CALFED Progress Report
California Sea Grant College Program

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Project Information

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

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 California's Central Valley. The proposed parentage-based tagging (PBT) experiment has four components: marker discovery, development of analysis tools, implementation of the parent database, and assignment of known offspring and mixed fishery samples. Utilizing new methods for large-scale parentage assignment, the collection of genetic information from a parental breeding generation can be used to "tag" 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. Offspring can be non-lethally sampled during their seaward migration, in fisheries, and upon return to spawn (at hatcheries or instream). Genotyping is followed by high-confidence parentage assignment wherein the inherited genetic tags are used to locate the parents of sampled individuals in the parent database, thereby identifying the stock and cohort of origin. Additionally, we will evaluate whether the same set of genetic markers for PBT are also effective for genetic stock identification (GSI). While PBT can identify the specific parents of an unknown individual (as long as their genetic data is in the parent database), GSI employs baseline samples from each population to which an unknown individual can be assigned.

Summary of progress in meeting each of these goals and objectives

ProgressSummary_11

The first year of the project launched a large scale DNA sequencing project to discover new single nucleotide polymorphism (SNP) markers for Chinook salmon and steelhead trout (Oncorhynchus mykiss), since few of these next-generation genetic markers existed for either species. SNPs have many tangible benefits over currently available microsatellites (no standardization required for use in different laboratories, low genotyping error rates, amenable to inexpensive, high-throughput genotyping). This initial effort yielded 16 novel Chinook TaqMan SNP assays and 17 new steelhead SNP TaqMan assays. SNP discovery for both species continued into the second year of the project as simulations indicated that ~100 SNPs would be required to achieve the desired level of power to perform a high confidence GSI analysis (Anderson and Garza 2006). Now after two years, we have discovered a total of 118 novel SNP markers for Chinook salmon and another 140 SNP markers for steelhead. All of these markers have been converted into TaqMan assays and validated in populations of interest. These markers comprise the majority of the SNPs now available for use in these species. The papers describing these loci are in preparation, however, some SNPs are already being used by GAPS consortium partner labs (Moran et al. 2005) in Oregon, Washington, Idaho and Alaska.

The Chinook salmon SNPs discovered here in Santa Cruz were then combined with others available from the literature (Campbell and Narum 2008, Smith et al. 2005a, Smith et al. 2005b) and 192 total markers were evaluated for PBT and GSI applications. SNPs were organized into panels of 96, the capacity of our current genotyping platform (the Fluidigm EP1), and their power for PBT and GSI determined. An optimal panel of 96 loci was selected, providing false positive rates for PBT on the order of 10E-12 (one incorrect parent-offspring trio in a trillion comparisons) and better resolution for population discrimination than current genetic tools. Dr. Eric Anderson (research mentor) has just released the software to rapidly perform PBT analyses (SNPPIT; Anderson, 2010).

With the optimal panel of 96 SNPs selected, we have begun the genotyping effort to populate the GSI baseline and PBT parent databases with genetic data. Already in 2010, over 7000 Chinook salmon have been genotyped at our 96 SNP markers. The GSI baseline currently includes over 2700 individuals from 30 populations representing California, Oregon, Idaho, Washington, British Columbia and Alaska. We have genotyped 4500 of 9000 samples collected at California ports over the last decade, which will be assigned to the GSI baseline populations providing unique insights into historical stock compositions. At the Feather River Hatchery

in the Central Valley, the spring-run Chinook salmon broodstock has been sampled and the matings recorded for the years 2006, 2007, 2008 and 2009. DNA has been extracted from the 2006 and 2007 collections and will be genotyped shortly. Samples from 2008 and 2009 will be acquired soon and inserted into the extraction and genotyping pipelines. The 2009 broodstock is expected to be composed primarily of offspring from 2006 matings. Using PBT, we will identify the parents of the 2009 broodstock in our 2006 parent database and compare these to recorded crosses.

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

As in 2008, the 2009 closure of the California and Oregon commercial and recreational salmon fisheries presented obstacles to the project as initially conceived. Historically, a majority of the Chinook salmon caught in these fisheries originated from California's Central Valley. We expected to recover offspring from the 2006 and 2007 Feather River hatchery spring-run parents in these ocean fisheries. Having missed the opportunity to sample offspring in mixed fisheries at sea, we were able to recover these offspring in 2009 at the hatchery where they were returning as 3 year old adults. Using PBT, we will identify the parents of these fish in the parent database and compare these to recorded crosses. It is also worth noting that broodstock sampling has expanded beyond the Feather River Hatchery and PBT programs will likely be implemented at all California hatcheries in the future.

Addition of the GSI component was a somewhat natural extension to the project. Once it was clear that the same set of markers could be used for both PBT and GSI applications, implementation of GSI required only selecting the baseline populations and genotyping the representative individuals. We utilized composition estimates from fisheries on the California and Oregon coasts to select the 30 populations currently in the baseline. To expand the baseline, a lab needs only to genotype individuals from the new population at the same 96 loci and add this genetic data to the GSI database. continued...

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 (Hankin etal. 2005). 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 "tag" 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. As comprehensive sampling of broodstock at California hatcheries increases, more and more hatchery fish will be tagged via PBT. Combined with the GSI baseline, we are moving towards a system where information can be obtained from every fish captured at sea. continued...

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 discovery associated with this project was extremely successful. These new markers now represent more than 50% of developed SNP assays for 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 in the peer-reviewed journals Molecular Ecology Resources, Conservation Genetics or Animal Genetics. These publications are in preparation.

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 have been shared with current state/federal/tribal multi-lab collaborative efforts such as the GAPS program for Chinook (funded by the Pacific Salmon Commission).

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 supplies and mentoring
UC Santa Cruz
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 salmon, Central Valley, spring-run, SNP, genetics, hatchery, parentage based tagging, PBT, genetic stock identification, GSI

PATENTS: List any patents associated with your project.

Patents_18

n/a

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

Additions_19

Modifications_12
Since stock composition estimates would not be available in 2008 and 2009 due to fishery closures, we acquired collections from California ports sampled over the last decade. These samples will provide managers a snapshot of historical stock composition for comparison as they monitor population recovery in the future. Ideally, fisheries will be open in 2010 allowing for estimates of current stock structure off the California coast using this GSI baseline and possibly "recapturing" offspring from our known Feather River Hatchery crosses.

BenefitsApplic_13
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
Anderson, EC. 2010. Computational algorithms and user-friendly software for parentage-based tagging of Pacific salmonids. Final report submitted to the Pacific Salmon Commission's Chinook Technical Committee (US Section). 46 p. http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=16021

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Moran, P. and 11 co-authors. 2005. Interlaboratory Standardization of Coast-wide Chinook Salmon Genetic Data for International Harvest Management. Progress report from the Genetic Analysis of Pacific Salmonids (GAPS) consortium to the Chinook Technical Committee of the Pacific Salmon Commission, FY2004, FY2005, 44 p. http://www.nwfsc.noaa.gov/research/divisions/cbd/documents/gaps_year2_final.pdf

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