Narrative

Genetic Assessment of Columbia River Stocks

Table 1. Proposal Metadata

Project Number	2008-907-00
Proposer	Columbia River Inter-Tribal Fish Commission
Short Description	Genetic Assessment of Columbia River Stocks
Province(s)	Basinwide
Subbasin(s)	Basinwide
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Information transfer:

A. Abstract

This proposal combines four inter-related projects from the Fish & Wildlife Program Accords that address Single Nucleotide Polymorphism (SNP) Discovery, Genetic Baseline Expansion, Genetic Stock ID (GSI) to Evaluate Catch, and GSI of salmon and steelhead passing Bonneville Dam. These four projects are highly related since SNP markers are needed to complete species specific baselines, and these baselines are requisite to complete GSI. The specific objectives are 1) discover and evaluate SNP markers in salmon and steelhead; 2) expand and create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho); 3) implement GSI programs for mainstem Chinook fisheries and 4) GSI of fish passing Bonneville Dam (steelhead and Chinook). These four objectives address needs for distinguishing specific stocks, determining genetic diversity, stock specific run timing, and estimating stock composition to provide information for fisheries management and harvest. Newly discovered SNP markers may also be useful for other applications such as pedigree studies for estimating reproductive success, and evaluating adaptive divergence of populations to specific environments. The work related to objectives 1 & 2 will be completed by CRITFC and University of Idaho staff at the Hagerman Fish Culture Experiment Station. Tissue samples to address objective 3 will be collected by staff from CRITFC, WDFW, and ODFW. Genotyping and GSI analysis for objective 3 will be completed by CRITFC and University of Idaho staff in Hagerman, ID. Geneticists with CRITFC have adequate expertise to complete each objective, and have published peer-reviewed papers on each of these topics (Narum et al. 2007, Campbell and Narum 2008a, Narum et al. 2008a, Narum et al. 2008b).

B. Technical and/or scientific background

RESEARCH

In this proposal we plan to discover and evaluate single nucleotide polymorphism (SNP) markers to expand existing baselines for genetic stock identification (GSI) of salmon and steelhead in the Columbia River Basin. The scientific background for each objective is included below.

SNP Discovery

One of the highest priorities in the full-scale implementation of SNPs for salmon genetics is the discovery and development of a sufficient number of these markers to characterize population variability. These polymorphisms represent the most abundant variation in the genome of most organisms, and are spread throughout the entire genome at high density (Morin et al. 2004). Thus SNPs can be discovered through sequencing known regions of DNA and converted to high throughput assays (e.g., Campbell and Narum 2008a). Further, mutation rates, mutation models and error rates for SNPs are generally well understood, providing a foundation for estimating genetic divergence between populations. SNP markers also offer the potential of a more cost-effective and less error-prone alternative to existing genetic tools that may be used independently or in tandem with existing microsatellite markers to improve accuracy and precision of stock assignments. The combined power of these two marker types is expected to improve stock composition accuracy (Narum et al. 2008a) and allow researchers to meet rigorous stock composition and assessment needs for timely management of fisheries. Thus we plan to proceed with SNP discovery in multiple species of salmon and steelhead. These markers will be genotyped in populations throughout the Columbia River Basin to expand and/or create genetic baselines for multiple species.

Baseline Expansion

Currently, genetic baselines of microsatellite markers are in place for Chinook salmon across the coastwide range (Seeb et al. 2007) and steelhead in the interior Columbia River Basin. Despite large, representative sample sizes from many populations and very high microsatellite allelic diversity, the resolution of specific stocks and populations in these baselines is limited in some cases. For example, fall Chinook salmon in the Columbia River are closely related and remain difficult to distinguish even with a powerful set of 13 microsatellite markers. Several other closely related populations in the Chinook salmon baseline are similarly difficult to distinguish and thus have been pooled into a single reporting unit for GSI applications. In some cases (i.e., mainstem Columbia R. Chinook fisheries), a finer level of stock discrimination is necessary for management of fisheries. Additional SNP loci will increase stock assignment reliability where greater resolution is required. Given the difficulty and expense of inter-laboratory standardization, additional microsatellite markers may not the most efficient choice. In this regard, SNP markers are the preferred option for additional loci since they offer many beneficial characteristics that make them amenable to adding loci to existing baselines.

Since SNP assays involve direct interrogation of genetic sequence variation, SNP loci are good candidates for standardization among laboratories and expanding existing baselines. In addition, it is expected that the cost of SNP genotyping will drop significantly, as has happened in human genetics. In addition to ease of standardization and decreasing costs, SNP markers may provide a technique to effectively genotype poorly preserved tissue samples (i.e., carcass tissues). Degradation of DNA occurs over time, particularly in sub-optimal conditions, resulting in the fragmentation of the DNA into increasingly smaller pieces. Since SNP assays amplify a much shorter segment of DNA than microsatellites, SNPs have a greater probability of producing results in degraded samples (Campbell and Narum 2008b). While SNPs will be used to augment

microsatellite baselines in most cases, there are some applications where SNP markers may produce the only viable baseline data for populations with only carcass samples available for genotyping. Furthermore, SNP development can focus on adaptive functional genes. The additive power of combining neutral (microsatellite) loci with adaptive loci like the Major Histocompatibility Complex (MHC) for stock identification was originally shown using the Canadian Department of Fisheries and Oceans (CDFO) baselines in sockeye and coho salmon (Beacham et al. 2001, 2004). Alaska Department of Fish & Game (ADFG) has also developed SNPs for MHC loci that have also been highly effective (Smith et al. 2005). Thus SNP genotypes will be added to existing microsatellite baselines (Chinook salmon and steelhead) to improve stock resolution in the Columbia River, and we will begin to construct baselines for species that lack genetic data (i.e., sockeye salmon).

Genetic Stock ID

Genetic Stock Identification (GSI) methods have proven to be effective in determining the proportion of stock origin in several mixed stock applications (Shaklee et al. 1999, Beacham et al. 2006, Narum et al. 2008b). This proposal includes two GSI projects that will utilize genetic baselines: 1) GSI to Evaluate Catch; and 2) GSI of fish passing Bonneville Dam.

This study will include GSI analysis of Chinook salmon collected from commercial, recreational, and tribal fisheries in the Columbia River. (Subsequent years of the study will include steelhead and coho fisheries as possible.) Implementation of GSI technology could make monitoring individual production units in mixed stock areas possible. Tissues will be sampled annually from fisheries with existing programs in place with Washington Department of Fish and Wildlife (WDFW) and Oregon Department of Fish and Wildlife (ODFW). We plan to genotype representative samples from fisheries of primary interest. Known origin coded-wire tag (CWT) fish will be used to provide a measure of accuracy for GSI estimates. The GSI estimates may help refine CWT based estimates of stock composition used in fishery management.

The second application of GSI analysis in this proposal includes sampling unknown origin salmon and steelhead at Bonneville Dam for genetic analysis. Samples will be collected over the entire length of the run on a weekly basis, and genetic baselines will be utilized to determine the stock composition of these runs. Few studies have been able to determine the extent of overlap among life history types of salmon and steelhead, but GSI of each life history type will allow us to determine the stock composition of the different runs through Bonneville Dam with greater accuracy than current methods. Population genetic methods and statistical assignment models have advanced dramatically in recent years, and estimating stock composition is now possible using either Bayesian or Maximum Likelihood methods (Anderson et al. 2008). Therefore, we plan to estimate stock composition of multiple species passing Bonneville Dam and provide this information on a timely basis to fisheries managers.

C. Rationale and significance to regional programs

This research proposal affects all stocks in the Columbia River Basin and therefore is considered a basinwide application. The four projects in this proposal address needs for distinguishing specific stocks, determining genetic diversity, stock specific run timing, and estimating stock composition for improved fisheries management and harvest. These needs have been identified in multiple “Reasonable and Prudent Alternatives” (RPA) in the BiOp:

-page 57, RPA No. 41, Preserve genetic resources
-page 69, RPA No. 50, Fish population status monitoring
-page 77, RPA No. 53, Monitor adult salmonids passing through FCRPS
-page 88, RPA No. 62, Fund selected harvest investigations (i.e., fifth bullet, Investigate the feasibility of genetic stock identification monitoring techniques).
-page 89, RPA No. 63, Monitor hatchery effectiveness
-page 89, RPA no. 64, Investigate hatchery critical uncertainties (i.e., Estimate relative reproductive success with genetic markers)

Other documentation that identifies the need for these projects include the Independent Scientific Advisory Board (ISAB) Report (2005), Columbia River Technical Advisory Committee (TAC), and in the 2000 Council Fish and Wildlife Program including:

-Basinwide Provisions – Strategies D. 5. Harvest: "Monitor inriver and ocean fisheries and routinely estimate stock composition and stock-specific abundance, escapement, catch, and age distribution. Expand monitoring programs as necessary to reduce critical uncertainties. Manage data so that it can be easily integrated and readily available in real time."
-Basinwide Provisions – Strategies D. 6. Hydrosystem: "…evaluation of escapement numbers to spawning grounds and hatcheries, research into water temperature effects on fish passage, and the connection between fish passage design and fish behavior."

D. Relationships to other projects

The genetic markers and baselines developed in this proposal will directly benefit both new and ongoing projects funded under the Accords and Fish & Wildlife Program. This includes the Kelt Reproductive Success and Snake River Kelt studies (200001700), and supplementation monitoring and evaluation projects (200852300). The GSI results from fish passing Bonneville Dam will be combined with PIT Tag results from migration studies (200851800). The sampling plan for Objective 3 (GSI to evaluate catch) will utilize input from "Power analysis to determine catch sampling rates" (200850800) and "Expanded tribal catch sampling" (200850200) .

Table 2. Relationship to existing projects

Funding Source	**Project #**	**Project Title**	**Relationship (brief)**
BPA	200851800	PIT Tags for migration montoring	Bonneville sampling effort and GSI results will be combined with PIT tag study.
BPA	200001700	Kelt Studies	Genetic markers and baselines will be used for pedigree and assignment purposes.
BPA	200852300	Supplementation monitoring evaluation	Genetic markers will be used for pedigree analysis.
BPA	200850800	Power analysis to determine catch sampling rates	Sampling design for Objective 3 will utilize input from this project.
BPA	200850200	Expanded tribal catch sampling	Samples from tribal fisheries will contribute to Objective 3.

E. Project history (for ongoing projects)

While the research projects in this proposal are newly funded under the Fish & Wildlife program, previously funded projects from the Pacific Salmon Commission and NOAAs FCRPS BiOp are applicable to these new projects. Most relevant, an existing coastwide microsatellite baseline for Chinook salmon will allow GSI analysis of fisheries, and an existing microsatellite baseline for interior Columbia River steelhead is in place for Bonneville GSI allowing these project to proceed immediately. The newly funded projects will allow us to build upon existing baselines by adding more genetic markers (SNPs) and provide more accurate and higher resolution GSI estimates for fisheries managers.

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

Related to all Objectives (1-4)
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 Progress Report for 1/1/2009 to 12/31/2009
The progress report will summarize the project objectives, hypotheses, completed and uncompleted deliverables, problems encountered, lessons learned, and long-term planning. Date range 1/1/2009 to 12/31/2009.

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.

185. Produce Pisces Status Reports: Periodic Status Reports for BPA
CRITFC shall report on the status of milestones and deliverables in Pisces. Reports shall be completed either monthly or quarterly as determined by the BPA COTR. Additionally, when indicating a deliverable milestone as COMPLETE, CRITFC shall provide metrics and the final location (latitude and longitude) prior to submitting the report to the BPA COTR.

Objective 1) Discover and develop SNP markers in salmon and steelhead.

Work Elements:
156. Develop RM&E Methods and Designs: SNP Ascertainment Strategy
Known genes from expressed sequence tag (EST) databases will be targeted as primary candidate regions for sequencing. Other candidates may include unknown genomic regions from bacterial artificial chromosome (BAC) libraries, “backbone” alignments from other large scale sequencing efforts, and microsatellite flanking regions. An ascertainment panel of 16-32 diverse individuals for steelhead, sockeye, and coho will be utilized for sequencing.

157. Collect/Generate/Validate Field and Lab Data: Sequencing and Assay Development
Once candidate regions are identified, primers will be developed to amplify the regions of interest. Primers will be tested for successful polymerase chain reaction (PCR) in each species, and primers with positive results will be used to amplify samples from ascertainment panels. PCR products will be sequenced, and subsequent sequence data will be aligned to identify SNPs. Putative SNP sites will be identified and attempts will be made to convert these into high-

throughput genotyping assays. The target number of new SNP assays for each species are: steelhead (10 assays), sockeye (15 assays), and coho (15 assays).

162. Analyze/Interpret Data: Analysis of SNP Frequencies
SNP sites will be evaluated to determine the frequency of minor alleles, and the potential utility of each SNP as a marker for broader genotyping for population structure, GSI, or species ID. The genomic position and functional effect of each SNP will be determined when possible (if SNP occurs in known gene region).

161. Disseminate Raw/Summary Data and Results: SNP Sites and Assays
SNP sites and genotyping assays will be made available in annual reports and publications. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 2) Expand and/or create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho).

Work Elements:
156. Develop RM&E Methods and Designs: Determine SNP Markers and Baseline Samples
Existing microsatellite baselines for steelhead and Chinook will be expanded by genotyping additional SNP markers for population samples. Populations will also be added as needed, especially for the steelhead baseline that is less developed than the Chinook baseline. Since there are not existing genetic baselines for sockeye and coho, markers for these species will be tested in Columbia River populations for use in future baseline genotyping (FY2010).

157. Collect/Generate/Validate Field and Lab Data: SNP Genotyping
Approximately 3,000 samples will be genotyped for baseline expansion. This will include about 2,000 steelhead and 1,000 Chinook samples, each typed with 75-96 existing SNPs. Existing and new SNP markers for sockeye and coho will be genotyped in a test set of approximately 96 samples for each species.

159. Transfer/Consolidate Regionally Standardized Data: Baseline Genotypes to Database
Standardized baseline genotypes will be submitted to an existing genetic database founded by a multi-agency consoritium called GAPS (Genetic Analysis of Pacific Salmonids).

162. Analyze/Interpret Data: Population Diversity and Differentiation in Baseline
Genotype data will be analyzed with standard population genetics procedures including estimating genetic diversity (heterozygosity), population differentiation (Fst and exact tests), assignment tests, and genetic relationships (neighbor-joining dendrograms). Mixture simulations will also be completed to determine the utility of the baseline for GSI of unknown samples.

161. Disseminate Raw/Summary Data and Results: Genotypes and Allele Frequencies
Genotypes and allele frequencies for baseline populations will be made available in annual reports and publications. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 3) Implement GSI to estimate stock composition of harvest in mainstem Columbia River fisheries.

Work Elements:

189. Regional Coordination: Coordination Among Agencies

Staff from CRITFC, ODFW, and WDFW will develop a stratified sampling program of Chinook fisheries to address management priorities.

156. Develop RM&E Methods and Designs: Develop Sampling Plan

Staff from CRITFC, ODFW, and WDFW will coordinate sampling effort of Chinook harvest from tribal and non-tribal fisheries in the Columbia River. Once seasons for fisheries are set, staff will coordinate sampling effort from harvested fish. Sampling effort will be stratified by time and fishery, with a target sample size of 200 individuals per strata, for a target of approximately 5,500 total samples. (Subsequent years of the study will include steelhead and coho as possible.)

157. Collect/Generate/Validate Field and Lab Data: Collect Tissue Samples from Fisheries

For each fishery and location sampled, biological data such as species, date, length, presence/absence of adipose fin and CWT, will be recorded for each individual along with a tissue sample for genetic analysis. We plan to collect tissue samples in the form of fin punches from each fish, and store in vials of non-denatured ethanol.

157. Collect/Generate/Validate Field and Lab Data: Genotype Tissue Samples

Tissue samples will be shipped to the Hagerman Fish Culture Experiment Station for genetic analysis. DNA will be extracted from approximately 5,500 tissue samples and genotyped with genetic markers. Genotype data will be utilized to estimate stock composition using existing genetic baselines for each species.

162. Analyze/Interpret Data: Estimate Stock Composition

GSI methods will be utilized to estimate stock composition of mixture samples, by fishery and strata.

161. Disseminate Raw/Summary Data and Results: Distribute GSI Estimates

Once GSI analysis is completed, estimates of fisheries stock composition will be discussed with fisheries managers and distributed in annual reports. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 4) Utilize GSI methods to estimate stock composition of salmon and steelhead passing Bonneville Dam.

Work Elements:

156. Develop RM&E Methods and Designs: Sampling period, size, and logistics

The sampling plan at the Bonneville Adult Fish Facility (AFF) will be altered according to actual fish returns, but initially includes non-lethal sampling of Chinook and steelhead from April 1 to October 31, 3-5 days per week. Field data to be collected will include biological data and tissues to be preserved for genotyping. Sampling effort will be coordinated with PIT tagging studies to limit impact to fish. Also involved is coordination of sampling plans with staff from the Army Corps of Engineers that operate the AFF.

157. Collect/Generate/Validate Field and Lab Data: Non-lethal sampling at Bonneville Dam
Chinook and steelhead will be sampled at the Bonneville AFF throughout the run. Biological data such as species, date, length, presence/absence of adipose fin, will be recorded for each individual along with a tissue sample for genetic analysis. We plan to collect tissue samples non-lethally, in the form of fin punches from each Chinook salmon and steelhead and then utilize GSI techniques to assign these to population of origin. After non-lethal sampling is completed, all fish will be released to a recovery pond and then to the fish ladder to continue upstream migration. Tissues samples will be shipped to the Hagerman Fish Culture Experiment Station for GSI and estimation of stock composition. This sampling effort is covered under Scientific Research Permit #1379 under Section 10 of the ESA (permit included in PISCES attachments).

157. Collect/Generate/Validate Field and Lab Data: Genotype tissue samples
Tissue samples will be shipped to the Hagerman Fish Culture Experiment Station for genetic analysis. DNA will be extracted from approximately 5,000 tissue samples and genotyped with genetic markers and assigned to population of origin using existing genetic baselines for each species.

162. Analyze/Interpret Data: GSI estimates
Genetic stock identification methods will be utilized to estimate stock composition of adults passing Bonneville Dam. Samples will be divided into bi-weekly or weekly mixture samples as collection size allows. We also plan to identify the stocks of origin in comparison to previous year's data to assist in determining annual variation in run timing and stock composition.

161. Disseminate Raw/Summary Data and Results: Post updates to CRITFC website
Stock composition estimates will be posted to the CRITFC website as they are available. Studies will also be presented at professional meetings and seminars as appropriate.

G. Research - Methods

Objective 1) Discover and develop SNP markers in salmon and steelhead.

Hypothesis:
- Single nucleotide polymorphisms (SNPs) are abundant throughout the genome of salmon and steelhead and can be identified by DNA sequencing.

Experimental Design
Known genes from EST databases will be targeted as primary candidate regions for sequencing. Other candidates may include unknown genomic regions from BAC libraries, "backbone" alignments from other large scale sequencing efforts, and microsatellite flanking regions. An ascertainment panel of 16-32 diverse individuals for steelhead, sockeye, and coho will be utilized for sequencing. Once candidate regions are identified, primers will be developed to amplify the regions of interest. Primers will be tested for successful PCR in each species, and primers with positive results will be used to amplify samples from ascertainment panels. PCR products will be sequenced, and subsequent sequence data will be aligned to identify SNPs. Putative SNP sites will be identified and attempts will be made to convert these into high-throughput genotyping assays. The target number of new SNP assays for each species are: steelhead (10 assays), sockeye (15 assays), and coho (15 assays).

Quality Control
In order to verify the accuracy of newly developed SNP assays, we will test for concordant results between sequencing and genotyping assay data.

Statistical Analyses
In order to confirm amplification of targeted gene fragments, BLAST (Basic Local Alignment Search Tool) searches will be completed at http://www.ncbi.nlm.nih.gov/. SNP sites will be evaluated to determine the frequency of alleles, and the potential utility of each SNP as a marker for broader genotyping for population structure, GSI, or species ID. The genomic position and functional effect of each SNP will be determined when possible (if SNP occurs in known gene region). To test for null alleles and linkage, Hardy Weinberg and linkage disequilibrium tests will be completed in GENEPOP and MICROCHECKER.

Communication of Results
Results from this project will be included in annual reports, and new markers will be submitted for publication as warranted. All newly discovered SNP sites will be identified by position in each gene, and assays will include appropriate primer/probe sequences. Studies will also be presented at professional meetings and seminars as appropriate.

Objective 2) Expand and/or create genetic baselines for multiple species (Chinook, steelhead, sockeye, and coho).

Hypothesis:
- Populations can be differentiated from one another based on multi-locus allele frequencies.

Experimental Design
Existing microsatellite baselines for steelhead and Chinook will be expanded by genotyping up to 96 additional SNP markers for population samples. Approximately 3,000 samples will be genotyped for baseline expansion. This will include about 2,000 steelhead and 1,000 Chinook samples, each typed with 75-96 existing SNPs.

Since there are not existing baselines for sockeye and coho, markers for these two species will be tested in Columbia River populations for use in future baseline genotyping (FY2010). Existing and new SNP markers for sockeye and coho will be genotyped in a test set of 96 samples for each species.

Quality Control
Genotype data will be checked for quality by utilizing positive and negative controls in each run. Further, repetitive genotyping of randomly selected individuals will be completed to ensure repeatability of genotyping results.

Statistical Analyses
Deviation from Hardy-Weinberg equilibrium will be evaluated at each locus and population using the Markov Chain Monte Carlo algorithm implemented in GENEPOP v. 3.3 (Raymond and Rousset 1995). Tests for linkage disequilibrium between all pairs of loci will also be performed using simulated exact tests in GENEPOP. Because multiple comparisons are involved, corrections were made against Type I error in both tests with the B-Y FDR method (Rice 1989). To estimate genetic diversity of each collection, unbiased heterozygosity (H_E),

observed heterozygosity (H_O), total number alleles, and allelic richness (average alleles per locus corrected for sample size) will be calculated for all microsatellite loci in FSTAT v.2.9.3.2 (Goudet 2001).

Pairwise genetic distance and differentiation (temporal and geographic) will be estimated in GENEPOP. Significance levels will be adjusted for multiple tests with a modified version of the False Discovery Rate referred to as the B-Y FDR (Narum 2006). In order to infer the degree of relatedness between sample collections, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) will be calculated between all sites with POPULATIONS software (Langella 2001) and displayed with the program TREEVIEW (Page 1996).

Communication of Results

Results from this project will be included in annual reports and submitted for publication as warranted. Studies will also be presented at professional meetings and seminars as appropriate. Standardized genotypes will be submitted to the online GAPS database for multi-agency use.

Objective 3) Implement GSI to estimate stock composition of harvest in mainstem Columbia River fisheries.

Hypothesis:

- Genetic stock ID methods can provide stock composition information for mainstem Chinook fisheries.

Experimental Design

Staff from CRITFC, ODFW, and WDFW will coordinate sampling effort of Chinook harvest from tribal and non-tribal fisheries in the mainstem Columbia River. Once seasons for fisheries are set, staff will coordinate sampling effort from harvested fish. Sampling effort will be stratified by time and fishery, with a target sample size of 200 individuals per strata, for a target of 5,500 total samples. As available, we will also utilize input from a project specifically designed to evaluate catch sampling rates, "Power analysis to determine catch sampling rates" (200850800). (Subsequent years of the study will include steelhead and coho as possible.)

For each fishery and location sampled, biological data such as species, date, length, presence/absence of adipose fin and CWT, will be recorded for each individual along with a tissue sample for genetic analysis. We plan to collect tissue samples in the form of fin punches from each fish, and store in vials of non-denatured ethanol.

Tissue samples will be shipped to the Hagerman Fish Culture Experiment Station for genetic analysis. DNA will be extracted from tissue samples and genotyped with genetic markers. Genotype data will be utilized to estimate stock composition using existing genetic baselines for each species.

Quality Control

Genotype data will be checked for quality by utilizing positive and negative controls in each run. Repetitive genotyping of randomly selected individuals will be completed to ensure repeatability of genotyping results. When possible, GSI and CWT stock composition results will be compared to estimate accuracy of each method.

Statistical Analyses
Genotype data will be utilized to estimate stock composition using existing genetic baselines for each species. Mixture simulations will be examined with the program ONCOR to determine reporting groups and evaluate the power of the baseline to analyze mixture samples. Genotypes from fisheries mixtures will be analyzed in ONCOR to estimate stock composition by fishery and strata.

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 4) Utilize GSI methods to estimate stock composition of salmon and steelhead passing Bonneville Dam.

Hypothesis:
- Genetic stock ID methods can provide estimates of stock composition for Chinook and steelhead throughout the run.

Experimental Design
Chinook and steelhead will be sampled at the Bonneville AFF from April 1 to October 31, 3-5 days per week. Biological data such as species, date, length, presence/absence of adipose fin, will be recorded for each individual along with a tissue sample for genetic analysis. We plan to collect tissue samples in the form of fin punches from each Chinook salmon and steelhead and then utilize GSI techniques to assign these to population of origin. After non-lethal sampling is completed, all fish will be released to a recovery pond and then to the fish ladder to continue upstream migration.

Tissue samples will be shipped to the Hagerman Fish Culture Experiment Station for genetic analysis. DNA will be extracted from approximately 5,000 tissue samples and genotyped with genetic markers and assigned to population of origin using existing genetic baselines for each species.

Quality Control
Genotype data will be checked for quality by utilizing positive and negative controls in each run. Repetitive genotyping of randomly selected individuals will be completed to ensure repeatability of genotyping results. When possible, GSI and passive integrated transmitter (PIT) tag results will be combined/compared.

Statistical Analyses
Genetic stock identification methods will be utilized to estimate stock composition of adults passing Bonneville Dam. Samples will be divided into bi-weekly or weekly mixture samples as collection size allows. We also plan to identify the stocks of origin in comparison to previous year's data to assist in determining annual variation in run timing.

Communication of Results

Stock composition estimates will be posted to the CRITFC website, 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 computers, consumable laboratory supplies, genotyping equipment, equipment maintenance, and vehicle lease for related travel. Due to the large number of samples to be processed, high-throughput genotyping equipment and automated instruments will need to be purchased to complete these projects. The largest piece of equipment is a SNP genotyping instrument and the complimentary items such as thermal cyclers and intergrated fluidic controllers (IFCs) involved in genotyping work-flow (total of $100,000). Additional lab benches may be needed to accommodate expanded staff and equipment.

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J. Key personnel

The Lead Geneticist (Dr. Shawn Narum) will oversee this project and each of the objectives. Assistant Department Manager (Doug Hatch) will facilitate coordination and administration of tasks related to the project. Key staff for completing objectives includes a Conservation Geneticist/Lab Manager (Jeff Stephenson), Genomics Researcher (Nate Campbell), Conservation Geneticist (Andrew Matala), Fishery Scientist (John Whiteaker), and Genetic Stock ID specialist (TBD). 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:

- Shawn Narum, Lead Geneticist, 6 months (FTE)
- Doug Hatch, 1 month (FTE)
- Jeff Stephenson, 2 months (FTE)
- Nate Campbell, 4 months (FTE)
- Andrew Matala, 7 months (FTE)
- John Whiteaker, 1 months (FTE)
- GSI Specialist (TBD), 9 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.

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B.S., Fishery Resources, University of Idaho, 1986

Appointments

1990-present Fisheries Scientist, Columbia River Inter-Tribal Fish Commission

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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.

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B.S., Fishery Resources, University of Idaho, 1997

Appointment

2004-present Conservation Geneticist/Lab Manager, Columbia River Inter-Tribal Fish Commission

Selected Publications

Narum, S. R., M. R. Campbell, and J. J. **Stephenson**. 2007. Genetic variation and structure of Chinook salmon life history types in the Snake River. Transactions of the American Fisheries Society 136:1252-1262.

Seeb, L. W, A. Antonovich, M.A. Banks, T.D. Beacham, M.R. Bellinger, S. M. Blankenship, M. Campbell, N.A. Decovich, J.C. Garza, C.M. Guthrie III, T. A. Lundrigan, P. Moran, S.R. Narum, J.J. **Stephenson**, K.J. Supernault, D.J. Teel, W.D. Templin, J.K.Wenburg, S.F. Young, C.T. Smith. 2007. Development of a Standardized DNA Database for Chinook Salmon. Fisheries 30:540-552.

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Education

B.S., Biology w/ Chemistry Minor, Eastern Michigan University

Appointments

2008-present Genomics Researcher, Columbia River Inter-Tribal Fish Commission
2006-2008 Genetics Laboratory Technician, University of Idaho

Publications

Fang M, Li J, Blauwkamp T, Bhambhani C, **Campbell** N, Cadigan K (2006) C-terminal-binding protein directly activates and represses Wnt transcriptional targets in Drosophila. *EMBO Journal* **25**: 2735-2745

Campbell N, and Narum S (2008) Identification of Novel Single-Nucleotide Polymorphisms in Chinook Salmon and Variation among Life History Types. *Transactions of the American Fisheries Society* **137**: 96-106

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Campbell N, and Narum S (2008) Quantitative PCR assessment of microsatellite and SNP genotyping with variable quality DNA extracts. Conservation Genetics, DOI 10.1007/s10592-008-9661-7.

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B.S., Microbiology, Washington State Unversity, 1995
M.S., Fisheries Genetics University of Alaska Fairbanks, 2002

Appointments

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

Publications

Microsatellite Variation Indicates Population Genetic Structure of Bocaccio. *North American Journal of Fisheries Management* 24:1189-1202, 2004.

Two Genetically Distinct Forms of Rougheye Rockfish (*Sebastes aleutianus*) are Different Species. *Transactions of the American Fisheries Society* 134:242-260, 2005.

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