Project Description
Dr. Bernie May, UC Davis

# Are 'Apparent' Sex Reversed Chinook Salmon a Symptom Of Genotoxicity?

## I. Executive Summary

The primary goal of this research project is to facilitate implementation of the CALFED Ecosystem Restoration goal for fall-run Chinook salmon with respect to the relative importance of chemical stressors on population viability and genetic diversity. We will determine the chromosomal mechanism responsible for producing phenotypic female fall-run Chinook salmon in the Central Valley that test positive for Y-chromosome specific genetic markers. Using established fish-rearing and molecular genetic protocols developed during a previous study we will perform genetic and cytogenetic analyses of offspring produced from genetically normal and 'apparent' XY-female fall-run Chinook salmon. This research project involves three tasks. First, we will harvest gametes and evaluate genotypic sex of phenotypic female and male fall-run Chinook salmon collected from the Merced River Fish Hatchery using two Y-chromosome specific markers, OtY1 and growth hormone pseudogene. By performing genetic screening of sex to determine which of the phenotypic females have a male genotype (XY-females) and which are genetically normal (XX) females, putative gamete donors will be selected for use in controlled breeding experiments. Second, we will evaluate the genetic sex of offspring produced from these crosses using the Y-chromosome markers, plus a recently published Y-chromosome marker, OtY2(WSU). Gross gonad morphology will be observed via necropsy to ascertain the phenotypic sex of offspring. In addition, whole blood will be collected from individuals to be used to create lymphocyte cultures for cytogenetic analyses. Third, the offspring produced between 'apparent' XY-female and normal male fish will be compared cytogenetically with the progeny of normal male and female fish using a previously established cytogenetic method. This will allow us to determine the chromosomal mechanism responsible for producing 'apparent' XY-female fall-run Chinook salmon. Evaluating the chromosomal changes incurred by XY-females should remove uncertainty regarding whether or not XY-female fish negatively impact population genetics and persistence and if they are a symptom of genotoxicity experienced by fall populations due to exposure to environmental contaminants.

We will use a hypothesis driven approach to reduce uncertainty regarding the negative impact that 'apparent' sex-reversed individuals have on populations of this at-risk species. We will address the following hypotheses:

1. Is the 'apparent' sex-reversed phenotype observed in fall-run Chinook salmon due to a Y- to X-chromosome/autosome translocation?

Ho: Male & female offspring of apparent XY-female fall-run Chinook salmon produce patterns of chromosome staining that are consistent with recombination of the male (Y-chromosome) specific markers to the X-chromosome/autosome.

2. Is the 'apparent sex-reversed' phenotype observed in fall-run Chinook salmon due to a Y-chromosome that has had the sex determining region deleted or inactivated?

Ho: Male & female offspring of apparent XY-female fall-run Chinook salmon produce patterns of chromosome staining that are consistent with a Y-chromosome that lacks a functional sex-determining region.

3. Does the OtY2(WSU) Y-chromosome specific marker exhibit the same pattern of inheritance as two other male-specific markers, OtY1 and the growth hormone pseudogene, in a controlled mating between an 'apparent' XY-female and a genetically normal male?

Ho: The Y-chromosome specific marker OtY2(WSU) is inherited in a manner consistent with Mendelian segregation. Specifically, does a controlled cross between a normal male (XY) and an 'apparent' XY-female produce a 1:3, female to male, genotypic sex ratio in the offspring?

The study of genetic mechanisms that alter sexual development of Central Valley Chinook salmon is within the CALFED Program goals of protecting and improving At-Risk Native Species populations and the CALFED Science Program goal of addressing uncertainties that influence management and developing performance measures. Our project will contribute information to the specific priority topic areas of life cycle models and population biology of, and relative stresses on target fish species.

We hope to provide management agencies with information regarding the impact that 'apparent' sex-reversed fish have on reproduction, population genetics, and thus population persistence of fall-run Chinook. In addition to providing quarterly reports the information produced will be disseminated to the management and scientific communities through poster and oral presentations at local and national meetings, as well as publication in peer-reviewed journals.