

Accord Project Sponsors ISRP Response Report

Date: March 8, 2010

Project Number	200850400
Proposer	CRITFC
Project Title & Brief Description	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
Contact Name	Blaine Parker
Contact email	parb@critfc.org

ISRP Review History:

Original Narrative submission date: *October 19, 2009*

<http://www.nwcouncil.org/fw/projectselection/accord/200850400.pdf>

Date ISRP Review comments were received: *December 10, 2009*

<http://www.nwcouncil.org/library/isrp/isrp2009-52.htm>

ISRP Review results: *[Check appropriate box]*

Meets scientific criteria.

Meets scientific criteria (qualified).

Response requested

Response requested – does not meet scientific criteria.

Response to ISRP Summary: *[Please check appropriate box and respond below in: Response to ISRP Comments]*

The narrative will be revised and resubmitted by **(insert target date)**.

A response to ISRP comments is provided in this document.

Response to ISRP Comments:

ISRP Overall Comments

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,

Smith et al. (2002; J. Appl. Ichthyol.18:307-312) –Population genetic analysis of white sturgeon (*Acipenser transmontanus*) in the Fraser River. **Summary:** barriers to migration resulted in differentiation of four populations of white sturgeon.

Ireland et al. (2002; J. Appl. Ichthyol. 18:642-650) - Success of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) following release in the Kootenai River, Idaho, USA.

Rodzen and May (2002; Genome 45:1064-1076) -Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*).

Rodzen and May (2004; Aquaculture 232:165-182) -Estimation of parentage and relatedness in white sturgeon.

Jager (2005; Can. J. Fish. Aquat. Sci. 62:1733-1745) -Genetic and demographic implications of aquaculture in white sturgeon (*Acipenser transmontanus*) conservation

(b) justification for use of microsatellite DNA versus other techniques (SNPs, mitochondrial DNA):

Currently, there is insufficient information available in the area of SNP discovery and development for white sturgeon. Although mtDNA has several advantageous qualities (maternally inherited, non-recombinant), in comparison microsatellite loci have a relatively high rate of mutation, and high allelic diversity or number of observed alleles (polymorphism). The Smith et al. 2002 example is limited in scope (4 loci) compared to our proposed study, but none-the-less found microsatellites to be useful, and both correlated and concordant with mtDNA. Wirgin et al. (2002; J. Appl. Ichthyol. 18:313-319) compared mtDNA and microsatellites to evaluate Atlantic sturgeon and found microsatellites to be particularly informative, while mtDNA were somewhat limited in estimating gene flow due to monomorphism in certain populations. The possibility of pedigree analyses, if it is implemented in this monitoring and evaluation, will necessarily disqualify the application of mtDNA marker data.

(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:

Our intention is to evaluate the population genetic structure of white sturgeon inhabiting four impoundments above Bonneville Dam. If significant gene flow occurs between the impoundments (i.e. movement of adults upstream or juveniles downstream) it is unlikely we will be able to distinguish fish from specific reservoirs. If on the other hand, there are few migrants then it is likely that demographic and genetic forces (e.g. random genetic drift, inbreeding, bottlenecks) have resulted in population distinctions that will be detectable.

(d) The rationale for analysis of fish of different ages.

Rationale for fish of different ages is based upon the premise that very young fish (i.e. ages 0 to 3+ years of age) are likely produced in? the reservoir of origin and that very old fish (> 30+ years) are likely to have been in the reservoir of origin for sometime. Unless the fish is very young, it will be difficult to determine if the reservoir of capture is the reservoir of origin. Based upon analysis of marked fish, nearly all sturgeon movement out of the reservoir of origin is downstream, with very little upstream movement (North et al. 1993). Collecting large numbers of samples across the broad spectrum of sizes/ages in the four study pools reduces the risk of low sample sizes for some sizes/ages and the potential for missing migrants from upstream reservoirs.

(e) A discussion of the sample sizes required to make interpretations under various assumptions about the breeding population sizes in particular reservoirs.

Samples will be collected from several sources including routine population assessments, sampling commercial fisheries and young of year monitoring samples. The goal is to collect as many samples as possible and then sub-sample based upon numbers of fish in representative size/age classes. Because the proposed project is intricately linked with ongoing Project 1986-50-00, getting many samples is relatively easy at virtually no cost to the proposed project. Since some populations are inherently much larger than others (i.e. Bonneville Reservoir population exceeds 330K, compared to The Dalles at 130K), and in some cases many times larger, sample sizes will need to be adjusted accordingly to glean the most information from the funding available.

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.

a) As a past and present contractor with the 1986-50-00 Project CRITFC staff have been closely involved with most aspects of the project since 1994. Correspondingly, this involvement has enabled the proposed project to coordinate for the collection of samples from stock assessment activities, young of the year indexing efforts, and from commercial fisheries monitoring that will yield many hundreds of samples annually and thousands over the length of the proposed project.

b) Genetic information would be useful and will be shared with the Yakama (note spelling) Sturgeon Project (#2008-455-00) as well as any other sturgeon cooperator in the Basin. The information derived from our proposed study will be very helpful to Project 2008-455-00 because of the unique information regarding population genetics, gene flow, and uniqueness (or lack thereof) of broodstock within and between different study reservoirs.

c) With regard to complementing the Kootenai project, the proposed project reports will be made available to Kootenai staff for their review. It is unknown at this time what benefits might be realized by the Kootenai or other researchers until we have begun our initial analysis and interpretation.

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:

We optimized 14 microsatellite loci by first establishing appropriate PCR multiplex groups to amplify multiple loci in a single reaction. Estimated allelic size ranges (measured in nucleotide basepairs) for each locus were provided by A. Drauch and B. May at UC Davis. Each locus was PCR amplified over a 12°C range and with variable primer concentrations to determine locus specific optimal annealing temperatures and optimal primer proportions in multiplex reactions. Ultimately, multiplex panels consisted of 1) loci sharing an optimal annealing temperature, 2) numbers of loci accommodating a four-color fluorescent dye protocol; labeled primers detected at different wavelengths in the same reaction, and 3) when needed, addition of loci with non-overlapping allelic size ranges to existing multiplex sets; two loci with the same color label. We established three working panels: two 4-locus multiplexes and one 6-plex, for a total of 14 microsatellite loci. Four sturgeon genetic samples were provided by UC Davis and analyzed in common by both laboratories. Sizes of observed alleles were aligned between research groups, and a nucleotide base pair shift was applied at the CRITFC lab to establish a standardized allele scoring method; this procedure allows data compatibility and sharing. We observed a total of 127 alleles across 14 loci, ranging from 5 to 16 alleles per locus, among a group of 53 sturgeon test samples from the Columbia River; we believe this level of diversity and polymorphism indicates ample power to evaluate population structure among populations outlined in this proposal.

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:

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.

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.

As previously stated, sampling will be conducted from three primary strategies; stock assessment tagging/monitoring, young of year index efforts, and commercial fisheries sampling. The stock assessment sampling results in a handle of many sturgeon sizes from small young fish ~ 40 cm FL to large old adults with fork lengths that exceed 230 cm. The young of year indexing efforts focus on sturgeon less than 40 cm FL. Finally, the commercial fishery sampling provides samples from these sub adult fish that are harvested during the commercial fishery. Regarding the analysis to sort out migrant individuals see our responses in Comment 1c for a detailed synopsis regarding our approach.

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.

The experimental design and statistical analysis are in reference to the objective of evaluating relatedness, which is a measure of productivity as well as demographics, since observed full-sibling groups and a limited number of families would in turn be indicative of the number of spawners. In addition, objective 2 also describes the possibility of conducting pedigree analyses to complement or substantiate relatedness by helping to clarify any apparent interpretations of relatedness; it is likely the use of pedigree analysis will become increasingly informative in later years as the data base builds. Pedigrees may be evaluated anachronistically, where candidate parents are sampled in years subsequent to their progeny. Across inclusive years in the monitoring and evaluation it will be possible to compare pedigrees, and estimations or trends in relatedness, against any existing sample/s in the genotypic database. The projected 1000 young-of-the-year sturgeon that will be sampled annually is a number that it is likely to be in excess of the actual number available, and the balance will therefore consist of older fish. Upon implementation of the planned integrated supplementation program for white sturgeon (see objective 3), these methods will also be employed to evaluate relatedness of candidate broodstock, and to track survival and natural productivity of hatchery outplants via pedigree analyses.

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.

This number is consistent with a large body of fisheries publications in the literature documenting genetic analyses of fish populations. One could argue that an accepted standard minimum sample size is $n=50$ per population per year when conducting analyses based on microsatellite data. Moreover, in any evaluation of populations in decline or of conservation concern it is doubtful that larger numbers of samples are reasonable given that low abundance is often the impetus for the study. For our efforts, the 1000 samples (yearly) will be represented in proportion to impoundment abundances, and analyzed from across locations and age classes in order to best characterize genetic structure throughout the study area.

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.

Objective 1) Describe population differentiation and migration of sturgeon –

The potential outcome(s) regarding this objective would be the possible genetic verification of the documented downstream movement of sturgeon in the reservoirs upstream of Bonneville Dam (North et. al. 1993). A more interesting and useful outcome might be the determination through genetic analysis that upstream populations are becoming less diverse genetically via the continuous loss of recruits over time, particularly for the populations further upstream. If this is occurring and the Hatchery Master Plan is implemented, then these data would be quite useful for hatchery managers seeking to utilize the best marriage of genetics and sturgeon culture to enhance reproductively depressed populations both in number and within their genome.

Objective 2) Estimate relatedness and effective population size within each reservoir –

Results from this objective would aid managers and operation of the facility from the Hatchery Master Plan (if built) by verifying the uniqueness (or not) of sturgeon populations within the project area of this proposal. If there is very little uniqueness then the potential exists to treat multiple populations in a similar manner; conversely if there are unique characteristics found in one or more populations, then appropriate steps could be utilized to aid in preserving those characteristics. Additionally, if genetic analysis could relate effective population size about individual reservoirs, then regional managers would have an additional tool with regard to assessing the genetic strength of the different populations throughout the Basin.

Objective 3) Implement hatchery broodstock characterization: Monitor supplementation—

This objective provides hatchery managers with a tool to readily identify wild broodstock by their genetic makeup and ensure that sibs or offspring of previously spawned broodstock are not being spawned and overwhelming the current genetic frequencies in the reservoir of interest. Additionally it can be utilized as a tool for monitoring supplementation efforts, particularly when release strategies use larval and small juvenile releases, sizes that prohibit tagging.

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.

Note: The sturgeon workshop was initiated sufficiently late in 2009 that it was not possible for its inclusion into the narrative for this project. Needless to say, the results of this workshop and any future workshop(s) will integrate this project and others into the Master Planning process for Project # 2007-155-00.

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,

Smith et al. (2002; J. Appl. Ichthyol.18:307-312) –Population genetic analysis of white sturgeon (*Acipenser transmontanus*) in the Fraser River. **Summary:** barriers to migration resulted in differentiation of four populations of white sturgeon.

Ireland et al. (2002; J. Appl. Ichthyol. 18:642-650) - Success of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) following release in the Kootenai River, Idaho, USA.

Rodzen and May (2002; Genome 45:1064-1076) -Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*).

Rodzen and May (2004; Aquaculture 232:165-182) -Estimation of parentage and relatedness in white sturgeon.

Jager (2005; Can. J. Fish. Aquat. Sci. 62:1733-1745) -Genetic and demographic implications of aquaculture in white sturgeon (*Acipenser transmontanus*) conservation

Wirgin et al. (2002; J. Appl. Ichthyol. 18:313-319) Comparison of mitochondrial DNA control region sequence and microsatellite DNA analyses in estimating population structure and gene flow rates in Atlantic sturgeon *Acipenser oxyrinchus*. *Journal of Applied Ichthyology* 18:313-319.

As evident from the citations listed, genetic research is gaining ground regarding the understanding of sturgeon genetics, which being an octoploid animal has taken some additional efforts when compared with the more commonly studied diploid species. As time has progressed the attention of genetic studies has shifted focused to include studies and research regarding supplementation and aquaculture of sturgeons, both white and other species. Given the continued impacts and declines of most sturgeon species, including white sturgeon, the focus on hatcheries, aquaculture, and gene flow is appropriate and our proposed efforts will add to this body of work and its ramifications for future supplementation efforts.

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,

Currently, there is insufficient information available in the area of SNP discovery and development for white sturgeon. Although mtDNA has several advantageous qualities (maternally inherited, non-recombinant), in comparison, microsatellite loci have a relatively high rate of mutation, and high allelic diversity or number of observed alleles (polymorphism). The Smith et al. 2002 example is limited in scope (4 loci) compared to our proposed study, but none-the-less found microsatellites to be useful, and both correlated and concordant with mtDNA. Wirgin et al. (2002; J. Appl. Ichthyol. 18:313-319) compared mtDNA and microsatellites to evaluate Atlantic sturgeon and found microsatellites to be particularly informative, while mtDNA were somewhat limited in estimating gene flow due to monomorphism in certain populations. The possibility of pedigree analyses, if it is implemented in this monitoring and evaluation, will necessarily disqualify the application of mtDNA marker data.

3. a description of potential pitfalls in meaningful interpretation if migration of individuals interferes with distinguishing fish from specific reservoirs,

Our intention is to evaluate the population genetic structure of white sturgeon inhabiting four impoundments above Bonneville Dam. If significant gene flow occurs between the impoundments (i.e. movement of adults upstream or juveniles downstream) it is unlikely we will

be able to distinguish fish from specific reservoirs. If on the other hand, there are few migrants then it is likely that demographic and genetic forces (e.g. random genetic drift, inbreeding, bottlenecks) have resulted in population distinctions that will be detectable.

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 is 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.

In the past, fisheries management agencies have realized (GAPS marker standardization for Chinook salmon, SPAN standardization for steelhead trout) that researchers working on the same species, particularly in the same regions would benefit in the long run by using the same core set of markers and allele scoring protocols. This process leaves the door open for future collaborations, and data sharing, and guards against duplication of efforts. In the case of proposed work outlined here, CRITFC has benefited from the groundwork completed by UC Davis researchers to develop markers, multiplexes, PCR protocols, (though some have been modified to accommodate specific chemistries, instrumentation, etc.) and scoring methods. Standardized methodologies for white sturgeon throughout the Columbia River basin and the

northwest provide future opportunities to compare populations, and population trends (e.g. with the Kootenai). Lastly, it is believed that certain existing broodstocks in the Columbia River Basin have been influenced by fish of Sacramento River origin. Those origins can only be evaluated if both stocks have been characterized with the same markers, using identical procedures for scoring alleles.

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.

We optimized 14 microsatellite loci by first establishing appropriate PCR multiplex groups to amplify multiple loci in a single reaction. Estimated allelic size ranges (measured in nucleotide basepairs) for each locus were provided by A. Drauch and B. May at UC Davis. Each locus was PCR amplified over a 120C range and with variable primer concentrations to determine locus specific optimal annealing temperatures and optimal primer proportions in multiplex reactions. Ultimately, multiplex panels consisted of 1) loci sharing an optimal annealing temperature, 2) numbers of loci accommodating a four-color fluorescent dye protocol; labeled primers detected at different wavelengths in the same reaction, and 3) when needed, addition of loci with non-overlapping allelic size ranges to existing multiplex sets; two loci with the same color label. We established three working panels: two 4-locus multiplexes and one 6-plex, for a total of 14 microsatellite loci. Four sturgeon genetic samples were provided by UC Davis and analyzed in common by both laboratories. Sizes of observed alleles were aligned between research groups, and a nucleotide base pair shift was applied at the CRITFC lab to establish a standardized allele scoring method; this procedure allows data compatibility and sharing. We observed a total of 127 alleles across 14 loci, ranging from 5 to 16 alleles per locus, among a group of 53 sturgeon test samples from the Columbia River; we believe this level of diversity and polymorphism indicates ample power to evaluate population structure among populations outlined in this proposal.

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.

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.

This number is consistent with a large body of fisheries publications in the literature documenting genetic analyses of fish populations. One could argue that an accepted standard minimum sample size is n=50 per population per year when conducting analyses based on

microsatellite data. Moreover, in any evaluation of populations in decline or of conservation concern it is doubtful that larger numbers of samples are reasonable given that low abundance is often the impetus for the study. For our efforts, the 1000 samples (yearly) will be represented in proportion to impoundment abundances, and analyzed from across locations and age classes in order to best characterize genetic structure throughout the study area.

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.

The analysis may not sort out migrant individuals (if there do not appear to be any). It will provide an informative perspective on levels of gene flow. If there is significant population structure it is also likely the methods in STRUCTURE will identify migrant individuals or those with mixed impoundment origin (which in this scenario would be a small proportion of the sample).

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.

The experimental design and statistical analysis are in reference to the objective of evaluating relatedness, which is a measure of productivity as well as demographics, since observed full-sibling groups and a limited number of families would in turn be indicative of the number of spawners. In addition, objective 2 describes the possibility of conducting pedigree analyses to complement or substantiate relatedness by helping to clarify any apparent interpretations of relatedness; it is likely the use of pedigree analysis will become increasingly informative in later years as the data base builds. Pedigrees may be evaluated anachronistically, where candidate parents are sampled in years subsequent to their progeny. Across inclusive years in the

monitoring and evaluation it will be possible to compare pedigrees, and estimations or trends in relatedness, against any existing sample/s in the genotypic database. The projected 1000 young-of-the-year sturgeon that will be sampled annually is a number that it is likely to be in excess of the actual number available, and the balance will therefore consist of older fish. Upon implementation of the planned integrated supplementation program for white sturgeon (see objective 3), these methods will also be employed to evaluate relatedness of candidate broodstock, and to track survival and natural productivity of hatchery outplants via pedigree analyses.

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 of 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.