

NPAFC
Doc. 996
Rev. _____

Report of the Second Annual SNP Workshop, Anchorage, Alaska, USA,
Sept. 21-22, 2006

by

L. W. Seeb and J. E. Seeb

*Gene Conservation Laboratory, Alaska Department of Fish and Game, 333 Raspberry Road,
Anchorage, AK, USA 99518*

Submitted to the
NORTH PACIFIC ANADROMOUS FISH COMMISSION
BY THE
UNITED STATES OF AMERICA

October, 2006

This document may be cited as:

Seeb, L.W and J.E. Seeb. 2006. Report of the Second Annual SNP Workshop, Anchorage, Alaska, USA, Sept. 21-22, 2006. (NPAFC Doc. 996). 11 p. Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518, USA.

Abstract

Genetic stock identification using a comprehensive allozyme baseline has been extremely valuable for high-seas and coastal migration studies from throughout the Pacific Rim. However, newer DNA techniques provide significant advantages over allozymes in sampling, sample handling, and the potential for improved resolution. Of the various DNA markers, single nucleotide polymorphisms (SNPs) assayed through high-throughput technologies are particularly appropriate for NPAFC applications. Unlike marker types based on fragment size, SNPs are based on the actual DNA sequence and, SNP data can be easily transferred between laboratories and instrument platforms. We review two SNP workshops held in Alaska in 2005 and 2006 that brought scientists from throughout the Pacific Rim together for research discussions on discovery, applications, laboratory techniques, and statistics. Scientists from four NPAFC Parties (Canada, Japan, Russia, and United States) attended both workshops. The status of current SNP baselines for sockeye, chum, Chinook, and coho salmon was reviewed at the workshops and is included here. Over 60 SNPs have been described for chum and Chinook salmon, nearly 40 for sockeye salmon, and 22 for coho salmon. The baseline for chum salmon is being used for BASIS research, while the Alaska Department of Fish and Game is extensively using the sockeye salmon baseline in Bristol Bay studies. SNPs are a simple and cost effective method that can be readily incorporated into NPAFC laboratories for BASIS and similar high-seas applications.

Background and SNP Workshop I, 2005

Genetic stock identification studies have become a central part of North Pacific Anadromous Fish Commission (NPAFC) research and BASIS activities. The NPAFC *ad hoc* Working Group on Stock Identification Studies has coordinated these studies with the goals to develop, standardize, and disseminate genetic databases among the parties. These databases must provide an appropriate level of accuracy and precision for stock identification studies while at the same time be easily shared and repeatable among the NPAFC Parties.

Recently researchers from the NPAFC Parties have begun developing single nucleotide polymorphisms (SNPs) as a marker of choice for many NPAFC studies (Seeb et al. 2004, 2005; Templin et al. 2005; Sato et al. 2006). Although many SNPs were identified over a decade ago, the chemistries available for applying these markers were relatively slow and cumbersome. Recent developments have produced rapid SNP genotyping technologies which are now faster than those for any other marker class and highly appropriate for high-throughput genotyping (Smith et al. 2004). SNP data can be standardized to external DNA sequences and are thus easily adapted across chemistries, instrument platforms, and laboratories (Zelenina et al. 2005). Many DNA assays estimate the size of fragment polymorphisms yielding data that tends to be lab-specific; in contrast, SNP scores of A, C, G, or T are unambiguous. In addition, the fact that all of these assays interrogate mtDNA as well as neutral and selected nuclear genes such as MHC provides an array of powerful opportunities for various types of study.

The Alaska Department of Fish and Game (ADFG) with the assistance of the Genetics Section of American Fisheries Society and Applied Biosystem hosted the first SNP workshop (*SNP Workshop, Developing Cooperative Databases for Pacific Salmon*) in Anchorage, Alaska, USA, April 28-29, 2005. Fifty-five participants traveled to Anchorage for the conference. Speakers described the advantages of SNPs for cooperative research and discussed the various different SNP assays that all offer high-throughput, relatively inexpensive data that is easily shared from laboratory to laboratory. A description of the workshop can be found at:

<http://www.genetics.cf.adfg.state.ak.us/workshop/snpworkshop05.php>

NPAFC scientists from NOAA Fisheries, Washington Department of Fish and Wildlife, ADFG, Canadian Department of Fisheries and Oceans, Hokkaido University, and the Russian Federal Research Institute of Fisheries attended, and Pacific Rim baselines for chum and sockeye salmon were discussed.

SNP Workshop II, 2006

ADFG and the Genetics Section of the American Fisheries Society, with sponsorship from Applied Biosystems, hosted a second SNP workshop (*SNP Workshop II: Applications of SNP Genotyping in Fisheries Management*) in Girdwood, Alaska, Sept. 21-22, 2006. The workshop explored applications, SNP discovery, statistics, laboratory techniques, and reviewed case studies of SNPs for fisheries management and conservation. Discussions were structured with a goal of building cooperative research and Pacific Rim baselines. The expanded scope of this year's workshop drew from a variety of related disciplines. Invited presentations included overviews and SNP applications from a variety of disciplines representing a diversity of non-model organisms. Representatives of Applied Biosystems presented recent developments on TaqMan genotyping and troubleshooting. Experience papers presented by participating laboratories generated group discussions of research priorities and opportunities for cooperation. The Workshop was well attended with over 85 scientists from eight nations. A description of the workshop can be found at:

<http://www.genetics.cf.adfg.state.ak.us/workshop/snpworkshop06.php>

NPAFC scientists from NOAA Fisheries, Washington Department of Fish and Wildlife, ADFG, Canadian Department of Fisheries and Oceans, Japan National Salmon Resources Center, and the Russian Federal Research Institute of Fisheries attended.

Status of SNP Baselines

Substantial progress has been made for chum, sockeye, and Chinook salmon in the past year. These databases were reviewed at the workshops. The U.S., Japanese, and Korean Parties have been working to expand and utilize the SNP baseline for chum salmon (Seeb et al. 2004, 2005; Sato et al. 2006). Japanese, Korean, and Russian scientists have visited the ADFG laboratory to coordinate techniques and collect Asian baselines. The current baseline includes over 104 populations (Figure 1a) each characterized for between 40-62 SNPs following the protocols of Smith et al. (2005a,b) and Elfstrom et al. (in prep.) Additional coverage is highly desirable. Coverage of Russia and Canada has been hampered by the lack of representative samples; however, access to Canadian samples is anticipated this fall.

The sockeye salmon baseline is also progressing with the majority of Alaska well characterized (Figure 1b) for 39 SNPs (Smith et al. 2005a; Elfstrom et al. 2006). Populations from Russia and Canada are lacking, although access to Canadian samples is also anticipated this year. A scientist from Federal Fisheries Agency of Russia has visited the laboratory to collaborate on sockeye salmon SNPs and is currently collecting data for some of the loci in Moscow (Zelenina et al. 2005).

Progress on a baseline for Chinook salmon has benefited from a recent research award from the Arctic-Yukon-Kuskokwim Sustainable Salmon Initiative (AYK SSI) to

characterize baseline populations and develop forecasting capabilities through the analysis of BASIS and bycatch samples from the walleye pollock fishery (Figure 1c). Additionally, development of SNPs for Chinook salmon has been supported by the Pacific Salmon Commission with funds for both U.S. and Canadian laboratories to develop SNPs and characterize populations from Southeast Alaska to California.

The first stages of SNP development has occurred for coho salmon (Smith et al. 2005d), but no coordinated effort is yet underway to develop a baseline. Discussions are underway for development of the SNP loci in steelhead and, possibly, pink salmon.

Conclusions

The two SNP workshops provided excellent opportunities for NPAFC scientists to exchange ideas and experiences concerning SNPs as well as to plan for future collaborations. It is anticipated that applications of the SNP genetic stock identification will continue to expand as the baselines become more comprehensive and SNP technologies become increasingly integrated into NPAFC laboratories. The number of available SNPs is rapidly approaching a point where locus selection will be required to refine applications and improve cost-effectiveness.

References

- Elfstrom, C.M., Smith, C.T. and Seeb, J.E. (2006) Thirty-two single nucleotide polymorphism markers for high-throughput genotyping of sockeye salmon. *Molecular Ecology Notes* **6**, (in press).
- Elfstrom, C. M., C. T. Smith, L. A. Stewart, and L. W. Seeb. In prep. Thirty-five single nucleotide polymorphism markers for high through-put genotyping of chum salmon. *Molecular Ecology Notes*.
- Sato, S., S. Takahashi, L. W. Seeb, J. E. Seeb, F. Fukuwaka, and S. Urawa. 2006. Stock identification of winter chum salmon by mitochondrial DNA and SNP analyses (NPAFC Doc. 963). 8 p. National Salmon Resources Center, Fisheries Research Agency, Toyohira-ku, Sapporo 062-0922, Japan.
- Seeb, L. W., C. T. Smith, W. D. Templin, R. L. Wilmot, and, J. E. Seeb. 2004b. Development of a Pacific Rim baseline for chum salmon based on single nucleotide polymorphism markers (SNPs). (NPAFC Doc. 824) 10 pages. Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK, USA 99518.
- Seeb, L. W, W. D. Templin, C. T. Smith, C. Elfstrom, S. Urawa, R. L. Wilmot, and, J. E. Seeb. 2005. SNPs provide an Easily-Standardized Baseline for NPAFC Studies of Chum Salmon (NPAFC Doc. 907) 12 pages. Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK, USA 99518.
- Smith, C.T., Baker, J., Park, L., Seeb, L.W., Elfstrom, C., Abe, S. and Seeb, J.E. 2005b. Characterization of 13 Single Nucleotide Polymorphism Markers for Chum Salmon. *Molecular Ecology Notes* **5**, 259-262.
- Smith, C.T., Elfstrom, C.M., Seeb, L.W. and Seeb, J.E. 2005a. Use of Sequence Data From Rainbow Trout and Atlantic Salmon for SNP Detection in Pacific Salmon. *Molecular Ecology* **14**, 4193-4203.
- Smith, C.T., Park, L., Vandoornik, D., Seeb, W. and Seeb, E. 2006. Characterization of 19 Single Nucleotide Polymorphism Markers for Coho Salmon. *Molecular Ecology Notes* **6**, 715-720.
- Smith, C. T., J. E. Seeb, and L. W. Seeb. 2004. Development of 5'-nuclease reactions for high-throughput SNP genotyping in salmon. N. Pac. Anadr. Fish Comm. Tech. Rep. 5: 45-46.
- Smith, C.T., Seeb, J.E., Schwenke, P. and Seeb, L.W. 2005c. Use of the 5'-Nuclease Reaction for Single Nucleotide Polymorphism Genotyping in Chinook Salmon. *Transactions of the American Fisheries Society* **134**, 207-217.

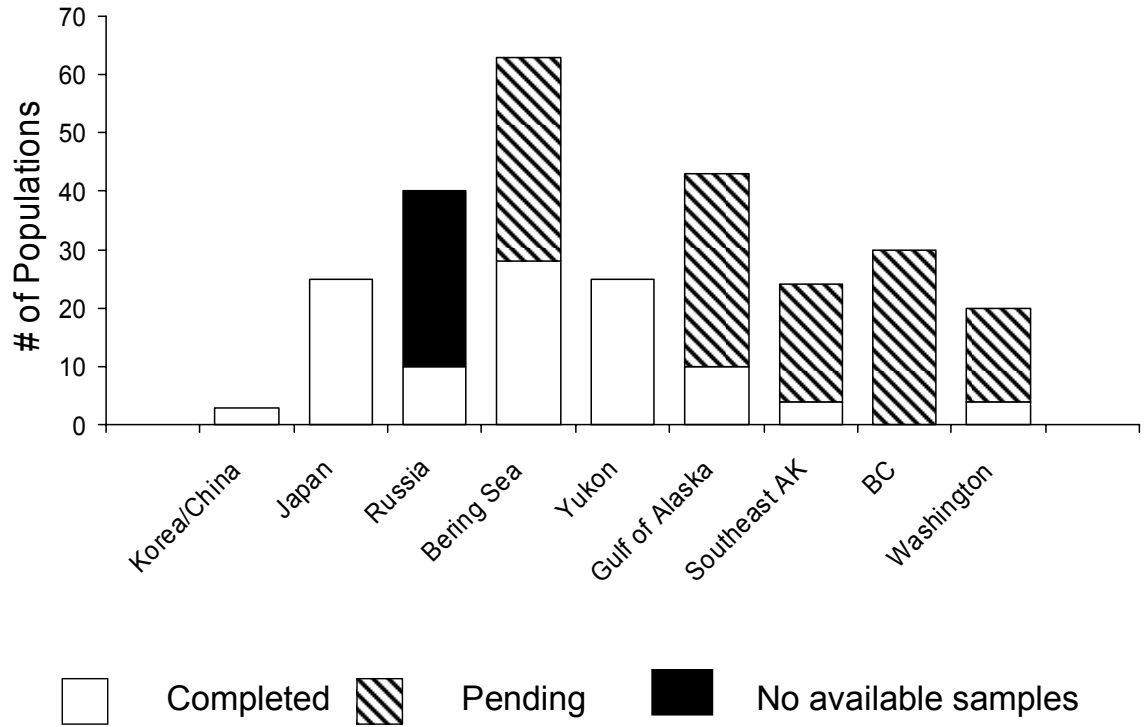
Smith, C.T., Templin, W.D., Seeb, J.E. and Seeb, L.W. 2005d. Single Nucleotide Polymorphisms Provide Rapid and Accurate Estimates of the Proportions of US and Canadian Chinook Salmon Caught in Yukon River Fisheries. *North American Journal of Fisheries Management* **25**, 944-953.

Templin, W. D., C. T. Smith, J. E. Seeb, and L. W. Seeb. 2005. SNPs provide high throughput resolution for migratory studies of Chinook salmon. (NPAFC Doc. 908) 10 p. Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK, USA 99518.

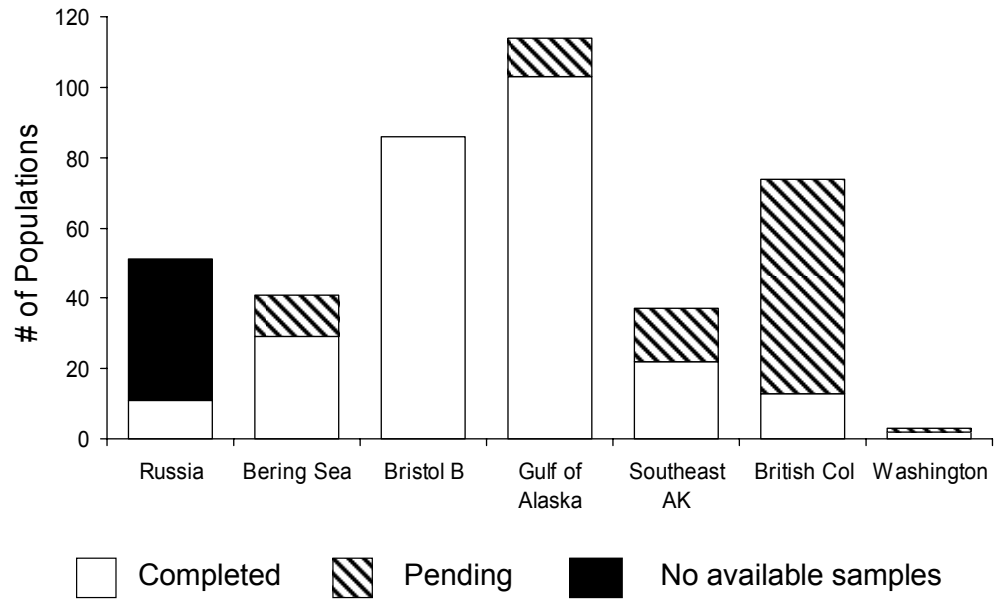
Zelenina, D., A. Khrustaleva, A. Volkov, C. Habicht, C. Smith, J. Seeb . 2005. A Case Study of Two Genetic Markers for Inter-Laboratory Collaboration: SNPs Provide Transportability without Standardization. (NPAFC Doc. 913) 14 p. Russian Federal Research Institute of Fisheries & Oceanography, Federal Fisheries Agency of Russia, VNIRO, 17 V. Krasnoselskaya, Moscow, Russia.

Figure 1. Status of SNP baselines for NPAFC and BASIS studies.

a. Chum salmon



b. Sockeye salmon



c. Chinook salmon (Russia and Alaska only)

