



Independent Scientific Review Panel
for the Northwest Power & Conservation Council
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Memorandum (ISRP 2010-11)

April 22, 2010

To: Bruce Measure, Chair, Northwest Power and Conservation Council

From: Eric Loudenslager, ISRP Chair

Subject: Final Review of CRITFC's Accord Proposal, Genetic stock structure, relative productivity and migration (gene flow) of white sturgeon among Bonneville, The Dalles, John Day and McNary reservoirs in the lower mid-Columbia River region (#2008-504-00)

Background

At the Council's October 20, 2009 request, the ISRP began a review of the Columbia River Inter-Tribal Fish Commission's Accord proposal *Genetic stock structure, relative productivity and migration (gene flow) of white sturgeon among Bonneville, The Dalles, John Day and McNary reservoirs in the lower mid-Columbia River region* (#2008-504-00). This project will use genetic markers to evaluate population structure of white sturgeon in the lower Columbia River and upstream of Bonneville Dam. The specific objectives are to 1) evaluate population differentiation and migration (gene flow) among reservoirs, 2) determine relatedness, mean productivity, and number of spawners within each reservoir, and 3) characterize broodstock including identifying origins (reservoir or population) and degree of relatedness among candidate broodstock fish for use in a potential restoration and enhancement facility. These three objectives are intended to address needs for determining genetic diversity, relative broodstock abundance, distribution and movement, and supplementation efforts.

On December 10, 2009, the ISRP released a review of the original project proposal, requested a response on eight specific items, and emphasized that the Sturgeon Strategic and Hatchery Master Plan document that is currently being prepared should guide this project ([ISRP 2009-52](#); attached). On March 19, 2010, the Council sent us CRITFC's response and requested our review. Our final review follows below.

ISRP Recommendation

Meets Scientific Review Criteria (In Part)

The first part of Objective 1 meets scientific review criteria. That part of Objective 1 is to describe the population differentiation of white sturgeon among Bonneville, The Dalles, John Day, and McNary reservoirs. If the results from implementing the first part of Objective 1

indicate distinct differences in populations among reservoirs, then the second part of Objective 1 would be justified – evaluate the migration (gene flow) among the reservoirs.

The ISRP appreciates the efforts in the response summary document to each of the ISRP's requests for more information, and many of the responses were adequate. However, the analytical study designs and proposed sample sizes need more detail and justification (see below). Therefore, after Objective 1 is complete, the proponent should develop a revised proposal for the ISRP to review that includes fully developed designs for the additional Objectives 2 and 3, which are contingent upon finding distinct genetic differentiation among populations in the four reservoirs.

ISRP Comments

The proponents addressed many of the ISRP's comments/questions with adequate responses (listed by comment numbers and letters):

(1) They provided (a) an annotated list of references describing the recent results of genetic assessments in the Basin, (b) a discussion of why the microsatellite DNA was chosen as a preferred technique, and (c) an honest discussion of potential outcomes of analysis based on amount of gene flow among reservoirs;

(2) Some details were provided regarding the relationships and coordination of this proposed project with other ongoing white sturgeon projects in the Basin;

(3) Information was provided giving the initial steps of DNA amplification and confirmation that sufficient genetic variation can be detected (as compatible results at both UC Davis and CRITFIC labs);

(8) General discussions were given for each of the Objectives to indicate various outcomes of project results which may show benefits to white sturgeon populations in the Basin.

However, in regards to ISRP comments (1e), (4), (5), (6), and (7), the proponents failed to provide adequate justifications for sample designs, sample sizes, methods of analysis and interpretation and management application of the data. For example, for ISRP comment "(4). For both Objectives (1) and (2), provide a better description of the samples available for analysis and provide evidence that the sampling of fish is consistent with a robust analysis of the genotypic data." The proponent response was:

"Samples will be gathered from sport and Tribal fisheries at catch reporting stations, and during gill net surveys conducted by, and in cooperation with WDFW and ODFW managers. We will be evaluating up to n=1000 samples across four impoundments per year. Surplus samples will be gathered and archived for future use when possible, from all additional fish interrogations during field seasons. Samples will include young-of-the-year, age-1+ juveniles, and adult fish when encountered. These numbers are consistent with those necessary for a robust analysis, and are

adequate in comparison to many genetic population structure analyses across fish species.”

We really can't tell what the sample size would be “up to n=1000 samples” and why and how that number was arrived at. The response also indicates that “These numbers are consistent with those necessary for a robust analysis...” how do we determine this? A reasonable way to arrive at realistic sample size per reservoir would be to use recent sampling, monitoring, and abundance data collected by WDFW, ODFW, USGS, or CRITFIC in their various research and monitoring efforts of white sturgeon in the four lower Columbia River reservoirs.

Specific information is needed on the methods, interpretation, and application of the relatedness and parentage analysis. It seems to the ISRP that extending the sturgeon genetics investigations into this realm is primarily to serve the management of artificial production programs. The consideration of artificial production and any design should follow evaluation of population structure of the sturgeon population using genetic and traditional methods. It is not clear yet how differentiation might be interpreted by managers and applied to an artificial production program design. The evaluation of migration might not only involve a clearer definition of the sampling design but a comprehensive review of existing tagging and recovery data. The ISRP has not been informed on how thoroughly the conventional tagging data has been assembled or even how much there is.

Parentage analysis in populations where the adult population is only partially sampled, where parents may have contributed to production over many years, and where individuals in the population are related present analytical challenges that are not fully explained in the proposal.

Attachment:

Memorandum (ISRP 2009-52)

December 10, 2009

To: Tony Grover, Fish and Wildlife Division Director, Northwest Power and Conservation Council

From: Eric Loudenslager, ISRP Chair

Subject: Response Request for Accord Proposal, Genetic stock structure, relative productivity and migration (gene flow) of white sturgeon among Bonneville, The Dalles, John Day and McNary reservoirs in the lower mid-Columbia River region (#2008-504-00)

At the Council's October 20 request, the ISRP reviewed the Columbia River Inter-Tribal Fish Commission's Accord proposal *Genetic stock structure, relative productivity and migration (gene flow) of white sturgeon among Bonneville, The Dalles, John Day and McNary reservoirs in the lower mid-Columbia River region* (#2008-504-00). This project will use genetic markers to evaluate population structure of white sturgeon in the lower Columbia River and upstream of Bonneville Dam. The specific objectives are to 1) evaluate population differentiation and migration (gene flow) among reservoirs, 2) determine relatedness, mean productivity, and number of spawners within each reservoir, and 3) characterize broodstock including identifying origins (reservoir or population) and degree of relatedness among candidate broodstock fish for use in a potential restoration and enhancement facility. These three objectives intend to address needs for determining genetic diversity, relative broodstock abundance, distribution and movement, and supplementation efforts. The ISRP's review follows below.

ISRP Recommendation

Response Requested

ISRP Overall Comments

An adequate response will address several ISRP recommendations found in Sections 1 and 2 and listed below.

1. Provide a more robust technical justification for the proposed genetic analyses, including: (a) a review of past and ongoing genetic assessments of white sturgeon in the Columbia River Basin, as well as relevant studies in other rivers outside the Basin, (b) justification for use of microsatellite DNA versus other techniques (SNPs, mitochondrial DNA), (c) an evaluation of potential pitfalls in meaningful interpretation of the results, for example, if migration of individuals interferes with distinguishing fish from specific reservoirs, (d) the rationale for analysis of fish of different ages, and (e) a discussion of the sample sizes required to make

interpretations under various assumptions about the breeding population sizes in particular reservoirs.

2. Provide specific details on the relations and coordination between the proposed project and other white sturgeon projects in the Basin: (a) #1986-050-000 (types of data collected, number of suitable fish, and how data for the proponent's Objective (2) will complement the current effort); (b) the Yakima Sturgeon Management Project (# 2008-455-00), and (c) other projects (e.g. Kootenai Tribe's genetics work) or additional avenues available to collect sturgeon samples.
3. Include the necessary first step that is missing from the proposal, that is, optimizing the amplification of DNA, genotyping the microsatellite DNA in Columbia River white sturgeon, and confirming that sufficient genetic variation can be detected.
4. For both Objectives (1) and (2), provide a better description of the samples available for analysis and provide evidence that the sampling of fish is consistent with a robust analysis of the genotypic data.
5. For Objective (1), clarify how sampling of various fish will provide complete coverage of potential contributing populations and that the approach to the analysis will be able to sort out migrant individuals.
6. For Objective (2), the experimental design (parentage analysis) and statistical analysis (relatedness analysis) appear to be for two different objectives. Provide an experimental design and statistical analysis for both. Discuss potential limitations of the proposed designs and analyses, for example, in the case that analyses are based only on young-of-the-year genotypes.
7. Provide justification that proposed sample sizes (up to 1000 fish per year for ten years) will be sufficient to yield useful results and interpretations of results.
8. To each of the objectives, add a sufficiently detailed description of potential outcomes of uses of project data that will result in measurable benefits to Columbia Basin fish and wildlife, more specifically white sturgeon.

This project has a reasonable likelihood of aiding management and conservation of white sturgeon in these lower Columbia River reservoirs. The Sturgeon Strategic and Hatchery Master Plan document that is currently being prepared should guide this project. It is somewhat surprising that the proponents did not make note of the sturgeon workshop conducted under the auspices of Project #2007-155-00, scheduled to take place the first week of December 2009. The work conducted for the current project needs to support the Master Plan and the analysis conducted under this MOA should reflect uncertainties that are documented in the Master Plan. Once the Master Plan is completed and preliminary data are available on genetic diversity of sturgeon in the mid-Columbia, more robust experimental designs for both fish collections and data analysis should be developed and peer reviewed.

ISRP Specific Comments:

1. Technical Justification, Program Significance and Consistency, and Project Relationships (sections B-D)

Technical Justification: White sturgeon populations are depleted in lower Columbia River reservoirs, and a white sturgeon Master Plan that may include artificial production for population restoration and harvest is being contemplated (MOA Project #2007-155-00). Genetic information is scientifically defensible for use in contemporary fishery management to understand stock dynamics, life histories, population structure, and in the design of mitigation and restoration actions. The proposal anticipates using microsatellite DNA variation to study features of the sturgeon populations in lower mainstem Columbia River reservoirs. The type of DNA variation to be studied and the framework for analysis reflects standard practices in the field of fishery genetics. However, the technical justification needs additional details in several areas including:

1. discussion of past and ongoing genetic assessments of sturgeon,
2. justification for the use of microsatellite DNA versus other techniques, for example, Smith et al. (2002; J. Appl. Ichthyol.18:307-312) discusses the complexity of the species' nuclear genome and found that mitochondrial DNA provided greater resolution and inferential power (unambiguous inheritance pattern) for describing population structure than microsatellite DNA,
3. a description of potential pitfalls in meaningful interpretation if migration of individuals interferes with distinguishing fish from specific reservoirs,
4. the rationale for analysis of fish of different ages, and
5. a discussion of the sample sizes required to make interpretations under various assumptions on the breeding population sizes in particular reservoirs.

Program Significance and Consistency: The proposal does not specifically describe how this project relates to a specific regional program (e.g. Fish and Wildlife Program, Subbasin plan, or Master Plan) and this should be described. White sturgeon is recognized as a focal species in the Lower Mid-Columbia Mainstem Subbasin Plan but the proponents did not identify this. The data collected by this project will support management consistent with the Fish and Wildlife Program. No mention was provided about whether the hydrosystem BiOp or Lower Mid-Columbia Mainstem subbasin plan called for collecting this type of information to design management actions, establish the status of the species, or monitor management actions.

Project Relationships: The proposal only describes the relationships in general terms, to existing project #1986-050-000 and the Sturgeon Strategic and Hatchery Master Plan (# 2007-155-00), which are still in development/review. A more detailed description of how this project will complement those projects is needed. Specifically, the types of data that are being collected by #1986-050-000 should be provided with an indication of how data for objective (#2) will complement the current effort. The proposal should also include descriptions of how this project relates to and will be coordinated with the Yakima Sturgeon Management Project (# 2008-455-00), which also includes genetic work. In addition, there is the potential that sturgeon from river segments above the three reservoirs of interest (The Dalles, John Day, and McNary) may be contributing juveniles to this river reach. How this will be sorted out is not discussed. It is also not clear how many suitable fish are captured by project #1986-050-00 and what additional avenues are available to collect sturgeon samples.

The ISRP recognizes the great importance that the stock situation of sturgeon in the Columbia Basin be understood. Specifically, how many stocks are distinct and thus to be conserved? Are there 2, 5, or 10 stocks? The past DNA research conducted in the Basin did not adequately

provide answers. So the use of the microsatellite approach is welcomed as an important and hopefully effective method in answering this and other stock questions in the basin. However, the ISRP does not understand the relationship between ongoing sturgeon microsatellite work conducted out of UC Davis for the Kootenai Tribe and this proposed research to be conducted by CRITFC out of the Hagerman facility. That is, it was not immediately apparent how this proposed research would be necessary or complement, rather than duplicate, ongoing research. It would be useful if the proponents further clarified the relationship between ongoing work at UC Davis and this proposed research.

2. Objectives, Work Elements, and Methods (section F)

The three primary objectives are appropriate and worthwhile: (1) Describe population differentiation and gene flow among reservoirs; (2) Estimate relatedness and effective population size with each reservoir; and (3) Implement broodstock characterization – origins and relatedness.

However, it seems that a necessary first step is missing – optimizing the amplification of DNA, genotyping the microsatellite DNA in Columbia River white sturgeon, and confirming that sufficient genetic variation can be detected.

In addition, for Objectives (1) and (2), there is a need to better describe the samples available for analysis and provide evidence that the sampling of fish is consistent with a robust analysis of the genotypic data. For Objective (1), proponents anticipate using STRUCTURE to evaluate migration. It is not clear that the sampling of various fish will provide complete coverage of potential contributing populations and that the approach to the analysis will be able to sort out migrant individuals.

For Objective (2), relatedness of individuals and estimates of effective population size, the full scope of the analysis is not clear. In the “experimental design” on page 7 the proposal states: *“Genotypic data will be recorded (as genetic tags) in a sturgeon database in order to track reproductive success through parentage analyses in future years, and to detect variation in levels of inbreeding and relatedness. Parentage will be based on full exclusion.”*

In the “statistical analysis” on page 7 the proposal states:

“Relatedness of individuals will be evaluated within- and among-year classes on an annual basis for sample collections within each reservoir. A matrix of estimated relatedness between all pairs of juveniles will be calculated using the equation described in Lynch and Mulligan (1994) and the relationship of individuals will be displayed as a dendrogram using PHYLIP v3.77 (Felsenstein 1993) as described in Rodzen and May (2004). Relative long-term effective population sizes (N_e) among reservoirs will be estimated using the relationship of heterozygosity (H_E), mutation rate (μ), and N_e as described by Nei (1987). Both the stepwise mutation model, $N_e = \{[1/(1-H_E)]^2 - 1\}/(8\mu)$ and the infinite allele model, $N_e = H_E/4\mu(1 - H_E)$ will be evaluated for a range of μ ”.

The experimental design and statistical analysis appear to be for different objectives – the first a parentage analysis, the second a relatedness analysis. There should be an experimental design and statistical analysis for both. If only yearling age fish are obtained it seems unlikely that the project will actually be collecting progeny produced from fish genotyped in the next few years. If the analysis will be conducted based on the genotypes of young-of-the-year, without potential parents included in the analysis, putative parents will have to be constructed based on the variation in progeny genotypes. A limitation of the approach is that parents that yield no, or very few, progeny will not be represented in the analysis.

Objective (3) on broodstock characterization meets review criteria only if the sturgeon strategic and hatchery Master Plan concludes that artificial production is a reasonable recovery strategy.

This project is described mostly as a basic research project and the Fish and Wildlife Program calls for projects that result in data showing measurable benefits for fish and wildlife. A list of potential outcomes or uses of project data should be added to each of the objectives to show potential uses/application of results. Therefore, the ISRP recommends that a subsection be added (or information expanded in the Communication of Results subsections) to each of the three objectives that describes in sufficient detail how the data/results will be applied to address white sturgeon conservation and management needs useful to the fisheries managers in this region and indicates what the benefits may be for this species. For example, one critical management need is information on "stock discreteness" for now-isolated groups of sturgeon between dams.

Although the microsatellite genetics analysis *per se* cannot be expected to unequivocally provide information to determine the number of stocks, it is important to know the protocol as to how the data will be used in conjunction with any other types of data to address this question effectively in the near future. Such other types of data may include life history information, information from tagging studies, movement studies, etc. if available. Similarly, in evaluating the within-pool genetic diversity, it is important to know how that data will be interpreted to aid management decisions. Information relevant to management is needed very soon because there is considerable interest in forging ahead with hatchery-based supplementation in the basin.

Methods: Using microsatellite DNA variation for population assessment is an acceptable method. Until SNPs are available, microsatellite or possibly mitochondrial DNA would be the next best choices. The models proposed to estimate various population attributes from the genotypic data follow commonly accepted practice. One challenge is whether there is sufficient genetic variation within populations and between populations for the analysis to yield useful interpretations. A second challenge is whether the migration of juvenile individuals from upper basin reaches that are not part of the assessment will confuse and obscure relationships and migration among the mid-Columbia River reservoirs. It would be worthwhile to consider additional sampling requirements that might be necessary to examine these questions. The number of samples required for various evaluations is not discussed and this is a shortcoming. The proponents plan to use opportunistic samples from 1986-050-00 rather than initiating a new sampling regime, which might conflict with 1986-050-00. The ISRP acknowledges that this is being proposed as an effort at efficiency and coordination. However, the proposal does not document that sufficient samples will be available and this should be discussed.