

# New genetic technology for the management of Snake River Chinook salmon and steelhead

BPA Project 2010-026-00:  
Genetic Stock identification of Snake River steelhead and Chinook salmon

BPA Project 2010-031-00:  
S Snake River Chinook Salmon and steelhead Parental Based Tagging

**Presentation to Pacific States Marine Fisheries Commission  
Annual Meeting – August 30<sup>th</sup>, 2011**

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# Genetic stock identification of Snake River steelhead at Lower Granite Dam



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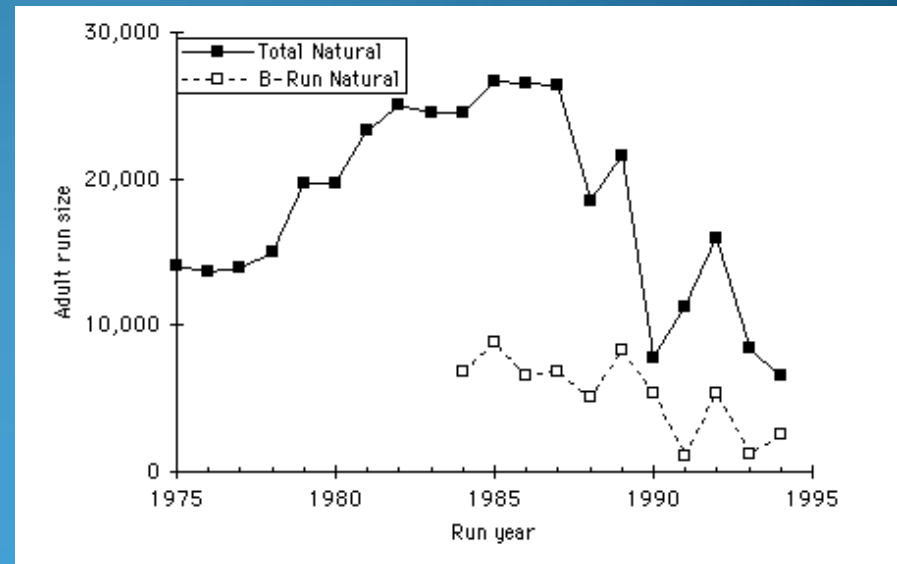
Shawn Narum, Jon Hess, Andrew Matala

Columbia River Inter-Tribal Fish Commission



# Introduction

- Abundance is a primary metric needed for monitoring the status of salmon and steelhead populations (McElhany et al. 2000)
- Estimates of abundance (combined with age and sex data) over time allows estimation of population growth and productivity parameters
- Estimates of these metrics provide indicators of the resiliency and viability of populations and allows extinction risk assessment



# Introduction

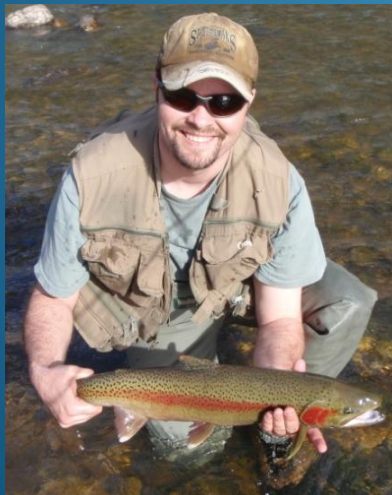
- HOWEVER, population level assessments of Snake River steelhead is often very difficult due to...
  - wide distribution
  - conditions at time of spawning
- Most steelhead populations in the Snake River have little to no abundance and productivity information
- Most status assessments rely on aggregate measurements of all adults at Lower Granite Dam



## “A-run” versus “B-run”

- In lieu of more detailed basin-level stock specific information, managers have relied primarily on a two stock classification system for Snake River steelhead adults characterized by life history variations in size and age at return, timing of migration into freshwater, spawn timing and spawning distribution

Stock	Ocean Residence	Timing over Bonneville	Size at return	Distribution	Spawn Timing
A-run	<b>1 year</b>	June 1 to August 25	<b>≤ 78 cm</b>	Tucannon, Grande Ronde, and Imnaha rivers, upper Salmon River, Snake River	March-April
B-run	<b>2 years</b>	August 25 and October 31	<b>&gt; 79 cm</b>	Clearwater River, S.F./M.F. Salmon Rivers	Late April-May



“A-run” steelhead  
Lower Snake River



“B-run” steelhead  
S.F. Clearwater

# "A-run" versus "B-run"

- Snake River "B-run" steelhead are currently identified as exhibiting a distinctive life-history component of the Snake River Distinct Population Segment (NOAA 2003)
- However, their management is confounded by the lack of a clear and detailed understanding of their actual spawning distribution and evolutionary structure
- Genetic studies have reported that steelhead in Snake River tributaries in Idaho exhibit a complicated pattern of genetic structuring with populations grouping genetically according to drainage locality, not simply by "A-run" or "B-run" designations (Moran 2003; Nielsen et al 2009)

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[Article]

## Steelhead Genetic Diversity at Multiple Spatial Scales in a Managed Basin: Snake River, Idaho

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**Abstract.**—We investigated the genetic diversity of steelhead *Oncorhynchus mykiss* in 74 wild populations and 5 hatchery stocks in Idaho's Snake River basin at the drainage, watershed, and population spatial scales using 11 microsatellite loci. We found significant genetic diversity at multiple spatial scales. Analysis of molecular variance showed that genetic diversity was greater among watersheds within drainages (3.66%) than among drainages (1.97%). Over 94% of the genetic diversity found in the Clearwater, Salmon, and Snake River drainages occurred within individual populations. Estimated effective population sizes ranged from 213 to 486.6 at the drainage scale, from 81.2 to 610.4 at the watershed scale, and from 8.4 to 4,845 at the population scale. The Middle Fork Salmon, South Fork Salmon, Lochsa, and Selway rivers—watersheds managed for wild fish—formed distinct groups in our consensus neighbor-joining (NJ) trees. At the watershed scale our analyses support differentiation of all hatchery and wild stocks. However, this was not the case for analyses at the population scale, where 236  $F_{ST}$  pairwise comparisons out of 3,081 (wild and hatchery) were not significantly different. The distribution of genetic diversity across the landscape does not appear to be organized by the A run or B run management designations for anadromous steelhead. The Dworshak hatchery stock was significantly different from all but one population (O'Hara Creek, Selway River) in pairwise  $F_{ST}$  comparisons and grouped with other Clearwater River drainage populations in our NJ trees. The Oxbow, Sawtooth, and Pahsimeroi hatchery stocks were indistinguishable from each other based on  $F_{ST}$  analysis. Currently, this study represents the most comprehensive evaluation of genetic diversity in Idaho's steelhead populations across multiple scales with different management histories.

The ability to adapt to highly variable conditions in unique environments is a critical element throughout the life cycle of anadromous salmonids. *Oncorhynchus mykiss* populations express a diversity of life history strategies from strongly anadromous (steelhead) to nonanadromous (resident or rainbow trout) throughout the species' natural range (Shapovalov and Taft 1954; Rybock et al. 1975; Taylor 1995). Many studies have shown that sympatric populations of anadromous and resident *O. mykiss* found within the same drainage cannot be separated taxonomically or genetically based on migration timing or different life histories (Allendorf and Utter 1979; Reisenbichler et al. 1992; Nielsen et al. 1994; Docker and Heath 2003; Nanum et al.

2004a; Olsen et al. 2006; Heath et al. 2008). Additional studies demonstrated that steelhead isolated as resident fish behind artificial barriers and dams contain components of the *O. mykiss* gene pool formerly found in geographically proximate anadromous populations (Gall et al. 1990; Nielsen et al. 1997; Deiner et al. 2007). Variation in reproductive success and genetic compensation between different life history forms can result in changes in effective population size and subsequent patterns of genetic diversity across the landscape (Heath et al. 2001; Araki et al. 2007).

Historically, steelhead were broadly distributed throughout most of the Columbia River basin, including populations in Oregon, Washington, Idaho, and British Columbia (Behnke 1992; reviewed in Busby et al. 1996). Mallet (1974) estimated that 55% of all steelhead in the lower Columbia River were produced in the Idaho portion of the Snake River.

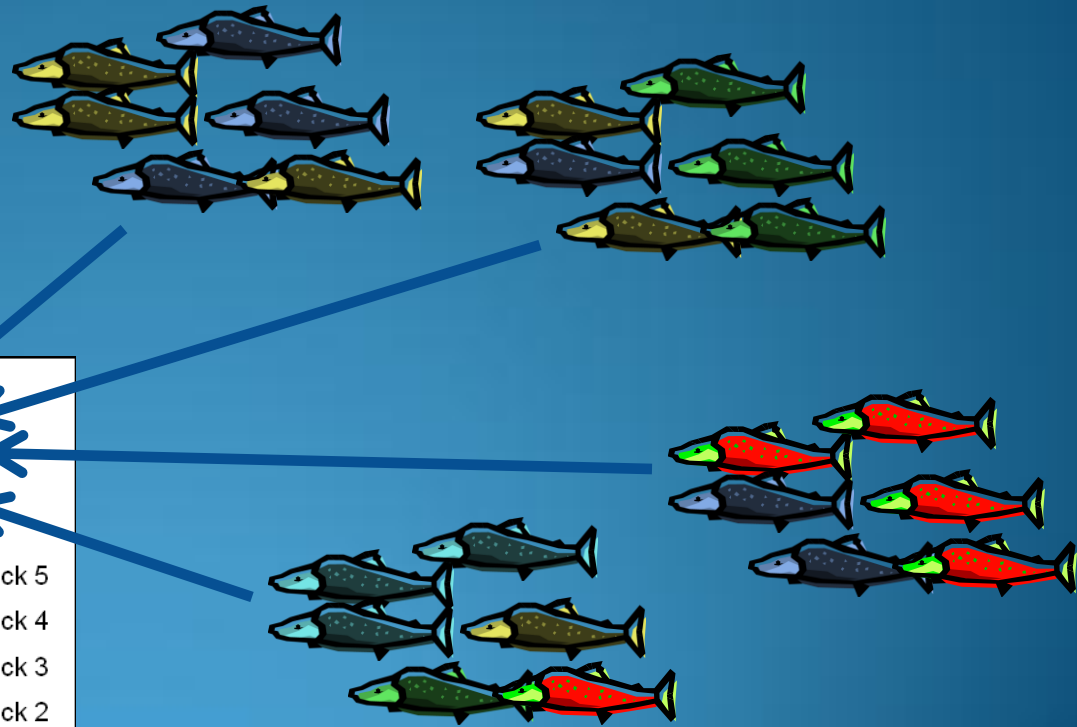
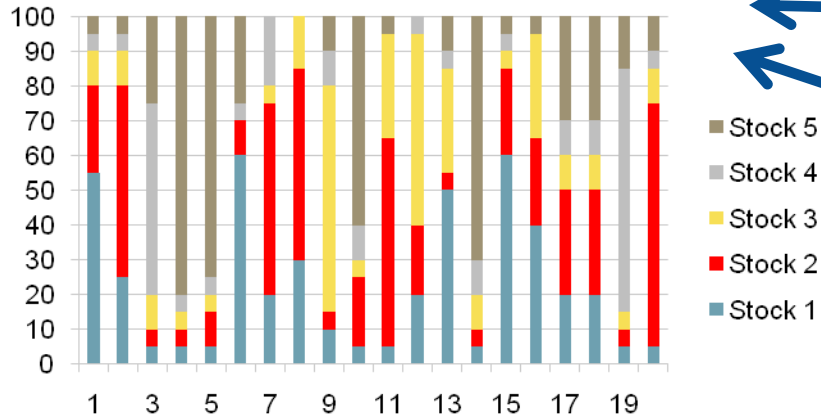
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# Genetic Stock Identification

- One technology that might improve our understanding of “A-run”/”B-run” life histories and providing a finer scale of stock management for Snake River steelhead is Genetic Stock Identification (GSI)
- GSI uses allele frequencies from baseline populations (from all major contributing stocks), and genotypes from mixture samples, to estimate stock proportions within mixed stock fisheries (Anderson et al 2008)

Stock Composition



# Genetic Stock Identification

- GSI technology has been used extensively to investigate and manage mixed stock fisheries for a variety of Pacific salmonids including Chinook salmon (Beacham et al. 2008), sockeye salmon (Habicht et al. 2007), chum salmon (Beacham et al. 2008) and steelhead (Beacham et al. 1999, 2000)
- In the Snake River basin, previous genetic studies have indicated that steelhead exhibit significant genetic structuring at the watershed (subbasin) level (Moran 2003; Nielsen et al. 2009) and GSI procedures have already been used successfully to identify the origin of kelt steelhead at lower granite dam (Narum et al. 2008)

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## Genetic Stock Identification of Steelhead in the Columbia River Basin: An Evaluation of Different Molecular Markers

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**Abstract.**—Protein genetic markers (allozymes) have been used during the last decade in a genetic stock identification (GSI) program by state and federal management agencies to monitor stocks of steelhead *Oncorhynchus mykiss* in the Columbia River basin. In this paper we report new data for five microsatellite and three intron loci from 32 steelhead populations in the three upriver evolutionarily significant units (ESUs) and compare the performance of allozyme, microsatellite, and intron markers for use in GSI mixture analyses. As expected, microsatellites and introns had high total heterozygosity ( $H_T$ ) values; but there was little difference among marker classes in the magnitude of population differentiation as estimated by Wright's fixation index ( $F_{ST}$ ), which ranged from 0.041 (microsatellite loci) to 0.047 (allozyme loci) and 0.050 (intron loci). For allozyme and microsatellite loci, the relationships among populations followed the pattern of geographic proximity. In computer-simulated mixture analyses, GSI estimates were more than 85% correct to the reporting group, the exact percentage depending on the marker data set and target group. Microsatellite loci provided the most accurate estimate (83%) in the 100% upper Columbia River ESU simulation, whereas simulation estimates for the 32-locus allozyme baseline were 93–94% for the 100% middle Columbia River ESU and two Snake River management groups. The simulations also showed that the estimates improved substantially up to a sample size of 50 fish per population. Technical advances will concomitantly increase the number of useful microsatellite loci and the rate of laboratory throughput, making this class of molecular marker more valuable for GSI mixture analyses in the near future. In the meantime, we recommend that steelhead management in the Columbia River rely on both allozyme and microsatellite data for GSI procedures.

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## Iteroparity in complex mating systems of steelhead *Oncorhynchus mykiss* (Walbaum)

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This study investigated diverse reproductive types in complex mating systems of steelhead *Oncorhynchus mykiss*. Postspawned steelhead (kelts) were sampled during attempted downstream migration over Lower Granite Dam on the Snake River, U.S.A. Multilocus microsatellite genotypes (14 loci) were used to assign unknown origin, kelt individuals to upstream populations of origin. Results indicated that iteroparity is a life-history trait that remains in several tributaries of the Snake River basin despite strong selection against downstream adult passage because of hydroelectric dams. The largest populations of steelhead in the Snake River, however, were only weakly represented (Clearwater River = 7.5% and Salmon River = 9.4%, respectively) in the kelt steelhead mixture relative to the Grande Ronde River (18.2%), Imnaha River (17.4%), Pahsimeroi Hatchery (25.2%) and Asotin Creek (22.2%). A lack of correlation between population escapement size and kelt proportions ( $P > 0.05$ ) suggests that iteroparity was not uniformly expressed across populations, but was significantly negatively correlated with body size ( $P < 0.05$ ). Iteroparity may be a valuable source of genetic variability and a conservation priority, especially in years with poor recruitment or in recently bottlenecked populations.

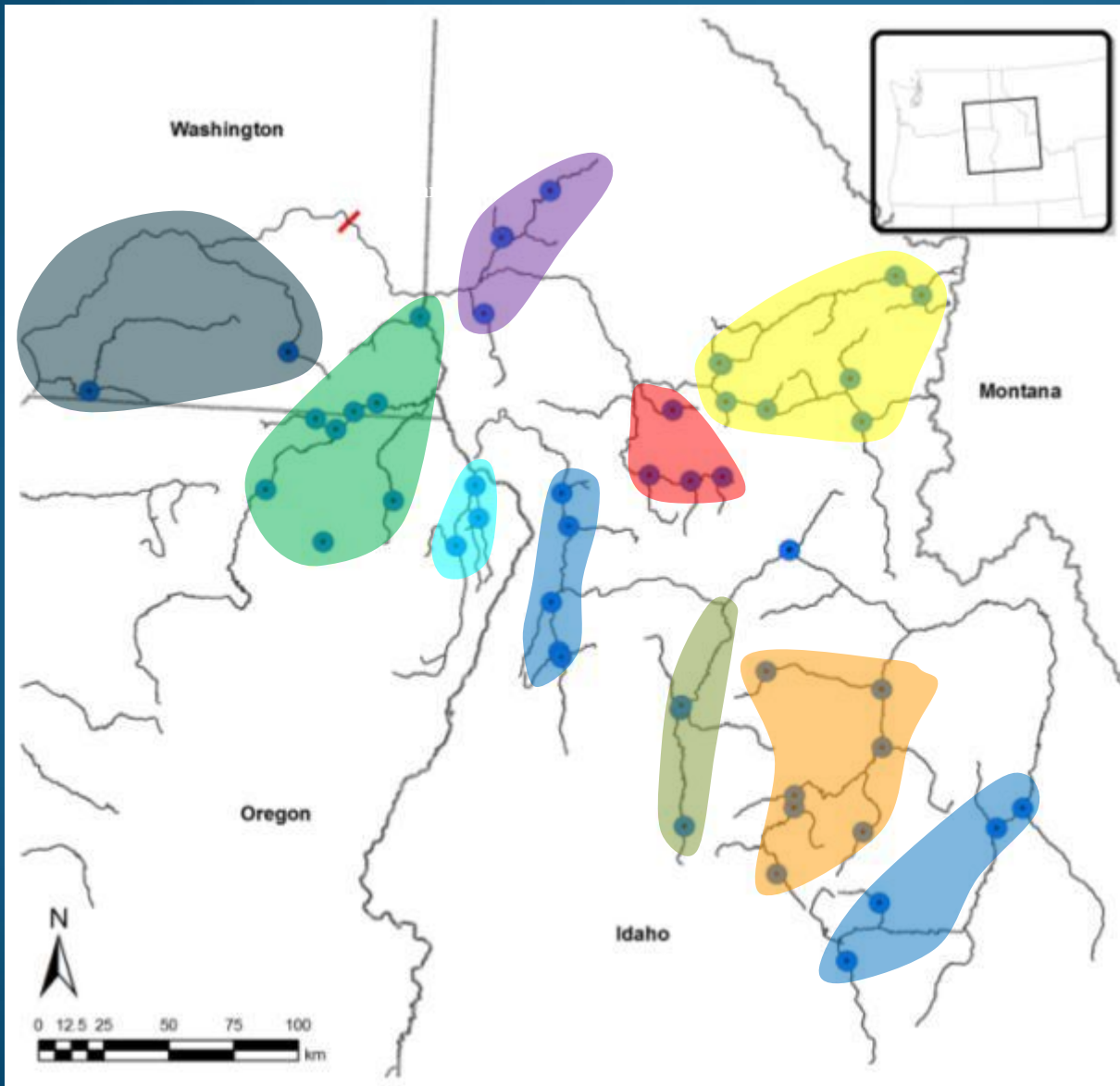
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Key words: iteroparity; mating systems; *Oncorhynchus mykiss*; semelparity; sex ratio.

SNP baseline: 49 populations,  
2,506 samples, 187 SNP loci

- Reporting groups: genetically discrete groups of populations



## 10 REPORTING REGIONS

- Lower Mainstem Salmon
- Upper Mainstem Salmon
- MF Salmon
- SF Salmon
- Upper Clearwater
- SF Clearwater
- Lower Clearwater
- Imnaha
- Grande Ronde
- Tucannon (BLWLGR)

# Methods – Adult Steelhead

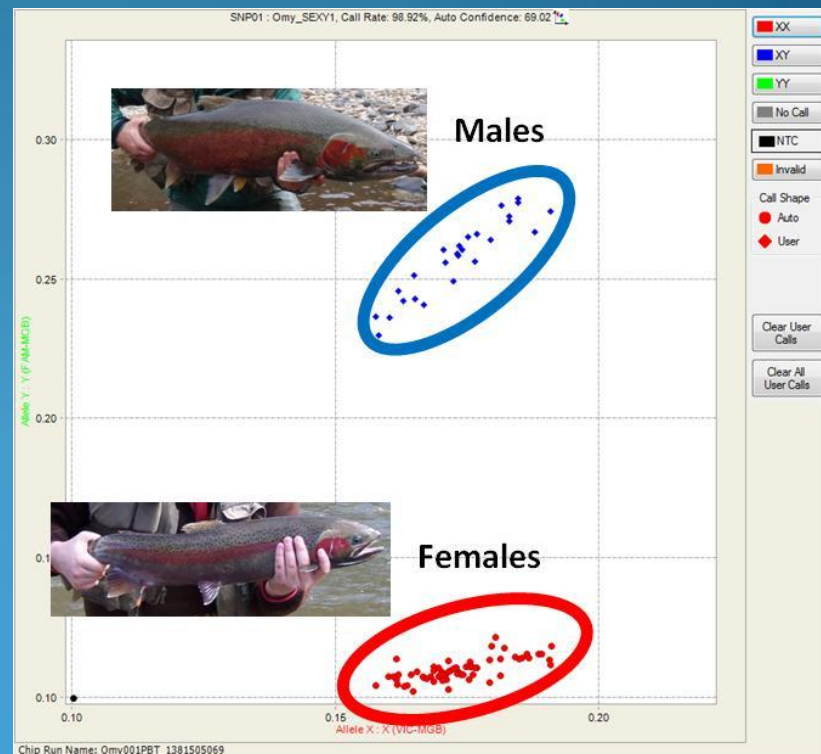
## Lower Granite Dam sampling:

- SY2009 = 1,083 samples
- SY2010 = 1,938 samples
- Biological data collected for each individual
  - ✓ length
  - ✓ tissue = genetics/gender
  - ✓ scale = age

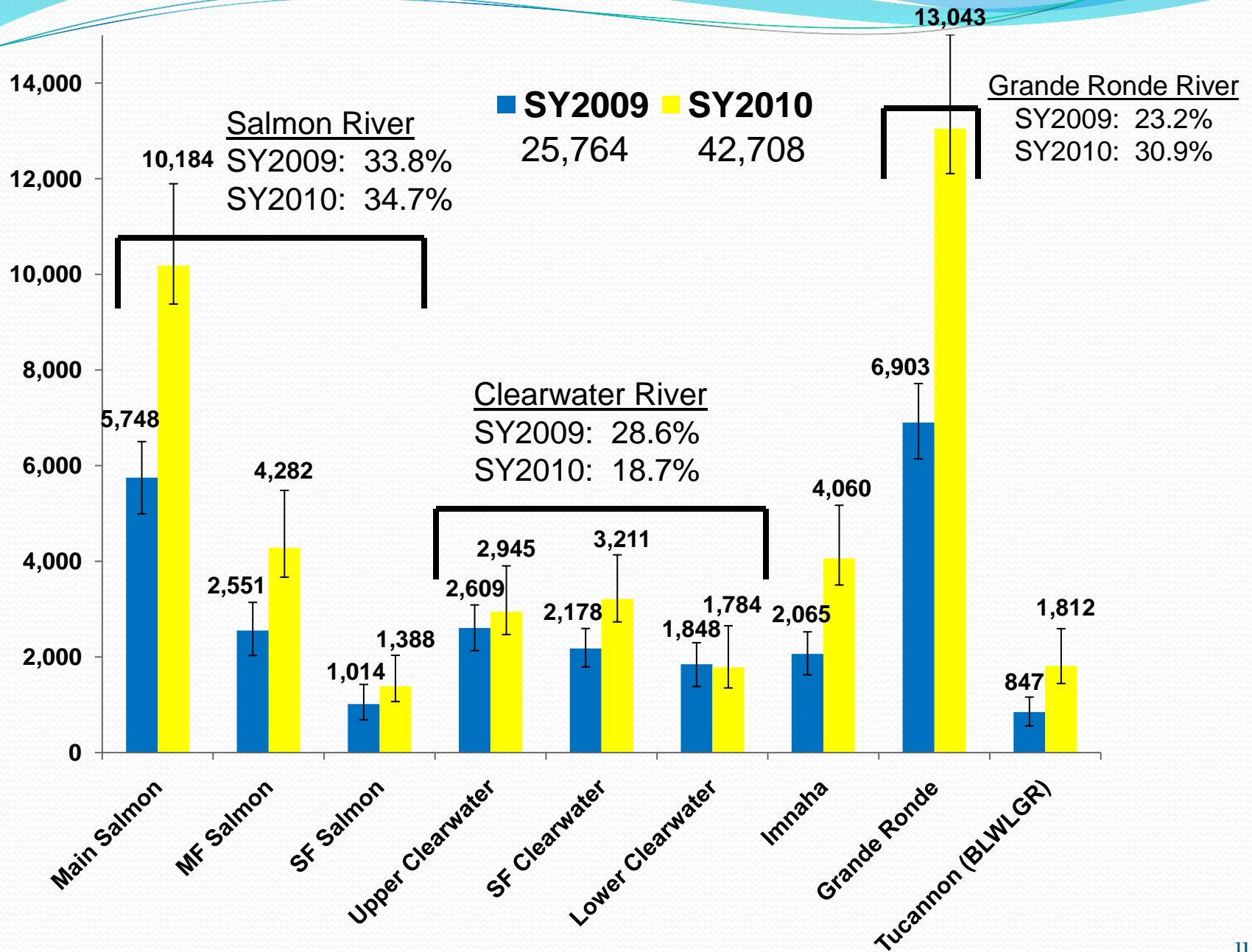


## Analysis

- 187 SNP loci
- including genetic sex
  - ✓ Y-chromosome specific assay
  - ✓ >98% accurate



# Stock escapement at Lower Granite Dam



# Sex ratios

## SY2009

- Males = 32%
- Females = 68%

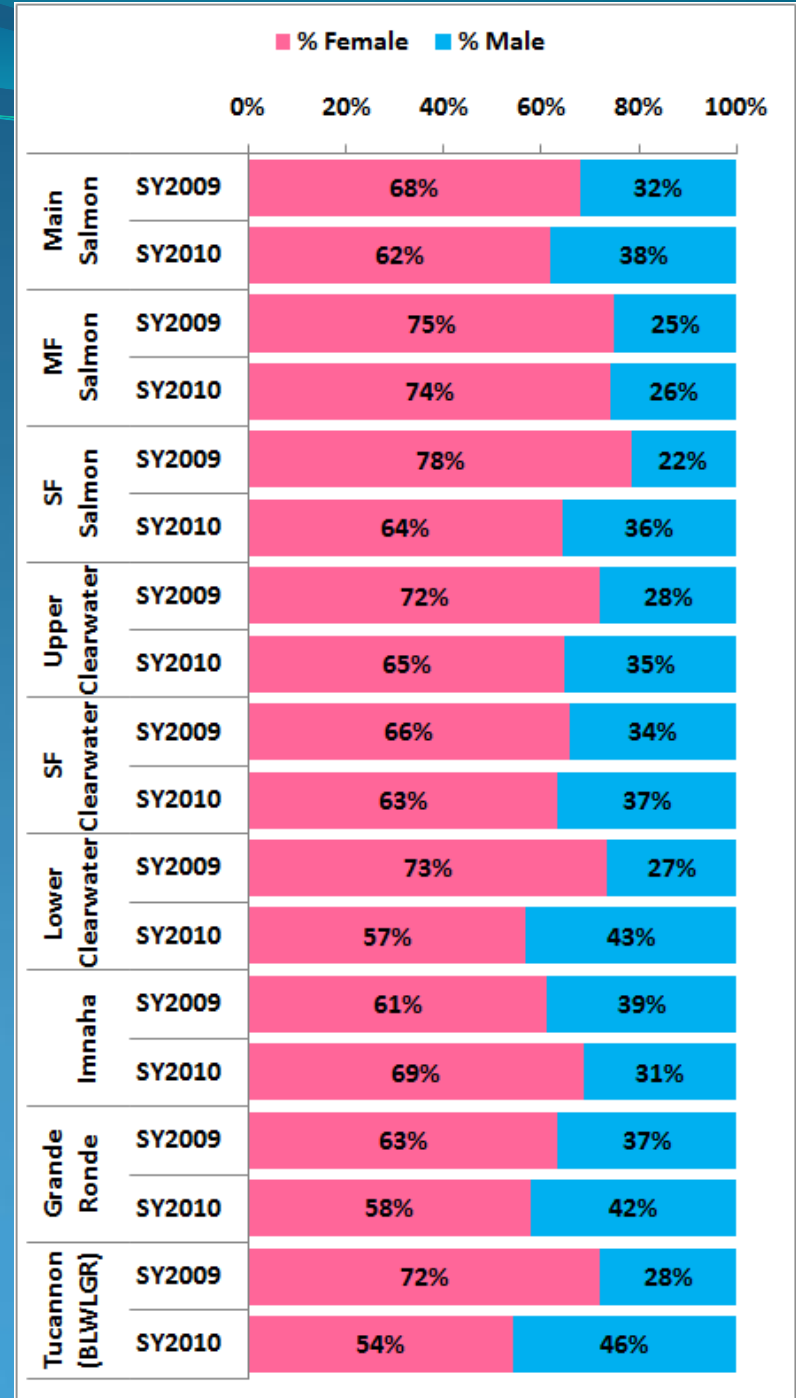
## SY2010

- Males = 38%
- Females = 62%

*Male*



*Female*

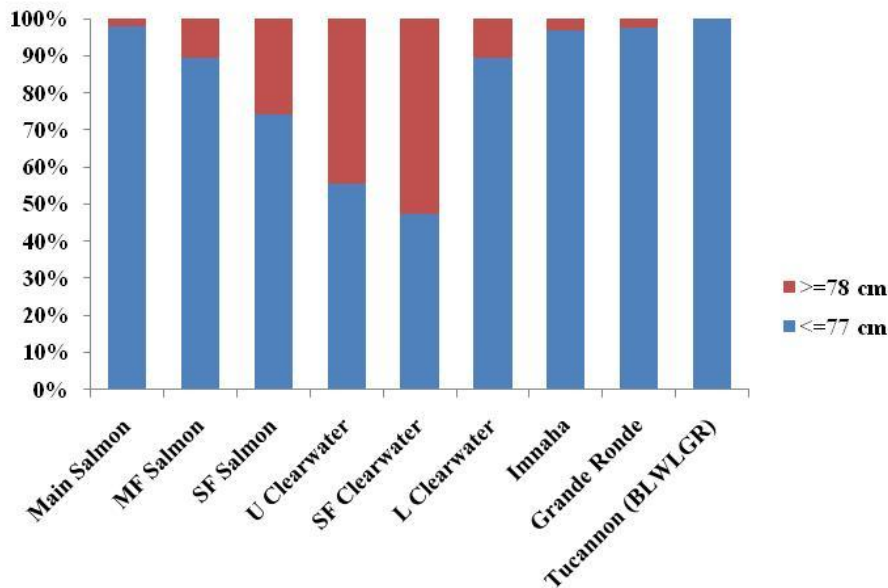


# Major Findings: Sex composition

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- Females comprise the majority of the run of adult steelhead passing Lower Granite Dam (>65%)
- Female-biased sex ratios in steelhead have been explained by two separate life history behaviors:
  1. The predominance of males to residualize
  2. The predominance of anadromous females to spawn more than once (Savvaitova et al. 1997; McMillan et al. 2007)
- Although kelts are observed passing LGR, successful iteroparity very rare in the Snake River basin (Keefer et al. 2008; Narum et al. 2008).
- One likely explanation for the skewed sex ratios that we are observing is the residualization of large numbers of males during freshwater rearing

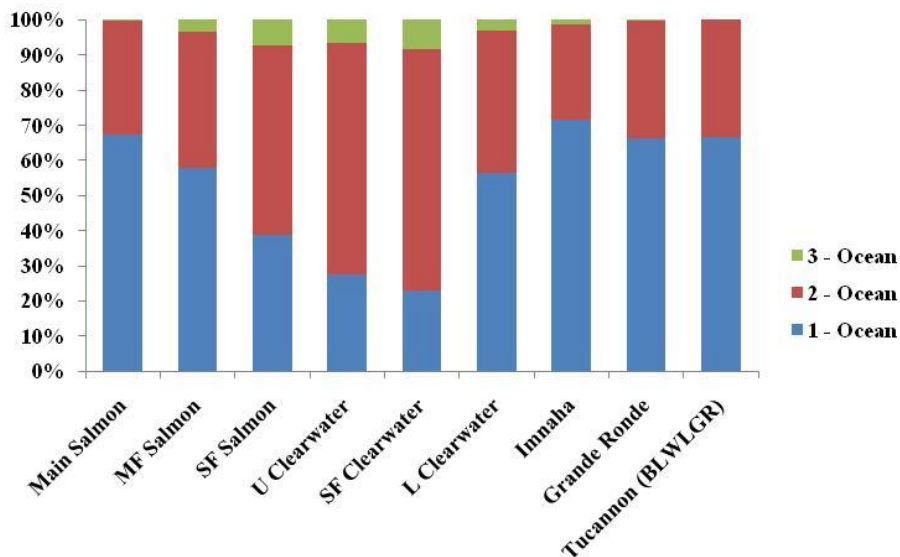
**SY2010: Length**



Proportion of “A-Run” (blue) and “B-run” (red) sized fish returning to these reporting groups in SY2010

Generally, length patterns conformed with A-run/B-run expectations, with proportionately smaller fish returning to areas that we consider “A-run” (Imnaha, Grande Ronde, Upper Salmon), and proportionately larger fish returning to areas we consider to be “B-run” (S.F. Clearwater, S.F. Salmon).

**SY2010: Ocean Age**



Proportion of 1-ocean (blue), 2-ocean (red) and 3-ocean (green) age adults returning to these reporting groups in SY2010

Similar patterns to what we saw before, but these results emphasize what some biologists have recognized for some time, that 2-ocean fish do not strictly return “B-run” sized fish. For example, in SY2010, although the composition of 2-ocean adults from the Imnaha and Grande Ronde Rivers was ~30-35%, less than 5% of the adults met “B-run” size length requirements.

# Major Findings: “A-run” versus “B-run”

- Drainage based differences in A-Run vs. B-Run composition (based on length)
  - A-Run: Upper Salmon, Grande Ronde, Imnaha
  - B-Run: Upper Clearwater, SF Clearwater, SF Salmon
- BUT, all regions appear to produce both smaller size/younger age returning adults (A-run) and larger size/older age returning adults (B-run)
- Relationship between run type (A vs. B) based on length and saltwater age (1-ocean vs. 2-ocean) not clear-cut
  - we report a higher proportion of 2-ocean steelhead than  $\geq 78$  cm fish in all regions



# 2011/2012 Projects/Analyses at LGR:

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## Adults

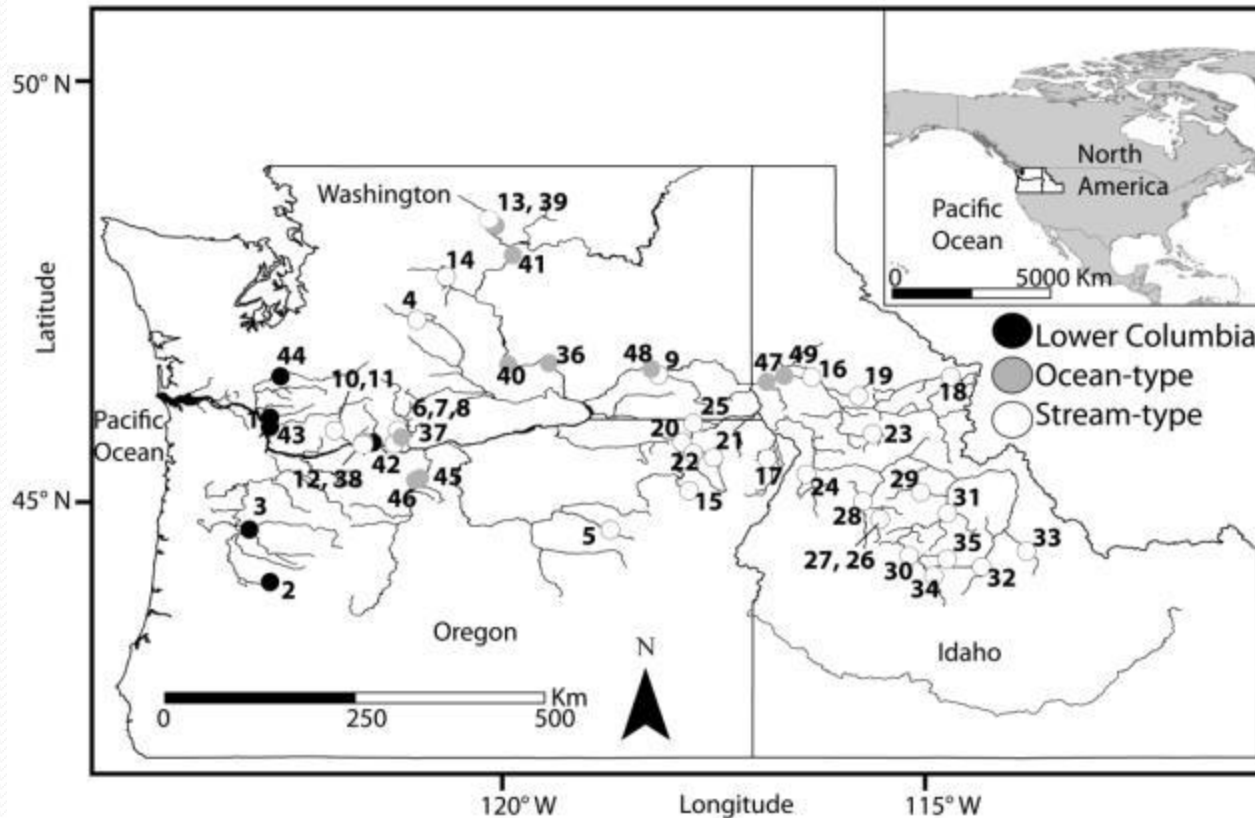
- Continue adult stock escapement estimation (both steelhead and Chinook Salmon)
  - ✓ How many unclipped hatchery steelhead adults are called wild?
    - Currently the identification of ad-intact hatchery steelhead is determined by fin erosion, but the accuracy of this determination needs to be tested.
  - ✓ Address wild versus unclipped hatchery differentiation for Chinook salmon adults
  - ✓ Identify % of fall Chinook salmon trapped prior to 8/17

## Juveniles

- Stock composition of steelhead and Chinook salmon juveniles (% hatchery called wild)
- Identify % sp/sum component of out-migrating sub-yearling Chinook salmon

# Chinook Salmon Columbia River Baseline

- 49 spawning aggregates
  - 5000+ fish
- Genetic markers
- 92 SNP loci



## 12 Reporting Groups

Lower Columbia R spring  
Willamette R spring  
Lower Columbia R fall

Mid Columbia R spring  
Upper Columbia R spring  
Rapid R/Clearwater R  
spring  
SF Salmon R spring  
MF Salmon R spring  
Upper Salmon R spring

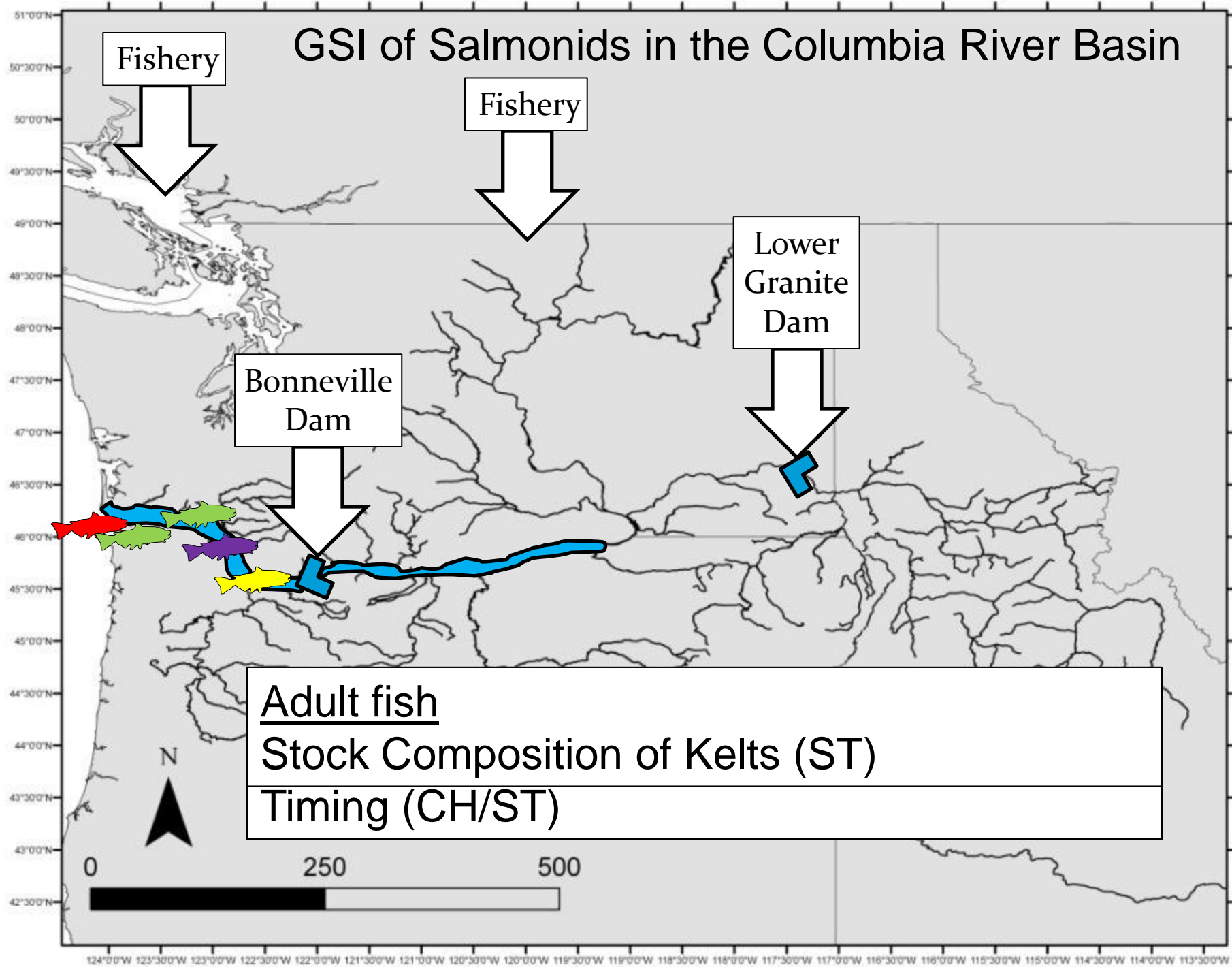
Upper Columbia R  
sum./fall  
Deschutes R fall  
Snake R fall

Baseline described in Matala et al. 2011 TAFS

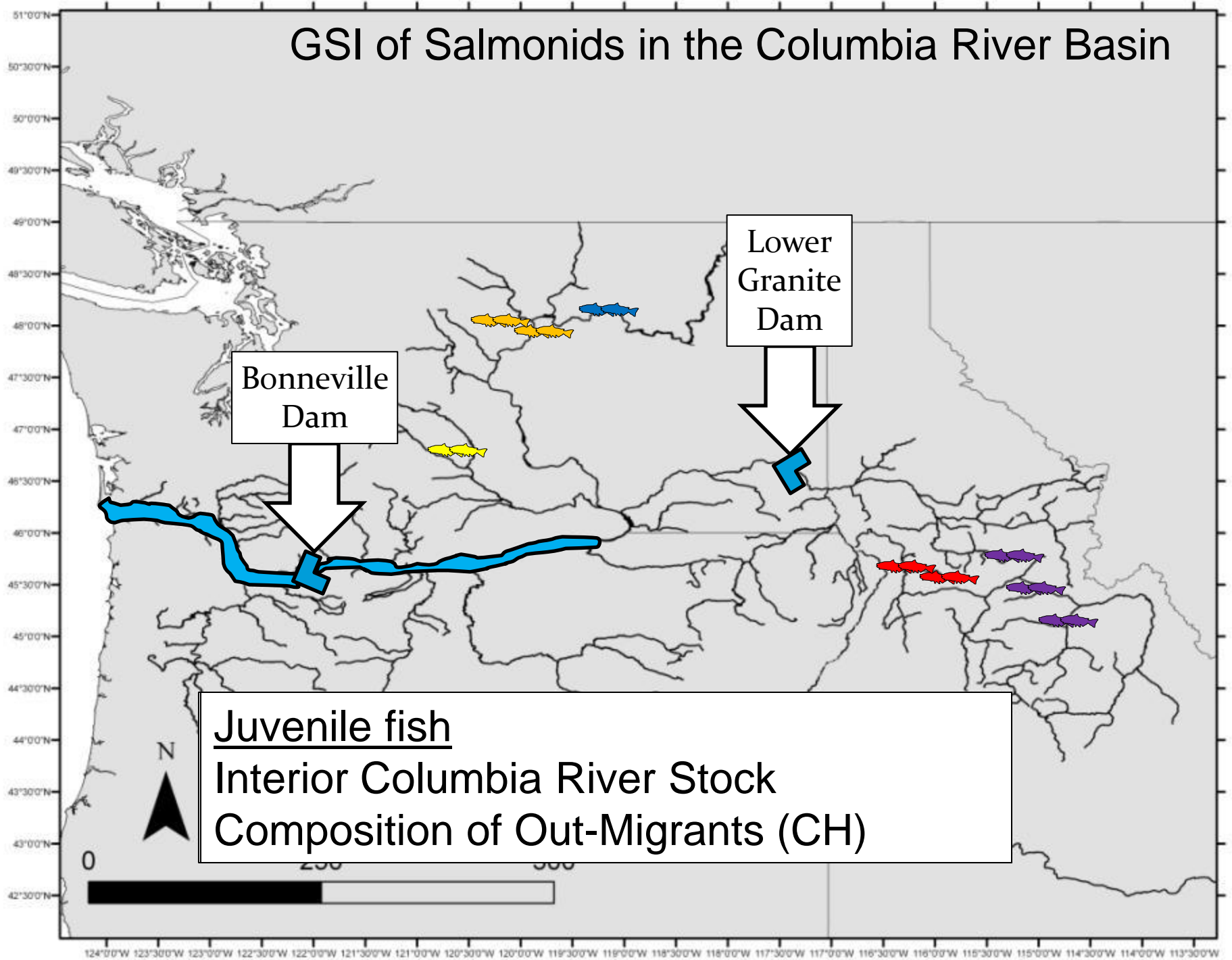
Accuracy of Reporting groups described in Hess et al. 2011 MER



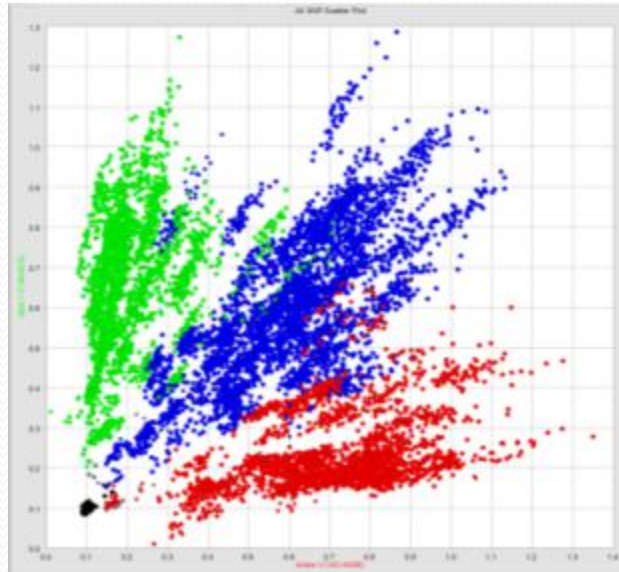
# GSI of Salmonids in the Columbia River Basin



# GSI of Salmonids in the Columbia River Basin



# PBT in the Snake River Basin: Evaluation and Implementation



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Maureen Hess, Nathan Campbell, Shawn Narum - Hagerman Genetics Lab

**Problem:** IDFG managers believe that annual coded-wire tags recoveries from statewide fisheries in Idaho are insufficient to reliably estimate stock contributions

- CWT tag more fish?
  - ✓logistically difficult
  - ✓labor intensive
  - ✓expensive



Sampling for coded wire tag with Wand Detector; checking for snout tag

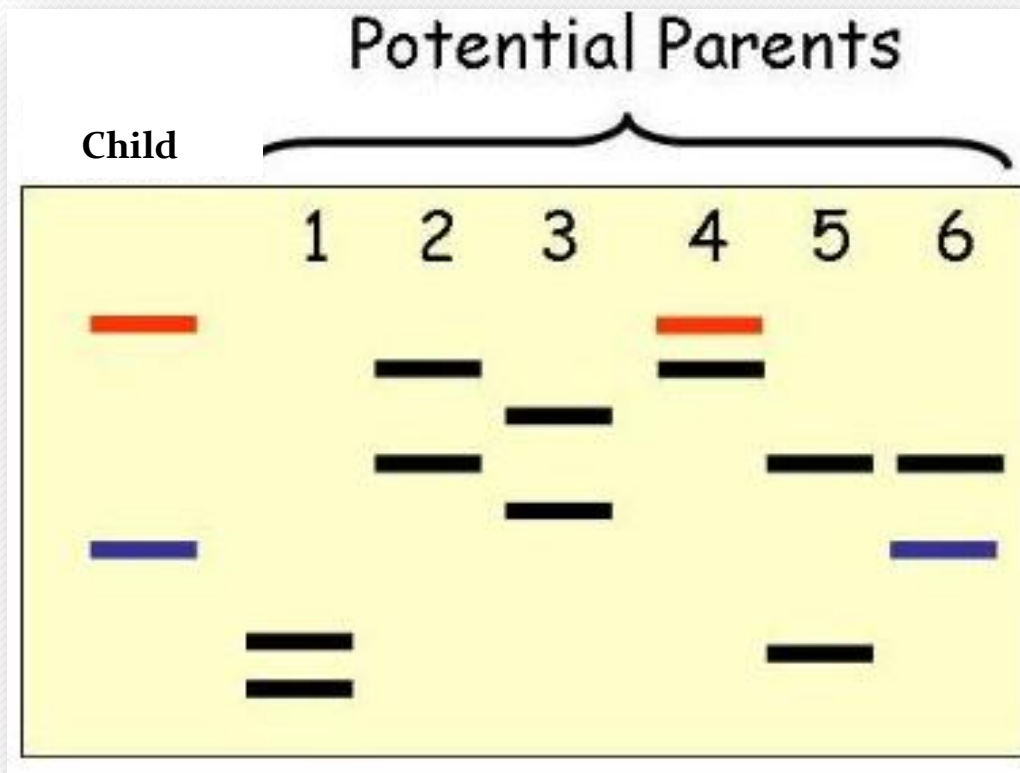


## Parentage-based genetic tagging - PBT (Anderson and Garza 2005)



Parentage-based tagging uses similar DNA techniques as those used in human parentage testing

# Parentage-based genetic tagging - PBT



- Every person receives a unique set of genetic information from their parents!
- If your parents have been genetically screened they can easily be identified from thousands of possible candidates!

# Genetic information can be obtained from all hatchery fish (broodstock) that are spawned



Lookingglass



Wallowa



		PIT T	Other info	103-1	103-2	111-1	111-2	112-1	112-2	114-1	114-2	104-1	104-2	108-1	108-2	115-1	115-2
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BY08	F	3D9.1BF11A0C428	219-220-294	204	232	226	298	176	188	255	255	181	185	210	210	189	197
BY08	M	3D9.1BF11A0C440	219-220-204	204	232	298	298	134	134	255	255	165	181	210	210	189	189
BY08	F	3D9.1BF11A0C4CF	219-220-287	204	232	226	298	176	188	255	255	181	185	210	210	189	197



Sawtooth



Oxbow

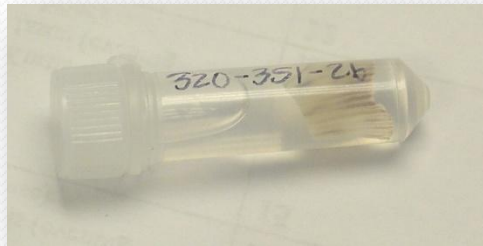


Pahsimeroi



Dworshak/Clearwater

Offspring from any of these parents (either collected as juveniles or returning adults), if genotyped, could be assigned back to their parents, thus identifying the hatchery they originated from and exact brood year they were produced in

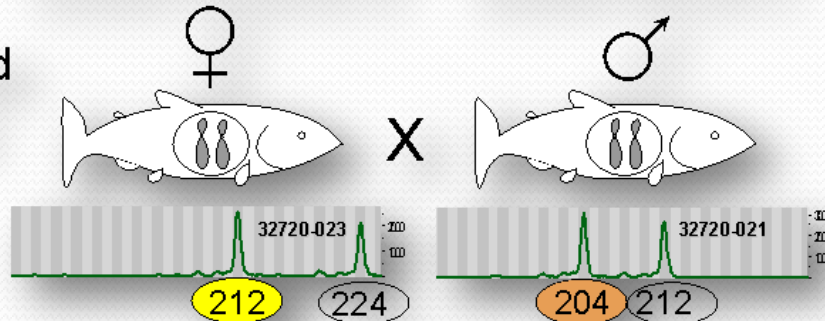


**Sawtooth BY2008**

**Sawtooth Female-08-121**

**Sawtooth Male-08-15**

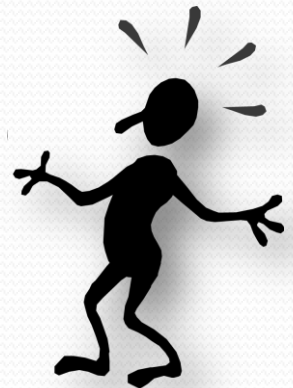
**Assigned  
parents**



While between 500-800 coded-wire tags have been recovered annually (2005-2007), ~ten times that many (~6,000) adults have been physically examined each year (Hansen 2007)

Potentially, under a PBT program, the hatchery origin and brood year of a much higher number of these adults could have been determined. This increase in sample size would have undoubtedly improved contribution estimates, especially from smaller mark groups

500-800 < 6,000



## Benefits of PBT

- Provides same information as CWTs (stock and cohort)
  - ✓ Run reconstruction (age, sex, stock of returning adults)
  - ✓ Harvest estimates
- Additionally, many issues associated with tagging studies all but go away
  - ✓ Tag loss
  - ✓ Tag detectability
  - ✓ Differential mortality



## Additional benefits of PBT

### PROS:

- It is a passive mark (no handling of juveniles needed)
- “Tagged” fish can be non-lethally interrogated

✓ Fin-clip

✓ Scales

✓ Biopsy hook

*“The hollow stainless steel tip of the hook collects a small amount of tissue as the fish strikes, and a special rasp holds the sample in place while the hook is in the water”*

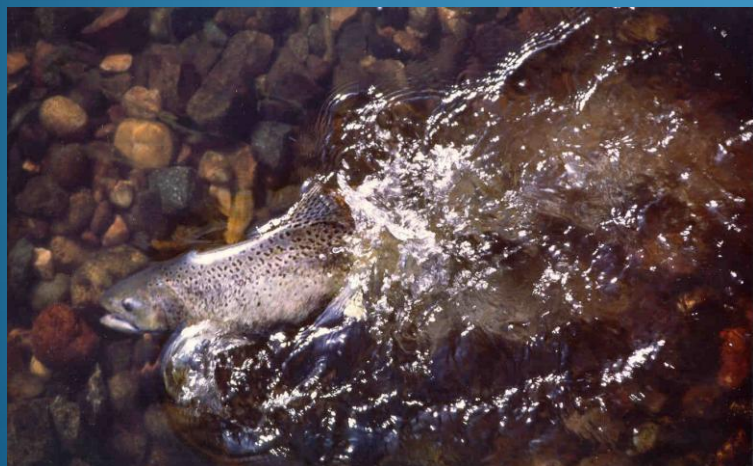
### “Genetag hook”



## Versatility of PBT

### PROS:

- Can potentially provide much more information than stock and cohort of origin
- Host of other life history, ecological and quantitative genetic questions



## Versatility of PBT

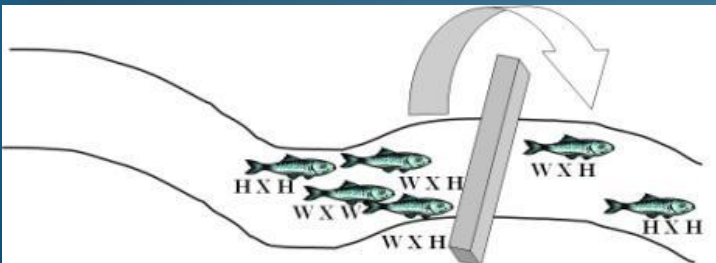
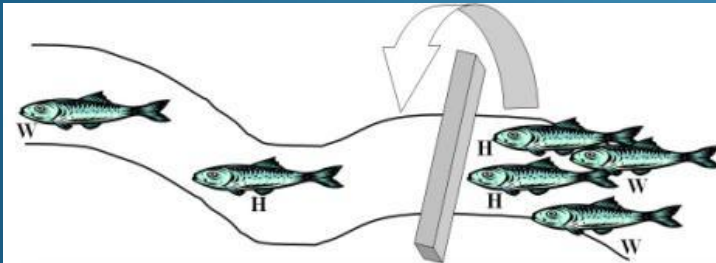
- Hatchery reform (Integrated programs)

- ✓ What is the reproductive success of hatchery-origin/wild-origin spawners? (both in the wild and in the hatchery)

- Hatchery reform (Segregated programs)

- Concern that straying hatchery fish reduce the fitness of wild populations
- Is it possible to manage a hatchery program so that it is truly “segregated” from wild populations?

- ✓ PBT technology can identify where straying hatchery fish were released, their stock of origin, and age



## Versatility of PBT

- Heritability estimates

To what extent are physical or behavioral traits (e.g. size and age of returning adults) controlled by genetics, environment or interactions between the two?

✓ Are hatchery spawning practices selecting for younger age at maturity in hatchery Chinook salmon populations



Hankin et al (2009) recommended that in the hatchery male length  $\geq$  female length

## Versatility of PBT

- Origin of kelt steelhead

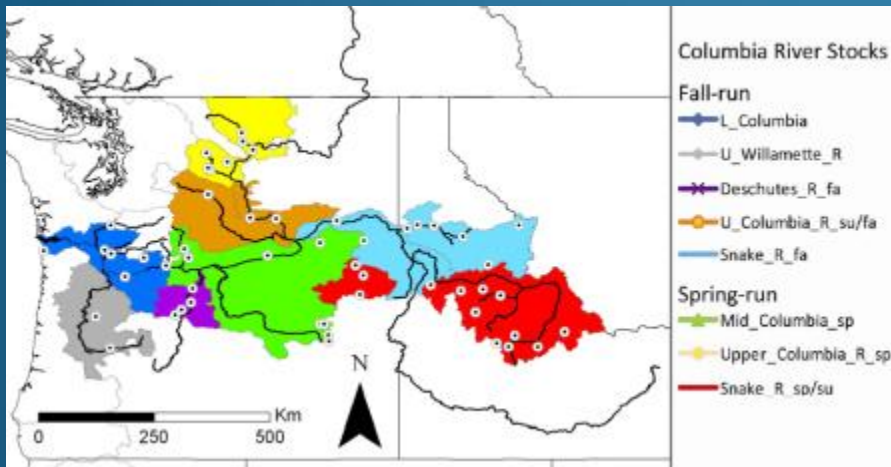
Thousands of kelt steelhead outmigrate past Lower Granite Dam each year (~50% hatchery origin)

- ✓Where are they from?
- ✓How old are they?
- ✓Is phenotypic sex correct?



## Versatility of PBT

- PBT program can easily be integrated into existing sampling/genetic programs at Bonneville Dam and in lower Columbia fisheries (CRITFC)



Bonneville Chinook weekly stock composition  
(2004-2006)



Collaborative Center for Applied Fish Science



PBT sounds great!!!.....

However, it has been largely theoretical up to this point...

- Needs to be empirically tested and validated on a large scale



These types of evaluations have been directly called for by both the Pacific Salmon Commission and the Independent Scientific Review Panel and Advisory Boards

**Recommendations for Application of  
Genetic Stock Identification (GSI)  
Methods to Management of Ocean  
Salmon Fisheries.**

Special Report of the GSI Steering Committee  
and the Pacific Salmon Commission's  
Committee on Scientific Cooperation.

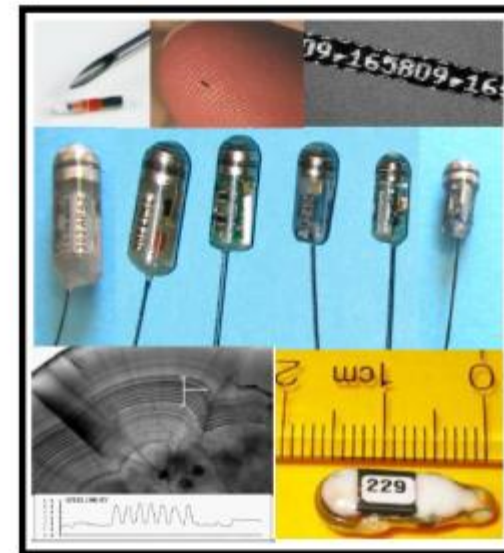
January 2008



**Pacific Salmon Commission  
Technical Report No. 23**

INDEPENDENT SCIENTIFIC REVIEW PANEL  
INDEPENDENT SCIENTIFIC ADVISORY BOARD

**TAGGING REPORT**



A comprehensive review of Columbia River  
Basin fish tagging technologies and programs

March 17, 2009  
ISRP/ISAB 2009-1

# Snake River Chinook and Steelhead Parentage Based Tagging-Proposal #201003100



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## Proposal 201003100: Snake River Chinook and Steelhead Parental Based Tagging

### Jump to:

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| 1. <a href="#">Administrative</a>       | 6. <a href="#">Objectives</a>    | <a href="#">Reviews</a> |
| 2. <a href="#">Location</a>             | 7. <a href="#">Work elements</a> |                         |
| 3. <a href="#">Species</a>              | 8. <a href="#">Budget</a>        |                         |
| 4. <a href="#">Past accomplishments</a> | 9. <a href="#">Future</a>        |                         |
| 5. <a href="#">Relationships</a>        | 10. <a href="#">Narrative</a>    |                         |

Perfect timing to conduct a large-scale test of this technology in the basin....



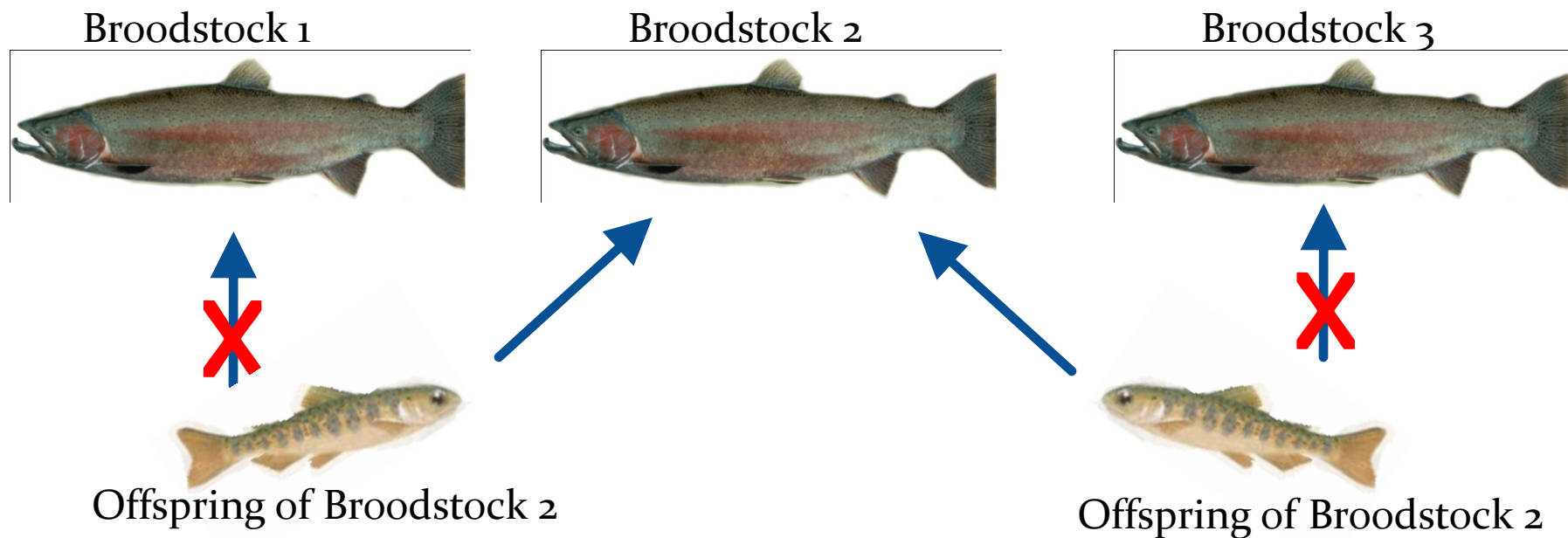
## Major objectives over the next several years:

- Demonstrate the accuracy and utility of PBT technology in addressing hatchery management and conservation issues in the Snake and Columbia River basin



## Objective 1: Accuracy

Evaluate accuracy and ability of PBT to assign offspring of known parentage back to their hatchery stock of origin (among several closely related stocks)



# PILOT STUDY - Adults

- Broodyear 2008
- Candidate Parent Dataset

Hatchery Stock	N
Squaw Crk Upper Salmon B	35
Sawtooth Hatchery	234
Dworshak Hatchery	393
East Fork Salmon River	119
Pahsimeroi Hatchery	255
Grande R./Cottonwood	169
Total	1205



# PILOT STUDY - Offspring

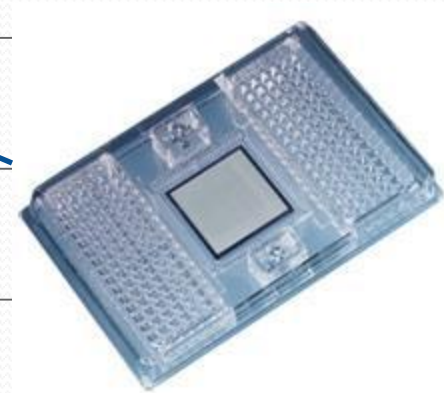
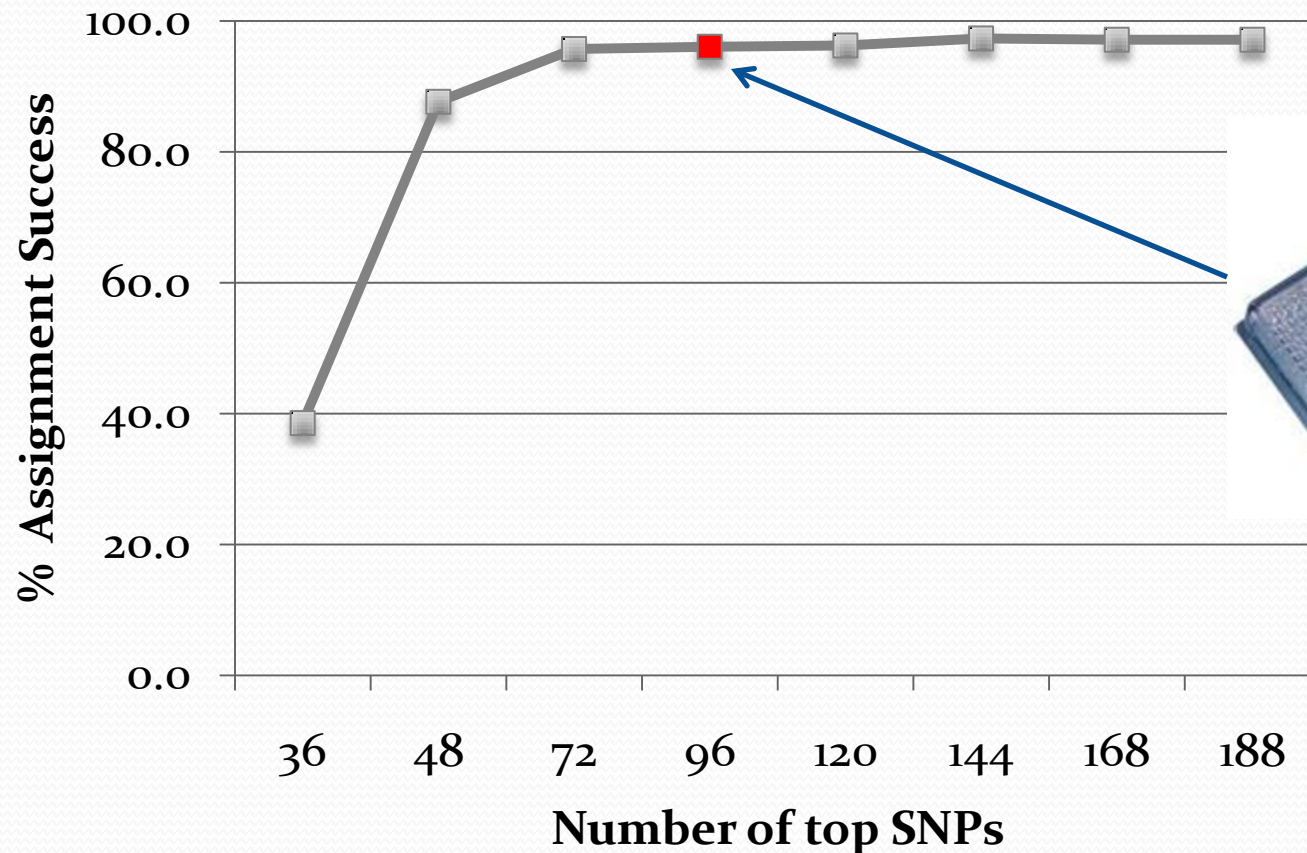
- Sampled at Magic Valley
- Juvenile Dataset

Juveniles Sampled	N
Squaw Crk Upper Salmon B	92
Sawtooth	93
Dworshak	93
EFSR	94
Pahsimeroi	93
Grande R./Cottonwood	93
Lyons Ferry*	93
Total	651

\* Individuals with no parents in the database



# The Power of SNPs



96 X 96 SNP Chip

# PILOT STUDY - Results

## Results: SNPs

- High number of juveniles assigned (97.3%)
- 100% accuracy to stock

Hatchery Stock	Juveniles Genotyped	Juveniles Assigned	% assigned correctly to stock
Squaw Crk	92	92	100.0%
Sawtooth	93	91	100.0%
Dworshak	93	93	100.0%
EF Salmon	94	88	100.0%
Pahsimeroi	93	88	100.0%
G.R. Cott.	93	91	100.0%
Lyons Ferry*	93	0	----

\* Individuals with no parents in the database

# Evaluating False Positives

Pilot study :

Tried to assign Lyon's Ferry individuals (93) to non-parents (1205)  
Resulted in no false assignment of the 93 Lyon's Ferry individuals

Experimental Assignments:

Tried to assign 2008 broodstock (5107) to 2009 broodstock (5672)  
Resulted in no false assignment of the 5107 individuals broodstock

# Snake River Basin PBT Milestones

FY2011 - Genotyped 2008 and 2009 broodstock for :  
Chinook (2008 = ~10,700; 2009 = ~8,700)  
Steelhead (2008 = ~5,100; 2009 = ~5,700)

FY2012 – Genotyping 2010 and 2011 broodstock for:  
Chinook (2010 = 8,400, 2011 = underway)  
Steelhead (2010 = ~5,700; 2011 = ~5,700)

# Implementation of PBT

- CWT comparisons
  - ✓ Zone 6 steelhead fishery
  - ✓ LSRCF Chinook
- Hatchery run reconstruction over Lower Granite Dam
- Origin of hatchery strays
- Origin of hatchery kelts



# Ultimate goal:

All hatchery salmon and steelhead hatchery in the Snake River basin!!!!



Where and when it was released

Identify parents

- ✓Diet
- ✓Disease
- ✓Rearing density
- ✓Temperature

Where it reared

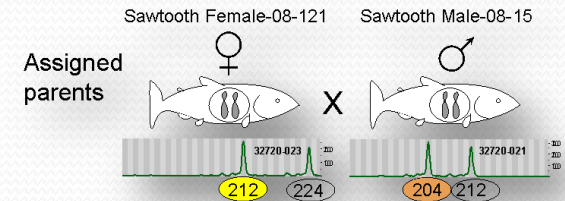
Stock and age

Where it incubated



<http://www.flickr.com/photos/natekay/4319654380/sizes/o/>

↓ ♀ ♂  
SEX



Sawtooth Fish Hatchery

This will require the development of similar types of databases that currently exist for CWTs and PIT tags.

# Current staff: 7 PSMFC employees!!!

- Fish Genetics Manager (IDFG) = 1
- Sockeye geneticist (IDFG) = 1
- **GSI geneticist (PSMFC) = 1**
- **PBT geneticist (PSMFC) = 1**
- **GSI/PBT data coordinator (PSMFC) = 1**
- **GSI/PBT genetic lab technicians (PSMFC) = 4**
- GSI/PBT genetic lab technicians (IDFG) = 4



Thank you:  
George Nandoor, Stan Allen



Eagle Fish Genetics Lab-2011

# Questions???



## **Idaho Department of Fish and Game Eagle Fish Genetics Lab**

