CALFED Progress Report
California Sea Grant College Program

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ProjectYear_2A: 1st Year

ProjectNo_2C: R/SF-24

TypeQuestionnaire_2B: Interim Questionnaire

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Project Information

ProjectNo_2C: R/SF-24 StartDate_3a: June 1, 2007 EndDate_3b: June 1, 2010

ProjectTitle_4: Validation of a new method for population assessment of paciﬁc salmonids using genetic markers

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Additional Research Mentors and Community Mentors

Additional Research Mentors_8

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Additional Community Mentors_9

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Project Objectives: Please type your responses, and answer the questions in a style appropriate for laymen.

ProjectObjectives_10

The goal of the proposed research project is to evaluate a novel method of efficient genetic tagging through an experiment with Chinook salmon (Oncorhynchus tshawytscha) from Californiaís Central Valley. Utilizing new methods for large-scale parentage assignment, the collection of genetic information from a parental breeding generation can be used to ìtagî the offspring cohort. When this is done at a hatchery or at a weir, the entire breeding population of a stock or population can be sampled, and the entire next generation tagged. Subsequent non-lethal sampling of fish during their seaward migration, in fisheries and upon return to spawn (hatcheries or instream) is followed by high-confidence parentage assignment. The inherited genetic tags are used to identify the parents of sampled individuals, thereby indicating the stock and cohort of origin. The proposed parentage-based tagging (PBT) experiment has three components: marker development, implementation of the parent database, and known offspring and mixed fishery assignments. Marker development is focussed on finding single nucleotide polymorphism (SNP) genetic markers for Chinook salmon. The parent database requires collection of tissue samples from the hatchery broodstock and then genotyping of these individuals at the selected genetic marker loci. Finally, the utility of the method is demonstrated by assigning sampled individuals (from known crosses and captured in mixed-population fisheries) back to their parents.

Summary of progress in meeting each of these goals and objectives

ProgressSummary_11

The first year of this project successfully focussed on the discovery of new SNP markers for use in a PBT experiment with Chinook salmon (Oncorhynchus tshawytscha) from the Central Valley in California. We also pursued the parallel development of new SNPs in California coastal steelhead trout (Oncorhynchus mykiss) populations, as few of these next-generation genetic markers currently exist for either Chinook or steelhead. SNP markers have the benefit of being both easily standardized between labs and relatively inexpensive to run compared with current genetic markers (e.g. microsatellites). Additionally, modest gains in analytical power are attained by adding small numbers of SNP loci. We compared the DNA sequences of 24 individuals per species at each candidate locus, and identified a total of 88 single base pair differences (SNPs) between the chinook individuals and 136 between the steelhead. The sequence information was used to design 16 chinook and 17 steelhead TaqMan assays, which allow for rapid ascertainment of genotype information with the ABI 3700 Real-Time PCR system. Using these new TaqMan assays we are assessing the variability of the new SNPs in large numbers of individuals from our target populations. We also assessed the potential utility of published SNP assays (e.g. Smith et al. 2005, Narum et al. 2007) in Central Valley chinook populations. These markers will be organized into panels to provide high resolution of potentially small genetic differences between closely related groups of Central Valley chinook salmon.

In addition to the SNP development, tissue samples were collected from the entire 2006 and 2007 broodstock of spring-run Chinook salmon (~3200 fish) from the Feather River Hatchery in Oroville, California. DNA has been extracted from these samples and we have begun to generate genotype data for inclusion in the parental database. The offspring of these parents will begin to be collected in downstream traps and estuarine nets and by 2009 in ocean fisheries. In-hatchery mortalities of young sac-fry were also collected in late 2007, providing a first opportunity to assign known offspring back to their parents. We continue to work on the discovery of new SNPs, as well as the development of analysis tools to expedite the accurate identification of parent-offspring families.

PROJECT MODIFICATIONS: Please explain any substantial modifications in research plans, including new directions pursued. Describe major problems encountered, especially problems with experimental protocols and how they were resolved. Describe any ancillary research topics developed.

Modifications_12

The only substantial modification to the project concerned the 2008 closure of the California and Oregon commercial and recreational salmon fisheries. A majority of the Chinook salmon caught in these fisheries originate from Californiaís Central Valley. Unexpected record low returns in 2008 forced these unprecedented closures. These ocean fisheries were to be the source of samples which we would attempt to assign back to Feather River hatchery spring-run parents. Fortunately, the offspring from the 2006 parental generation are not expected to compose a large percentage of ocean stocks until 2009 and 2010 (as three and four year old fish, respectively). Unfortunately, the prospects for commercial and recreational fishing in 2009 look even worse than those for 2008. An open fishery in 2009 already appears unlikely. CDFG denied a proposal for a limited test fishery (10,000 fish) in 2008, however we have already begun making preparations for implementing such a test fishery in 2009 if a fishery closure is again necessary. Additionally, we have begun exploring options for catching likely Feather River hatchery offspring in the bay-delta (i.e. Chipps Island) or during estuarine seining projects.

BENEFITS AND APPLICATIONS: Suggest the relevance of these new findings to management. Describe any accomplishment, that is significant effects your project has had on resource management or user group behavior. CALFED is looking for "management cue" (see http://science.calwater.ca.gov/pdf/soemgmtcues.pdf).

BenefitsApplic_13

Parentage-Based Tagging (PBT) has numerous advantages over the CWT program currently in place on the West Coast. From a practical standpoint, collection of DNA from returning adults at the hatchery requires much less effort than physically tagging the much more numerous offspring. Normally, coded wire tagging necessitates the capture, transport and tagging of juvenile fish, whereas adult fish would already be in-hand for breeding purposes. Additionally, juvenile fish are more susceptible to disease and stress than adult chinook that are destined to die after spawning anyway. Moreover, only a small fin clip needs be taken, so that a PBT tag could potentially be recovered and the fish released alive. Tag loss, which plagues CWT to an uncertain but substantial degree (Johnson 2004), is not an issue for PBT. The itagî is simply the sequence of genomic DNA and therefore cannot fall out or be expelled from the fish. By collecting DNA from and genotyping the entire spawning stock, one can tag the entire next generation. A higher percentage of marked fish inevitably results in a higher percentage of recaptures, which drastically improves the power of estimates in mixed population analyses. If applied over a large spatial extent (i.e. at many hatcheries, on many rivers), PBT could also be used for genetic stock identification (GSI).

cont'd in Additions_19

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PUBLICATIONS: List any publications, presentations, or posters that have resulted from this funded research. Give as many details as possible, including status of paper (e.g., in review; in press), journal name, conference location and date of presentation. Please note (as outlined in the conditions of the award) that each fellow is required to submit an abstract for an oral or poster presentation at each State of the Estuary conference and CALFED Science Conference during the duration of the fellowship.

Publications_14

The SNP development associated with this project was extremely successful. These new markers now represent almost 20% of confirmed SNP markers in each of the target species. This is a valuable contribution to future genetic studies of both Chinook salmon and steelhead trout in California. The information that has resulted from this project has been or will be shared with the greater scientific community through the following channels.

1) Some of these results were presented in a poster (of the same title) that won honorable mention at the 2007 State of the Estuary Conference in Oakland, CA.

2) The project was publicly outlined by the fellow as a panelist at the Spring-run Chinook Salmon Symposium, hosted by the Salmonid Restoration Federation and South Yuba River Citizens League

3) Confirmed SNPs will be published via a Primer Note in the peer-reviewed journals Molecular Ecology or Animal Genetics.

4) Unique genetic sequences will be submitted to GenBank, an online database of publicly available DNA sequences maintained by the NIH (http://www.ncbi.nlm.nih.gov/).

5) SNP allele frequency data will be submitted back to the Harvard Gene Index for inclusion in SNP reports.

6) New SNP markers will be shared with current state/federal/tribal multi-lab collaborative efforts such as the GAPS program for chinook (funded by the Pacific Salmon Commission).

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COOPERATING ORGANIZATIONS: List those agencies and/or persons who provided financial, technical or other assistance to your project since inception. Describe the nature of their collaboration.

CoopOrganiz_15

NOAA Fisheries - laboratory space and supplies, mentoring
UC Santa Cruz - fellow support and education
CA DFG - sample collection at the Feather River Hatchery

AWARDS: List any special awards or honors that you, or mentor or members of the research team, have received during the duration of this project.

Awards_16

Student Poster Award Honorable Mention @ 8th Biennial State of the Estuary Conference (2007)

KEYWORDS: List keywords that will be useful in indexing your project.

Keywords_17

Chinook, spring-run, SNP, parentage, PBT, genetics, population, hatchery

PATENTS: List any patents associated with your project.

Patents_18

does not apply

Additions: Additional information can be added here. Please begin the text with the number of the question you are adding to.

Additions_19

BenefitsApplic_13 cont'd

GSI enables managers to identify source populations of ocean-caught salmon in almost real time, without the need to collect, store and transport fish heads. GSI can also be used to accurately estimate straying (migration) rates, manage individual chinook salmon stocks and estimate ocean distribution. Finally, PBT offers the potential to identify the inherited components of physical traits through genetic mapping. This powerful technique requires large known pedigrees, which are a collateral benefit of the PBT methodology.

References - all sections

Johnson, J.K. 2004. Regional review of coded wire tagging of anadromous salmon and steelhead in northwest America. Paper updated from 1989 to current year 2004. Available from: http://www.psc.org/pubs/CWT/CWTWebPapers/SpecificForWorkshop/johnson2004.pdf

Narum, S.R., M. Banks, T.D. Beacham, R. Bellinger, M. Campbell, J. DeKoning, A. Elz, C. Guthrie, C. Kozfkay, K.M. Miller, P. Moran, R. Phillips, L. Seeb, C. T. Smith, K. Warheit, S. Young, and J.C. Garza (In revision) Differentiating populations at broad and fine geographic scales with microsatellites and SNPs. Molecular Ecology

Smith CT, Templin WD, Seeb JE, Seeb LW. 2005. Single Nucleotide Polymorphisms (SNPs) provide rapid and accurate estimates of the proportions of U.S. and Canadian Chinook salmon caught in Yukon River fisheries. North American Journal of Fisheries Management 25:944-953.

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