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DETERMINING THE IMPORTANCE OF STOCK STRUCTURE, AND PRODUCTION SOURCES TO POPULATION DYNAMICS OF CALIFORNIA CHINOOK SALMON USING OTOLITHS AS GEOCHEMICAL SIGNATURES

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ABSTRACT

Among the many populations of Pacific coast salmonids whose numbers have declined markedly over recent decades, populations of California Central Valley Chinook salmon, *Oncorhynchus tshawytscha,* are listed or proposed for listing as endangered or threatened under the U.S. Endangered Species Act. Although California's natural stocks are declining, mass production of Central Valley fall-run Chinook salmon through hatchery production has supplemented the commercial and recreational fisheries. One challenge central to understanding the dynamics and persistence of California Central Valley Chinook and any other salmon stocks, is understanding the relative contribution of hatchery-reared populations as well as each of the tributaries that provide spawning and nursery habitat for juveniles that recruit to oceanic adult stocks. Restoring exploited salmon stocks relies on understanding the links between these sources of young that replenish adult stocks, and how their relative contributions vary in time, depending on temporal variation in both the riverine and marine environments. Our ability to estimate the contribution of these different source populations (hatchery or natural runs form the many tributaries that contribute the adult stock) has been prevented by our inability to distinguish individuals produced in each.

The research goals funded through the UC Marine Council focused on developing novel techniques to determine whether otoliths (fish earbones) can be used asnatural population markers to identify individual sources of salmon from the California Central Valley in adults caught in the ocean. My research shows that otolith microstructure and geochemical composition provide discrete tags for determining production source (hatchery vs. wild) and individual hatchery and stream-of-origin for adult Chinook salmon. Hatchery and wild individuals can be distinguished with 90% correct classification based on differences in otolith microstructure formed during early growth. Sr isotopes ($^{87}Sr/^{86}Sr$) in fish from the ten natural spawning rivers in the CCV are significantly different from one another and can be used to identify the natal origin of wild adults with 95% accuracy. In addition, Sr isotopes ($^{87}Sr/^{86}Sr$) are distinct among juveniles from each of the five hatcheries and these distinctive markers are identifiable in otoliths from adults captured in the ocean fishery. This match between natal sources and otolith signatures in ocean-caught adults was ground-truthed by examining otoliths of adults that had been tagged with coded wire at their natal tributary.

We are using these techniques to identify the origin of fishes caught in the ocean fishery to determine whether some river/ hatchery sources are contributing disproportionately to the fishery, which has direct implications for targeting restoration efforts on critical salmon habitat and quantifying the role of hatcheries in supplementing natural populations. A final analysis of this data is being used in a mixed-stock fisheries model to elucidate movement patterns and how different source populations contribute to

fisheries distributed along the coast. The results from this research will act to inform current fishery ocean harvest models critical for sustainable management of the fishery. Our ability to correctly identify the origin of adults in ocean fisheries and reconstruct their freshwater environmental histories demonstrates that this technique has the potential to link juvenile and adult life histories and elucidate influences of specific habitats to subsequent growth and perhaps survival in a broad range of fishes.

INTRODUCTION

A central problem in fisheries ecology lies in our inability to identify the relative contribution of juveniles from different natal sources (i.e., tributaries) to adult populations. Such information would extend our understanding of spatial mechanisms of persistence for anadromous fishes and aid in determining critical habitats for survival and growth for commercially valuable salmonids like Chinook salmon (*Oncorhynchus tshawytscha*) from the California Central Valley (CCV; MacFarlane and Norton 2002). Current use of coded wire tags provides limited insight into the role of individual natal sources to Chinook salmon population dynamics due to small numbers of tagged fish and even fewer recoveries (<10% of hatchery releases and far fewer wild fish are tagged). In addition, few techniques can reconstruct fish movement into different habitats to elucidate the role of habitat in determining which individuals successfully enter the ocean fishery. To better understand the importance of natal origin and habitat to salmon population dynamics, we build upon recent studies using $^{87}Sr/^{86}Sr$ variations in otoliths to identify natal environments and track movement of individual fish (Ingram and Weber 1999; Kennedy et al. 2000; 2002).

The chemical and isotopic composition of the otoliths has been used in a variety of ways to aid in stock identification of fish populations. Otoliths are formed by the daily deposition of a layer of a calcium carbonate and protein matrix. Because ninety percent of the calcium carbonate and trace elements that comprise otolith material is derived from surrounding water, the chemical and isotopic composition of otoliths provides a signature map of specific water masses. Unlike scales and bone, otoliths are metabolically inert and therefore provide a permanent record of isotopic signature from different habitats. Strontium (Sr) readily substitutes for calcium in otoliths allowing for small amounts of material to be used, and thus, for resolution over small spatial and temporal intervals. $^{87}Sr/^{86}Sr$ ratios of river waters, derived from underlying bedrock, are not fractionated when incorporated into otoliths and are generally stable across seasons and years (e.g., Kennedy et al. 2000). Variations in rock type and age in watersheds of the CCV produce a gradient in $^{87}Sr/^{86}Sr$ for salmon spawning rivers with low $^{87}Sr/^{86}Sr$ in Sacramento River tributaries (0.7039-0.7063) and high $^{87}Sr/^{86}Sr$ in San Joaquin tributaries (0.7068-0.7092; Ingram and Weber 1999, and references within). To identify hatchery fish from wild fish that co-occur on the same rivers and therefore are predicted to reflect similar isotopic chemistry, we developed additional population markers using otolith microstructure.

Otolith microstructure, the pattern in concentric bands in otoliths has also been used in stock identification especially when growth rates among populations are known to occur. Like tree rings, otoliths provide a record of age and growth in fishes and therefore can be used in juvenile salmon to record growth rates during the life of the fish. Environmental factors that effect fish growth such as temperature, photoperiod, stress, developmental changes and food resources have been demonstrated to influence otolith

microstructure (Campana and Neilson 1985). Otolith microstructure was used to discriminate between hatchery and wild Chinook salmon in British Columbia based on wider and less variable increment widths found in hatchery produced individuals (Zhang et al. 1995). The potential differences in rearing environments between hatcheries and natural rivers, with hatcheries providing a more constant and abundant feeding environment, may contribute to differences in microstructure between production sources.

In this study we measure Sr isotopes in hatchery and wild juvenile Chinook salmon to determine whether $^{87}Sr/^{86}Sr$ natal signatures are distinguishable among geographic locations. In addition, we ground-truthed techniques by examining otoliths of hatchery adults caught in the ocean fishery that had been tagged with coded wire at their natal hatchery. Finally, we measure the banding patterns in hatchery and wild otoliths to determine whether microstructure can be used to identify hatchery and wild fish reared on the same rivers.

METHODS

Juvenile Chinook salmon from the fall run were collected from all five CCV hatcheries (Coleman National Fish Hatchery (CNH), Feather River Fish Hatchery (FRH), Nimbus River Fish Hatchery (NRH), Mokelumne River Fish Hatchery (MOH), and Merced River Fish Hatchery (MEH) and ten natural spawning tributaries (Battle Creek (BC), Deer Creek (DEE), Mill Creek (MIL), Yuba River (YUB), Feather River (FEA), American River (AME), Mokelumne River (MOK), Tuolumne River (TUO), Stanislaus River (STA) and Merced River (MER)) in April 1999 and 2002 comprising all potential spawning sites in the CCV river system. Adults from five hatcheries, identified by coded wire tags, were caught in the ocean fishery off the Central California coast. The average age and size of juveniles was 100 days and 74mm FL. Sagittal otoliths were extracted, washed, and stored in dry vials prior to cleaning and mounting.

Isotopic composition

The left otolith from juvenile Chinook salmon was soaked for 6 hours in 30% Suprapur[®] H_2O_2 to remove organic material. Otoliths were then double-rinsed in ultrafiltered water, triple-washed in 0.01N HNO_3, and double-rinsed again in ultrafiltered water. Otoliths were mounted on clean microscope slides with the sulcus side facing up and polished on both sides using Al_2O_3 lapping paper of 30, 15, and 6μm grit until the primordia and daily increments were exposed. For adults, the additional step of using 250μm grit lapping paper to expose the juvenile core of the otolith was included. Strontium isotopes in the natal growth portion of juvenile and adult otoliths were analyzed by laser ablation multi-collector inductively coupled plasma mass spectrometry (LA-MC-ICPMS). Otoliths were sampled using 60x500x80μm (WxLxD) laser tracks in the dorsal region along the longest axis parallel to daily increments (5μm/increment) where the plane of growth exhibits the least curvature. Beam widths of ~60μm reflect temporal resolution of ~12 days. A total of 30 ratios were measured in each analysis (laser track) with each ratio integrated for 8 seconds. We used a New Wave[TM]UP213nm Laser and ThermoFinnigan Neptune[TM] MC-ICPMS with specific instrument, laser, and interference corrections described in Ramos et al. (2004). $^{87}Sr/^{86}Sr$ differences among natal sites were tested using a one-way ANOVA, with post hoc pair-wise comparisons

tested using Tukey's HSD. A univariate linear discriminant function analysis was used to test the accuracy of using the discriminant rule for classifying single $^{87}Sr/^{86}Sr$ observations to the correct natal origin for juveniles. Assignment of adults to river-of-origin was accomplished by comparing the $^{87}Sr/^{86}Sr$ value of the natal portion of the adult otolith to the population means of the juvenile $^{87}Sr/^{86}Sr$ from the five natal sites. Correct assignment of the adults occurred when the $^{87}Sr/^{86}Sr$ value for the adult was closest to the mean $^{87}Sr/^{86}Sr$ of its natal origin, confirmed by wire tags.

Microstructure

Otoliths were mounted on microscope slides with the sulcus side facing up and polished on both sides using Al_2O_3 lapping paper of 30, 15, and 6μm grit until the primordia and daily increments were exposed. For adults, the additional step of using 250μm grit lapping paper to expose the juvenile core of the otolith was included. For each otolith, microstructural features were measured and counted to determine potential differences in otolith formation between hatchery-reared and wild fish. Otolith sections were visually observed using a compound microscope under transmitted polarized light in conjunction with a digital camera, Optimas imaging software and the aid of a customized macro program. Increment widths were measured and enumerated for age determination along dorso-posterior quadrant orient along a 90 degree transect, relative to the longest growth axis. Along the same transect, other landmarks of ontogeny were measured such as the hatch check and exogenous feeding check as described by Zhang et al. (1995). A distinct dark band and formation of secondary primordia characterized the hatch check, formed during the transition in development from embryo to fry. Similarly, the exogenous check is identified by a characteristic dark band that forms during the metamorphous from fry to parr, where the maternal yolk sac is fully absorbed and exogenous feeding begins. The prominence of the exogenous feeding check was categorized as distinct, intermediate, or not distinct based on whether it was visible along the entire circumference and isolated from other surrounding dark bands.

Otolith measurements from known hatchery-reared and wild juveniles collected in 1999 were compared using an ANOVA to determine whether there was a significant difference in parameters between hatchery and wild origin fish. All variables met the assumptions of normality and homogeneity of variance of an ANOVA. A multivariate discriminant function analysis (DFA) was used to determine whether the relationship among several independent variables could be used to predict the origin of an individual fish (hatchery-reared verses wild). All variables were tested for co linearity, and the variables retained in the final model all explained a significant portion of the model variance. To test the spatial and temporal robustness of the DFA across years, otolith parameters were measured on fish collected in 2002 and the original model was used to test the percent correct classification of the fish collected in a different year.   Finally, the combined model was used to classify unknown origin fish collected in a mixed fishery off the Central California coast. Some adult otoliths of known hatchery were blindly read with the unknown adult otoliths to test the robustness of the classification matrix to detect the correct origin for known adults.

RESULTS AND DISCUSSION

Isotopic composition

Sr isotopes in wild juvenile otoliths are distinct across geographic regions (ANOVA, $F_{9,73} = 2998$, p<0.001). All pair-wise comparisons are significantly different among rivers with the exception of Deer Creek and Mill Creek (Tukey's HSD, p<0.001). Individuals from Feather River (n=8), Mokelumne River (n=10), Merced (n=10), and American (n=6) are 100% correctly classified to their natal tributary with one individual incorrectly classified from Stanislaus (90%, n=9), Tuolumne (83%, n=6) and Yuba (88%, n=8). Individuals from Battle Creek have a 78% correct classification rate (n=9). When Sr concentrations measured in each otolith are added as parameters in the model, 100% of Deer Creek individuals (n=7) and 90% Mill Creek (10) are discriminated.

Hatchery juveniles have Sr isotopes that are distinct across geographic regions (ANOVA, $F_{4,50} = 819.3$, p<0.001). All pair-wise comparisons are significantly different among hatcheries (Tukey's HSD, p<0.001). Individuals from CNH (n=13), MOH (n=11), MEH (n=9), and NRH (n=9) are 100% correctly classified to their natal tributary and 92% from FRH are correctly assigned (n=12 with one fish incorrectly classified as MOH). Our mean $^{87}Sr/^{86}Sr$ values for juveniles measured by LA-MC-ICPMS for CNH, MOH, MEH, and NRH agree with $^{87}Sr/^{86}Sr$ estimates reported by Ingram and Weber (1999) for fish collected in 1997 from the same natal sites suggesting robustness of natal values across years. Our $^{87}Sr/^{86}Sr$ value for FRH is significantly higher than Ingram and Weber (1999), but should not be directly compared given the inclusion of both wild and hatchery fish in their FRH estimate.

This technique was ground-truthed using adults caught in the ocean fishery that had been tagged with coded wire at their natal hatchery as juveniles, so their true hatchery of origin was known. Sr isotope ratios in the natal river portion of adult otoliths fall within the population values of juveniles from the same natal hatchery. All adults are correctly classified into their correct hatchery of origin (n=40) with the exception of two adults, indicating our analytical techniques developed with juveniles are also successful in categorizing natal origins of adults.

Microstructure

We averaged thirty increment widths post exogenous feeding and quantified the variability of the widths (standard deviation). The mean increment width between hatchery fish and wild fish were significantly different (ANOVA, $F_{1,144} = 44.3$, p<0.001). However, the variability in increment widths for a given individual was not significantly different between hatchery and wild fish (ANOVA, $F_{1,144} = 2.12$, p= 0.148). Hatchery fish on average had larger increments (2.887μm $\pm$ 0.345μm, n= 64) that were less variable (0.413μm $\pm$ 0.092μm, n= 64) than wild fish (increment widths 2.492μm $\pm$ 0.365, n= 82; SD 0.444μm $\pm$ 0.149, n= 82). In addition, there was a significant relationship between production type (hatchery verses wild) and the prominence of the exogenous feeding check (Chi-square, $X_{2,146} = 85$, p<.001). Hatchery fish did not exhibit a distinct exogenous feeding check. This is likely due to hatchery fish feeding on supplemental food prior to fully depleting maternal yolk, which would result in a smooth transition from fry to parr with no distinct metamorphoses recorded in the otolith.

A multivariate DFA using otolith parameters (mean increment width, standard deviation of increment widths, and distinctness of exogenous feeding check) resulted in

correct classification of 90% of hatchery fish (n=39) and 96% of wild fish (n=51) for fish collected in 1999. We tested the robustness of the DFA developed on fish in 1999 to assign fish collected in 2002, which resulted in 84% of hatchery (n= 25) and 90% of wild fish (n= 33) correctly classified. The results were identical using the 1999 or 2002 algorithm. Therefore, we combined the two models for the final model resulting in 87% of hatchery fish correctly classified (n=64) and 93% of wild fish (n=84).

Our research shows that otolith microstructure and microchemistry can be used to identify the natal origin of salmon collected in the ocean stocks. This is the first study to identify natural tags at the scale of individual natal environments to track the contribution of sources to a fishery stock. We are using these techniques to identify the origin of fishes caught in the ocean fishery to determine whether some river/ hatchery sources are contributing disproportionately to the fishery, which has direct implications for targeting restoration efforts on critical salmon habitat and quantifying the role of hatcheries in supplementing natural populations. A final analysis of this data is being used in a mixed-stock fisheries model to elucidate movement patterns and how different source populations contribute to fisheries distributed along the coast. The results from this research will act to inform current fishery ocean harvest models critical for sustainable management of the fishery. Our ability to correctly identify the origin of adults in ocean fisheries and reconstruct their freshwater environmental histories demonstrates that this technique has the potential to link juvenile and adult life histories and elucidate influences of specific habitats to subsequent growth and perhaps survival in a broad range of fishes.

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NAMES OF PARTICIPANTS
Graduate support
Rachel Barnett-Johnson
Undergraduate training
Chantell Royer, Abby Nickels, and Morgan Kilgour

PUBLICATIONS AND MANUSCRIPTS IN PREPERATION

Barnett-Johnson. Sources of Salmon. *In PISCO Coastal Connections 2004*: 3:13.

Barnett-Johnson, R., F.C. Ramos, C.G. Grimes, and R.B. MacFarlane. Identifying the natal origin and migration history of adult salmon using Sr isotopes in otoliths by laser ablation MC-ICPMS. In review for *Canadian Journal of Fisheries and Aquatic Sciences.*

Barnett-Johnson, R. Identification of natal origin of Chinook salmon off the central California coast using strontium isotopes and trace elements. To be submitted to *Science.*

Barnett-Johnson, R., C.B. Grimes, C.F. Royer and C.J. Donohoe. The role of hatchery supplementation to the fishery: assessing the contribution of hatchery and naturally produced Chinook salmon using otolith microstructure. To be submitted to *Canadian Journal of Fisheries and Aquatic Sciences.*

Barnett-Johnson, R.C. Spatial and temporal structuring of adult Chinook salmon in the ocean with respect to river and hatchery origin. To be submitted to *Ecology.*

PROFESSIONAL SOCIETY PRESENTATIONS

• Western Society of Naturalists
    2004, 2002, 2000
• Third International Symposium Fish Otolith Research and Application
              2004 *
    * Received honor of Best Student Talk
• Larval Fish Conference, American Fisheries Society
    2003, 2001
• Ocean Salmon Ecology Conference
    2002
• American Fisheries Society, California-Nevada Chapter
    2001