CALFED BAY-DELTA PROGRAM

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

FINANCIAL SUMMARY

First Year CALFED Funds Requested:	$43,125
Total CALFED Funds Requested:	$129,375
Duration:	3 Years
Proposed Start/Completion Dates:	11/1/06-10/31/09

APPROVAL SIGNATURES

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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

CALFED SCIENCE FELLOWS PROGRAM

Proposed Research

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. Utilizing new methodology for large-scale parentage inference, collection of genetic information from the 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. Subsequent sampling of fish during their seaward migration, in fisheries and upon return to spawn (either at hatcheries or instream) is followed by parentage assignment with high confidence, thereby allowing accurate pedigree reconstruction, identifying stock and cohort of origin in the process. Hankin et al. (2005) have described this novel genetic method as an alternative or complement to the current coded wire tag (CWT) program, which provides the bulk of fishery population dynamic data for salmon in the northeast Pacific. Anderson and Garza (2006) have described the statistical genetic framework for the large-scale parentage analyses the method requires. The ability to accurately identify offspring of spawning fish through parentage analyses means that a pair of parental genotypes translates into many genetic tags in the next generation and has broad potential application for population assessments of fish and other high fecundity species. In addition, the FPG method can be used to identify family groups and, therefore, to estimate heritability and eventually map genes involved in the inheritance of physical traits (e.g. size, growth, age-at-return) to their chromosomal locations. Such data can provide a predictive framework for assessing the effects of different management and conservation actions.

I propose to work collaboratively with NMFS scientists to evaluate this novel method for population assessment. I will help to develop molecular assays and then use them to identify Chinook salmon descended from broodstock at one of the largest hatcheries in California when they are captured by NMFS and state biologists during juvenile outward migration and upon return to spawn. I will evaluate the relative costs and benefits of the FPG genetic method with traditional coded wire tagging, as well as evaluate statistical power and lay the foundation for future investigation of inheritance of important life history traits.

Chinook salmon (*O. tshawytscha*) are the largest species of Pacific salmon and constitute the largest fishery. These anadromous fish home to their natal streams, which can lead to populations that are adapted to local environmental conditions. In California, this has led to separate stocks in the major river systems, and different temporal runs that spawn (at least historically) in different parts of the two largest basins, the Sacramento and the Klamath. In the ocean, stocks from different river systems, including both wild and hatchery fish, comingle, making it difficult to limit commercial harvest of at-risk or depressed populations, which can result in large scale fishery closures. The need to identify stock-specific fishery impacts led, in the 1950s, to clipping of particular fins (adipose, anal, maxillary), in an attempt to identify production from specific hatcheries or regions. By the 1970s, managing agencies had turned to the use of coded wire tags (CWTs) in juvenile fish to indicate stock and cohort of origin.

CWTs are mechanically implanted into the heads of juvenile fish. Each tag bears a unique code that identifies the release cohort and source hatchery (or stock). Tag recovery is

accomplished through identification of fish carrying a tag, followed by removal of its head for shipping to a laboratory, where it is manually extracted and read under a microscope. It is estimated that over 50 state, federal, tribal, and private entities in the U.S. and Canada tag approximately 50 million juvenile salmon and steelhead per year (about 39 million of which are Chinook). Total tagging costs exceed $7.5 million annually, while another $12-13 million is spent for tag recovery programs in U.S. and Canadian commercial and recreational fisheries (Johnson 2004). Tag recovery estimates are generally less than one percent. The resulting CWT data, though limited, is then used in a variety of stock and fishery assessment methods. For example, CWT data has been used to estimate "exploitation rates by age, maturation rates, adult equivalents, marine survival rates, total mortality" and even to infer exploitation patterns of natural stocks (Morishima 2004). However, there is frequently great uncertainty associated with these estimates, due to limited tagging and recovery rates, which calls into question the effectiveness of managing specific fisheries with such data.

Another major challenge to the continuing use of CWTs is state and federal regulations which require adipose fin clips on a majority of hatchery production (Hankin et al. 2005). Prior to 1996, only fish with CWTs were given adipose clips. This has resulted in a large number of adipose fin-clipped but untagged salmon and has "decreased the effectiveness of the current program, added costs without gaining information, increased the numbers of fish that samplers handle and mutilate[,] and decreased the value [of] these fish to retailers (Alexandersdottir *et. al* 2004)." This problem has necessitated the use of a secondary, electronic tag detection method at considerable increased cost and effort to the entire program. CWTs are also subject to loss at uncertain rates, which effectively increases the number of clipped but untagged fish.

While serving on a binational expert panel convened by the Pacific Salmon Commission (PSC) to evaluate the future of the CWT system for stock assessment of Pacific salmon, my proposed NMFS mentor, Dr. Carlos Garza, and his colleague, Dr. Eric Anderson, proposed a novel method for tagging at the indicator hatcheries that provide the CWT data used in stock assessment by the PSC. The collection of genetic information from the entire broodstock population used at these indicator hatcheries would tag all of their offspring, with tags recovered through genetic parentage analysis. This method, which they termed full parental genotyping (FPG), is fully outlined in a chapter of the final Expert Panel report submitted to the Pacific Salmon Commission (Hankin et al. 2005) and its validation in experiments with Chinook salmon is one of the panel's recommendations (No. 13).

In short, an FPG experiment begins with the genotyping of the parental broodstock generation at a hatchery (or a weir). This information is then included in a "parent" database. While no additional information is required, addition of the date of spawning for each fish reduces the amount of genetic data and computation time necessary for tag recovery, by dramatically limiting the number of possible parental pairs to evaluate (Anderson and Garza 2006). Inclusion of other physical characteristics of each spawner, such as age-at-reproduction, length and weight, allows eventual estimation of the stock-specific heritability of these traits. The offspring of these fish are then sampled in subsequent years during migration, in fisheries, or when they return to spawn, and their genotypes compared to those in the parent database. Anderson and Garza (2006) describe a likelihood-based method by which the exact parents of any sampled fish can be identified with 60 to 100 SNP markers, as long as their genotypes are contained in the parent database. Importantly, they showed that the number of parental pairs that can be analyzed increases exponentially with the number of SNP markers, so a large increase in the number of potential parents in the database requires only a few additional markers to achieve

the same statistical power. Because of this, and also that identification of the parent pair for a recovered fish also identifies its stock and cohort of origin, this method provides the same information provided by a coded wire tag system and is appropriate for coastwide implementation in stock assessment.

In addition, an FPG program would have considerable collateral benefits. From the final expert panel report (Hankin et al. 2005):

- The per-fish tagging cost of FPG would be considerably lower than that of the CWT program, and a much higher proportion of adipose-clipped production could be tagged; All juvenile production from FPG hatcheries would be tagged.
- The higher proportion of tagged fish may eliminate the need for a secondary, electronic tag detection method in sampling of harvest or in escapement.
- Heads of recovered fish need not be removed; only a small piece of fin must be collected and sent for genetic analysis.
- The parent database could easily be integrated into a genetic stock identification (GSI) database that shares molecular markers, allowing both adipose-clipped, but non-FPG, and naturally spawned fish to be identified to stock, and sometimes cohort, of origin. Such an integrated FPG and GSI system would provide information about stock of origin for every sampled fish, marked or unmarked.

FPG thus has numerous advantages over the CWT program currently in place on the West Coast. From a practical standpoint, collection of DNA from returning adults in the terminal fishery 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. Moreover, only a small fin clip needs be taken, so that an FPG tag could potentially be recovered and the fish released alive. Additionally, juvenile fish are more susceptible to disease and stress than adult Chinook that are destined to die after spawning anyway. Tag loss, which plagues CWT to an uncertain but substantial degree (Johnson 2004), is not an issue for FPG. 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 virtually 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), FPG could also be used for genetic stock identification (GSI). 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 rates, manage individual Chinook salmon stocks and estimate ocean distribution. Finally, FPG 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 FPG methodology.

In the first year of the project, the focus will be on the optimization and selection of 60-100 SNP markers for use in FPG of Chinook salmon using published assays (e.g. Smith et al. 2005), as well as those currently being developed by a multi-laboratory collaboration funded by the PSC, of which my proposed NMFS mentor is a co-investigator. The markers will be chosen and organized into panels to provide high resolution of potentially small genetic differences between closely related groups of Chinook salmon from the Central Valley. At this time, the "parent" database will be implemented for the storage and accession of genotype data, in addition to a

statistical evaluation of the power, costs and benefits of genetic tags and CWTs for estimating population parameters. Further analysis tools may also be developed to expedite the accurate identification of parent-offspring families.

In year two of this project, the panels of SNP markers will be used to genotype the adult broodstock for fall-run Chinook salmon at the Feather River Hatchery (FRH) in the California Central Valley. The FRH contributes up to 20% of the salmon caught off of California. The California Department of Fish and Game, who runs the FRH, is excited about the potential of the new method and has agreed to work with the NMFS mentor to collect the tissue samples. Returning spawners will be bred according to hatchery protocol, the offspring reared and smolts released for seaward migration. In addition, some portion of these will receive CWTs.

NMFS Science Center biologists (P.I., Bruce MacFarlane, NMFS-Santa Cruz) already capture juvenile salmon during out-migration and early ocean entry. The first real evaluation of the FPG method will be assessed in the third year by identifying offspring of FRH fish through parentage analysis of these juvenile fish. Parentage analysis may also be performed with the age 2 fish that return to spawn at the FRH, evaluating FPG through an entire ocean migration. The subsequent returning cohort (age 3 fish) will be analyzed as time and funding permit. Ocean fishery catches for FRH fish will also be targeted, through analysis of port-sampled fish in the final two years.

Assuming that we are able to successfully reconstruct large pedigrees, this project will set the stage for future estimation of heritability of physical and life history traits in Chinook salmon, which will allow the prediction of the consequences of different hatchery protocols and fishery regimes. This is also the first step in the mapping and identification of the genes responsible for characters such as size, growth, and run-timing, which will be of great interest to both geneticists and fishery managers. As a postdoctoral scientist, I will pursue some combination of these investigations with an eye towards providing the highest quality data to fishery management agencies and councils for use in stock assessment and other biological inference.

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