Title
Validation of a New Method for Population Assessment of Pacific Salmonids Using Genetic Markers

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Validation of a new method for population assessment
of Pacific salmonids using genetic markers

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Project Objectives:
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
The first year and a half of this project was dedicated to a large-scale DNA sequencing effort to identify the singlenucleotide polymorphism (SNP) markers required for an integrated PBT and GSI program (Anderson and Garza 2006). The paper describing the resulting 117 novel SNP markers has now been published in Molecular Ecology Resources (vol. 11, supp. 1, March 2011). A parallel effort for discovering steelhead SNPs appears in the same issue (Abadia-Cardoso 2011). At the end of year 2, we combined the SNPs discovered here in Santa Cruz with others available from the literature (Campbell and Narum 2008, Smith et al. 2005a, Smith et al. 2005b) and composed an optimal panel of 96 loci which provides appropriate false positive rates for PBT (on the order of 10E-12, or one incorrect parent-offspring trio in a trillion comparisons) and sufficient population discrimination for GSI applications. The software to rapidly and accurately perform PBT analyses (SNPPIT; Anderson 2010), has also now been released by Dr. Eric Anderson (research mentor).

As described, GSI relies on a baseline comprised of populations to which unknown samples may be assigned.

The current SNP baseline now contains 5926 individuals representing 41 populations from California, Oregon, Washington, Canada and Alaska. We estimate that this baseline should allow for the accurate assignment of over 99% of Chinook caught off California and Oregon to the appropriate reporting group. With the resumption of sport and commercial Chinook fisheries in California in 2010, samples were finally available to perform a proper GSI analysis of a mixed ocean fishery using the new baseline. We have also genotyped over 10000 Chinook samples collected at California ports over the last decade. These have been assigned using the new SNP GSI baseline, and are providing managers with unique insights into historical stock compositions and temporal trends.

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 2006-2010. All except the 2010 fish have now been genotyped with the our 96 SNP panel and the data entered into the parent database. Previous work suggests that this stock is dominated by 3-year old adult returners (Grover and Kormos 2006, 2007), which means that the 2009 broodstock is expected to be composed primarily of offspring from 2006 matings. Using PBT, we have indeed identified the parents of a majority of the 2009 broodstock in our 2006/2007 parent database and are in the process of validating these with recorded crosses. Genotyping is currently under way for the 2010 broodstock, for which the parents of 2, 3 and 4 year old fish should be found in the 2008, 2007 and 2006 broodstock collections, respectively. We have also identified Feather River spring-run parents for some of the fish captured in the 2010 ocean fisheries, and have verified these recoveries with coded-wire tag data. The success of these preliminary analyses in unambiguously identifying parent-offspring trios is extremely encouraging, not only in verifying the utility of the PBT method but potentially moving management efforts away from the inefficient coded-wire tag paradigm.

PROJECT MODIFICATIONS:
No significant modifications were made to the project with the exception of expanding both the number of populations and the size of each in the GSI baseline. As more collaborators transition to SNPs, the baseline will likely continue to grow, expanding its resolution and utility in other parts of the species' range.

BENEFITS AND APPLICATIONS:
PBT has numerous advantages over the coded-wire tag (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. 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.

PUBLICATIONS:
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. Abadia-Cardoso A, AJ Clemento, JC Garza. 2011. Discovery and characterization of single-nucleotide
2. polymorphisms in steelhead/rainbow trout, *Oncorhynchus mykiss*. *Molecular Ecology Resources* 11(supp.1):31-49
3. Clemento AJ, A Abadia-Cardoso, HA Starks, JC Garza. 2011. Discovery and characterization of single-nucleotide polymorphisms in Chinook salmon, *Oncorhynchus tshawytscha*. *Molecular Ecology Resources* 11(supp.1):50-66
4. Unique genetic sequences have been submitted to GenBank, an online database of publicly available DNA
5. sequences maintained by the NIH (http://www.ncbi.nlm.nih.gov/), while unique polymorphisms were added to dbSNP, a related public database which tracks known SNPs in a variety of species.
6. New SNP markers have been shared with current state/federal/tribal multi-lab collaborative efforts such as the GAPS program for Chinook (moran et al 2005), funded by the Pacific Salmon Commission.
7. The fellow outlined an integrated GSI and PBT program for California using SNPs in an oral presentation at the 6th Biennial Bay-Delta Science Conference 2010 in Sacramento, CA.
8. Some results were presented in a poster (of the same title) that won honorable mention at the 2007 State of the
9. Estuary Conference in Oakland, CA.
10. 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 in 2006.

COOPERATING ORGANIZATIONS:
NOAA Fisheries - laboratory supplies and mentoring
UC Santa Cruz
CA DFG - sample collection at-sea, in ports and at the Feather River Hatchery

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

KEYWORDS:
Chinook salmon, Central Valley, spring-run, SNP, genetics, hatchery, parentage-based tagging, PBT, genetic stock identification, GSI

PATENTS:
N/A

Additions:
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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 pp. http://www.nwfsc.noaa.gov/research/divisions/cbd/documents/gaps_year2_final.pdf

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