshwater habitats.

Ocean harvest models aim to quantify impacts of fishing mortality in the marine environment, with specific mandates to manage the overall resource to meet conservation objectives for endangered or threatened populations. The abundance of the Klamath ESU is frequently the limiting factor in determining the areas of fishing opportunities and the duration of the Chinook salmon seasons off the north-central California coast, which has large socio-economic impacts (PFMC 2006). Results from this project (molecular and CWT analyses) will inform the spatial scales of mixing of Klamath ESU with other salmon stocks, including the more abundant CENTRAL VALLEY Fall ESU. Identifying the locations where salmon from the Klamath ESU may concentrate could be used to better manage the fishery (i.e., spatially and temporally) to limit impacts on Klamath ESU fish while targeting CENTRAL VALLEY Fall ESU individuals.

The role of artificial propagation in managing fisheries and recovering threatened and endangered populations to sustainable levels is one of the more controversial issues in applied ecology. The difficulty in distinguishing hatchery from wild salmon has led to contentious debates over policy decisions on whether to count hatchery along with wild salmon in assessing the endangered designation of populations and the effectiveness of recovering depleted wild stocks by artificial enhancement. Results from this work would provide independent estimates of the status of wild populations within the Central Valley fall ESUs and quantify the extent to which hatcheries are supplementing the ocean fishery off the north-central California coast over time. The sample provided by our collaborators at CDFG- Ocean Salmon Project are particularly valuable because they represent samples from a year with very high marine mortality. These additional analyses will provide an estimate of interannual variability in the estimate of hatchery vs. wild fish, data which will be critical for the ESA status update for the fall-run scheduled for 2010. The Central Valley fall-run is currently listed as a 'species of concern' largely due to a lack of information on the status of wild populations (CDFG 2001). A change in this listing could have large socio-economic impacts.

Understanding the spatial structure of salmon populations in the ocean provides information necessary to ensure the sustainable use and long-term productivity. Overall, this research will generate critical information for improving both our understanding of salmon ecology and management of our coastal resources. As a result, a variety of stakeholders such as federal, state, tribal and local marine resource managers, legislators, scientists, academic research institutions, commercial and recreational businesses, nongovernmental and conservation agencies, and the general public would benefit from the results of this work.

Fig. 1: Conceptual diagram of the resolution of Chinook salmon identification using different physical and natural tags for mixed-stock fisheries analysis in the coastal ocean. Chinook salmon freshwater structure is a hierarchy with three levels nested within each other with increasing resolution. (1) Molecular tools can identify the Evolutionarily Significant Unit (ESUs) of fish caught in the ocean (e.g., Central Valley fall-run). (2) Otolith microstructure and sulfur isotopes in otoliths can identify whether salmon from the Central Valley fall-run ESU were reared in hatcheries or in the wild. (3) Coded wire tags (when recovered) identify fish to individual hatcheries and Sr isotopes in otoliths can identify all fish from the California Central Valley caught in the ocean fishery to individual rivers and hatcheries of origin. For mixed-stock analyses in the ocean fishery, we propose the integration of molecular, otolith and physical tags.

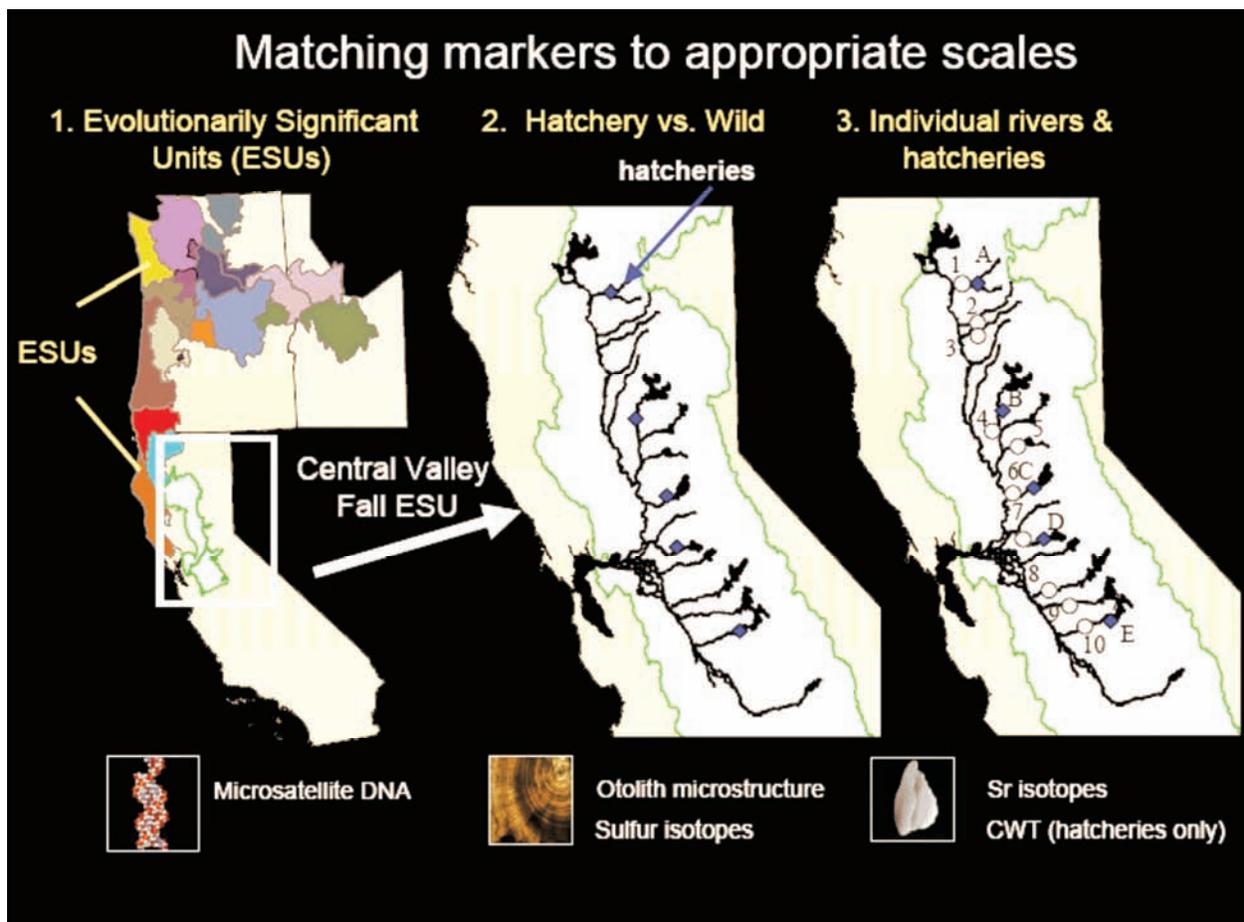


Fig. 2: Natal river  $^{87}\text{Sr}:$  $^{86}\text{Sr}$  values in otoliths from wild juvenile Chinook salmon collected from California Central Valley river. The majority of individuals are correctly reclassified to their natal river of origin using a discriminant function analysis (green circles) while some were misclassified into neighboring sites (blue circles). All pairwise comparisons are significantly different (Tukey's HSD,  $p < 0.001$ ) with the exception of DEE and MIL, which were combined in final assignment algorithm. Percent correct classification is displayed for each river with overall classification success of 94%.

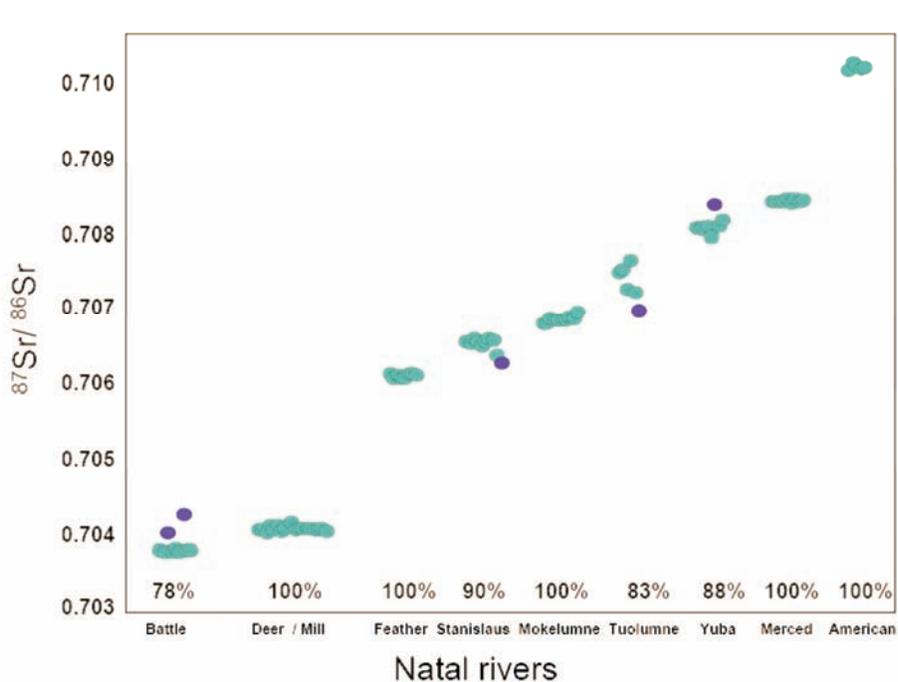


Fig. 3: (A) Maximum likelihood proportion of fish from different ESUs (color coded on map) caught from Point Lobos to Fort Bragg in 2002 using molecular techniques. Central Valley fall-run ESU fish were analyzed to determine the (B) proportion of fish from different rivers and hatcheries (in parentheses) using otolith microstructure and strontium isotopes. Most of the fish (43%) originated from a single hatchery source- Coleman National Fish Hatchery. Ninety percent ( $\pm 6\%$ ) of fish from the California Central Valley fall ESU originated from a hatchery (Barnett-Johnson et al. 2007).

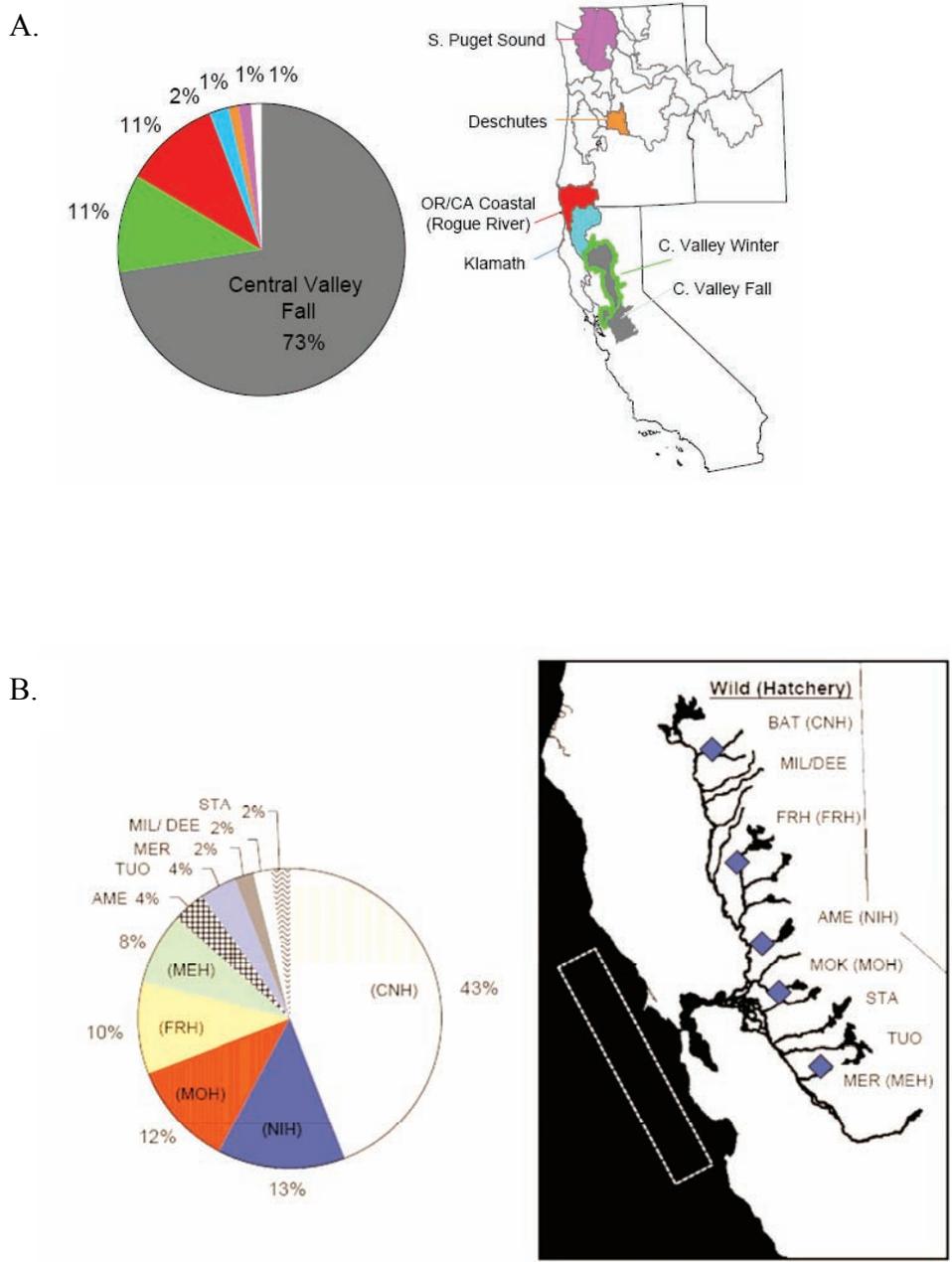
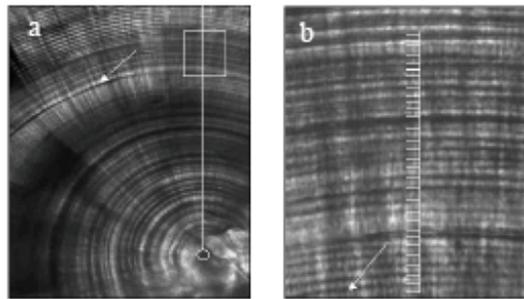
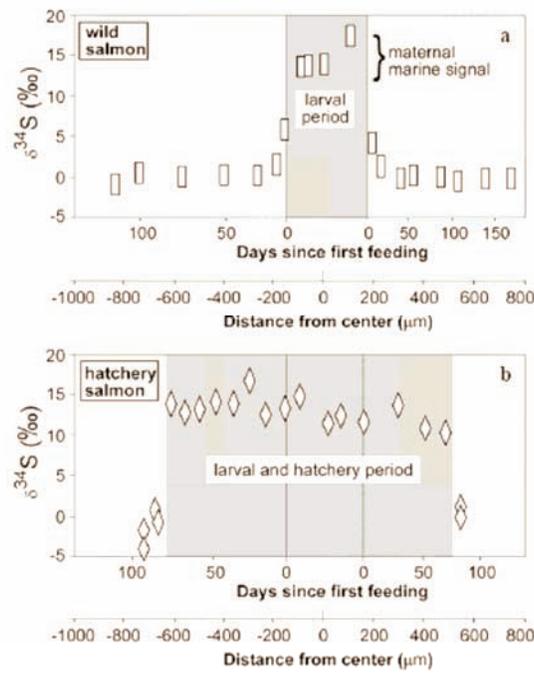


Fig. 4: Otolith microstructure (A; Barnett-Johnson et al. 2007) and sulfur isotopes (B; Weber et al. 2002) methods used to identify hatchery and wild origin Chinook salmon from the California Central Valley. (A) Dorso-posterior quadrant of otolith from naturally produced juvenile Chinook salmon. Widths of the first 30 increments (short lines) post exogenous check (arrow) were measured along a 90 degree transect (line) from most posterior primordial (circle). Magnified boxed area (b) illustrates transect with narrow and variable increment widths in a natural salmon otolith. (B) Spatially resolved otolith  $\delta^{34}\text{S}$  values for a (a) juvenile naturally spawned (wild) salmon and (b) a recaptured juvenile hatchery-raised salmon show an unambiguous difference in the distribution of  $\delta^{34}\text{S}$  values, correlated with salmon diet and lifehistory. Elevated  $\delta^{34}\text{S}$  values in the core of the wild salmon otolith, reflecting the marine S signal transmitted to the fish from the mother via the egg, decreases to values near zero once the hatchling begins to feed in the river. The hatchery-raised salmon otolith exhibits high  $\delta^{34}\text{S}$  values until release to freshwater.

A.



B.



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