

Narrative

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.

Table 1. Proposal Metadata

Project Number	2008-504-00
Proposer	Columbia River Inter-Tribal Fish Commission
Short Description	Genetic Assessment of Columbia River Sturgeon
Province(s)	Middle Columbia Basin
Subbasin(s)	Middle Columbia Basin
Contact Name	Andrew Matala
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Information transfer:

A. Abstract

This project from the Fish Accords 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 address needs for determining genetic diversity, relative broodstock abundance, distribution and movement, and supplementation efforts. A panel of microsatellite (μ SAT) loci have been compiled and optimized with a locus scoring protocol adopted from researchers at the University of California at Davis (UCD; Andrea Drauch pers. comm.) for collection of standardized multilocus genotypic data. The protocol provides the opportunity for future collaboration and complementary efforts among interested research groups. Beginning with the long-term monitoring effort proposed here, establishing a data repository will allow implementation of future pedigree studies for monitoring survival of hatchery releases, and ultimately for estimating natural reproductive success of both wild and released hatchery reared sturgeon. The work related to these objectives will be completed by CRITFC and University of Idaho (UI) staff at the Hagerman Fish Culture Experiment Station. Tissue samples will be collected by staff from CRITFC, WDFW, and ODFW. Genotyping and genetic analysis will be completed by CRITFC and UI staff in Hagerman, ID. Geneticists with CRITFC have adequate expertise to complete each objective as evidenced in published peer-reviewed papers (e.g. Matala *et al.* 2008, Narum *et al.* 2007, Narum *et al.* 2008).

B. Technical and/or scientific background

RESEARCH

In this proposal, we describe our plan to conduct a thorough genetic evaluation of white sturgeon that will be a valuable component in monitoring the need for, and success of, conservation and restoration activities in upstream reservoirs, particularly The Dalles, John Day and McNary reservoirs; efforts may also be expanded to the Snake River reservoirs downstream of Lower Granite Dam. This proposed genetic evaluation complements the Sturgeon Strategic and Hatchery Master Plan Accords Project (proposal #2007-155-00). This project will be carried out as a complementary project to existing BPA Project 1986-050-00 (Evaluate Sturgeon Populations in the Lower Columbia) and its associated tasks. As such, this project will opportunistically collect genetic material produced during ongoing tasks in Project 1986-050-00 and will not influence, alter, or modify existing procedures and protocol used by those cooperators. This relationship allows this project to conduct extensive genetic analyses without the prohibitive costs of collecting the raw genetic materials from the population at large. The scientific background for each associated objective in the genetic evaluation is included below.

Population differentiation and migration (gene flow) among reservoirs

Decreased abundance and loss of productivity affects the biological viability of fish populations (McElhany *et al.* 2000). Overall viability of Columbia River white sturgeon is largely represented by populations downstream of Bonneville Dam that maintain a large migration corridor and an undisrupted diadromous life cycle. The dams of the lower mid-Columbia River region represent a significant barrier to upstream and downstream movement for white sturgeon, where passage is largely deterred by fish ladders designed for salmon migration (North *et al.* 1993, Parsley *et al.* 2007). Small numbers of effective breeders live in each section of impounded habitat, each of which sustains a population that is below carrying capacity. The small effective numbers, associated low genetic diversity, and restricted gene flow are likely to have profound effects on population distinctions among reservoirs. In other words, the combination of founder effect, genetic drift, and restricted gene flow (migration) may result in small but highly differentiated effective populations among impoundments, even with the recognized downstream movement of tagged subadult fish (North *et al.* 1993). We will use allele frequency data from a suite of 14 microsatellite loci, and standard indices including pairwise F_{st} and G-tests to analyze population homogeneity and relative levels of genetic variation, and infer movement of fish between populations or reservoirs. Bayesian methods will be used to infer proportional population membership of individuals from among reservoir collections. The multilocus genotypes collected among young-of-the-year fish sampled from each reservoir will also serve as genetic tags. Such tags can be used to directly evaluate migration by identifying genetic matches observed among subsequent recaptures of sampled fish. Some collection of genetic material from juvenile fish (age <10 years) may also be necessary for John Day and McNary reservoir populations due to the low capture numbers of young of year sturgeon from these reservoirs.

Relatedness and effective population size within each reservoir

Multilocus genotypic comparisons among young-of-the-year fish sampled from each reservoir will provide relatedness (full and half sibling group) information that can be used in turn to estimate the numbers of reproductively successful adult female sturgeon, numbers of recruits per

spawner, and relative reproductive success (productivity) among reservoirs. This can be achieved by estimating pairwise relatedness values from allele frequency data (proportion shared alleles among individuals) and using likelihood methods to infer sibship; the relationship between samples is then displayed graphically as a relatedness tree. In addition, the genetic data will be utilized to estimate effective size in each reservoir and below Bonneville Dam. Tagging methods as described in the previous section allow the possibility of future efforts to directly measure annual variability in numbers of spawners, and relative reproductive success through parentage analysis when adult fish are sampled, or as juvenile fish become sexually mature.

Broodstock characterization: Origins and Relatedness

Hatchery supplementation may provide alternative or additional restoration and conservation benefits for recovering depleted sturgeon populations (Munro *et al.* 2007). The primary goal of implementing an integrated hatchery program is to provide a demographic boost to the natural population/s (e.g. utilization of habitat that is below carrying capacity within reservoirs) and minimize genetic differences between the hatchery and natural population components, while maintaining high genetic variability within the supplementation broodstock (Mobrand *et al.* 2005). However, candidate broodstock fish will require genetic screening to confirm origin (e.g. reservoir) and relatedness, and to employ genetic tagging of all broodstocks in the lower mid-Columbia program. Providing the means to genetically identify outplanted fish will ultimately allow managers to monitor survival and abundance that is a direct result of supplementation, as well as natural productivity of hatchery fish through the use of pedigree analysis to estimate relative reproductive success of outplanted hatchery reared fish.

C. Rationale and significance to regional programs

This research proposal affects all stocks in the lower and mid-Columbia River impoundments and therefore is considered a regional application that addresses population declines throughout the Columbia River Basin above Bonneville Dam. Objectives of the genetic evaluation outlined in this proposal address several needs related to restrictions imposed by Bonneville, The Dalles, John Day and McNary barriers. These include describing genetic diversity, estimating migration and gene flow between impounded habitats, and characterizing composition of candidate hatchery broodstocks (population and family group) to help identify an appropriate stocking protocol among reservoirs. These needs have been presented in documentation that identifies the issues related to rehabilitation and monitoring of sturgeon populations above Bonneville Dam in the Columbia River Basin (Accords project #2007-155-00; Sturgeon strategic and hatchery master plan).

D. Relationships to other projects

The baseline of multilocus (14 microsatellites) data gathered during implementation of the proposed work will directly benefit both new and ongoing white sturgeon projects funded under the Fish Accords. This includes projected supplementation monitoring and the Sturgeon Strategic and Hatchery Master Plan project noted above (project #2007-155-00). Moreover, because each genotype collected (from both adult and juvenile fish) serves as a genetic “fingerprint”, all fish sampled and subsequently recaptured can be monitored for survival, movement and reproductive success in the natural environment using parentage studies, and genetic assignment tests in future years of this project. All sturgeon will be genotyped using a standardized marker scoring protocol allowing for consolidation of data contributed by other

research sources that also conduct sturgeon studies in the Columbia River Basin (i.e. staff at UCD). We have previously optimized and used this suite of microsatellite markers to characterize sturgeon broodstock of unknown origin at a Yakama Nation rearing facility that was maintaining a broodstock of unknown origin.

As noted above, this project also relates to project #1986-050-00, Evaluate Sturgeon Populations in the Lower Columbia, in that this project will opportunistically collect genetic material produced during ongoing tasks in project 1986-050-00 but will not influence, alter, or modify existing procedures and protocol used by those cooperators. This relationship allows this project to conduct extensive genetic analyses without the prohibitive costs of collecting the raw genetic materials from the population at large.

E. Project history (for ongoing projects)

The research projects in this proposal are newly funded under the Fish Accords. The newly funded projects will allow us to build upon existing baselines by adding more genetic markers (potentially SNPs) in the future, and provide more accurate evaluation of temporal genetic structure, population estimates, and parentage based tagging utility for fisheries managers.

F. Proposal biological/physical objectives, work elements, methods, and metrics

Related to all Objectives (1-3)

Work Elements:

119. Manage and Administer Projects: Project Management

This will include project administration, internal coordination, and contract development.

132. Produce (Annual) Progress Report: Submit Annual Progress Reports beginning 12/31/2009

The progress report will summarize the project objectives, hypotheses, completed and uncompleted deliverables, problems encountered, lessons learned, and long-term planning.

156. Develop RM&E Methods and Designs: Optimize a standardized suite of uSAT loci

A suite of 14 microsatellite loci has been identified for optimization of polymerase chain reaction (PCR) multiplex panels. The loci are: μ AciG2, μ AciG35, μ AciG52, μ AciG53, μ AciG110, μ AciG140 (Bork *et al.* 2008), μ Atr105, μ Atr107, μ Atr109, μ Atr1117, μ Atr1101, μ Atr1173 (Rodzen and May 2002), μ As015 (Zhu *et al.* 2005) and μ AciG43 (unpublished). Results of fragment analysis and genotype scoring will be standardized with the laboratory of Dr. Bernie May at the UCD.

157. Collect/Generate/Validate Field and Lab Data: dealing with octaploid data

Fragment analysis of polyploidy organisms such as white sturgeon requires transformation of data to accommodate the majority of analysis software that is generally used and structured for input of codominant or diploid genotypes. To allow for the presence of up to eight alleles at each locus, allele size scores will be converted into a binary system of presence (1) or absence (0), treating each allele as a unique locus.

159. Transfer/Consolidate Regionally Standardized Data: parentage database

Standardized genotypes will be managed for long-term use and collaboration. Temporal replicate or stratified data will be added annually to the existing database for use in conducting

multi-generational pedigree analyses. The data in its standardized format may be managed alongside data contributed by outside or collaborative sources, potentially allowing for extended coverage in ongoing genetic monitoring efforts.

161. Disseminate Raw/Summary Data and Results: Descriptive statistics

Compiled genotypic data including allele frequencies will be made available in annual reports and publications. Studies will also be presented at professional meetings and seminars as appropriate.

162. Analyze/Interpret Data: Analysis of μ SAT Frequencies

Allelic diversity among populations (impoundments) will be evaluated to identify locus specific resolving power and population attributes in association with the 14 locus suite. Specific data analyses and interpretation including observed private alleles, allelic richness, and gene diversity analyses will provide indication of the utility of each locus in parentage evaluations, genetic stock identification, and estimating relative abundances (effective numbers of spawners) within each reservoir. In addition, such analyses may also be useful toward identifying out-of-basin influences (presence of Sacramento River lineages) where they may occur.

183. Produce Journal Articles: Submit for Publication

As deemed appropriate by project leaders, results from studies will be submitted for publication in peer-reviewed journals.

G. Research - Methods

Objective 1) *Describe Population differentiation and migration of sturgeon among reservoirs*

Hypothesis:

- Relative population abundances in impoundments are low and migration is limited in comparison to the lower Columbia River; populations exhibit low genetic diversity and significant heterogeneity among populations as a result of restricted gene flow among reservoirs.

Experimental Design

Habitat alterations and restricted movement between reservoirs in the middle Columbia River upstream of Bonneville Dam is likely to have resulted in low genetic diversity within reservoirs and high population differentiation among them. Populations founded by a few individuals (effective breeders) are characterized by increased relatedness and relatively low allelic diversity among individuals (i.e. a bottleneck effect) in subsequent cohorts. Restricted movement of fish between impoundments leads to greater population differentiation as genetic forces such as mutation and local selection regimes operate in the absence of migration-drift equilibrium. Up to 1000 juvenile sturgeon will be sampled annually for a ten year period from the lower Columbia River and upstream pools in Bonneville, The Dalles, John Day and McNary reservoirs to evaluate genetic diversity, population differentiation, and migration. Fish will be genotyped at 14 standardized μ SAT loci. Genotypic data will be recorded in a sturgeon database in order to track temporal variation and to monitor changes or population influences resulting from proposed supplementation in subsequent inclusive years.

Quality Control

In order to verify the accurate scoring of octaploid genotypes (up to eight alleles per locus), we will test concordance of results between the data set and a random set of control samples. Control samples will be replicate DNA extractions and PCR amplifications from the original sample set. Numbers of observed mismatches (allele scores) when original and control multilocus genotypes are aligned, will provide an error rate that can be applied in subsequent likelihood based analyses (e.g. parentage assignments).

Statistical Analyses

Polyplidy μ SAT data requires conversion of alleles or base pair fragment size to a binary format (presence [1] or absence [0]) where each allele is treated as an independent dominant marker; the evaluation is similar to that for Random Amplified Polymorphic DNA (RAPD). As a result, some analyses (e.g. Hardy-Weinberg equilibrium probabilities) are necessarily precluded for this data set. The genetic analysis program GenAlEx version 6.1 (Peakall and Smouse 2006) will be used to calculate population allele frequencies, mean heterozygosity (Nei 1978) and private alleles among populations. Pairwise genetic distances will be estimated in GenAlEx following the method of Huff *et al.* (1993). The genetic distance matrix will subsequently be used to depict phylogenetic tree topology using PHYLIP version 3.68 (Felsenstein 1992) and TREEVIEW (Page 1996). To further describe population similarities and distinctions, principle coordinates analysis (PCA) will be performed (Orlaci 1978) using a standardized (converted) covariance matrix of genetic distance. Partitioning of within- and among- components of total genetic variation will be evaluated with analysis of molecular variance (AMOVA) and pairwise genetic distance (Φ_{pt}) in GenAlEx. The Φ_{pt} statistic is a Euclidean metric analogous to Wrights F_{st} , and appropriate for binary data. An exact test of population homogeneity will be performed in ARLEQUIN version 3.1 (Excoffier *et al.* 2005). Bayesian methods in the program STRUCTURE version 2 (Pritchard and Donnelly 2000) will be used to estimate the membership coefficients (\hat{Q}), or fractional membership in K inferred populations for each individual sample in each population. The STRUCTURE results will be used to infer levels of gene flow or proportional migration between reservoirs.

Communication of Results

Results from this project will be discussed with fishery managers, included in annual reports, and submitted for publication as warranted. Studies will also be presented at professional meetings and seminars as appropriate.

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

Hypothesis:

- Relatedness and effective size can be estimated from samples collected from each site.

Experimental Design

Habitat alterations and restricted movement between reservoirs in the middle Columbia River upstream of Bonneville Dam is likely to have resulted in decreased abundance of female spawners and contributed significantly to declining productivity (Beamesderfer *et al.* 1995). Up to 1000 juvenile sturgeon will be sampled annually for a ten year period from among lower Columbia River, Bonneville, The Dalles, John Day and McNary reservoirs to evaluate numbers of effective spawning females, and numbers of progeny per female by relatedness

measurements. Fish will be genotyped at 14 standardized μ SAT loci. 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, or allowing a limited number of mismatch loci among assignments that exceed an estimated confidence threshold using the program PARENTS (Rodzen et al. unpublished). All age appropriate parent-progeny comparisons will be considered across years (i.e. mature adults and all YOY).

Quality Control

In order to verify the accurate scoring of octaploid genotypes (up to eight alleles per locus), we will test concordance of results between the data set and a random set of control samples. Control samples will be replicate DNA extractions and PCR amplifications from the original sample set. Numbers of observed mismatches (allele scores) when original and control multilocus genotypes are aligned, will provide an error rate that can be applied in subsequent likelihood based analyses (e.g. parentage assignments).

Statistical Analyses

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 .

Communication of Results

Results from this project will be discussed with fishery managers, included in annual reports and submitted for publication as warranted. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 3) Implement hatchery broodstock characterization: Monitor supplementation

Hypothesis:

- Supplementation broodstocks will be genetically similar to their respective natural populations of origin, thus this project will help hatchery programs avoid mated crosses among closely related individuals, and will maintain high genetic variability within broodstock relative to naturally spawning populations.

Experimental Design

Integrated supplementation programs are comprised of broodstock that are collected from natural populations. Successful contributions to population abundance and productivity through supplementation specifically require that hatchery fish be genetically integrated into the natural population. Minimizing domestication effects and maintaining (in hatcheries) the adaptive advantages of sturgeon populations allows selective pressures in nature to shape the

genetic character of hatchery-origin fish, and benefit long-term natural population fitness (Moberg et al 2005). All groups of fish selected for enhancement and restoration broodstocks will be genotyped at 14 standardized μ SAT loci. They will be genetically characterized to estimate levels of diversity (e.g. heterozygosity), relatedness, and population homogeneity with source populations among lower Columbia River, Bonneville, The Dalles, John Day and McNary reservoirs. Genotypic data will also be recorded (as genetic tags) to monitor subsequent survival, and evaluate natural reproductive success of supplementation fish through parentage analyses.

Quality Control

In order to verify the accurate scoring of octaploid genotypes (up to eight alleles per locus), we will test concordance of results between the data set and a random set of control samples. Control samples will be replicate DNA extractions and PCR amplifications from the original sample set. Numbers of observed mismatches (allele scores) when original and control multilocus genotypes are aligned, will provide an error rate that can be applied in subsequent likelihood based analyses (e.g. parentage assignments).

Statistical Analyses

Specific analyses and analysis software for evaluating population structure, migration and gene flow, genetic diversity, relatedness, and productivity of sturgeon in hatchery programs have been described under the methods outlines for objectives one and two.

Communication of Results

Results from this project will be discussed with fishery managers, included in annual reports, and submitted for publication as warranted. Studies will also be presented at professional meetings and seminars as appropriate.

H. Facilities and equipment

Genetic analysis will be completed at the Hagerman Fish Culture Experiment Station in Hagerman, ID, operated by Columbia River Intertribal Fish Commission and University of Idaho staff. The Hagerman site houses multiple laboratories (including fish genetics, nutrition, and culture) and sufficient office space for the staff in this project. In addition to salaries, funding from this project will provide money for analysis of 1000 samples annually for a period of ten years.

I. References

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Department of Genetics, University of Washington, Box 357360, Seattle, WA 98105, USA.
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- Narum, S. R., M. Banks, T.D. Beacham, M.R. Bellinger, M.R. Campbell, J. DeKoning, A. Elz, C.M. Guthrie III, C. Kozfkay, K.M. Miller, P. Moran, R. Phillips, L.W. Seeb, C.T. Smith, K. Warheit, S.F. Young, J.C. Garza. 2008. Differentiating salmon populations at broad and fine geographic scales with microsatellites and SNPs. *Molecular Ecology* 17:3464-3477.
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J. Key personnel

The Lead Geneticist (Dr. Shawn Narum) will oversee this project and each of the objectives. Key staff for completing objectives includes a Conservation Geneticist (Andrew Matala), and Fishery Scientist (Blaine Parker). Additional field and lab technicians will be also be important to completing objectives, but are not listed individually (see budget spreadsheet). Time allocation to this project for each key staff member is below:

- Andrew Matala & Shawn Narum, 1.65 months (FTE)
- Blaine Parker, 0.4 months (FTE)

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Education

Ph.D., Natural Resources, University of Idaho, 2006
M.S., Marine Science, University of San Diego, 2000
B.S., Fishery Biology, Colorado State University, 1996

Appointment

2002-present Lead Geneticist, Columbia River Inter-Tribal Fish Commission

Selected Publications

- Narum, S. R.,** M. Banks, T.D. Beacham, M.R. Bellinger, M.R. Campbell, J. DeKoning, A. Elz, C.M. Guthrie III, C. Kozfkay, K.M. Miller, P. Moran, R. Phillips, L.W. Seeb, C.T. Smith, K. Warheit, S.F. Young, J.C. Garza. 2008. Differentiating salmon populations at broad and fine geographic scales with microsatellites and SNPs. *Molecular Ecology* 17:3464-3477.
- Narum S. R.,** D. Hatch, A. J. Talbot, P. Moran, and M. S. Powell. 2008. Conservation of iteroparous salmonids in complex mating systems. *Journal of Fish Biology* 72:45-60.
- Campbell, N. R., and S. R. **Narum.** 2008. Identification of novel SNPs in Chinook salmon and variation among life history types. *Transactions of the American Fisheries Society* 137:96-106.
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Education

B.S. in Fish and Wildlife Management, 1985, Montana State University
M.S. in Zoology 1990, Idaho State University

Appointments

1989-1991: United States Forest Service, Mt. Hood National Forest
1991-Present: Columbia River Inter-Tribal Fish Commission (White Sturgeon Program Manager 1993-Present)

Selected Publications

Author/Co-author on numerous project reports including mid-Columbia sturgeon research and mitigation project (BPA #1986-50) since 1994

Kappenman, K.M. and B.L. **Parker**. 2007. Ghost nets in the Columbia River: Methods for locating and removing derelict gill nets in a large river and an assessment of impact to white sturgeon. *North American Journal of Fisheries Management* 27:804-809.

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Education

B.S., Biology, Pacific Lutheran University, 1990
B.S., Microbiology, Washington State University, 1995
M.S., Fisheries Genetics, University of Alaska Fairbanks, 2002

Appointments

2008-present	Conservation Geneticist, CRITFC
2004-2008	Fishery Biologist/Geneticist, USFWS, Abernathy

Publications

- Matala**, A. P., A. K. Gray, J. Heifetz, and A. J. Gharrett. 2004. Population structure of Alaskan shorttraker rockfish *Sebastes borealis* inferred from microsatellite variation. *Environmental Biology of Fishes*. 69:201–210.
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