**Project Title: Genotype chinook salmon (adults and juveniles) to assist in characterization and maintenance of the genetic integrity of hatchery- and natural-origin winter-run chinook salmon.**

**Scope**: Genetic research and analysis in support of the U.S. Fish and Wildlife Service=s winter-run chinook captive propagation and captive broodstock program will continue at U.C. Davis= Bodega Marine Laboratory (BML)in Bodega Bay, California. This work is also expected to characterize and assist in maintaining the genetic integrity of the natural population of endangered winter-run chinook salmon. Objectives of the research and analysis include: 1) identify individual salmon adults for use in the Service=s winter-run chinook salmon captive propagation and captive brood stock programs, 2) determine genetic impacts of the program on the wild population through genetic analysis and verification and refinement of an effective population size (Ne) model, 3) genotype and identify to run origin (e.g., winter/non-winter) salmon carcasses collected in mainstem Sacramento River or Battle Creek carcass surveys for Ne validation and/or population assessments, and 4) genotype and identify to run origin juveniles collected from rotary screw trap operations at the Red Bluff Diversion Dam or in Battle Creek for Ne validation and/or population assessments.

At BML, research will continue related to the genetic characterization and identification of winter-run chinook salmon. Genetic analyses are made possible by the development of highly variable, simply inherited, microsatellite DNA markers. These markers have core DNA sequences from 2-6 nucleotides in length that may be repeated from 10 to 100 times at a particular chromosomal site. These markers are transmitted from Aboth@ parents thus providing a more detailed record of past breeding activity than mtDNA. Microsatellite DNA markers can be amplified by the polymerase chain reaction (PCR) from small tissue samples (fin clips) and rapidly typed fluorescence imaging (Hedgecock et al. 1995; Banks et al. 1996). The genotype of individual fish may be determined for a series of polymorphic DNA markers. However, different numbers and kinds of loci are needed for different tasks (see **Work to be Performed and Deliverables** Section below for specific tasks of this project). For example assignment to run can be accomplished with an existing set of 5-7 moderately polymorphic markers. Determining parentage, however, requires 3-5 or more highly polymorphic markers, and estimating average pairwise disequilibrium has an inherently high variance that can only be reduced by using as many markers as possible. Loci are still in the process of being optimized for each task.

The funding requested through this proposal will generally provide for a continuation of ongoing work associated with the winter-run chinook salmon captive propagation and captive broodstock program. However, upon completion of the above tasks, additional information critical to the recovery of the natural population of this endangered species (e.g., assisting in assessing the run size/strength, and existing genetic integrity/variability) will also be generated. Funding requested through this proposal will be applied in the year 2001 (Jan 1, 2001 through Dec 31, 2001).

This project has been highly supported in the past by all the resource agencies (USFWS, NMFS, CDFG), the California Department of Water Resources, the Bureau of Reclamation, the California Water Commission as well as the commercial and sport fishing groups (e.g., PCFFA).

**Justification for the project:**
The funding requested through this, and previous proposals, is justified as it supports the following AFRP Actions or Evaluations as described in the Anadromous Fish Restoration Program Plan (p108, May 1997):

**Central Valley-Wide Evaluation #4:**

*Evaluate and implement specific hatchery spawning protocols and genetic evaluation programs to maintain genetic diversity in hatchery and natural stocks.*

and

**Central Valley-Wide Evaluation #2:**

*Evaluate the potential to modify hatchery procedures to benefit native stocks of salmonids.*

The U.S. Fish and Wildlife Service conducts propagation and captive broodstock programs for endangered winter-run chinook salmon at the Livingston Stone National Fish Hatchery[1] located at the

[1]The winter-run chinook salmon propagation of winter-run chinook salmon was moved from Coleman National Fish Hatchery to the Livingston Stone National Fish Hatchery. U.S. Fish and Wildlife Service monitoring programs found that despite a release strategy to imprint juvenile winter-run chinook salmon reared at Coleman NFH to the mainstem Sacramento River, the resultant adults returned instead to Battle Creek. This finding resulted in the construction of the Livingston Stone National Fish Hatchery at the base of Shasta Dam in 1997. Rearing of juvenile rwinter-run chinook at this location is fully expected to result in adult hatchery-origin winter-run chinook salmon returning to the upper mainstem Sacramento River and commingling and spawning with naturally-produced winter-run adults. A task of this proposal also deals with genotyping hatchery-origin winter-run chinook salmon captured in Battle Creek (resulting from releases in previous years) to justify their relocation to the

base of Shasta Dam on Battle Creek. The program consists of collecting adult winter-run chinook salmon from the mainstem Sacramento River, holding and spawning the adults, rearing the juveniles in the hatchery environment, then releasing them back into the mainstem Sacramento River. The overriding goal of the programs is to supplement the endangered population and provide an insurance policy against extinction. The propagation program (initiated in 1989), and the captive broodstock program (initiated in 1991) are recognized in the National Marine Fisheries Service=s draft Recovery Plan for this endangered species.

As this program is designed to supplement an endangered population, attention to genetic considerations has remained a high priority since the inception of the programs, and since FY97, funding for genetic investigations has been sought and acquired through AFRP. Initial brood stock selection for the propagation program is obviously critical to the maintenance of the genetic integrity of the winter-run population. Genetic analyses of tissue samples from winter-run chinook salmon are made possible by the development of highly variable, simply inherited, marker loci. In 1996, the Service initiated a self imposed moratorium on the collection of naturally-produced adults from the Sacramento River. This was done in light of genetic integrity concerns of the captured adults and the imprinting problem (see footnote 1). A separate stock discrimination project supported with funding from the California Department of Water Resources, allowed accumulation micro-satellite-DNA allele frequency data for the four chinook salmon spawning stocks in the Sacramento River. The stock discrimination project, in conjunction with work funded through the winter-run chinook salmon captive broodstock program (1991 - 1997) and AFRP (1997 - 2000) resulted in the development of a set of DNA markers highly useful to discriminate winter-run chinook salmon from other runs of salmon. Additionally, AFRP funding supported investigations of the heritability and evidence of non-linkage of the most discriminatory loci. These investigations provide definitive support in justifying the discriminatory capability of the DNA marker set. The combination of genetic data generated by the winter-run chinook salmon captive propagation/captive brood stock programs and from the stock discrimination project, is now be used to confirm run-identity of the broodstock collected for the propagation program and parentage of any offspring subsequently produced.

A model to assess the genetic impact of the winter chinook salmon propagation program was presented in the Service's 1993 *Biological Assessment on the Effects of Coleman National Fish Hatchery Operations on Winter-Run Chinook Salmon* (USFWS 1993) and formalized by Hedrick et al. (1995). The model estimates the genetic impact of the hatchery program on the wild population by examining the effective population sizes of and the estimated proportion of juveniles produced by the hatchery and wild population. Genetic analysis of tissue collected from naturally-produced winter-run chinook salmon (live adults, carcasses of adults and juveniles) may allow a more precise estimate of the effective population size, strengthening the predictive ability of the model. The effective population size

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mainstem Sacramento River.

and the number of juveniles produced in the hatchery program can be fairly precisely measured or calculated. However, the model uses an assumed value of 25% of the estimated run-size to derive an estimate of the effective population size. Based on the genetic analysis of tissue samples collected from known winter chinook salmon juveniles in the upper Sacramento River, various techniques such as the use of linkage disequilibrium data (see Bartley et al. 1992), or temporal methods (e.g., Waples and Teel 1990) can be used to produce actual estimates of the effective population size for the wild/natural winter chinook salmon population. Genetic analysis of tissue for run identity purposes can also be used on juveniles and the adult carcass samples to refine run-size estimates generated in adult carcass and juvenile monitoring surveys in the mainstem Sacramento River and in Battle Creek.

As previously stated, genetic maintenance of this endangered population is critical to future recovery of this species. Collation of all information resulting from activities to maintain the genetic variation within this population will likely become the basis for the development of a Genetic Management Plan for winter-run chinook salmon. Information provided through the funding of this proposal will continue to provide information on the genetic integrity of the winter-run chinook salmon population. Additionally, the information will allow assessment of the effect of the hatchery program on the potential recovery of the species. Continued research, applied genetic analysis and general genetic guidance in this area is critical to the overall success of the program and the genetic resources of the species.

**Monitoring and data evaluation:**

Tissue samples from a variety of monitoring and trapping programs (Keswick Dam and Red Bluff Diversion Dam adult collection, mainstem and Battle Creek carcass surveys, Battle Creek rotary screw traps, Red Bluff Diversion Dam rotary screw traps) will be submitted to BML for genetic analysis. The results of the genetic analysis increases the effectiveness of the restoration activity supported in this proposal through genetic validation of selected broodstock and evaluation of potential genetic impacts. Additionally, the proposed work continues to improve the scientific understanding of the endangered population of winter-run chinook salmon through identification and maintenance of the genetic integrity of the population, and assists in providing information useful in the estimation of population strength/status (i.e., run-size).

**Work to be performed and deliverables:**

| Task | Milestone | Description |
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| Task 1 | Ongoing | Continue dialog between the Service and the Bodega Marine Laboratory=s Genetics lab through direct contact and through the Genetic Project Work Team, Winter-Run Chinook Salmon Captive Brood stock Committee, and the Technook and Genook sub-groups. Provide status reports on completed work and research advances during |

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| | | PWT and committee meetings.<br>. |
| Task 2 | Jan 1, 2001 - August 2001 | Genotype adult salmon (including hatchery-origin fish) captured in 2001 in the mainstem Sacramento River (Keswick Dam or Red Bluff Diversion Dam) for potential use in the artificial propagation program and to determine the parentage of hatchery-origin fish for verifying Effective Population Size (Ne) predictions. (ARapid Response@ genetic analysis). Approximate number of samples to analyze = 200 at 5 microsatellite loci, or 7 loci if 0 < LOD < 2. Provide written and electronic copies of the results of the analyses |
| Task 3 | March 2001 - June 2001 | Genotype natural and hatchery-origin salmon collected in Battle Creek trapping operations in 2001 (at the Coleman National Fish Hatchery Barrier Weir) to run and or family group to justify relocation of hatchery-origin fish to the mainstem Sacramento River and to determine the parentage of hatchery-origin fish or second generation hatchery-origin fish for verifying Effective Population Size (Ne) predictions.(ARapid Response@ genetic analysis). Approximate number of samples to analyze = 100 at 5 microsatellite loci, or 7 loci if 0 < LOD < 2. . Provide written and electronic copies of the results of the analyses.<br>. |
| Task 4 | May 2001 - August 2001 | Genotype all carcasses obtained in the Sacramento River in 2001 from tissue or scale samples (a) to estimate the number/proportion of winter-run collected in the mainstem carcass survey (b) to determine the parentage of hatchery-origin fish for verifying Ne predictions, and (c) and to assess temporal variation also for Ne verification. Approximate number of samples to analyze = 500. Exploration of methods to improve DNA extraction and use tissues other than fin clips, such as scales or operculum, which undergo less degradation is an identified sub task or see results of Task 5. Provide written and electronic copies of the results of the analyses |
| Task 5 | Early 2001 | Genotype all carcasses obtained in the Sacramento River in 1998 from tissue or scale samples (a) to estimate the number/proportion of winter-run collected in the mainstem carcass survey and (b) to determine the parentage of hatchery-origin fish for verifying Ne predictions and (c) and to assess temporal variation also for Ne verification. Use a blind set of scale collections to verify run call based on scale collection/analysis vs. tissue collection/analysis. Approximate number of samples to analyze = |

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| | | 640 (590 tissue samples and 40 scale samples). Exploration of methods to improve DNA extraction and use tissues other than fin clips, such as scales or operculum, which undergo less degradation is an identified sub task. Provide written and electronic copies of the results of the analyses |
| Task 6 | Dec 2001 | Develop a method to provide error estimates associated with AWHICH_RUN@ genetic calls for individual run origin assignments. |
| Task 7 | May 2001 - August 2001 | Continue to assist in the design of pedigree mating strategies for naturally-produced, collected hatchery-origin and captive broodstock winter-run chinook salmon to avoid inbreeding, ensure the genetic integrity of progeny, and avoid impacts on the wild/natural population. Provide a written copy of the recommendations |
| Task 8 | Dec 2001 | Genotype a subsample of outmigrating juveniles caught at the RBDD screw traps in 2001 (a) to verify the proportion of winter-run chinook salmon (individual ID or MSA) and (b) to estimate the effective size of the natural population from linkage disequilibrium and kinship analyses. Provide written and electronic copies of the results of the analyses. Approximate number of samples = 300. Loci required for efficient parentage analysis and estimation of average pairwise disequilibrium will be optimized to complete part b of this task. |
| Task 9 | Aug 2001 - Dec 2001 | Assist in providing recommendations for combining broodyear 2001 hatchery-origin winter-run chinook salmon progeny from different family groups. These combinations should allow for future differentiation through genetic analysis. Provide written and electronic copies of the results of the analyses. |
| Task 10 | Dec 2001 | Assist in the development and drafting of a winter-run chinook salmon genetic management plan which collates available genetic information/methodologies and technologies, documents the current genetic variance present in current and baseline winter-run chinook salmon population to document the genetic integrity and include recommendations to maintain genetic variance. Written plan completed by Dec 2001. |
| Task 11 | Jan 2001 - | Assist in the refinement/verification of effective population size models |

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| | Dec 2001 | through genetic analyses (e.g., temporal method, disequilibrium data). ($N_e$) to monitor potential genetic impacts of the artificial propagation program on the natural population. (This task will require information from tasks 1,2,3 and 5, as well as work completed in previous broodyears). Final report due December 2001. |
| Task 12 | Dec 31, 2001 | Provide a complete write-up of activities associated with the funded proposals and resultant cooperative agreement for all years (FY 97 through FY 2000). Written report due Dec 2001. |

**Budget:**

| Project Phase and Task | Direct Labor Hours and Salary | Benefits | Overhead Labor (General, Admin. and fee) | Service Contracts | Material and Acquisition Contracts | Misc. Costs | Totals |
|---|---|---|---|---|---|---|---|
| Complete Project (all tasks) | | | | $150,000 | | | $150,000 |

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Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1996. Research on chinook salmon structure using micro satellite DNA. Bull. Natl. Res. Inst. Aquaculture., Supplement 2:5-9

Bartley, D., M. Bagley, G. Gall, and B. Bentley. 1992. Use of disequilibrium data to estimate effective population size of hatchery and natural fish populations. Conservation Biology 6:365-375.

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Waples, R.S., and D.J. Teel. 1990. Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. Conservation Biology 4:144-156.

Also see:

U.S. Fish and Wildlife Service. 1996. Application for a permit to enhance the propagation or survival of endangered or threatened species under the Endangered Species Act of 1973. Northern Central Valley Fish and Wildlife Office, Red Bluff, California.

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