Extension of the Chinook Salmon Microsatellite Baseline

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by

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Summary:

Genetic stock identification (GSI) is a tool with great potential to provide stock composition data for managing mixed fisheries. Thorough baseline representation is one of the key components to successful application of GSI. The objective of this project was to add further hatchery stocks to the current GAPS (Genetic Analysis of Pacific Salmonids) baseline. We focused on hatchery stocks from the Columbia River basin, including spring, summer, and fall run populations. A total of 1098 individuals representing Chinook salmon hatcheries from the Columbia River were genotyped with standardized protocols for contribution to the baseline. We successfully completed this objective and closely followed the anticipated time schedule from the proposal. This additional data will be incorporated into the genetic baseline and all baseline genotypes will be added to the new online database (Beta version currently accessible).

Introduction

Current GAPS Baseline

The current Chinook salmon microsatellite baseline constructed by the GAPS (Genetic Analysis of Pacific Salmonids) consortium for the CTC provides a powerful tool for evaluating mixed stock fisheries. The initial version of the baseline (v. 1.1) was recently expanded to version 2.1 with additional coastwide populations that now total 165 populations (~22,000 samples) that have been genotyped with 13 highly polymorphic microsatellite loci (~500 alleles). Initial power analyses indicate that 41 of 43 reporting groups in the baseline have at least 90% accuracy in 100% mixture simulations. Further baseline expansion and maintenance planned in 2007 by GAPS laboratories will lead to a microsatellite baseline (v. 3.1) with even more dense geographic coverage (greater than 200 populations) with at least 51 populations from the Columbia River. Thus, a highly effective tool for mixed stock analysis of Chinook salmon is now in place and is available to co-managers through an online database.

Despite large, representative sample sizes from many populations and very high microsatellite allelic diversity, the resolution of specific stocks and populations in the baseline is limited in some cases. For example, fall Chinook salmon in the Columbia River are closely related and remain difficult to distinguish even with this powerful set of 13 microsatellite markers. Several other closely related populations in the baseline are similarly difficult to distinguish and thus have been pooled into a single reporting unit for GSI applications. In some cases (i.e., Washington coast troll fishery), a finer level of stock discrimination is necessary for management of fisheries. Additional baseline coverage

should increase stock assignment reliability when greater resolution is required (Banks 2005).

Current Project Objectives

As a contributing GAPS laboratory, CRITFC recognized the need to better represent hatchery stocks in the baseline since assignments of unknown origin fisheries are limited to populations included in the baseline. The objective of this project was to genotype additional hatchery stocks Chinook salmon for inclusion in the microsatellite baseline. Further, additional samples provided the opportunity to evaluate if new alleles are observed from the Columbia River, and indication of how well the basin has been represented in the baseline.

Methods

The objective of this project was to fill holes in the current coverage provided in the GAPS (Genetic Analysis of Pacific Salmonids) baseline. Hatchery stocks of Chinook salmon from Umatilla Hatchery (fall run), Little White Salmon Hatchery (fall run), Little White Salmon Hatchery (spring run), Carson Hatchery (spring run), and hatchery (ad-clipped) fish passing Bonneville Dam in spring, summer, and fall run were genotyped with CTC standardized microsatellite loci (Moran et al. 2005). The process included DNA extraction from tissue samples, polymerase chain reaction (PCR) to amplify 13 microsatellite loci (Table 1), and fragment analysis to detect fluorescently labeled PCR products. Raw genotype data was converted to standardized CTC alleles. The converted data will be incorporated to the microsatellite baseline for improved mixed fishery genetic stock identification. In order to maintain sample exchange among GAPS labs, an aliquot of DNA from each new sample will be available for distribution upon request from other GAPS laboratories.

Table 1. Microsatellite loci standardized for Chinook salmon

Locus	Primer Sequence (5'→ 3') **F** > Forward, **R** > Reverse	Citation	Curator Agency[1]
Ots201b	F- CAGGGCGTGACAATTATGC R- TGGACATCTGTGCGTTGC	OSU unpublished[2]	ADFG
Ots208b	F- GGATGAACTGCAGCTTGTTATG R- GGCAATCACATACTTCAACTTCC	Grieg et al. 2003	CRITFC
Ots211	F - TAGGTTACTGCTTCCGTCAATG R - GAGAGGTGGTAGGATTTGCAG	Grieg et al. 2003	ADFG
Ots212	F- TCTTTCCCTGTTCTCGCTTC R- CCGATGAAGAGCAGAAGAGAC	Grieg et al. 2003	OSU
Ogo4	F- GTCGTCACTGGCATCAGCTA R- GAGTGGAGATGCAGCCAAAG	Olsen et al. 1998	WDFW
Ogo2	F- ACATCGCACACCATAAGCAT R- GTTTCTTCGACTGTTTCCTCTGTGTTGAG	Olsen et al. 1998	ADFG
Ots3M	F- TGTCACTCACACTCTTTCAGGAG R- GAGAGTGCTGTCCAAAGGTGA	Banks et al. 1999	WDFW
Ots213	F- CCCTACTCATGTCTCTATTTGGTG R- AGCCAAGGCATTTCTAAGTGAC	Grieg et al. 2003	OSU
Omm1080	F- GAGACTGACACGGGTATTGA R- GTTATGTTGTCATGCCTAGGG	Rexroad et al. 2001	SWFSC
Ssa408UOS	F- AATGGATTACGGGTACGTTAGACA R- CTCTTGTGCAGGTTCTTCATCTGT	Cairney et al. 2000	NWFSC
Ots9	F- ATCAGGGAAAGCTTTGGAGA R- CCCTCTGTTCACAGCTAGCA	Banks et al. 1999	DFO
OtsG474	F- TTAGCTTTGGACATTTTATCACAC R- CCAGAGCAGGGACCAGAAC	Williamson et al. 2002	CRITFC
Oki100	F- CCAGCACTCTCACTATTT R- CCAGAGTAGTCATCTCTG	DFO unpublished	DFO

[1]Laboratory abbreviations: OSU, Oregon State University; SWFSC, Southwest Fisheries Science Center – National Marine Fisheries Service; DFO, Department of Fisheries and Oceans Canada; NWFSC, Northwest Fisheries Science Center – National Marine Fisheries Service; CRITFC, Columbia River Inter-Tribal Fish Commission; ADFG, Alaska Department of Fish & Game; WDFW, Washington Department of Fish & Wildlife.

Results

A total of 1115 Chinook salmon samples were genotyped from the Columbia River basin for addition to the microsatellite baseline. Of those, 17 samples did not provide adequate genotypes (failures or duplicates), and were removed from the data set leaving 1098 samples that were analyzed. Across all 13 loci, a total of 384 alleles were observed with five new alleles not previously included in the baseline. Those new alleles are being verified among GAPS labs to ensure standardized nomenclature. Of 130 tests for Hardy-Weinberg equilibrium, six were statistically significant after Bonferroni corrections. Populations with loci that deviated from equilibrium were: Little White Salmon Hatchery with one significant locus (Ots211 – heterozygote excess), Carson National Fish Hatchery (Ots208b – heterozygote deficit), 05BonnJune (Ogo4 and OtsG474 – heterozygote deficits), and 06BonnJune (Ogo4 and OtsG474 – heterozygote deficits). Significant heterozygote deficiences at OtsG474 and Ogo4 in the June samples from 2005 and 2006 are consistent with mixtures of ocean- and stream-type life histories (Narum et al. 2004). This suggests that samples in these collections should be sorted by assignment tests to life history type, and this will be completed for addition to the baseline.

Genetic diversity as measured by observed and expected heterozygosity, total alleles, average alleles per locus, and allelic richness was consistently higher in collections of ocean-type than collections of stream-type Chinook salmon (Table 2).

Tests of genetic differentiation with pairwise F_{ST} indicated that all collections were significantly different from one another ($P < 0.0114$) with the exception of Chinook

salmon passing Bonneville Dam in June and July of 2006 (P = 0.331). Overall, population relationships were consistent with expectations and suggest the data can be merged with the existing GAPS baseline.

Table 2. Estimates of genetic diversity for 10 collections of Chinook salmon added to the genetic baseline.

Population	Sample size	Unbiased Hz	Obs Hz	Total Alleles	Avg alleles/locus	Allelic Richness
Umatilla Hatchery (fall)	87	0.871	0.870	290	22.31	18.7
Little White Salmon Hat. (fall)	94	0.876	0.881	290	22.31	18.7
Little White Salmon Hat. (spring)	93	0.785	0.777	212	16.31	13.9
Carson NFH (spring)	94	0.798	0.791	214	16.46	14.3
05BonnApril	177	0.785	0.777	247	19.00	15.0
05BonnMay	220	0.808	0.786	279	21.46	16.0
05BonnJuneE	105	0.876	0.826	288	22.15	18.0
06BonnJuneL	113	0.870	0.844	296	22.77	18.4
06BonnJuly	74	0.864	0.855	279	21.46	18.4
06BonnSept	41	0.871	0.867	257	19.77	19.6

Discussion

The objective of this project was to genotype hatchery stocks of Chinook salmon with standardized microsatellite loci to extend the genetic baseline. We successfully completed this objective and closely followed the anticipated time schedule from the proposal. This additional data will now be incorporated into the genetic baseline with plans to post all baseline genotypes to an online database (currently under construction).

Quality Control

Genetic data was tested under standard quality control procedures in CRITFC's genetic laboratory. This includes confirmation of raw genotypes through repetitive genotyping, positive and negative controls, and automated allele conversion. Further, data was compared to initial baseline data generated in our laboratory to ensure consistency of new baseline data.

Project Benefits

This project was intended to enhance PSC genetic stock identification of mixed stock fisheries since this is essential information related to the Pacific Salmon Treaty. The baseline populations genotyped in this project add important hatchery stocks, and determine that new alleles are still being detected with further additions to the baseline. We expect the microsatellite baseline to be a long standing tool for genetic stock identification of mixed fisheries under the Pacific Salmon Treaty. All agencies and organizations that utilize the microsatellite baseline for genetic stock identification will benefit from a more complete baseline. The CRITFC genetics lab is currently one of the members of the coast-wide Chinook salmon microsatellite standardization effort supported by the PSC.

Future directions for the Genetic Baseline

In order to achieve the high level of precision and accuracy desired for mixed stock analysis, many loci are required (Kalinowski 2004) and this can be done most cost effectively for the CTC using SNP markers in tandem with existing microsatellite data. Stock assignments and Full Parental Genotyping (FPG) will likely require a dramatic

increase in the number of loci, although the actual number is still under investigation. In order to achieve incremental increases in discrimination power, we will need to add significantly more SNPs than the number of microsatellites now included in the current baseline data. In summary, SNP discovery efforts followed by baseline genotyping of these SNP markers will supplement the baseline to provide a significant increase in power for mixed fisheries analysis (Hankin et al. 2005). Baseline genotyping with SNP markers is progressing with funds received from the PSC, and SNP genotypes for up to 26 Columbia River populations are expected to be available in the baseline by early 2008.

References

Banks, M.A. 2005. Stock identification for the conservation of threatened or endangered species. *In*: Stock identification methods Eds: Cadrin, S.X., K.D. Friedland and J.R. Waldman. Elsevier Press. Pgs. 609-629.

Hankin, D.G., Clark, J.H., Deriso, R.B., Garza, J.C., Morishima, G.S., Riddell, B.E., Schwarz, C., Scott, J.B. 2005. Report of the expert panel on the future of the coded wire tag recovery program for Pacific salmon. Final Report to the Pacific Salmon Commission. 243pp. Available from: www.psc.org/info_codedwiretagreview.htm

Kalinowski, S. 2004. Genetic polymorphism and mixed stock fisheries analysis. Canadian Journal of Fisheries & Aquatic Sciences 61:1075-1082.

Moran, P., and 27 other authors. 2005. Interlaboratory Standardization of Coast-wide Chinook Salmon Genetic Data for International Harvest Management. LOA Technical Report prepared for the Pacific Salmon Commission Chinook Technical Committee. 44 pp.

Narum, S.R, M. Powell, and A. Talbot. 2004. A distinctive microsatellite locus that differentiates ocean from stream type Chinook salmon (*Oncorhynchus tshawytscha*) in the Columbia River Basin. Transactions of the American Fisheries Society 133:1051-1055.