Draft Individual Review Form

Proposal number: 2001-K201-3 Short Proposal Title: Population structure of chinook salmon

1a) Are the objectives and hypotheses clearly stated?
YES, IN GENERAL – A primary goal of the proposed study is to provide a comprehensive, standardized database of allozyme and microsatellite DNA data both to assess basic population characteristics, population subdivision, and stock interrelationships and to contribute to an integrated ecosystem-level model of population structure that can be applied to recovery and restoration efforts. While this goal is clearly stated, there is no explanation of how the genetic data will be incorporated into an integrated ecosystem-level model or what the other components of this model will be. The three hypotheses to be tested [1) genetic data can be used to describe population structure at ecosystem, ESU, and local levels; 2) population structure is complex with old and recent lineages; and 3) some populations are completely reproductively isolated from one another while others are not] are clearly stated. However, hypothesis #1 for chinook salmon in various areas, including the Central Valley of California, has previously been tested and validated in several published biochemical/molecular genetic studies, as noted by the authors of this proposal. Thus, its inclusion as a significant experimental component of the proposed study seems questionable to me.

1b1) Does the conceptual model clearly explain the underlying basis for the proposed work?
YES – The proposal does a good job of articulating the need for the proposed genetic investigations to provide essential information about subpopulation structure and differentiation and to generate a comprehensive baseline database (of genetic and selected life history data) to support future monitoring and evaluation of proposed or potential recovery and restoration actions. *This reviewer believes that the information resulting from the proposed study is critically needed for recovery planning, monitoring, and evaluation.*

1b2) Is the approach well designed and appropriate for meeting the objectives of the project?
YES – However, the descriptions of the approach in the proposal and the listings in Table 1 are vague on the issue of whether or not the tissue samples (or isolated DNA from them) used in past DNA studies and in the "pilot" allozyme study (1998 & 1999 collections; N = 1,850 samples) are available to the investigators and whether or not they will be analyzed for the microsatellite DNA loci to be screened in the proposed study. This is an important point because, collectively, they represent a substantial sampling effort and would be a significant portion of the fish that should be included in the study. If these will not be included in the proposed analysis of microsatellite DNA variation, why not? The proposed study design to include both allozyme and microsatellite DNA data is a definite and significant strength of the proposal. The allozyme data will be informative on their own, and can be integrated directly into existing, extensive datasets for both California and the entire west coast (from California to Alaska) both to enhance the understanding of population structure and evolution of chinook salmon and to analyze and manage West Coast chinook mixed-stock fishery harvests. The inclusion of microsatellite DNA data will broaden and strengthen the analysis by providing a second, independent dataset with which to assess population structure and interrelationships and, as pointed out in the proposal, by furnishing data that will likely be more powerful and informative for assessing population differentiation on a microgeographic scale and/or due to evolutionarily recent reproductive isolation.

1c1) Has the applicant justified the selection of research, pilot or demonstration project, or a full-scale implementation project?
YES – This is clearly a research project intended to provide baseline information needed for assessing existing population structure.

1c2) Is the project likely to generate information that can be used to inform future decision making?
YES – I expect the project to generate invaluable data to support future decision making with regard to the design, implementation, and evaluation of recovery actions.

2a) Are the monitoring and information assessment plans adequate to assess the outcome of the project?
N/A – Monitoring is not a relevant component of this research project. The proposed study appears to be well designed. Past chinook allozyme studies by D. Teel and his colleagues at the NMFS-Seattle laboratory have been characterized by a high standard of quality assurance/quality control so that the allozyme data resulting from the proposed study can be expected to be accurate and precise. I don't know about the microsatellite DNA analysis team's past performance so I am unable to comment on the expected quality of the DNA data resulting from this study (but I assume that project direction by Dr. Garza and adequate oversight of the project will assure a high standard).

2b) Are data collection, data management, data analysis, and reporting plans well-described, scientifically sound and adequate to meet the proposed objectives?
GENERALLY YES – The methods for otolith analysis seem adequate, although there is no explicit description of how the identification of hatchery-origin fish will be done or what criteria will be used to confirm the run timing of each fish or verify smolt outmigration timing.

The methods for allozyme data collection are now well established for chinook and the investigators are experienced and skilled in applying them. The proposed methods for allozyme data analysis are generally good, but should include tests of Hardy-Weinberg equilibrium and linkage disequilibrium. However, I doubt that the proposed analysis of allozyme data to estimate levels of gene flow, effective population sizes, and times of divergence will be accurate, precise or very informative. Estimates of gene flow from genetic data are likely to be inaccurate and misleading (see: Whitlock M.C., and D.E. McCauley. 1998. Indirect measures of gene flow and migration: F_{ST} …1 / (4Nm + 1). Heredity 82:117-125.) largely because the model they are based on (Wright's island model) has many simplifying assumptions, most of which are violated by real populations. Furthermore, using this approach (with Fst or theta) to estimate migration rates assumes that the observed patterns of allele frequencies among populations are due entirely to gene flow, an unlikely circumstance. Thus, I doubt that the proposed use of Weir's methods will yield useful estimates of gene flow. The estimation of effective population sizes from allozyme data often yields values whose standard errors include infinity and/or sometimes include zero. Finally, allozyme loci accumulate mutations at a relatively slow rate so that they are often not very useful in estimating times of divergence at the level of populations.

There is no description of the proposed microsatellite DNA data collection methods beyond the statement that at least 25 loci will be screened. The proposal to include all loci previously screened by Banks et al. and Nielsen et al. in the proposed study seems somewhat naïve given that even the Bodega Bay group (Banks, Hedgecock et al.) that developed the Ots-1 series of microsatellite primers no longer screens several of these loci because of technical problems with some of them or the limited variation observed at others. These same investigators are in the process of developing a number of new tetranucleotide chinook microsatellite DNA markers (M. Banks, UC Davis, Bodega Bay Laboratory, personal communication) that the DNA investigators in the present proposal should seek to obtain and use in order to have greater consistency between their data and the data being generated for California chinook populations by the Bodega Bay group. *The present proposal should contain an explicit description of the microsatellite DNA data collection equipment and methods to be used* (e.g., automated sequencer vs. other apparatus; in-lane size standards vs. another approach for estimating allele sizes; software and/or methods to be used in allele calling; etc.). [Some inferences about equipment and methods can be drawn from the list of existing equipment in the Cost Sharing section of the supporting documentation but that shouldn't be necessary.]. The proposed statistical analyses of the microsatellite DNA data seem to be robust and appropriate. A particular strength is the proposed population-wide immigrant assignment tests to identify hybrids between hatchery and naturally spawned fish and to identify strays. This analysis could provide very useful insights into population integrity vs. mixing. However, for this analysis to have any value, the baseline data set must be complete, with representation of all existing stocks in the region – will the proposed sampling and microsatellite DNA analysis yield such a complete database of spawning populations? Note that the proposed estimation of levels of gene flow (and possibly the estimation of effective population sizes) using microsatellite DNA data will likely be limited or compromised by some of the same factors discussed above for allozyme data, depending on what models and statistical approaches are used.

Also, a major strength of the allozyme portion of the proposed study is the previously achieved level of interlab standardization in locus coverage and allele identification that will allow data collected in the proposed study to be incorporated with data from other studies and laboratories to allow more comprehensive and extensive use of the data. The proposed microsatellite DNA data collection activities suffer from an apparent absence of an intent to seek/achieve a similar level of interlab standardization (at least with the Bodega Bay group) in terms of locus coverage and allele calling. *I see this as a critical shortcoming of the proposed study that should be corrected by inclusion of provisions to pursue/achieve such interlab standardization prior to approval of this proposal.*

3) Is the proposed work likely to be technically feasible?
YES – The otolith, allozyme, and microsatellite DNA approaches have all been successfully used in other studies of various salmonid fishes to address the same issues targeted in the present proposal. There is no reason to expect technical limitations to any of these approaches, especially with regard to the primary objective of defining subpopulation structure and stock interrelationships. But, note the concerns regarding the estimation of certain parameters (e.g., levels of gene flow and effective population sizes) raised in the previous section.

4) Is the proposed project team qualified to efficiently and effectively implement the proposed project?
YES & ?? – The allozyme team has an extensive and strong record of conducting studies such as that proposed here and of managing a large database of chinook salmon allozyme data. I am unfamiliar with the microsatellite DNA team's abilities and experience and do not see much in the citations included in this proposal (only one cited publication and that one is 'in press') to base a judgement regarding their qualifications on. This (microsatellite DNA) is a theoretically and technically demanding area of investigation and it is important that the investigators have the knowledge and skills necessary to conduct this study successfully. It is too bad that more detailed CVs or at least lists of relevant publications and/or similar studies successfully completed by the key project staff were not included in the proposal or supporting materials to allow a more informed assessment of personnel qualifications. Similarly, I am unable to comment on the skills and abilities of the otolith analysis team

Miscellaneous comments
Proposed Budget: The field collecting portion of the budget requests $30,000 for the purchase of three ultrafreezers. I am skeptical that successful completion of the proposed study will require the purchase of three ultrafreezers. I think the proposal should include justification for these items. Why are three ultrafreezers needed? Can samples be stored in large coolers with dry ice or in standard freezers for short periods in the field and then transported (weekly or every two weeks?) to a central ultrafreezer? Can ultrafreezers or ultrafreezer space be borrowed from other projects or leased?

The proposal states that approximately 350 chinook (Task 4, p.5) will be analyzed for allozymes each year and that a comprehensive data set including the results of relevant previous studies (incl. the 1998 & 1999 collections being analyzed by NMFS staff now) will be assembled and analyzed. The budget identifies service contract direct costs (excluding the 18.54% CA overhead rate or the 20% Federal overhead) for the allozyme analysis of $136,395, for supplies and expenses of $30,000, and for report generation of $20,394; for a total of $186,788 for the allozyme portion of the entire study (not including an additional $7,560 for travel) (Table 3a). If one assumes an actual cost of $60/fish to collect allozyme data for 70 loci in chinook salmon (for personnel and supplies & expenses) the total for this aspect of the study would be only $63,000 (350 fish/yr x 3 yrs x $60/fish). That leaves approximately $123,788 in the proposed budget for data management, analysis, and reporting (the difference between the $186,788 budgeted and the estimate of $63,000 to collect the allozyme data for 1,050 fish). I personally feel that this is far too much money for data analysis and reporting activities. While I support this proposed project, I believe that the portion of the budget for allozyme activities is significantly inflated. ***I think that a total of $120,000 for all aspects of the allozyme activities would be more than adequate to cover all real costs for the proposed work.***

The microsatellite DNA portion of the study requests $69,206 to purchase analytical equipment in year one. This is a large enough item that I think it should be described and justification for its purchase provided.

Overall Evaluation Summary Rating	Provide a brief explanation of your summary rating
☐ Excellent ■ Very Good ☐ Good ☐ Fair ☐ Poor	The information on genetic aspects of population structure and stock interrelationships (and possibly on migration rates [levels of straying] and effective population sizes) that will be provided by the proposed study will be of critical importance for planning, monitoring, and evaluating recovery actions for chinook salmon. The combination of both allozyme and microsatellite DNA data should be particularly informative and useful in this regard. This design represents a combination of a well established approach (allozymes) with an accompanying body of existing data that provides a perspective for interpretation and an opportunity for additional applications outside the proposed area and a state-of-the-art approach (microsatellite DNA) that promises increased resolution and power (including the likely ability to identify strays and assign them to their population of origin). While this is a large-scale, intensive study, I believe that the budget is unjustifiably large and that it should be reduced in at least two areas, and justification provided in another.