



Genetic stock identification of Pacific salmon

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Salmon DNA stock identification







What is a genetic marker?

A variable sequence of DNA nucleotides useful for identifying species, a regional stock group, a stock, or individual.

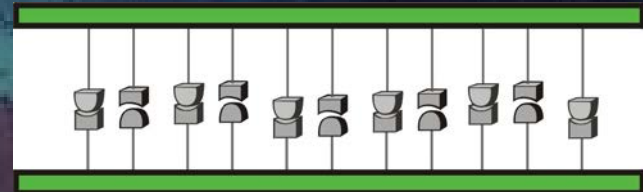
Microsatellites

- 2 or 4 base pair repeat
- alleles differ by size

Single nucleotide polymorphism (SNPs)

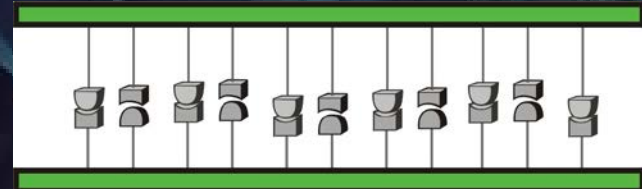
- alleles differ by sequence at a single base pair

GATC GATC



Ots103

GAAC TTCGAA

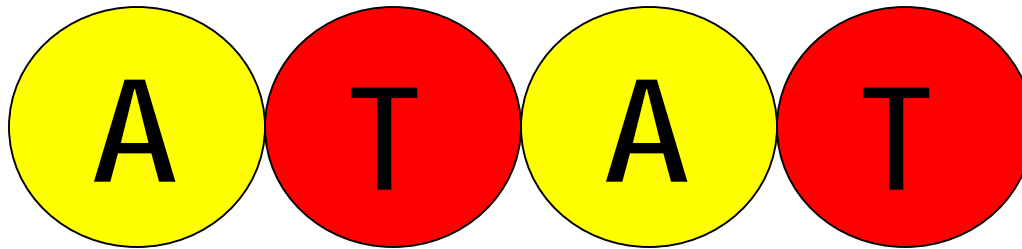


SNP



Microsatellite

- Short repeat of non-coding DNA

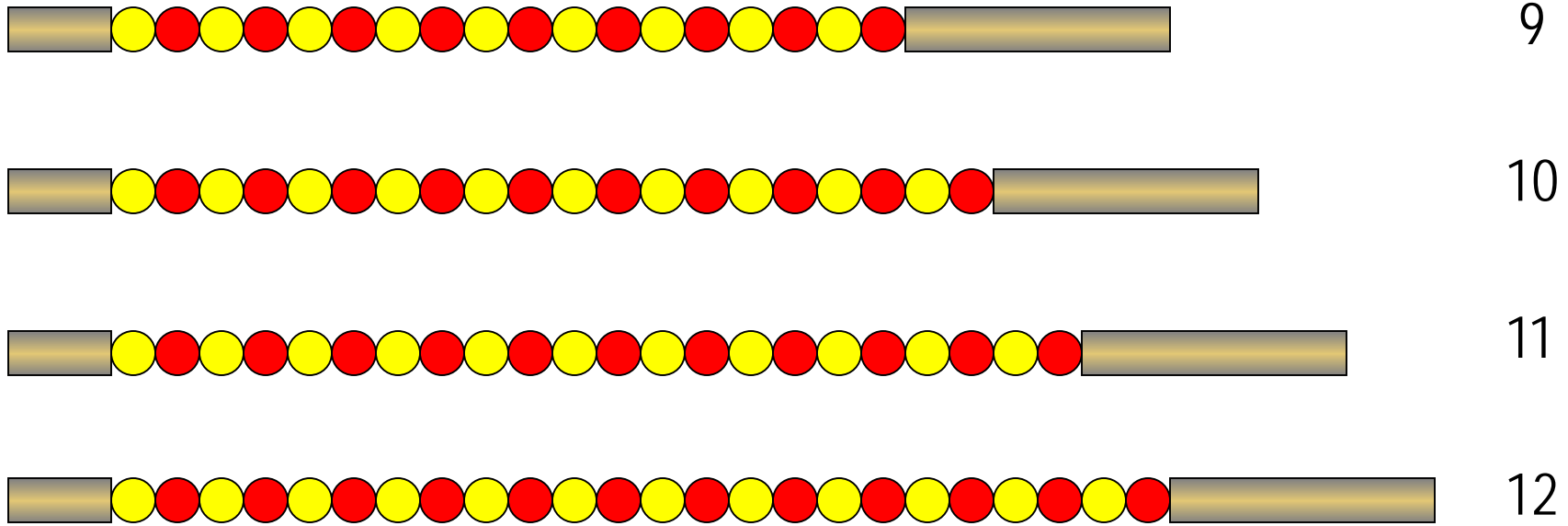


DNA sequence



Microsatellite

- Variation scored by estimating repeats



DNA sequence



Microsatellites

- DFO Molecular Genetics Laboratory (MGL) has used microsatellites for the past 25 years for salmon stock identification.
- Microsatellites applied exclusively to Yukon River Chinook and Chum Salmon in Canadian applications.



SNP

- Single nucleotide polymorphism

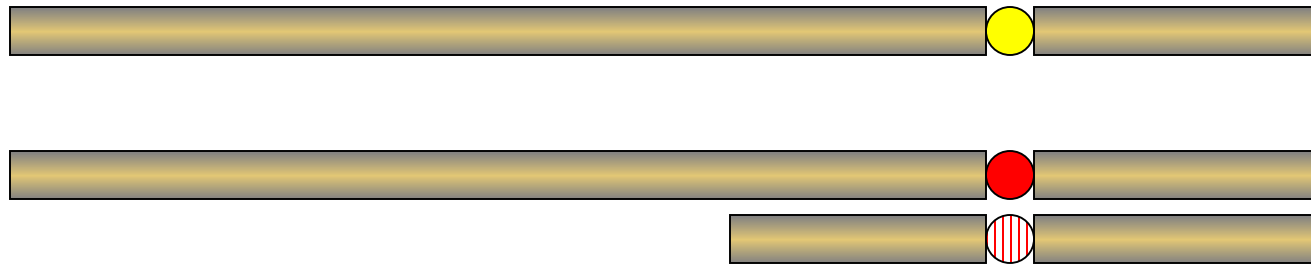


DNA sequence

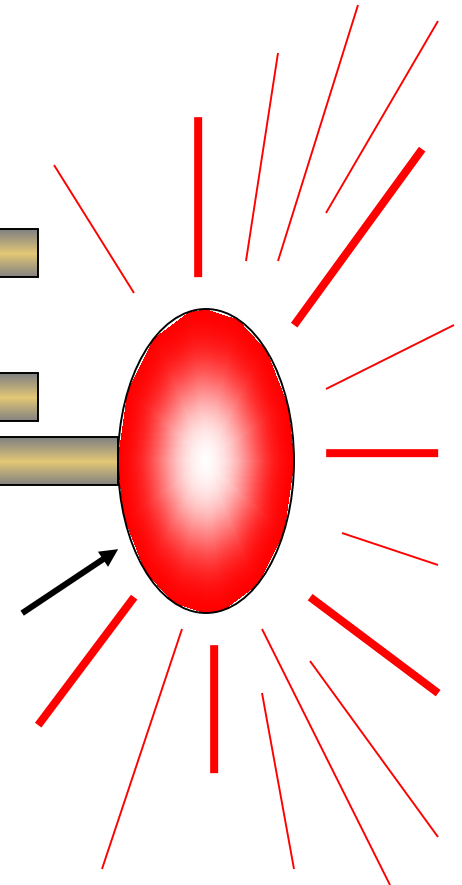


SNP

- Scored by probing actual base



Automated scoring by fluorescent probes





SNPs

- Used by many laboratories for stock identification applications.
- Use of fluorescent probes was the technology commonly associated with SNP genotyping.
- ADF&G genetics laboratory has used SNPs for many years in Yukon River stock identification applications.

Use of Genetic Variation

Task

- Species identification
- Regional structure
- Estimate of stock composition
- Individual identification to population

Degree of Difficulty

EASY

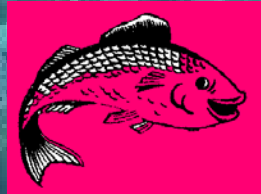
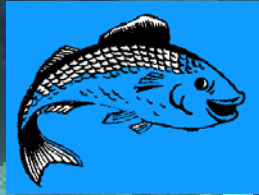


Hard

Two components to mixed stock analysis

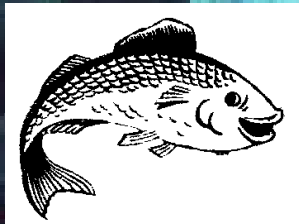
Baseline data

- genotypes from known populations
- usually spawning ground samples

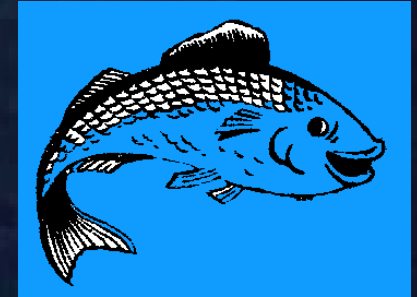


Mixture data

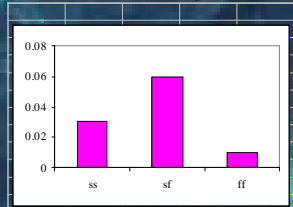
- genotypes from unknown populations
- usually samples from fisheries



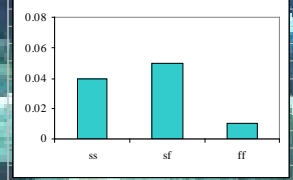
Mixed Stock Analysis



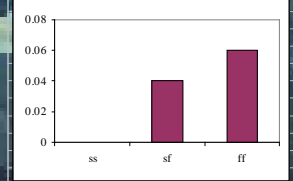
Frequency of Baseline Genotypes



Population 1

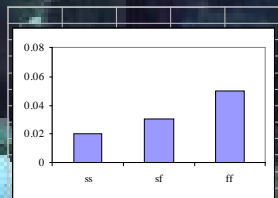


Population 2



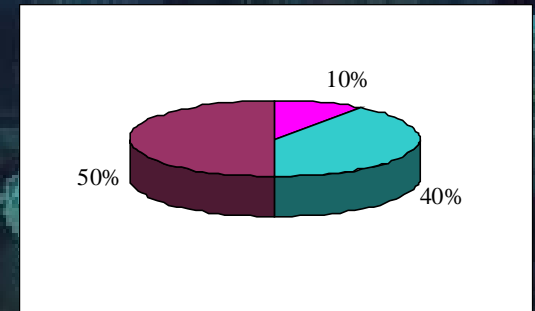
Population 3

Frequency of Mixture Genotypes



Computer Analysis

Estimation of the most likely composition of the mixed fishery





Two main classes of genetic variants used in stock identification

- Microsatellites and single nucleotide polymorphisms (SNPs).
- Microsatellites were the initial class of genetic variants used in stock identification applications, with SNPs subsequently used in many instances.
- Both classes of genetic variants have been applied to estimate stock composition in mixed-stock salmon fisheries under the Pacific Salmon Treaty (PST), and results from both classes of variants are considered reliable.



Why didn't the Molecular Genetics Laboratory (MGL) convert to SNPs for stock identification work?

- MGL evaluated the number of SNPs that would be required to produce results of equivalent quality to microsatellites.
- For the MGL, cost of analysis for an individual fish was higher for SNPs than for microsatellites.
- No reason for the MGL to switch to SNPs based on the fluorescent probe technology.



Genetic Stock Identification (GSI) analysis

- Sample from a mixed-stock fishery. How do you determine stock composition?
- Need a baseline of populations to apply to estimate stock composition. The more complete the baseline, potentially the better the stock identification results.
- Use genetic differentiation among populations in baseline to estimate stock composition in the fishery sample.



Stock identification, baseline reporting groups and populations within groups

- Want to be sure that estimates of stock composition are accurate when reported.
- Look at genetic structure of populations sampled. Arrange genetically similar populations into a single group.
- Report estimates based upon arranging individual populations into groups that provide at least 90% accuracy when that group is the only group in the mixture (100% is correct answer).
- A reporting group may contain several populations that are difficult to distinguish genetically.



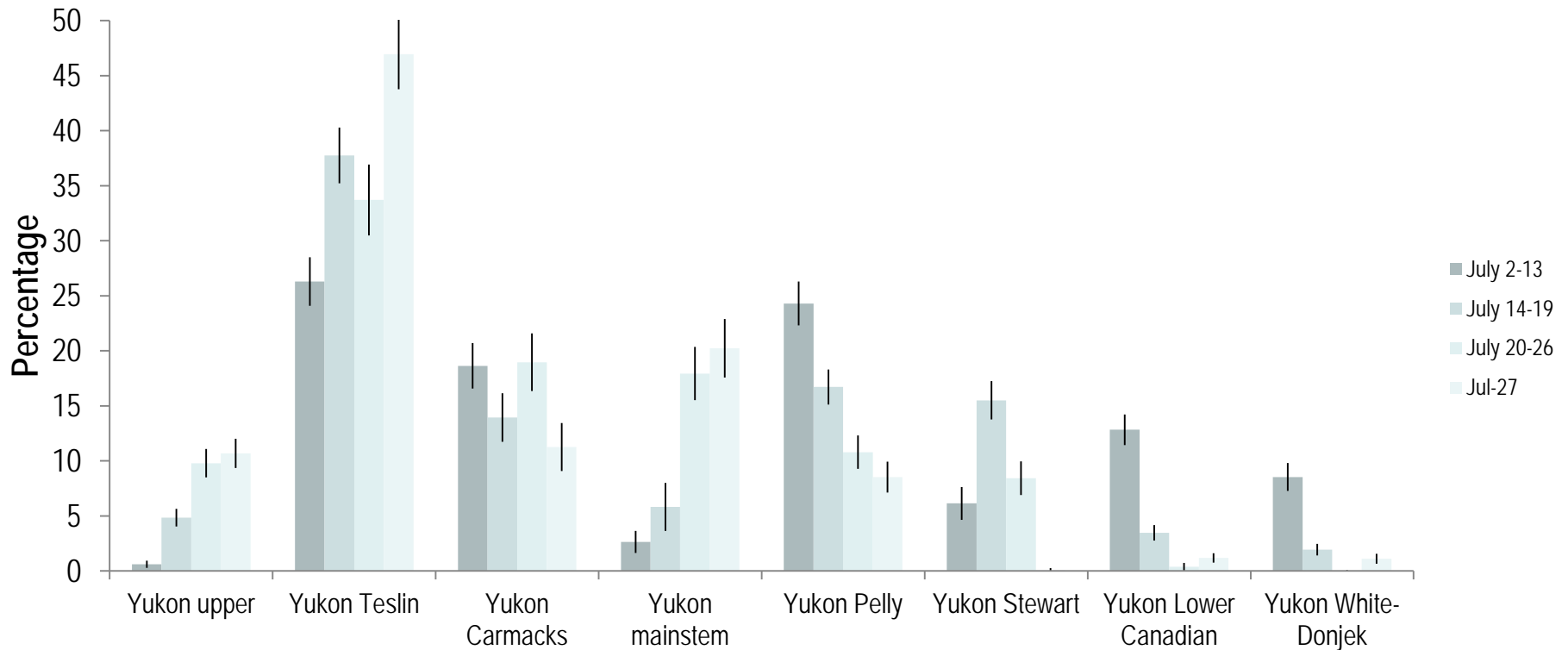
Regions and populations within regions for Chinook Salmon microsatellite baseline

Region	Population
Yukon_upper	Takhini
Yukon_upper	Whitehorse
Yukon_Teslin	Wolf_R
Yukon_Teslin	100_Mile_R
Yukon_Teslin	Morley
Yukon_Teslin	Nisutlin
Yukon_Teslin	Teslin
Yukon_Teslin	Teslin_Lake
Yukon_Carmacks	Big_Salmon
Yukon_Carmacks	L_Salmon
Yukon_Carmacks	N_Big_Salmon
Yukon_Carmacks	Tatchun
Yukon_mainstem	Nordenskiold
Yukon_mainstem	Yukon_main
Yukon_Pelly	Blind_Cr
Yukon_Pelly	Earn
Yukon_Pelly	Glenlyon
Yukon_Pelly	Hoole
Yukon_Pelly	Kalzas
Yukon_Pelly	L_and B_Kalzas
Yukon_Pelly	Pelly
Yukon_Pelly	Ross_YT
Yukon_Stewart	Mayo
Yukon_Stewart	McQuesten
Yukon_Stewart	Stewart
Yukon_LowerCAN	Chandindu
Yukon_LowerCAN	Klondike
Yukon_White-Donjek	Kluane
Yukon_White-Donjek	Nisling



Eagle 2016 Chinook stock identification by regional stock

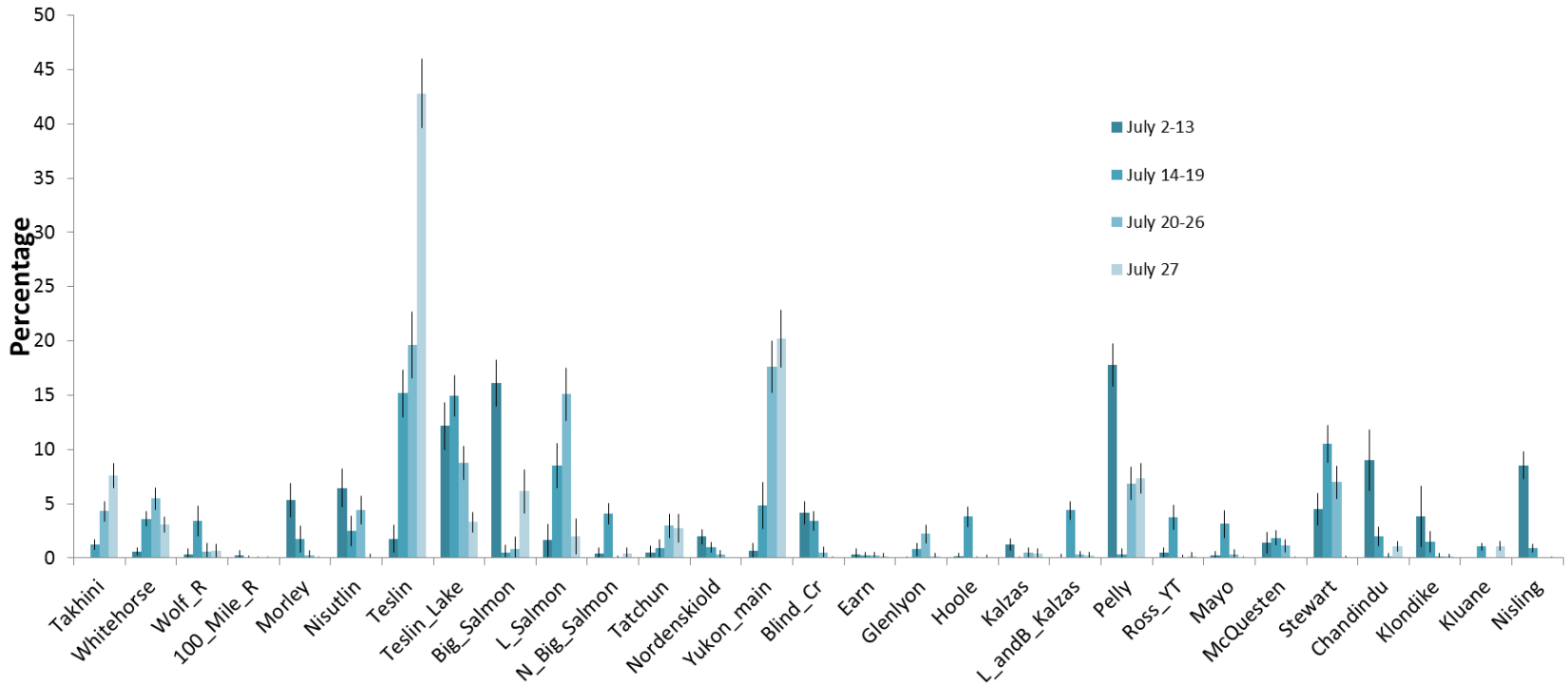
Regional Compositions Chinook Yukon Eagle 2016





Eagle 2016 Chinook stock identification by population

Stock Compositions Chinook Yukon Eagle 2016





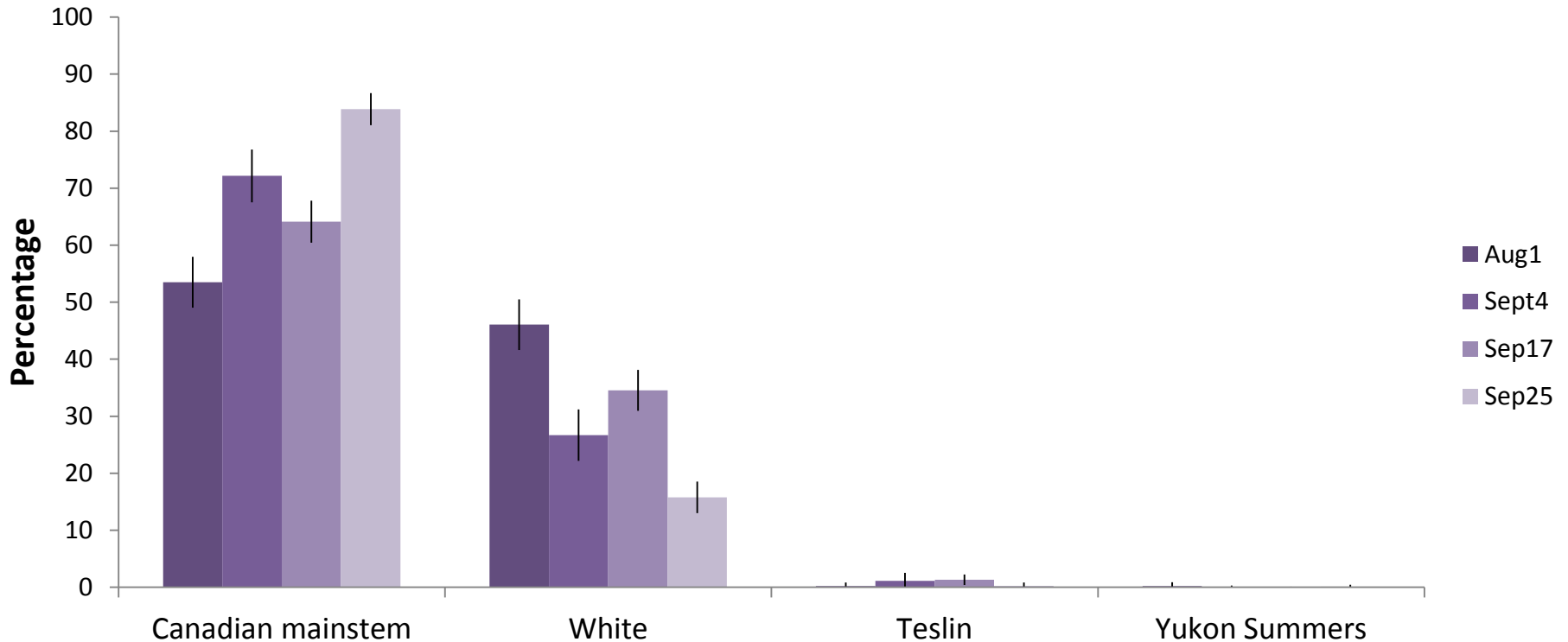
Regions and populations within regions for Chum Salmon microsatellite baseline

Region	Population
Canadian mainstem	Big Creek
Canadian mainstem	Minto
Canadian mainstem	Pelly
Canadian mainstem	Tatchun
White	Donjek
White	Kluane
White	Kluane Lake
Teslin	Teslin
Yukon Summers	Chandindu



Eagle 2016 Chum stock identification by regional stock

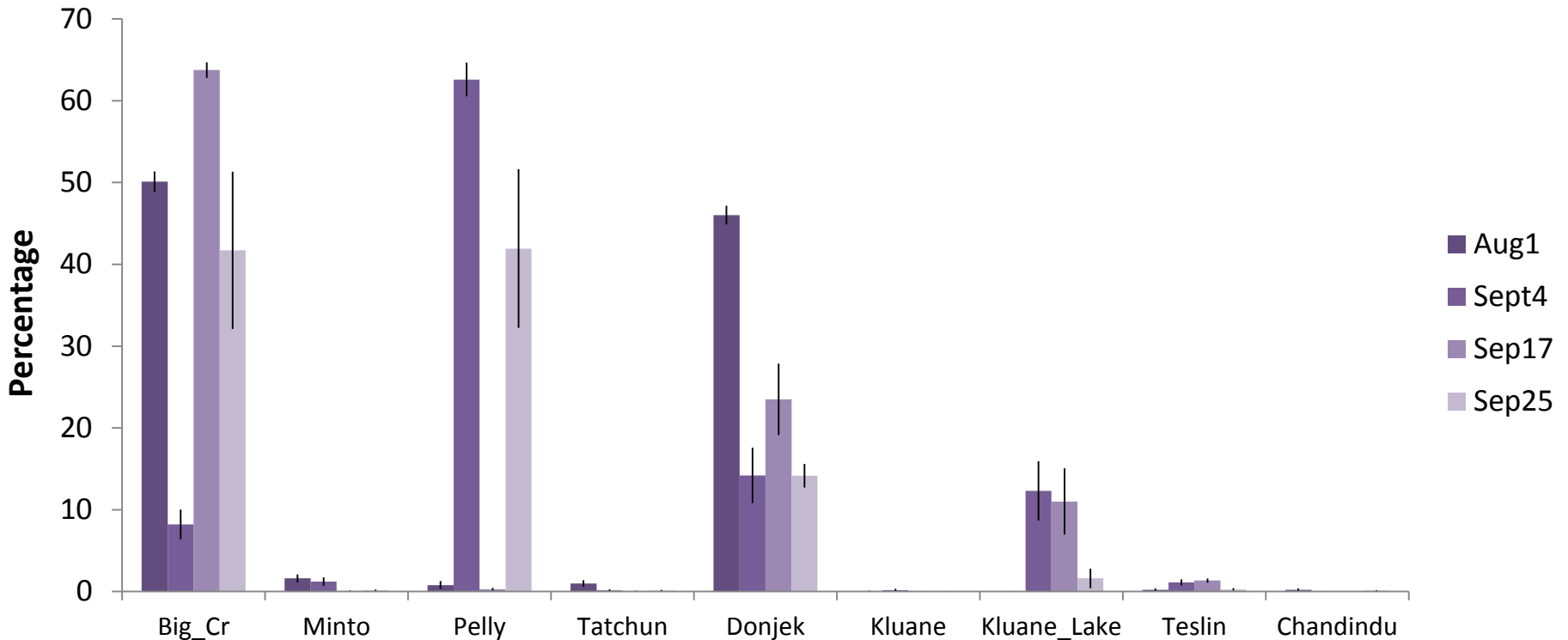
Regional Compositions Chum Yukon Eagle 2016





Eagle 2016 Chum stock identification by population

Stock Compositions Chum Yukon Eagle 2016





Why do stock identification analysis?

- When a fishery is sampled over the course of a season (like the Eagle sonar site), stock identification information combined with abundance can provide timing information on specific reporting groups or perhaps specific populations.
- When multiple fisheries are sampled, can track when and where a specific population or reporting group is caught.
- Can use stock identification information to manage fisheries to provide protection to stocks of conservation concern, and shift harvest to more abundant stocks.



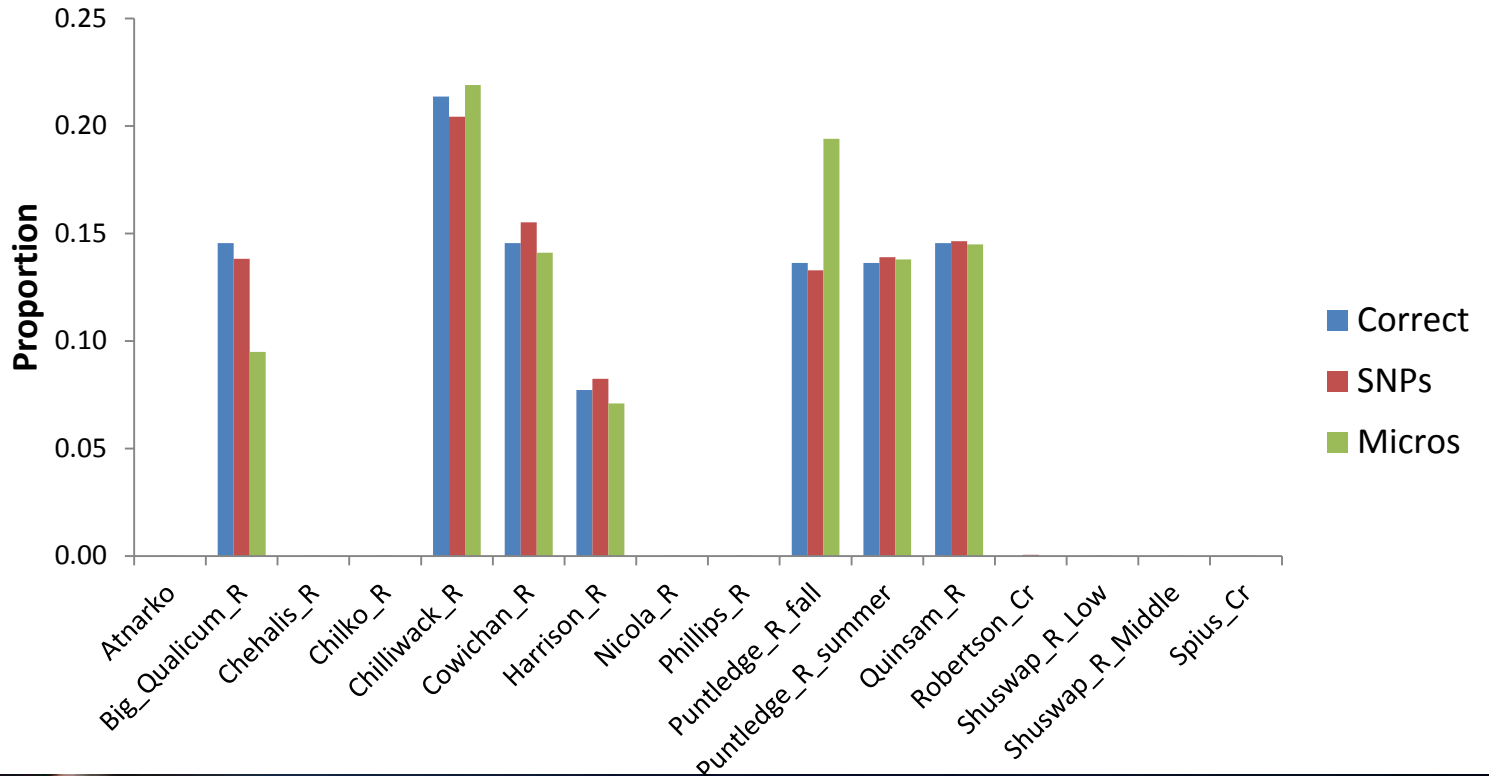
Why switch to SNPs now?

- Cost of SNP analysis has declined substantially with the use of direct sequencing of DNA to genotype SNPs.
- No longer have to use fluorescent probes to genotype SNPs.
- Can incorporate hundreds of SNPs into stock identification applications so that higher resolution results are produced at no additional cost relative to microsatellites.
- Need to develop baseline of Canadian populations.



How do we know that higher resolution stock identification results may be available with hundreds of SNPs?

- Example from British Columbia, 660 fish in mixture sample





What is the Yukon application?

- Maybe finer scale ability to separate out populations (90% accuracy rule) than currently available.

Region	Population
Yukon Carmacks	Big Salmon
Yukon Carmacks	Little Salmon
Yukon Carmacks	Tatchun
Teslin	Morley
Teslin	Nisutlin
Teslin	Teslin
Teslin	Teslin Lake
Teslin	Wolf



Advantages of new Canadian SNP baseline for Chinook

- May be able to increase resolution of stock ID estimates so that some individual populations can be reliably identified in mixed-stock samples.
- Provide managers with increased flexibility to structure fisheries to reduce catch of populations of conservation concern.
- May be able to estimate escapements of specific populations at the Eagle sonar site.