

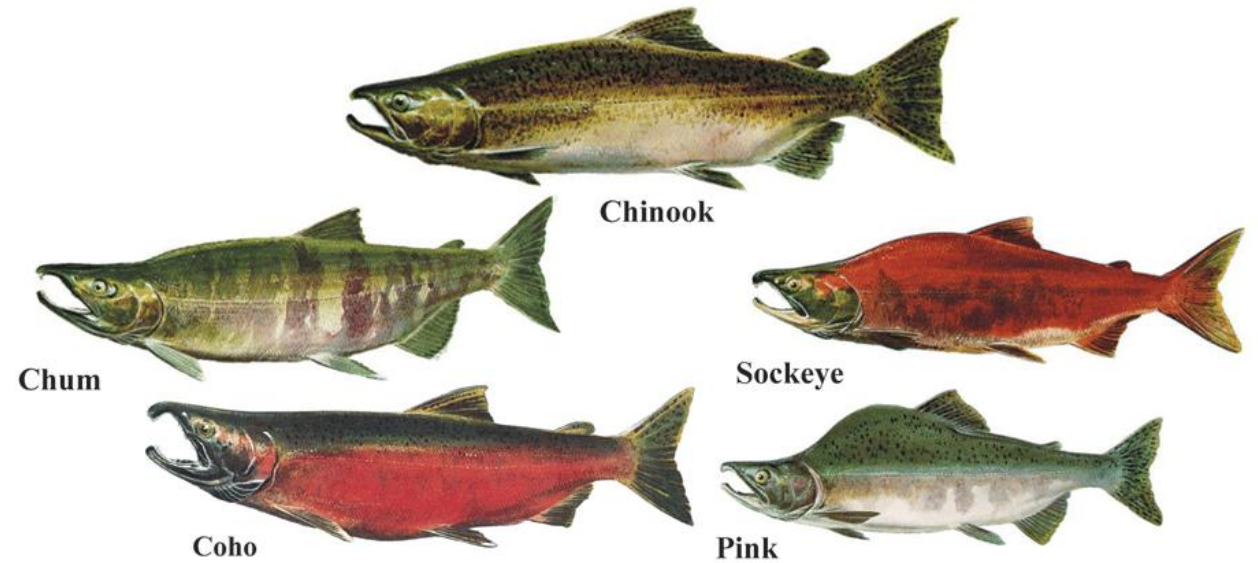


Hatchery effects on trait variation in Pacific salmon

*I respectfully acknowledge that I work and live on the unceded territory of the Coast Salish Peoples,
the traditional territory of the Snuneymuxw First Nation*

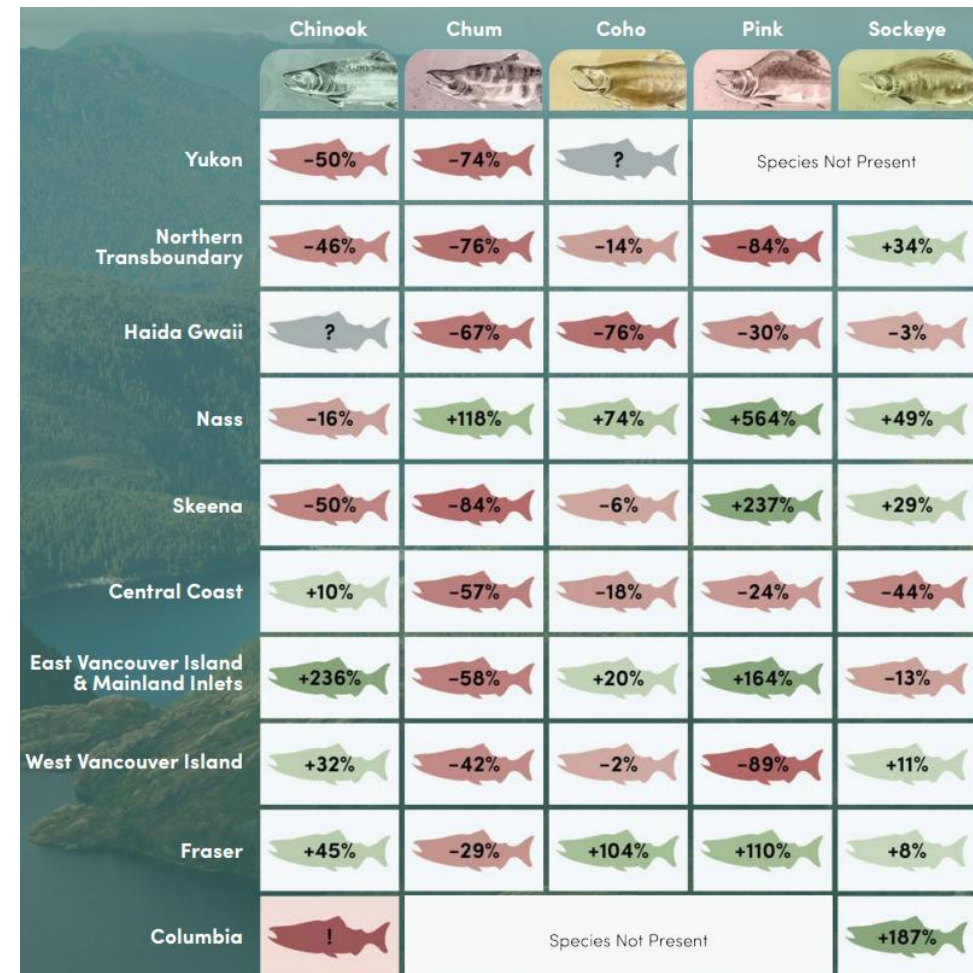
Pacific salmon

- *Oncorhynchus spp.* are large anadromous fish that traditionally return to freshwater environments in high numbers to spawn
- Very important in the Salish Sea ecologically, culturally, traditionally and economically



Pacific salmon population declines

- Pacific salmon abundances are generally lower than historic averages
- Regional patterns in spawner abundances anomalies shown on the right
 - Red = below average
 - Green = above average



Salmonid Enhancement Program (SEP)



Big Qualicum Hatchery
(one of 17 major operation hatcheries)

- DFO's SEP produces ~328 million juvenile salmon in BC annually
 - 38.6M Chinook
 - 7.9M Coho
- Objectives include: conservation, rebuilding, harvest, assessment and education/stewardship
 - Support vulnerable stocks and harvest

Pacific salmon population declines

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	Chinook	Chum	Coho	Pink	Sockeye
Yukon	-50%	-74%	?	Species Not Present	
Northern Transboundary	-46%	-76%	-14%	-84%	+34%
Haida Gwaii	?	-67%	-76%	-30%	-3%
Nass	-16%	+118%	+74%	+564%	+49%
Skeena	-50%	-84%	-6%	+237%	+29%
Central Coast	+10%	-57%	-18%	-24%	-44%
East Vancouver Island & Mainland Inlets	+236%	-58%	+20%	+164%	-13%
West Vancouver Island	+32%	-42%	-2%	-89%	+11%
Fraser	+45%	-29%	+104%	+110%	+8%
Columbia	!	Species Not Present			+187%

Hatcheries versus natural habitats



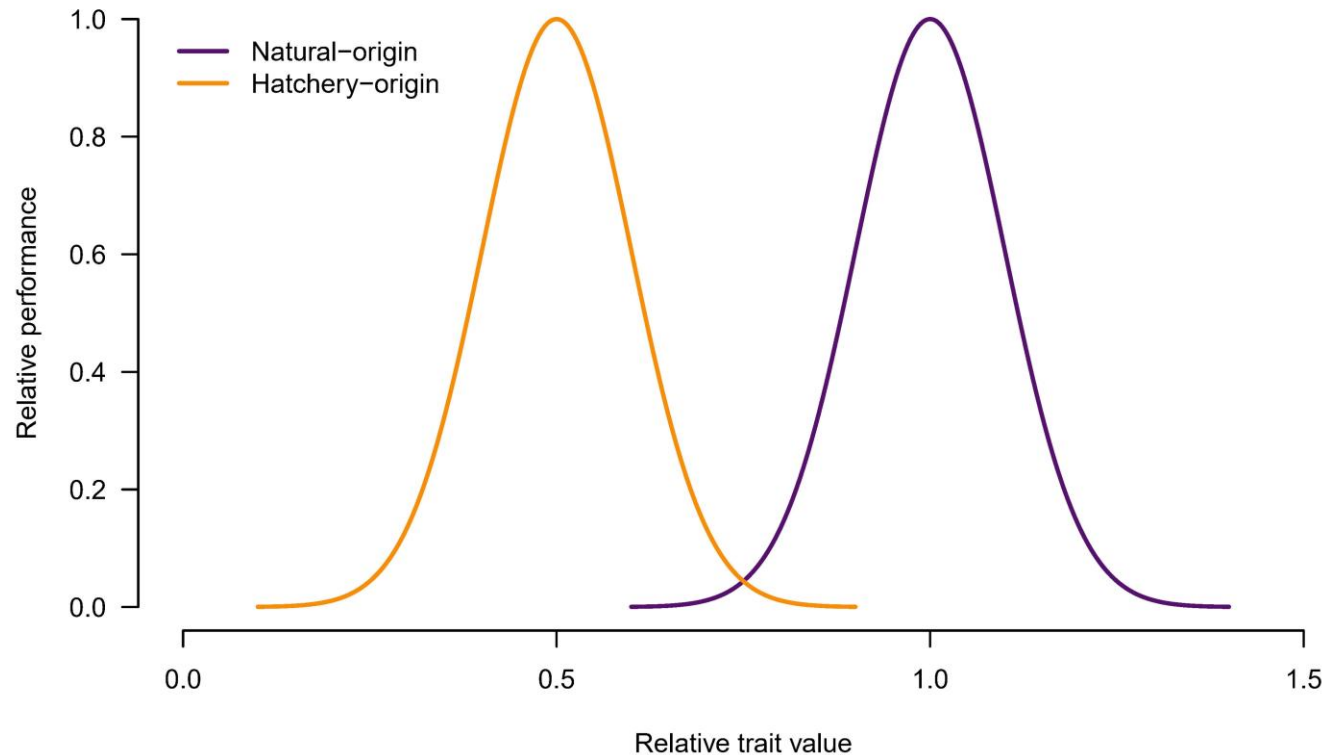
Nanaimo River Hatchery



Nanaimo River Spawning Stream

- Hatcheries add artificial habitat with high egg-to-release survival

Optimal traits differ between environments



- Different environments select for different traits
 - e.g., swimming performance, predator avoidance, etc.
- Optimal hatchery traits may be suboptimal in natural habitats
- Overwhelming majority of studies suggest hatchery-origin fish have relatively low performance in nature (e.g., McMillan et al. [2023](#))

Genetic guidelines for SEP hatcheries

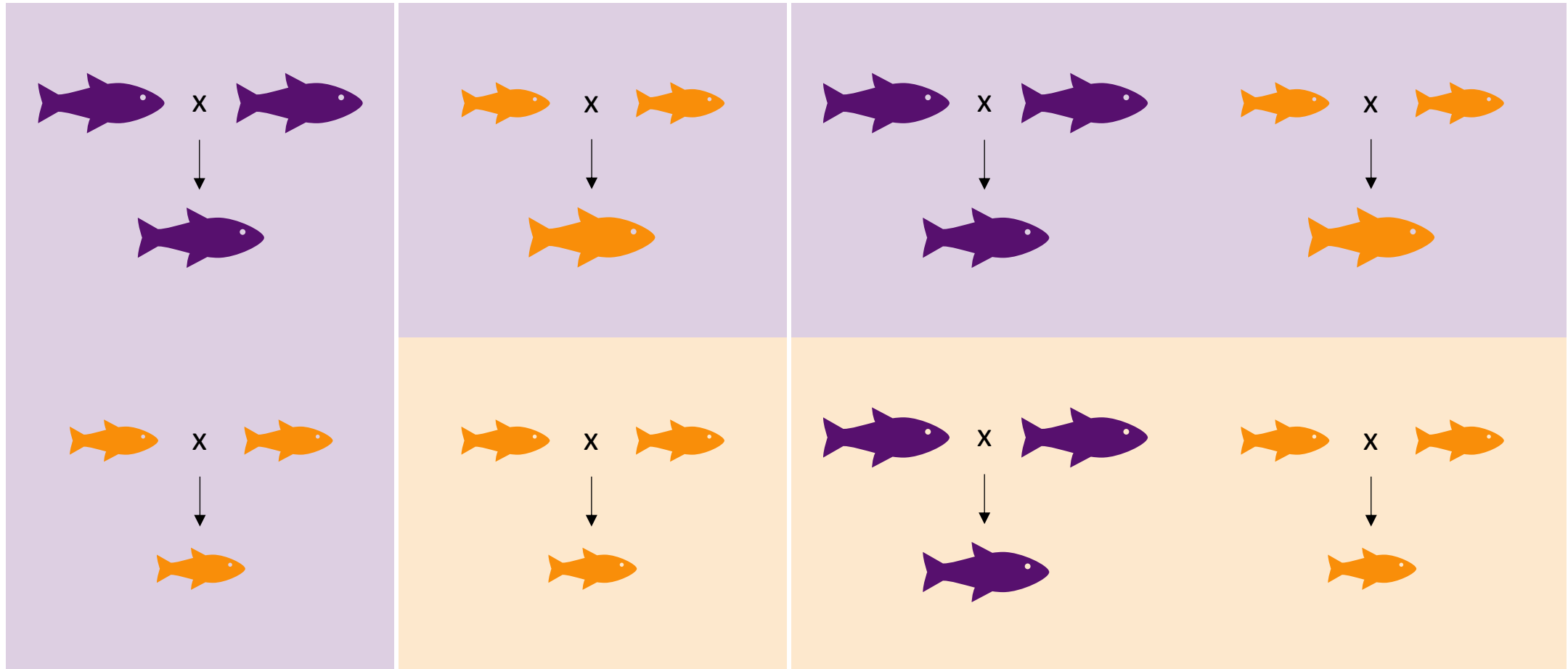
- DFO has had genetic guidelines for removal of fish from escapements for broodstock and hatchery spawning since the 1980s (DFO [2016](#))
- These guidelines were reviewed through the Canadian Science Advisory Secretariat (CSAS) recently with few recommended adjustments (DFO [2024b](#))
- Another recent CSAS process provided advice on incorporating proportionate natural influence (PNI) into hatchery guidelines to account for differences in selection between hatchery and natural environments (Withler et al. [2018](#); DFO [2024a](#))

Genotype, environment and interactions

Genetic effects

Environment effects

Interactive effects



 = natural genetics
 = hatchery genetics

 = natural habitat
 = hatchery habitat

Hatcheries versus natural habitats



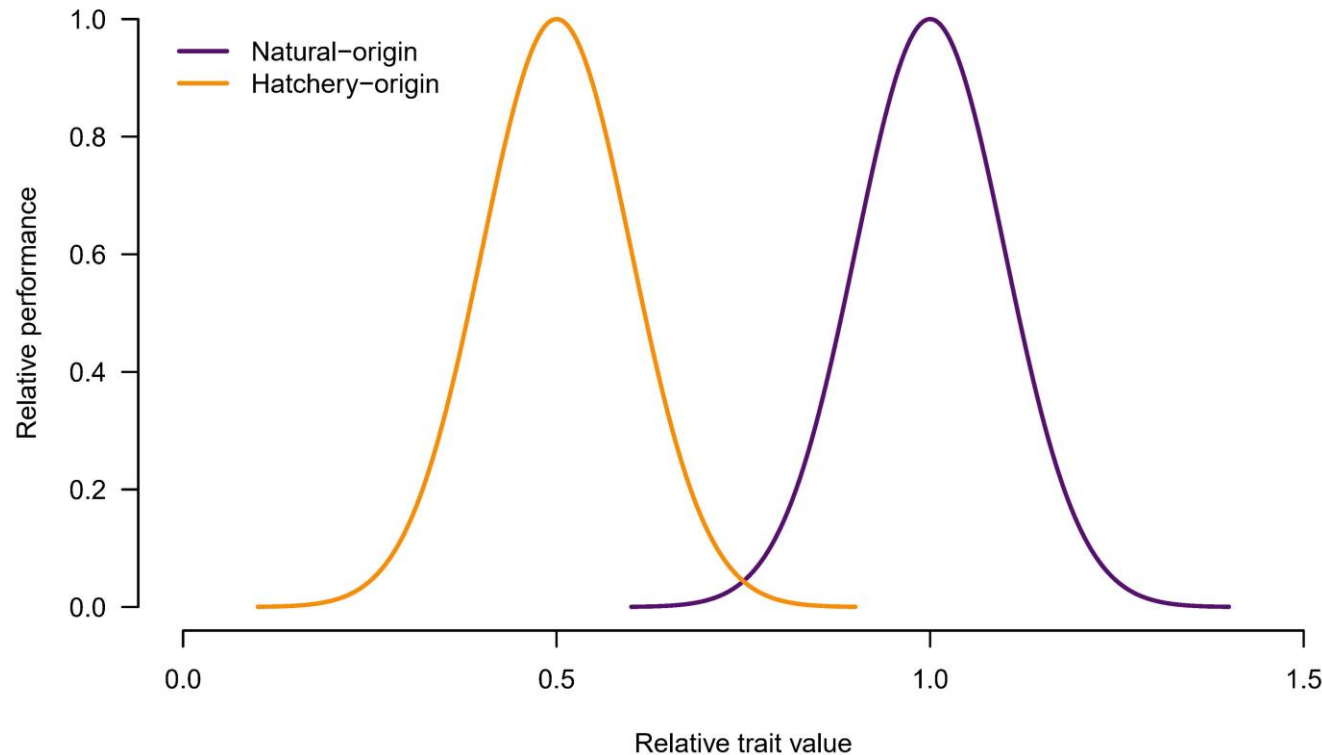
Nanaimo River Hatchery



Nanaimo River Spawning Stream

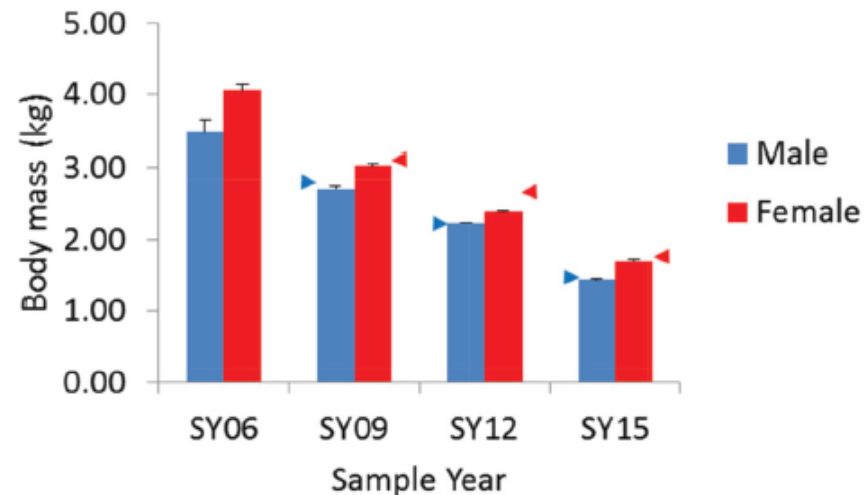
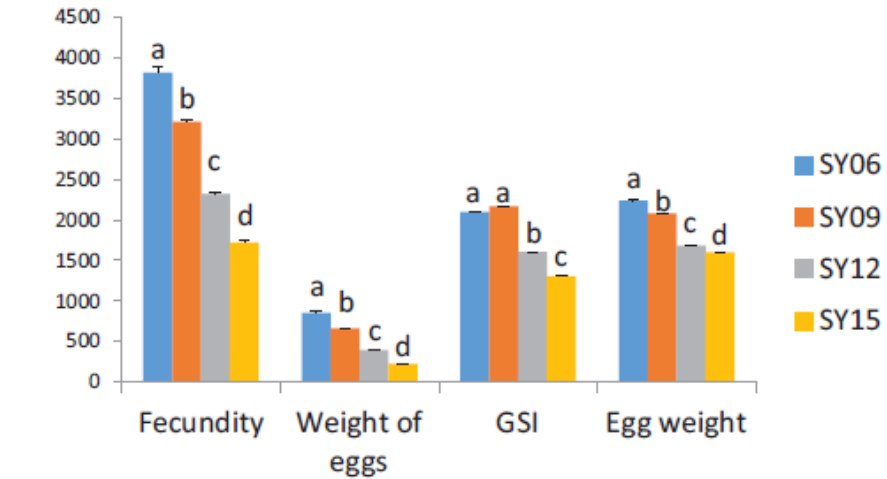
- Hatcheries add artificial habitat with high egg-to-release survival
- This artificial habitat can have genetic and environmental effects on traits

Evidence for genetic effects and fitness costs



- Hatchery-origin fish have low relative reproductive success (RRS) in nature (e.g., Araki et al. 2008)
- One study has shown reduced RRS persists over a generation (Araki et al. 2009)
- Models suggest performance changes may be irreversible (Willoughby & Christie 2019)

Trait monitoring in Inch Creek Coho Salmon



- Reductions in several fitness-related traits from 2006-2015
- The changes were heritable (i.e., at least some genetic basis)
- However, the “population harbours substantial heritability for fitness and reproductive traits and thus likely retains substantial capacity for adaptation despite many years of hatchery propagation”

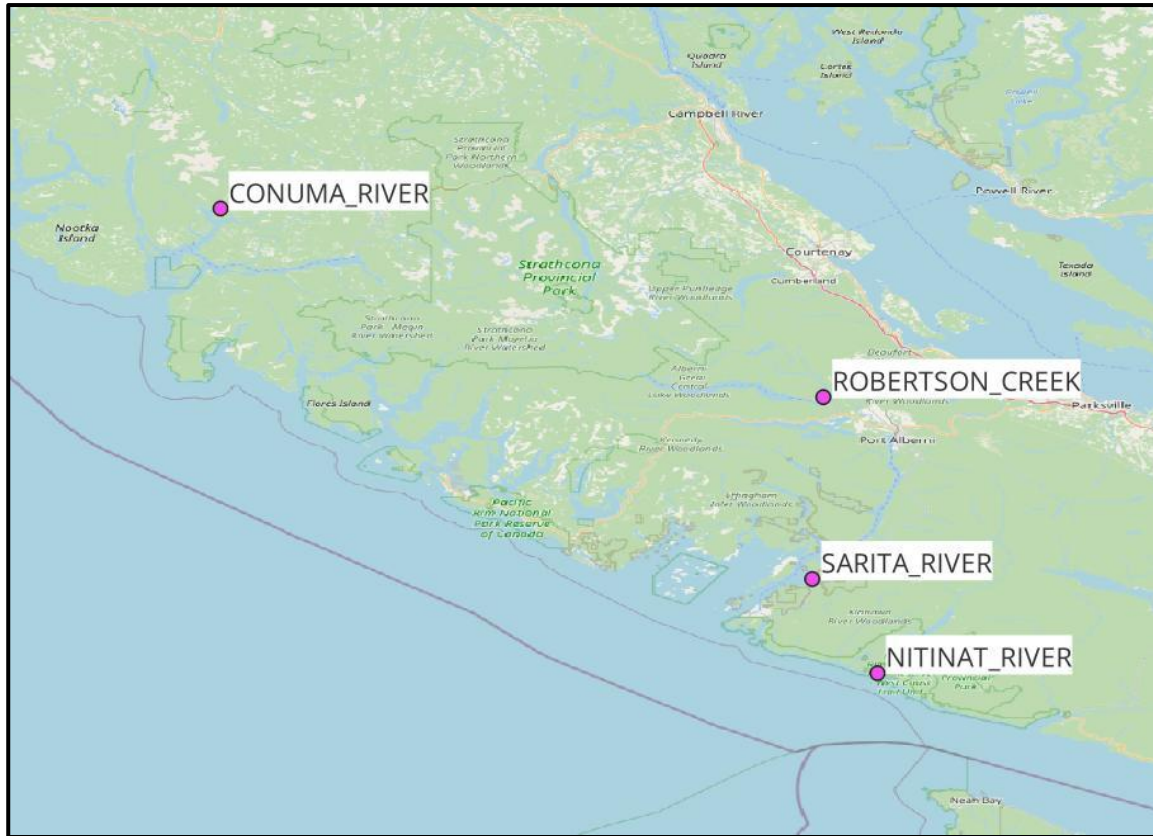
Approaches to understand trait variation

1. Screen genetic variation between hatchery- and natural-origin Pacific salmon, generate hypotheses based on the genomic locations of differences, and perform functional tests of those hypotheses
2. Examine genetic associations with variation in traits that are putatively linked with fitness differences between hatchery- and natural-origin Pacific salmon

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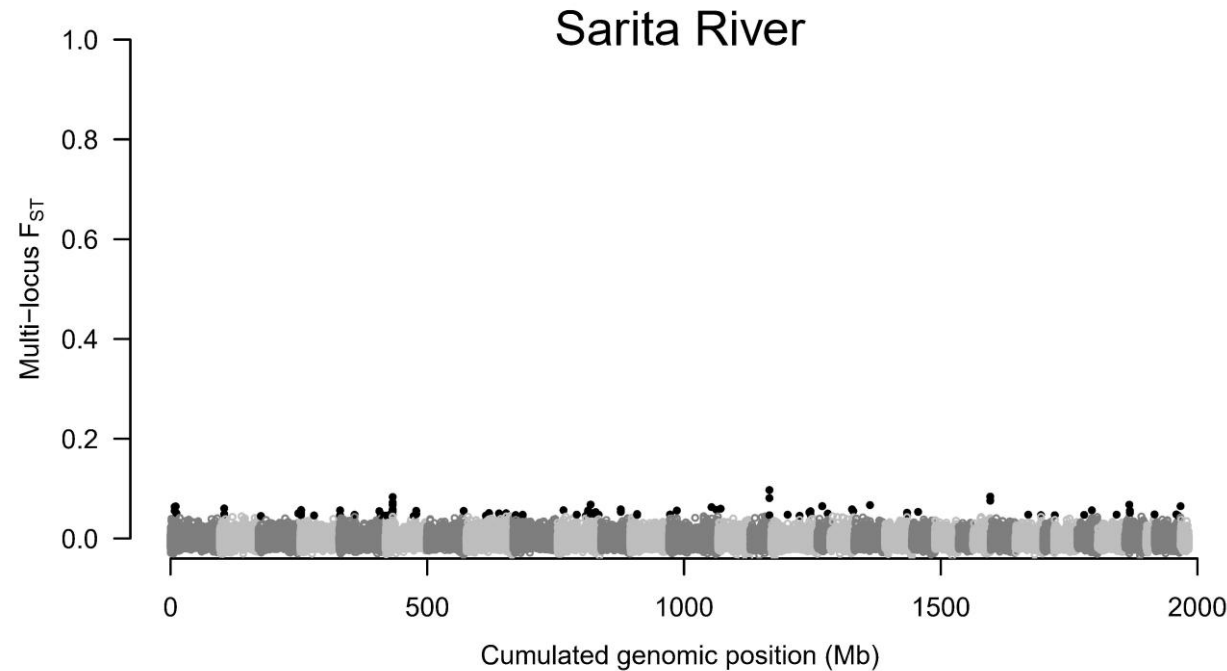
Is there genetic differentiation?



Study design:

- Four Chinook Salmon hatchery stocks
 - Fin tissue
 - Origin from parentage-based tagging
- Whole-genome sequencing by Pool-seq
 - Hatchery- vs natural-origin
 - $n = 80-90$ per group

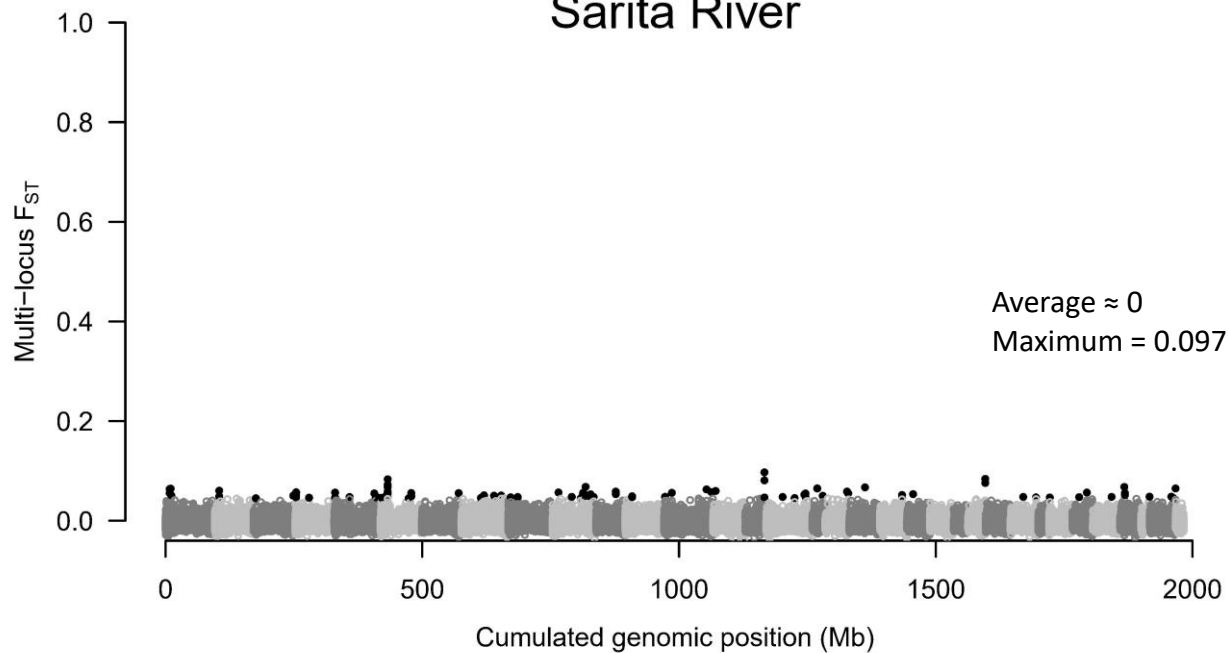
Lack of genetic differentiation



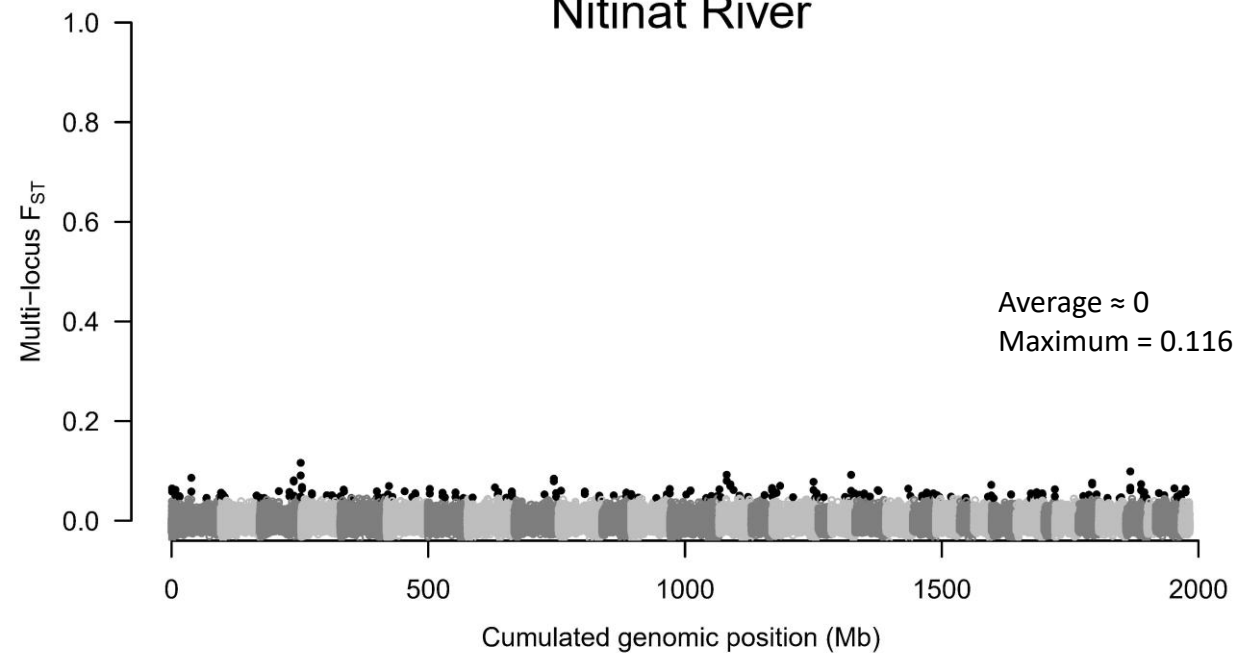
- Fixation index (F_{ST} – vertical axis) is the proportion of genetic variation that occurs between groups
- Limited differentiation between hatchery- and natural-origin Sarita River Chinook Salmon
(mean $F_{ST} \approx 0$, max $F_{ST} = 0.097$)

- Suggests limited genetic differences despite performance differences within a generation; likely polygenic traits, possibly weak selection

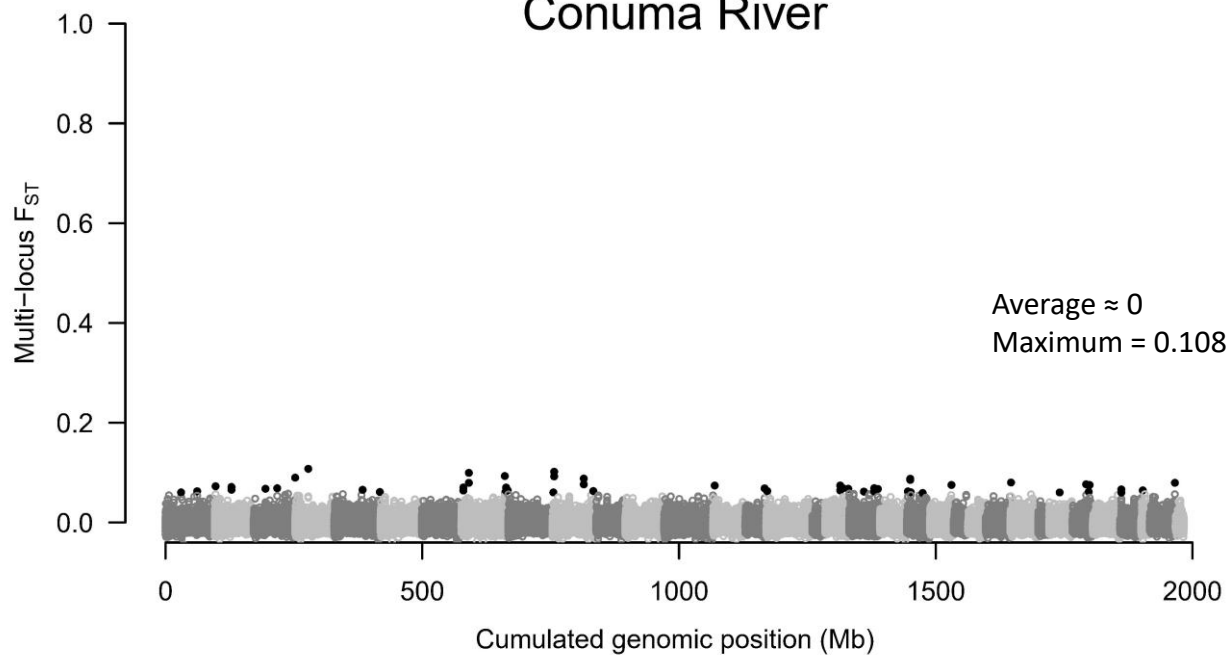
Sarita River



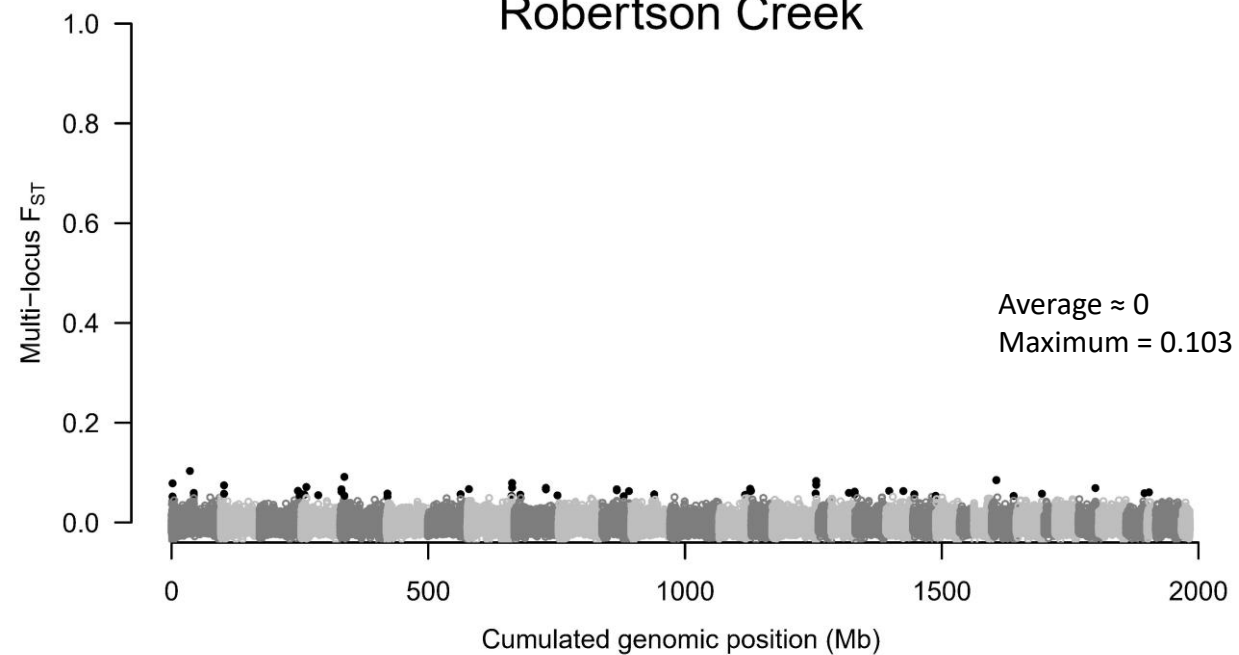
Nitinat River



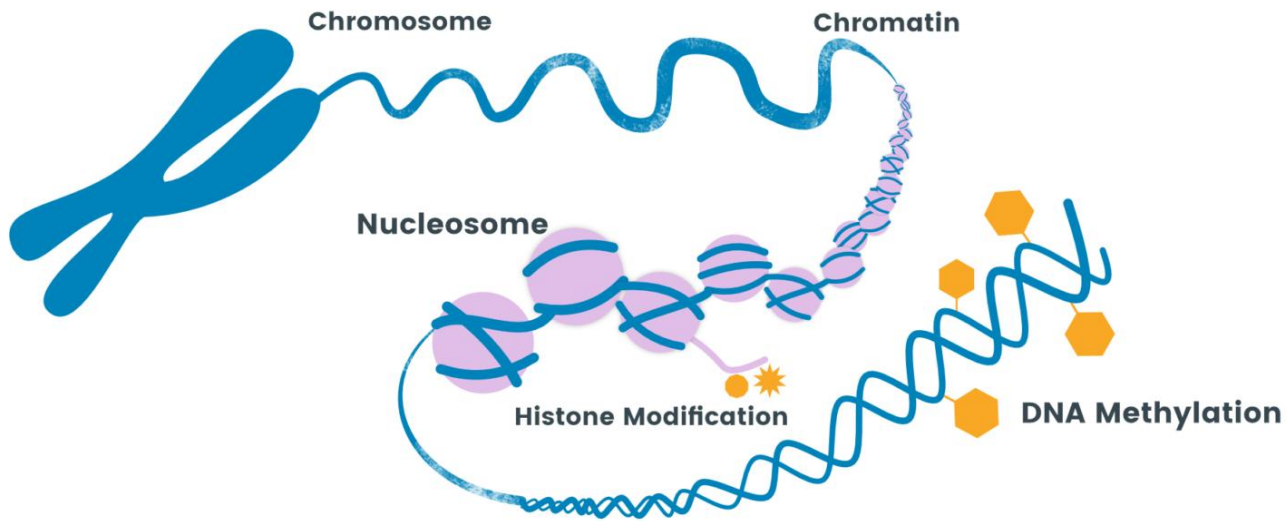
Conuma River



Robertson Creek

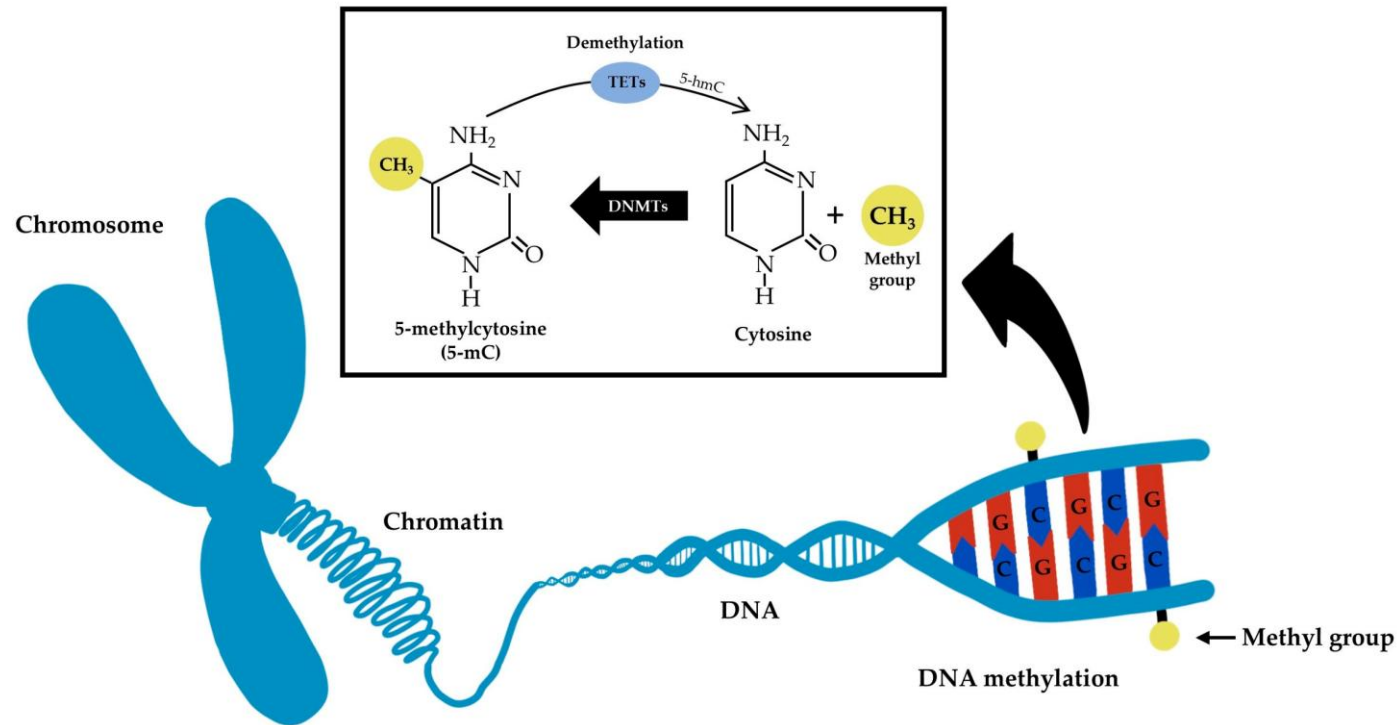


Alternative possibility: epigenetics



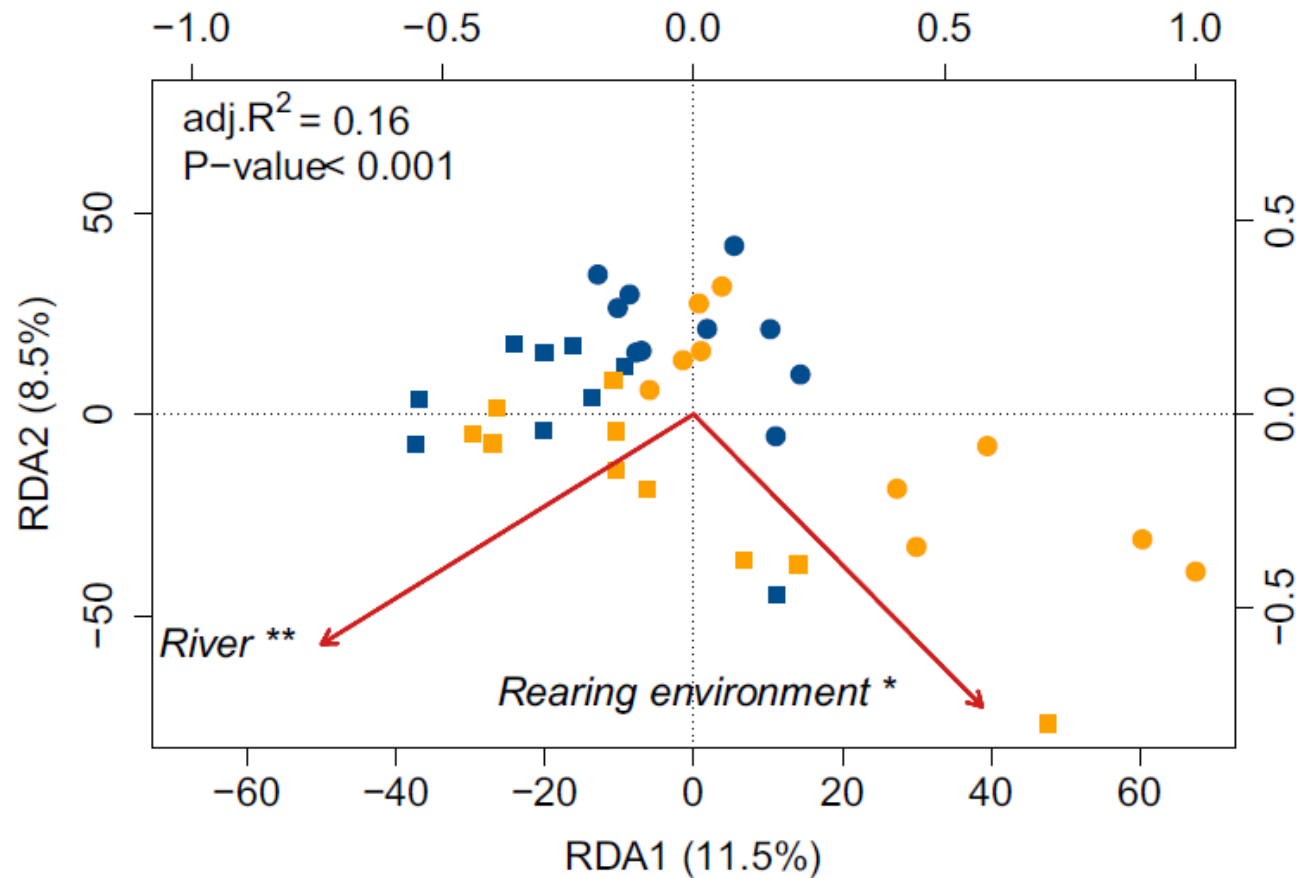
- Epigenetics = “on DNA”
- Potentially heritable changes in DNA structure and organization
- Best characterized epigenetic modification is DNA methylation

DNA methylation



- Addition of a methyl side group ($-\text{CH}_3$) to a cytosine base at specific DNA sequences (CpG)
- Enzymatic process resulting in a covalent bond
- Can change gene regulation and expression

Hatchery-associated epigenetic differences

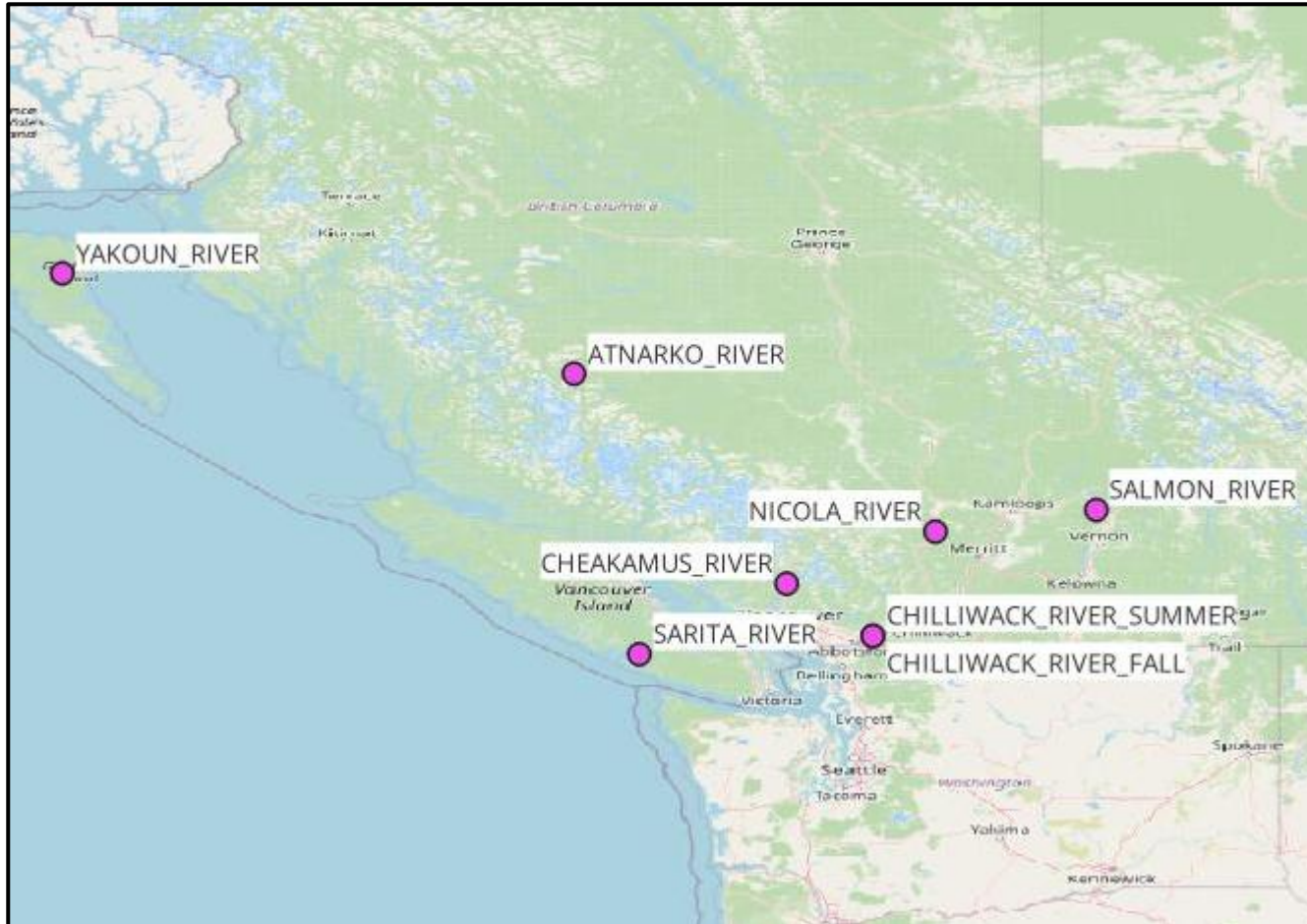


- Capilano (circles) and Quinsam (squares) Coho
 - Hatchery = blue
 - Natural = orange
- Significant effect of origin in white muscle in both stocks

Questions for today

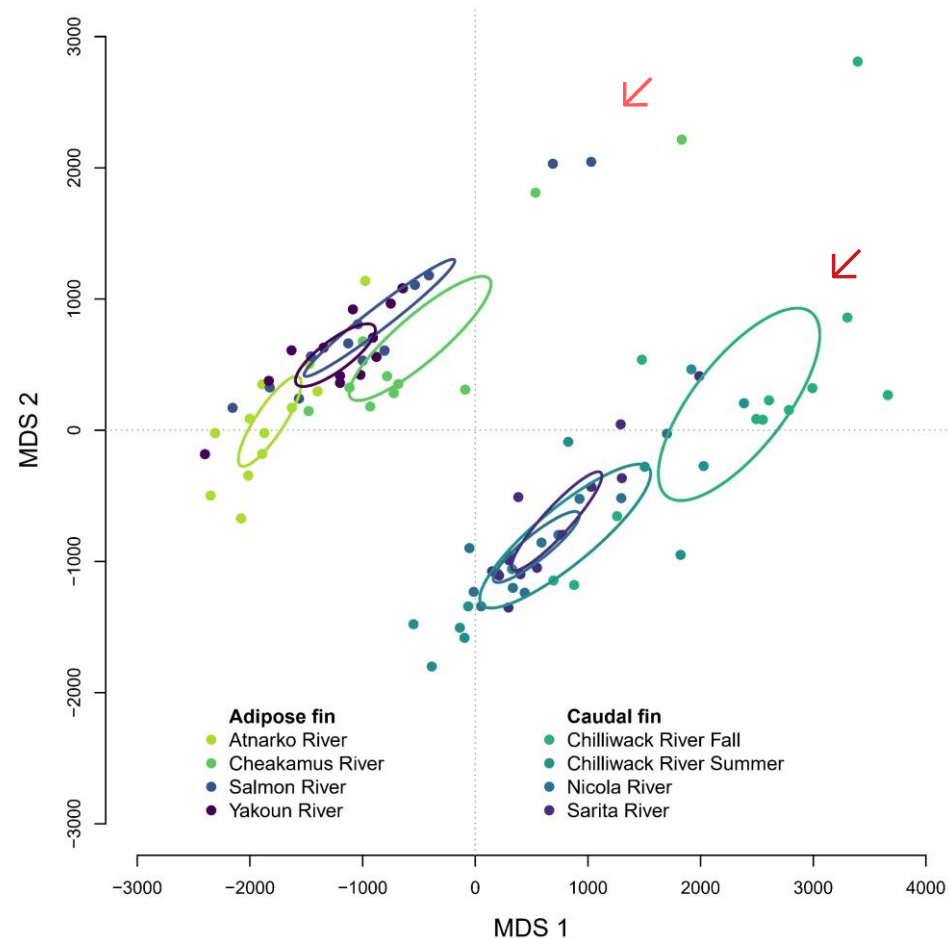
1. Are there epigenetic differences between hatchery- and natural-origin Chinook Salmon?
2. Are there consistent effects of hatchery-origin across many hatcheries?

Epigenetics study design



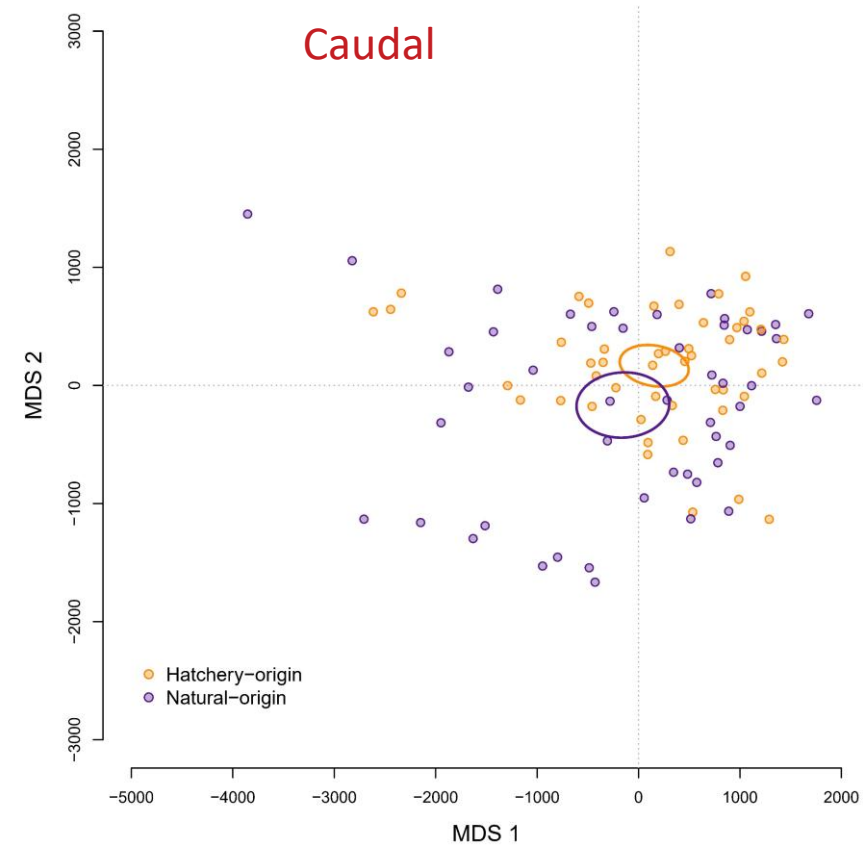
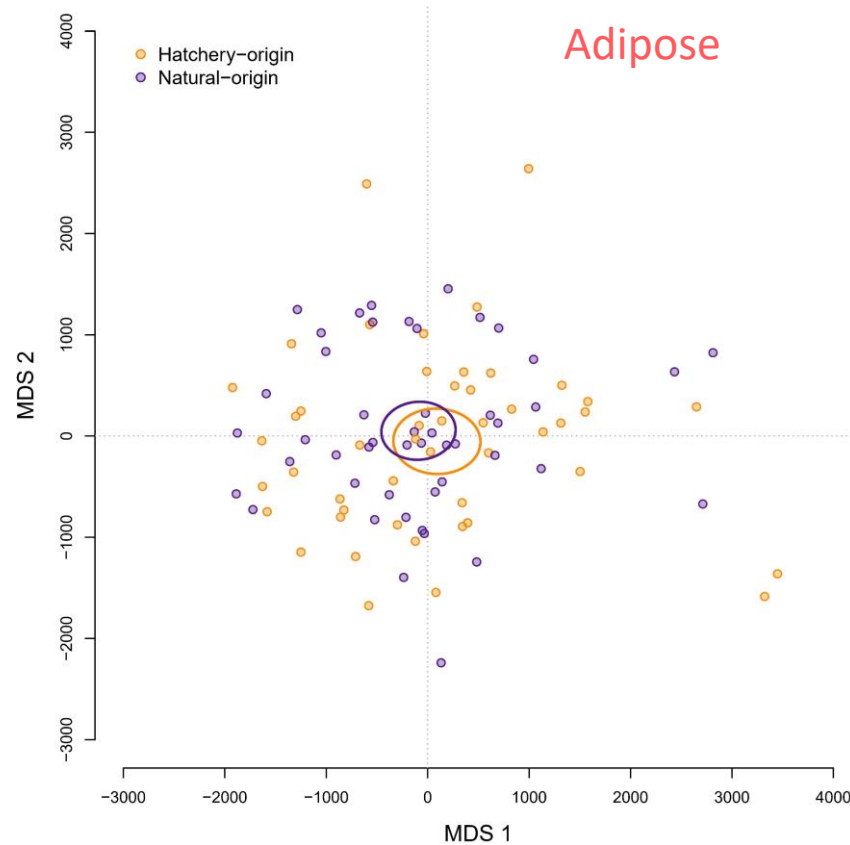
- Eight Chinook Salmon hatchery stocks
 - Fin tissue
 - Origin from parentage-based tagging
- Whole-genome methylation sequencing
 - Hatchery- vs natural-origin within and across hatcheries

DNA methylation in Chinook Salmon

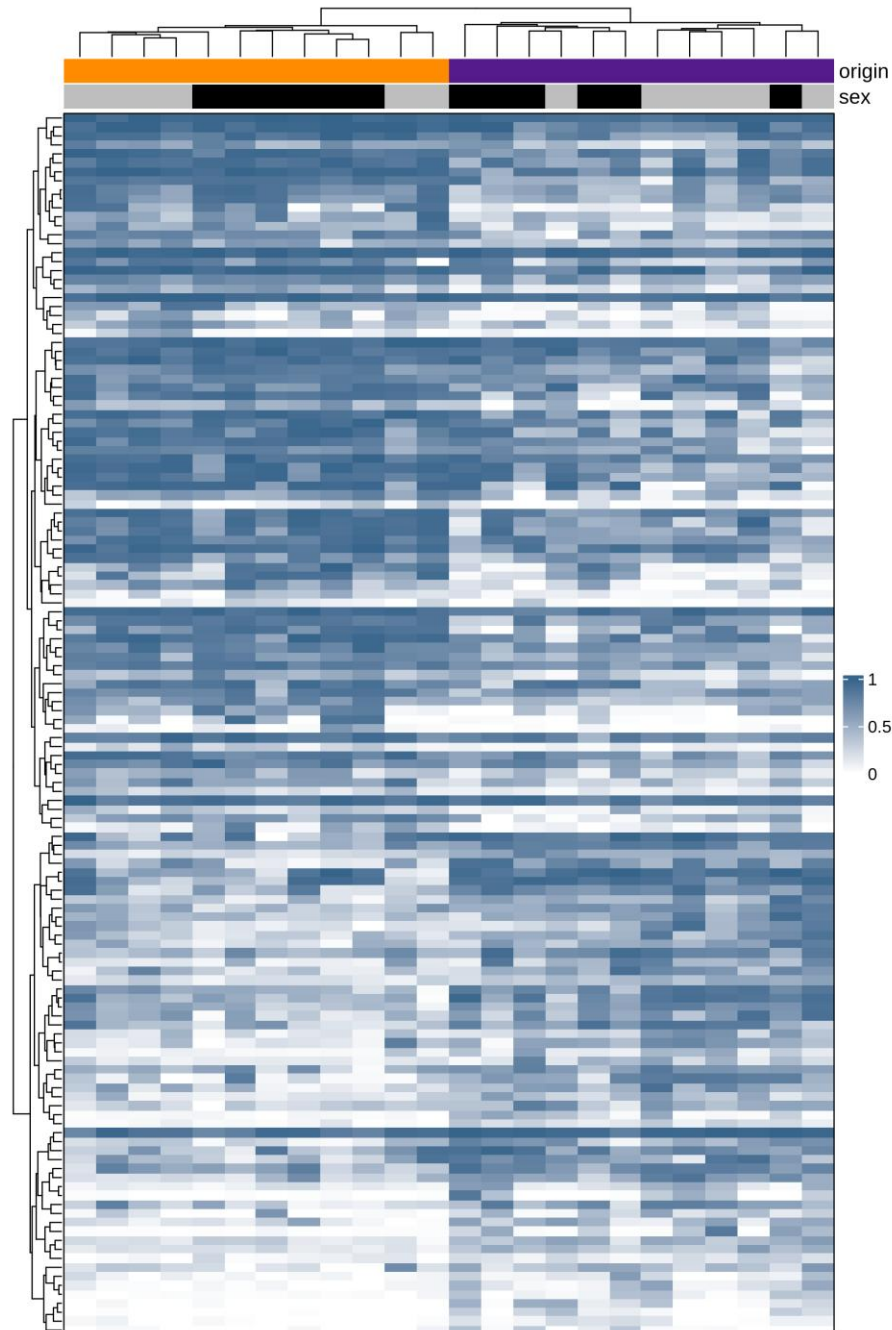


- Colours indicate stocks
- Ellipses = 95% confidence
- Stocks have distinct patterns of DNA methylation
- Four hatcheries prioritize sampling **adipose fin**, whereas the other four hatcheries prioritize **caudal fin**

Hatchery- vs natural-origin patterns



- Essentially no evidence of an overall difference between hatchery- and natural-origin Chinook Salmon across hatcheries



Cheakamus River

- Left to right:
 - Hatchery-origin to natural-origin
- Howe Sound – stream-type
- **136 Differentially methylated regions (DMRs) between origins**
- DMRs spread throughout genome
 - 31/34 chromosomes
 - 13 maximum on any chromosome

Variation in the effect of origin

	Atnarko	Cheakamus	Salmon	Yakoun	Chilliwack F	Chilliwack S	Nicola	Sarita
Atnarko	55							
Cheakamus	0	136						
Salmon	0	0	119					
Yakoun	0	0	3	131				
Chilliwack F	0	0	0	0	82			
Chilliwack S	0	0	0	1	0	373		
Nicola	0	3	2	0	0	0	52	
Sarita	0	0	3	1	0	0	0	70

- Vast majority of differential methylation associated with hatchery- or natural-origin is specific to stock

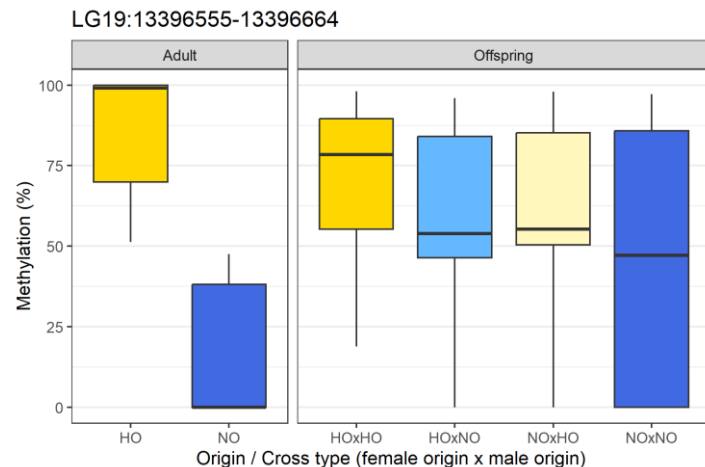
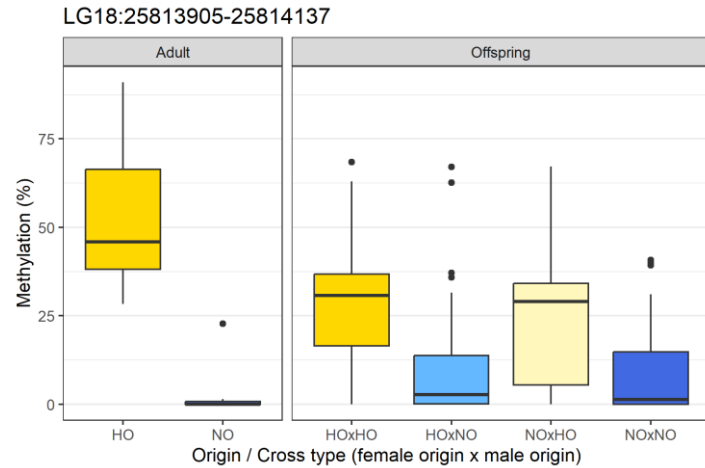
Questions for today

1. Are there epigenetic differences between hatchery- and natural-origin Chinook Salmon? **YES**
2. Are there consistent effects of hatchery-origin across many hatcheries? **NO for Chinook (and Coho)**

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1. Are there epigenetic differences between hatchery- and natural-origin Chinook Salmon? **YES**
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3. Is there evidence of inheritance of epigenetic variation over generations in Pacific salmon?

DNA methylation is heritable in Coho Salmon



- Crosses among **hatchery-** and **natural-origin** Chehalis River Coho Salmon
- DNA methylation of candidate genomic regions in offspring from pure crosses is similar to parental methylation patterns
- Patterns from crosses between hatchery and natural parents show additive or paternal inheritance

Questions for today

1. Are there epigenetic differences between hatchery- and natural-origin Chinook Salmon? **YES**
2. Are there consistent effects of hatchery-origin across many hatcheries? **NO for Chinook (and Coho)**
3. Is there evidence of inheritance of epigenetic variation over generations in Pacific salmon? **YES, but pattern depends on region**

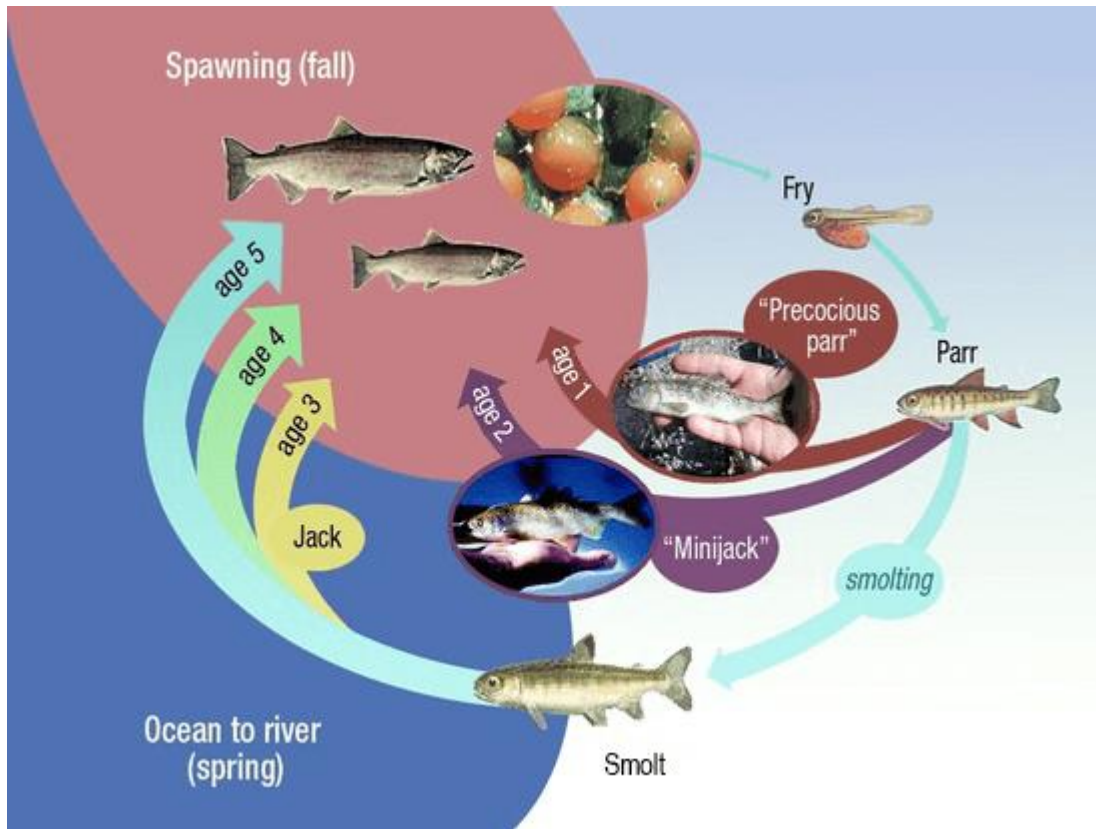
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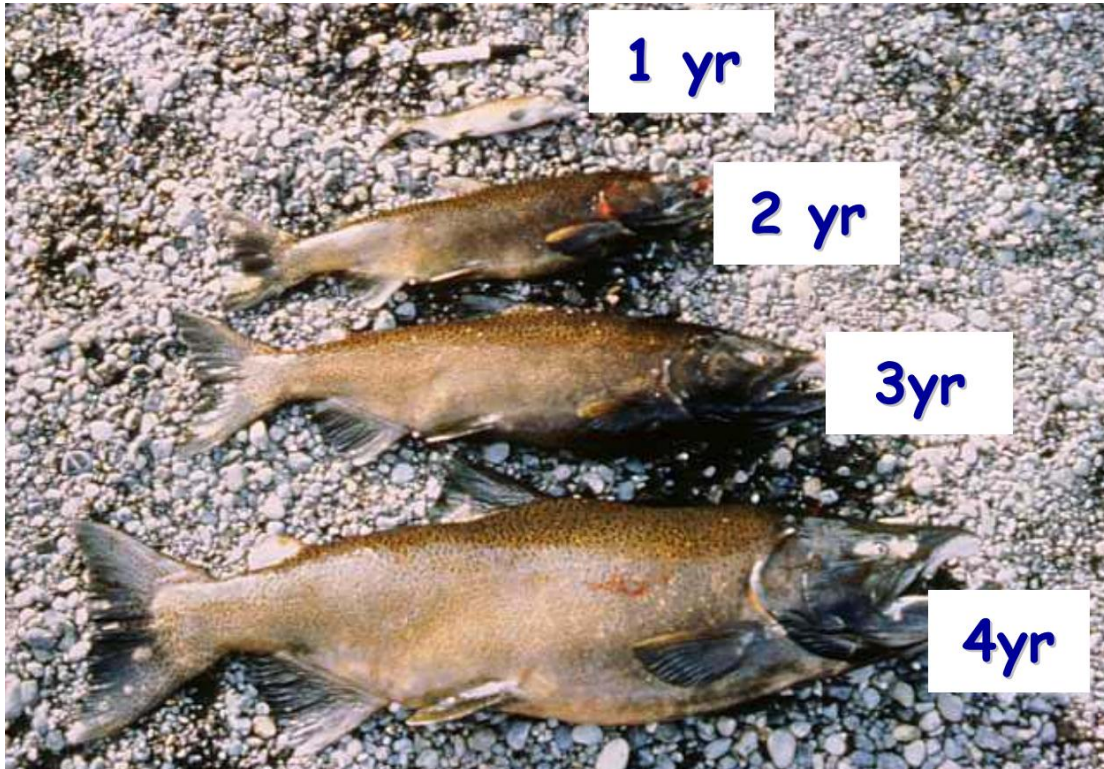
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Variation in seawater residency time



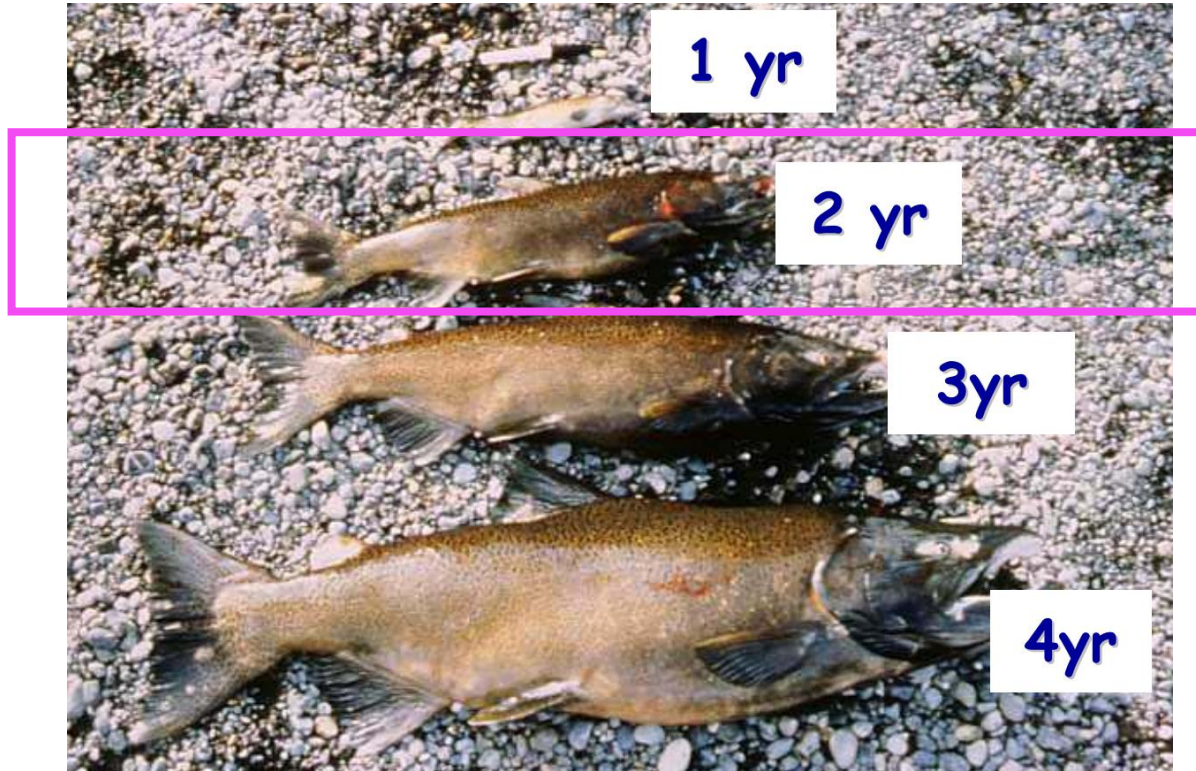
- Chinook Salmon mature and return to freshwater at different ages, particularly in males
- This variation in age-of-return has a substantial impact on size at spawning
- Older returns tend to be larger returns

Chinook salmon age and size



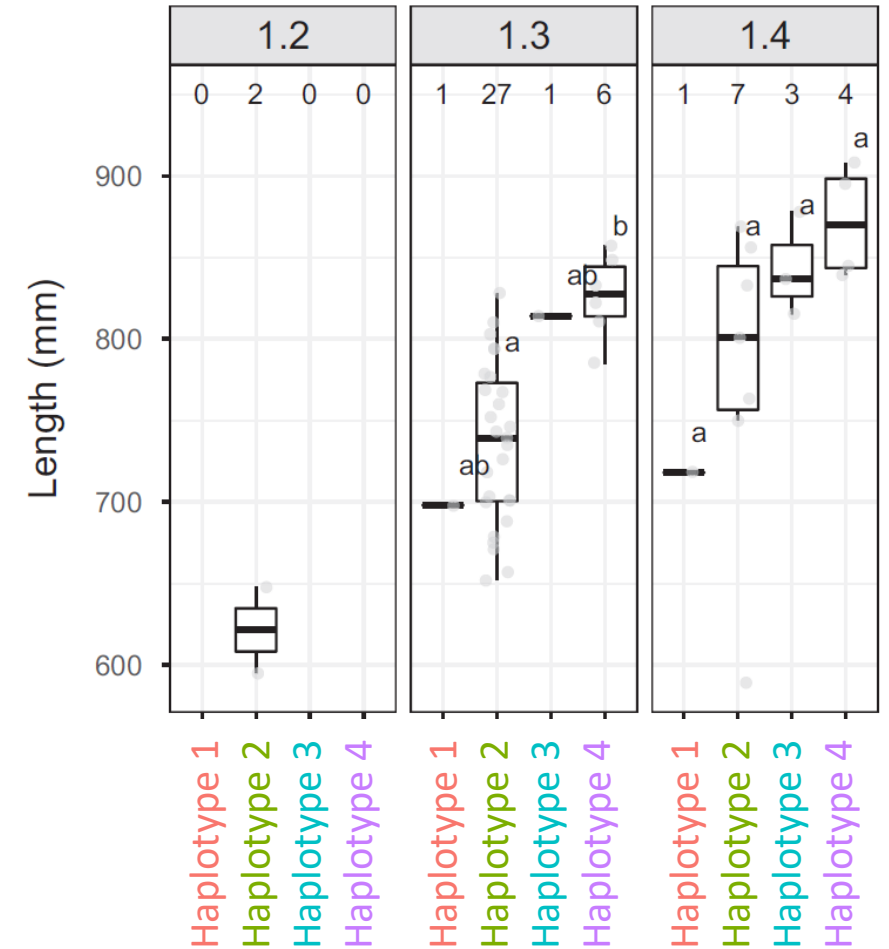
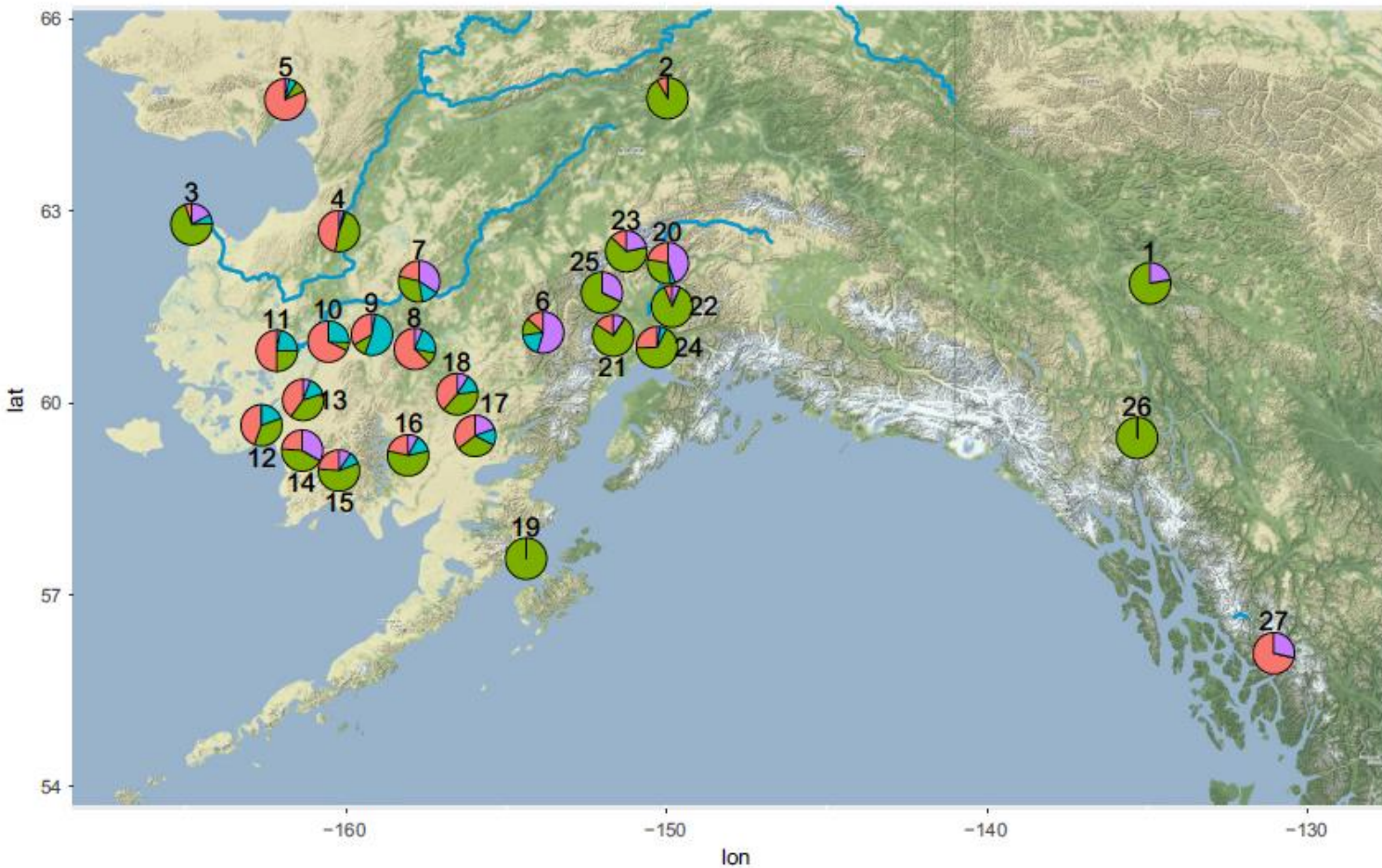
- Large size is generally a competitive advantage:
 - Males = mate competition
 - Females = higher fecundity, redd site competition
- The advantages of size are likely balanced against costs of time in the ocean and mortality
 - No return = zero fitness

Alternative reproductive strategy in males

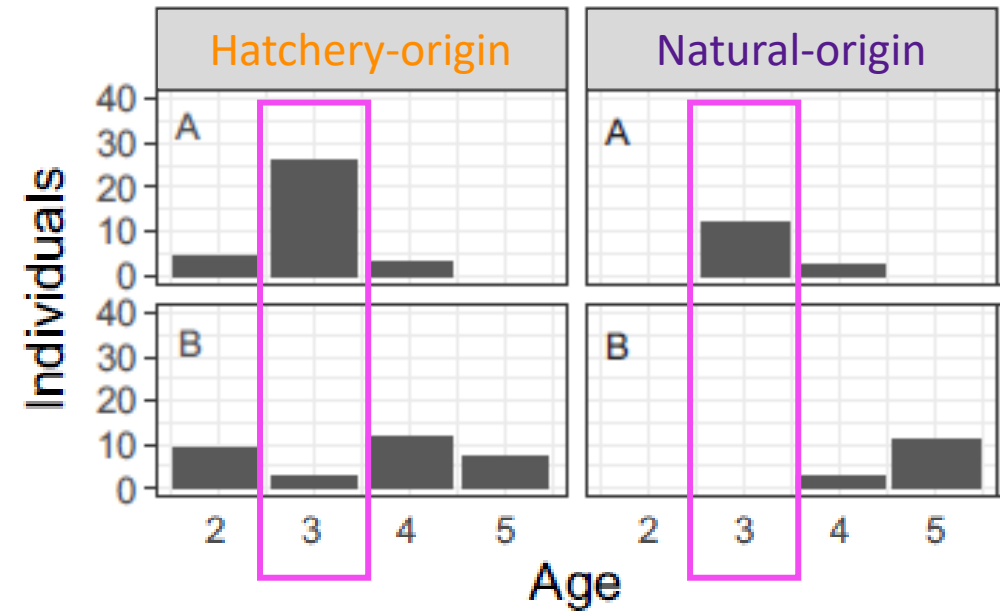
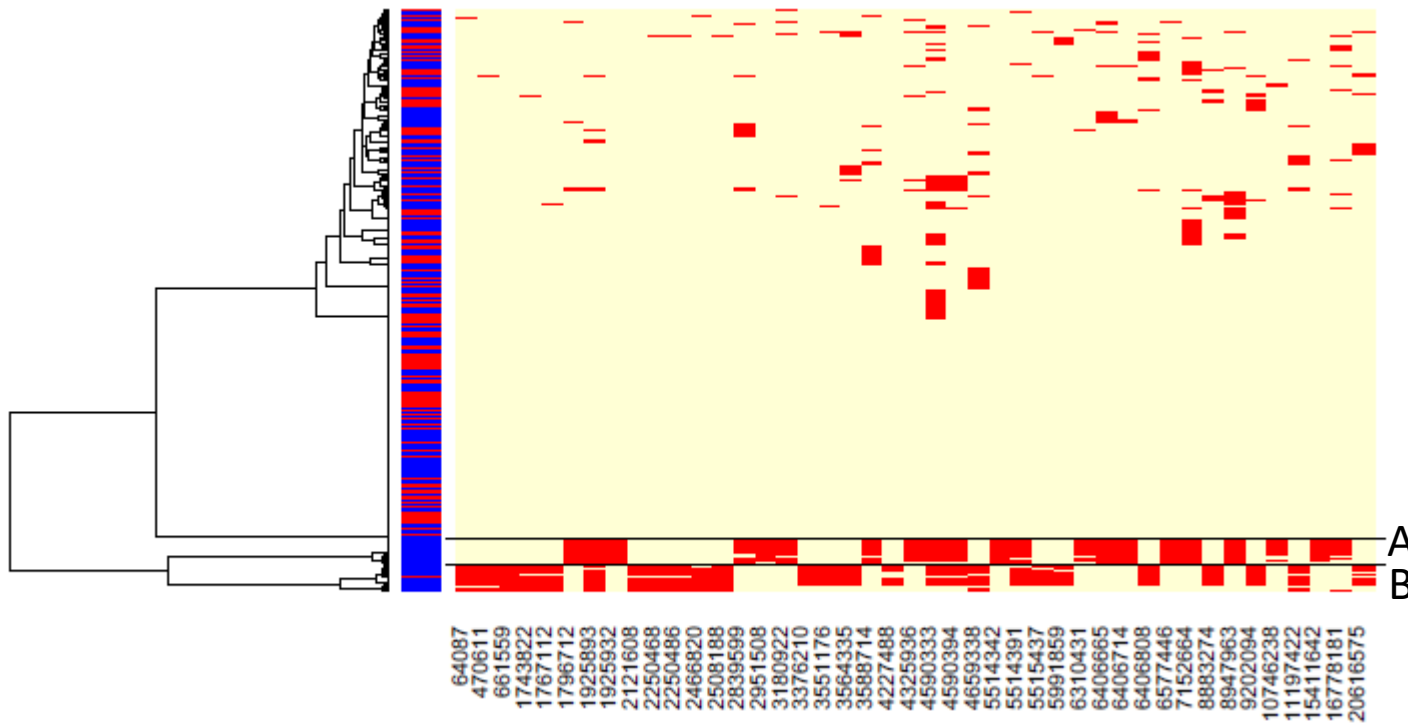


- **Jacks** are males that return one year younger than the earliest returning females
 - “Sneaker” males
- Negative frequency dependent selection
- Relatively high heritability ($h^2 \approx 0.4$) (e.g., Heath et al. [1994](https://www.dfw.state.or.us/fish/hatchery/docs/Berejikian.pdf))

Putative Y-chromosome genetic variation

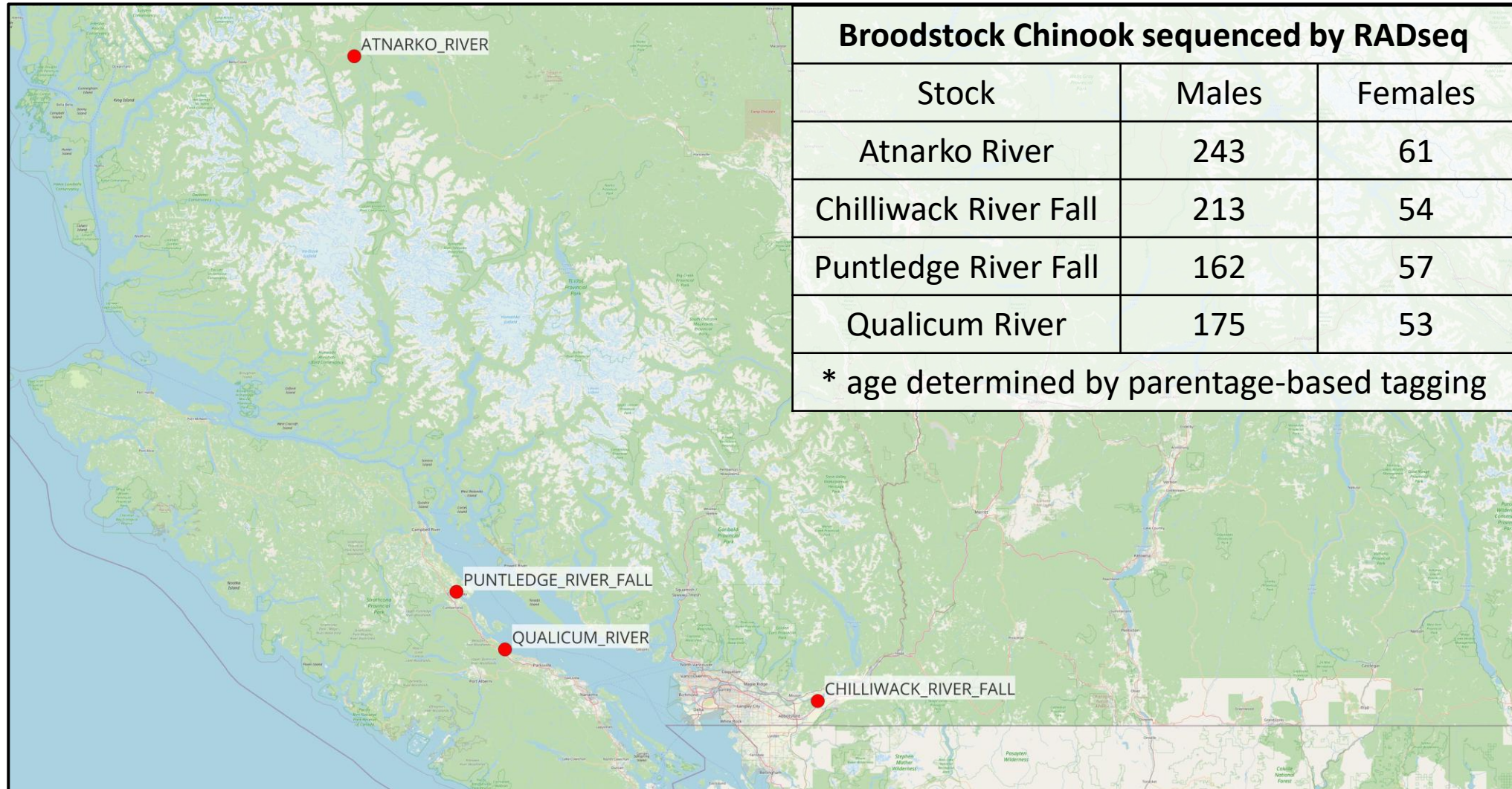


Putative Y-chromosome haplotype in jacks

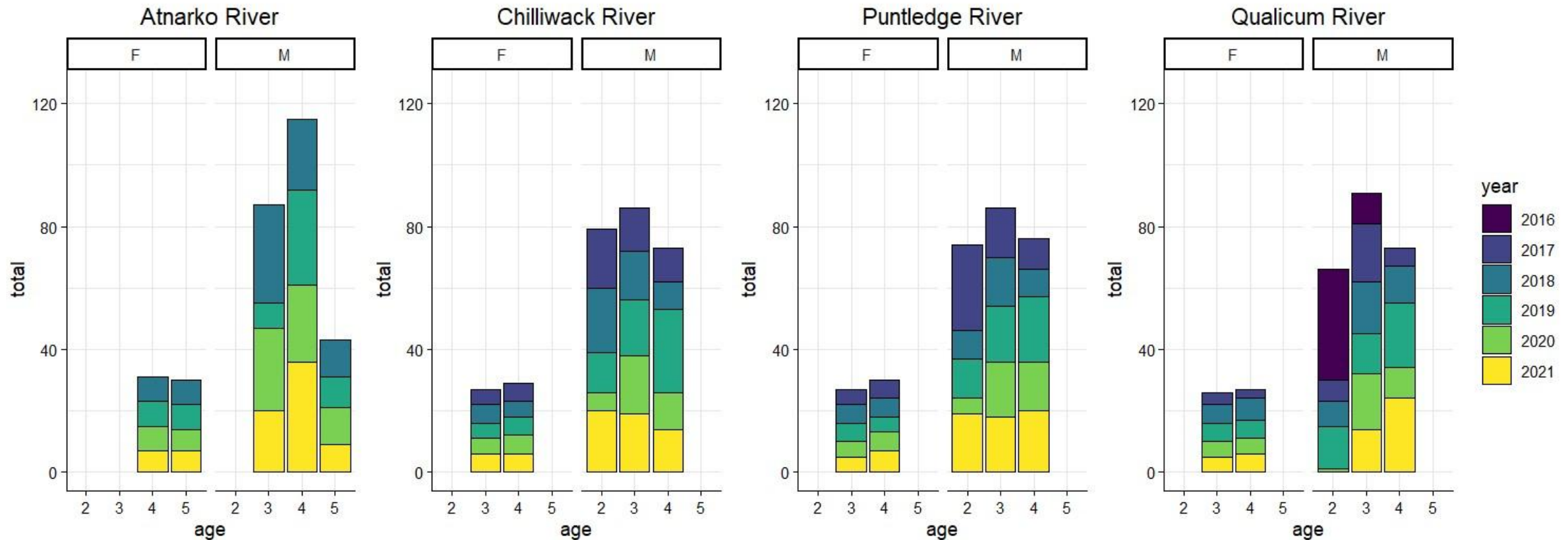


- Male-specific sex chromosome haplotypes are associated with jacks (age 3) and age-of-return in a Columbia River stock

Age association study design

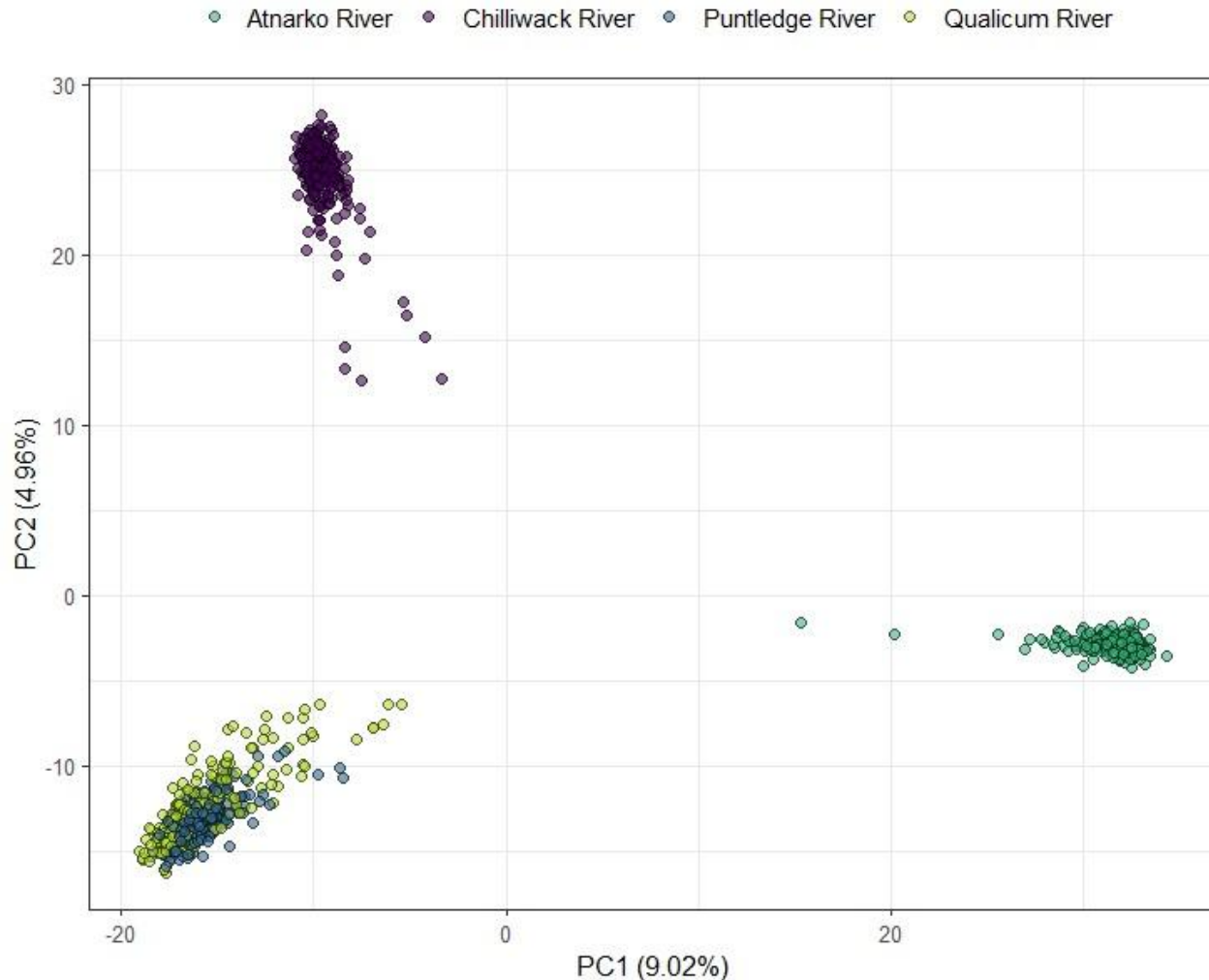


Samples sequenced by age and brood year



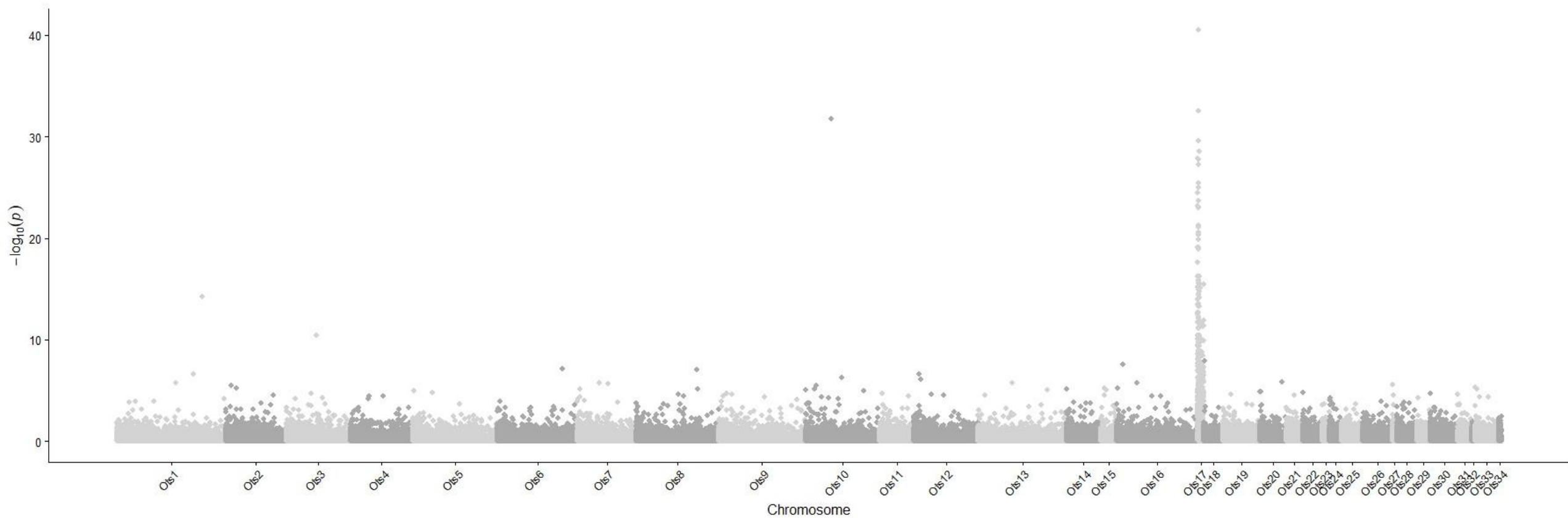
- Atnarko: females 4-5 years old $\therefore$ jacks 3 years old
- Other stocks: females 3-4 years old $\therefore$ jacks 2 years old

Genetic variation among stocks



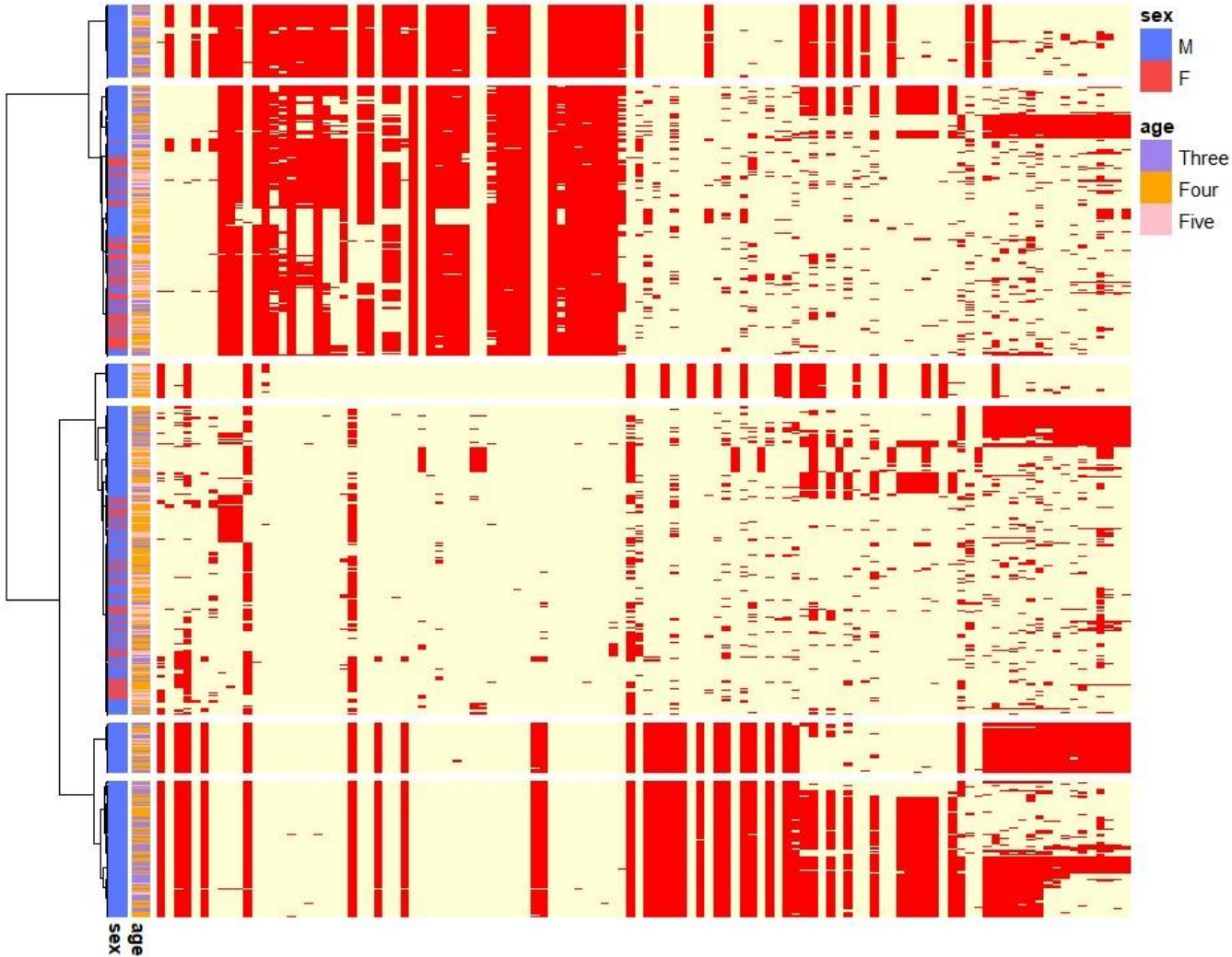
- RADseq approach:
 - 56,094 single-nucleotide polymorphisms (SNPs)
- Atnarko and Chilliwack Fall are genetically distinct
- Puntledge Fall and Qualicum cluster together
 - History of transplanting

Genetic associations with sex

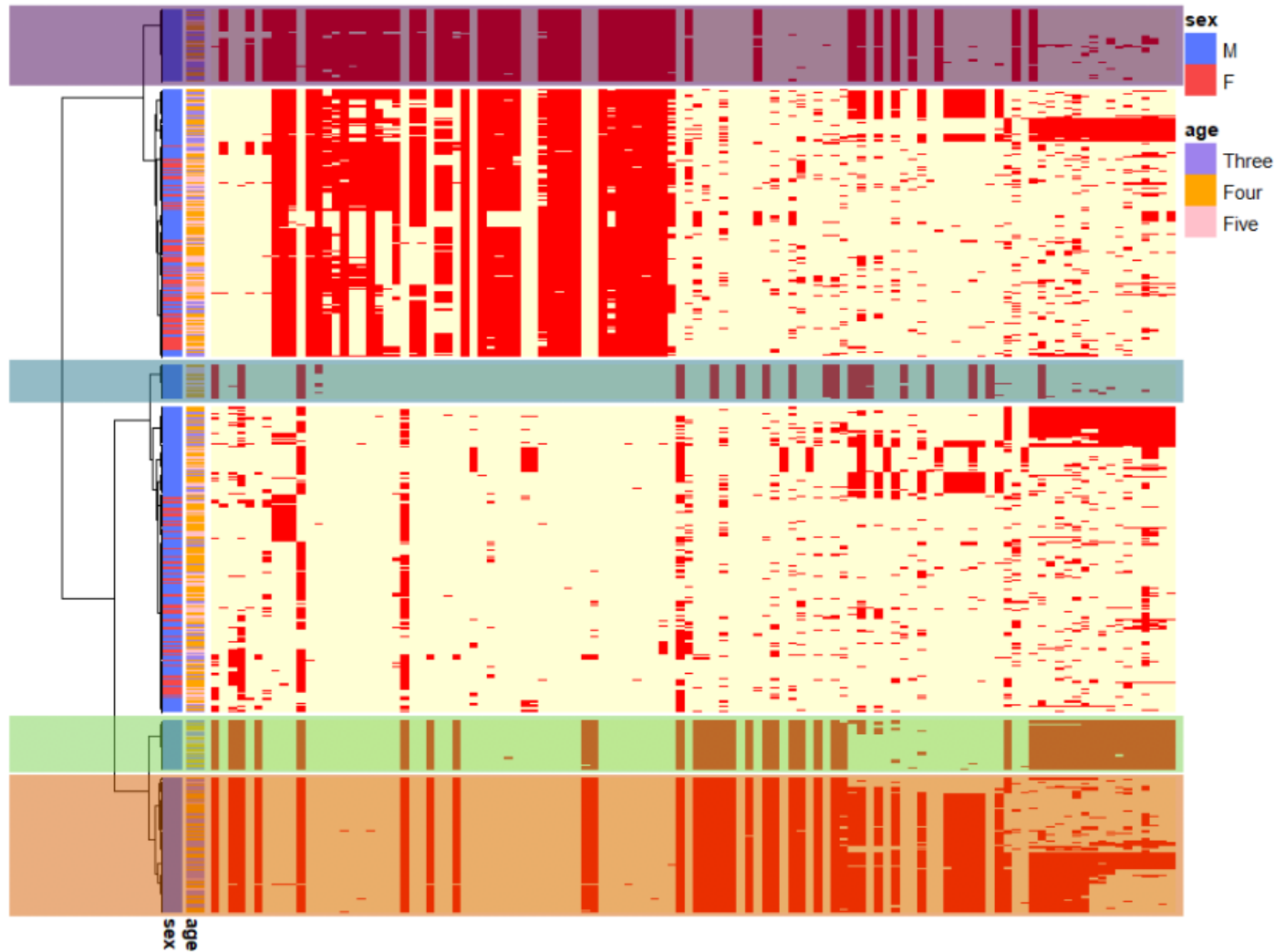


- Ots17 is clearly the sex chromosome in these stocks

Atnarko Ots17 phased genetic variation



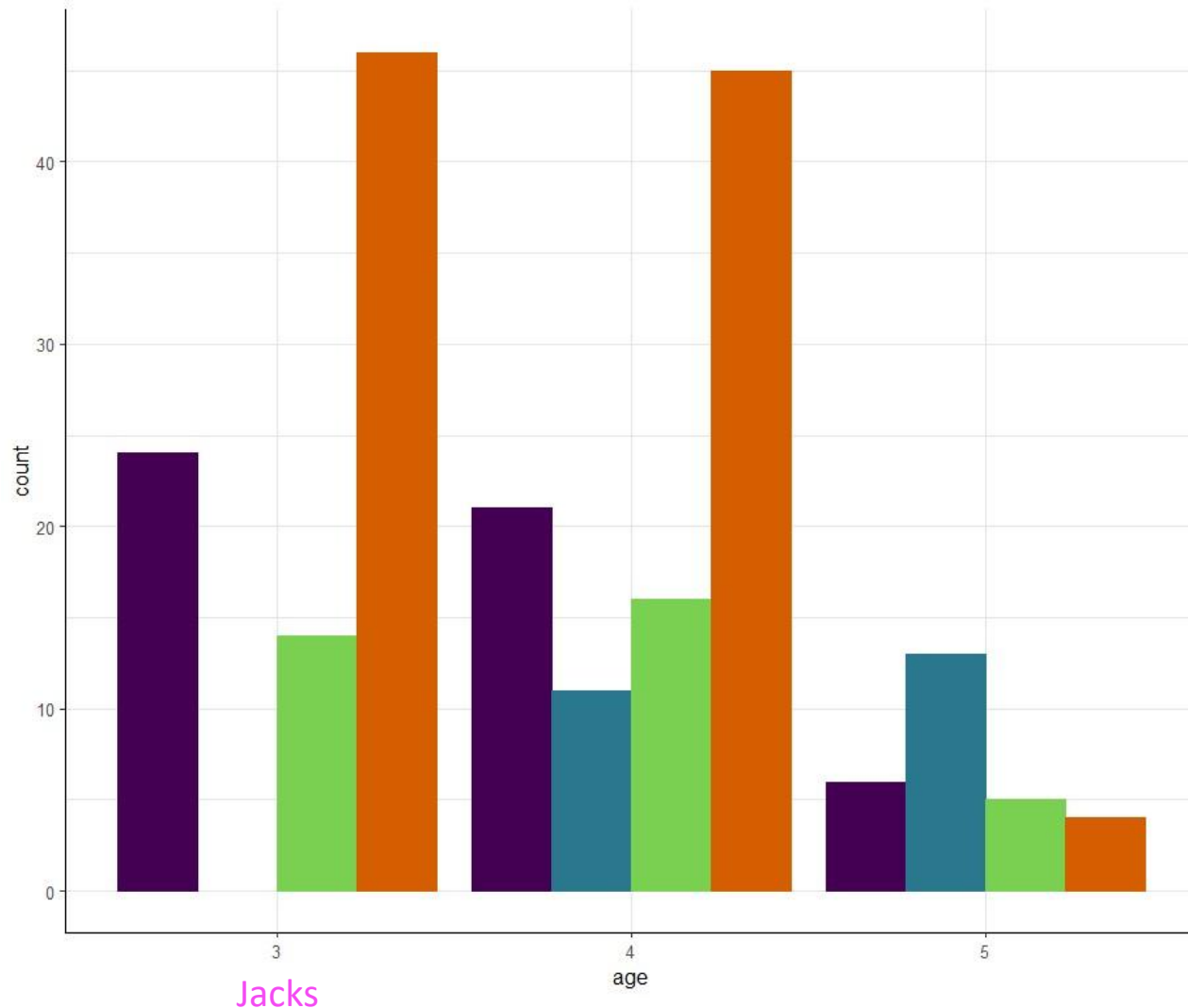
Atnarko male-specific haplotypes



Genotype	Expected in males	Observed in males
XY	243	205
XX	0	38
YY	0	0

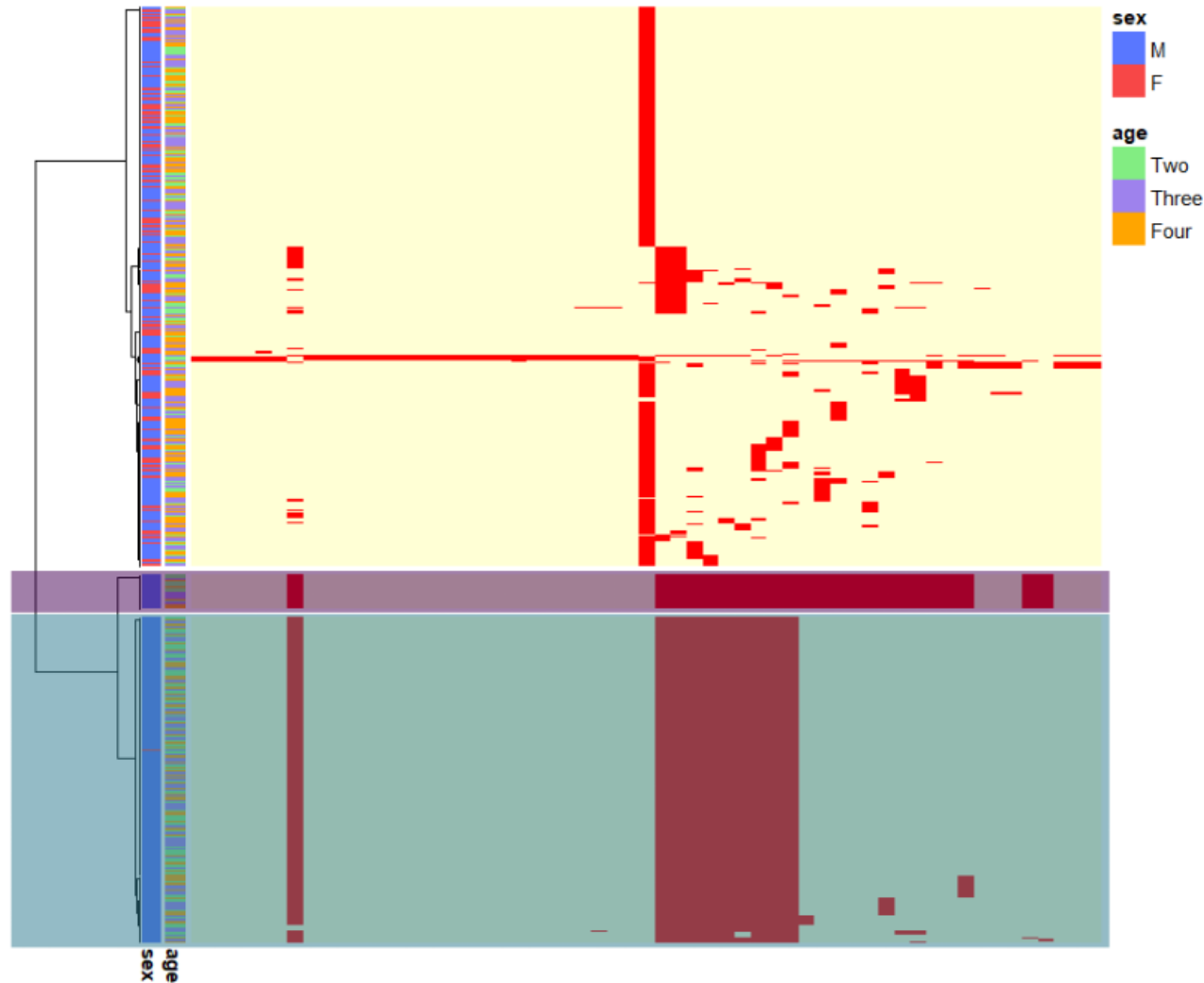
- Four male-specific haplotypes or putative y-chromosomes

Atnarko haplotypes by age-of-return



- Variation in age-of-return is associated with different haplotypes
 - One haplotype (■) is absent in **jacks** (age 3) and is the most common in age-5 males
- No haplotype is specifically associated with **jacks** (age 3)

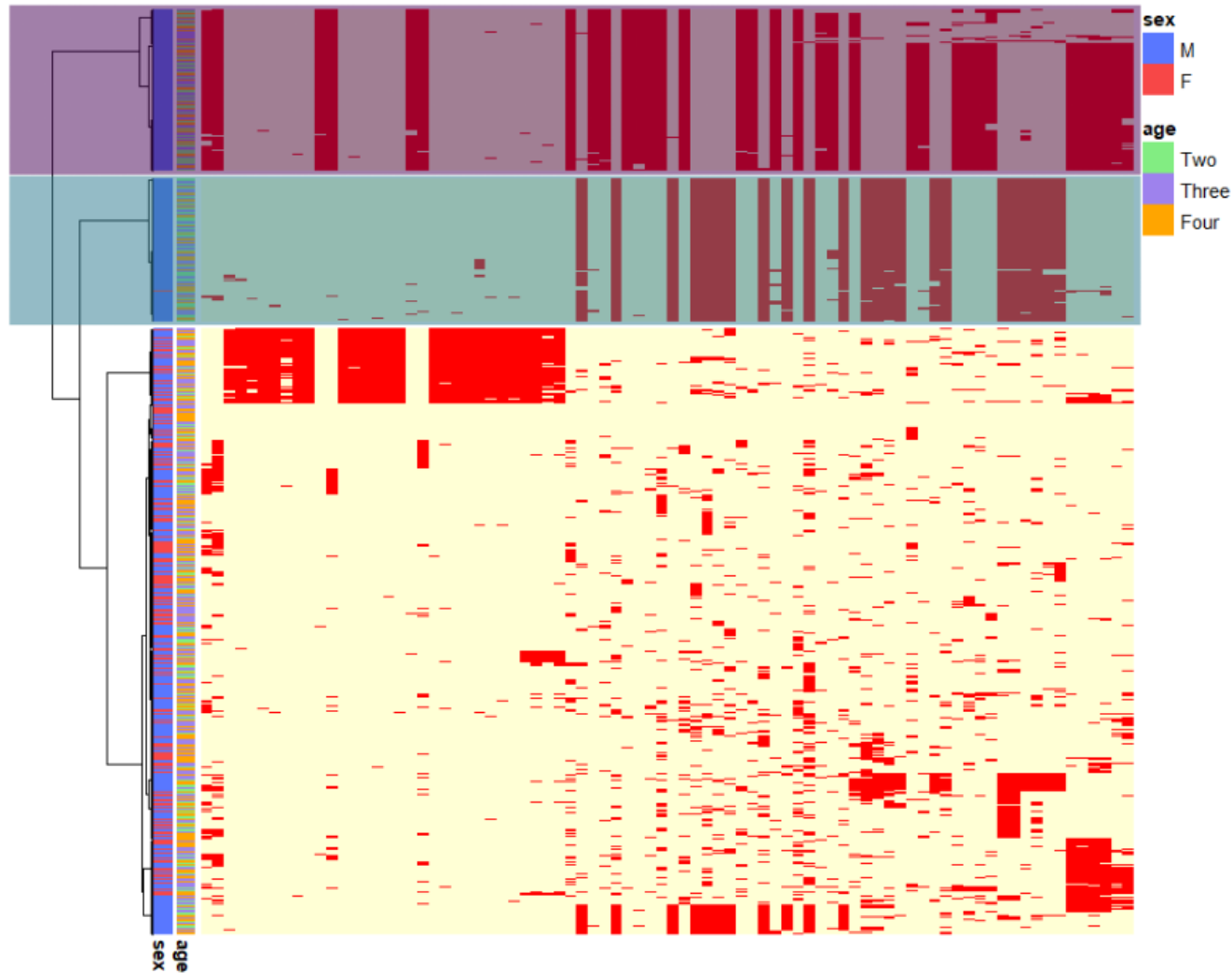
Chilliwack male-specific haplotypes



Genotype	Expected in males	Observed in males
XY	213	208
XX	0	5
YY	0	0

- Two male-specific haplotypes or putative y-chromosomes

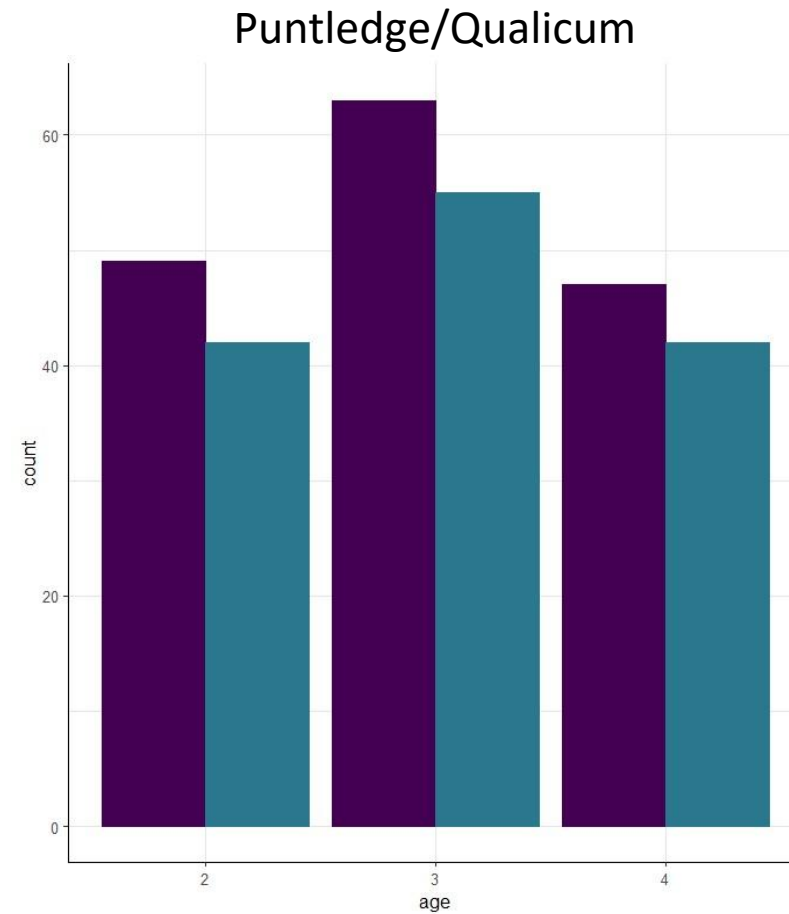
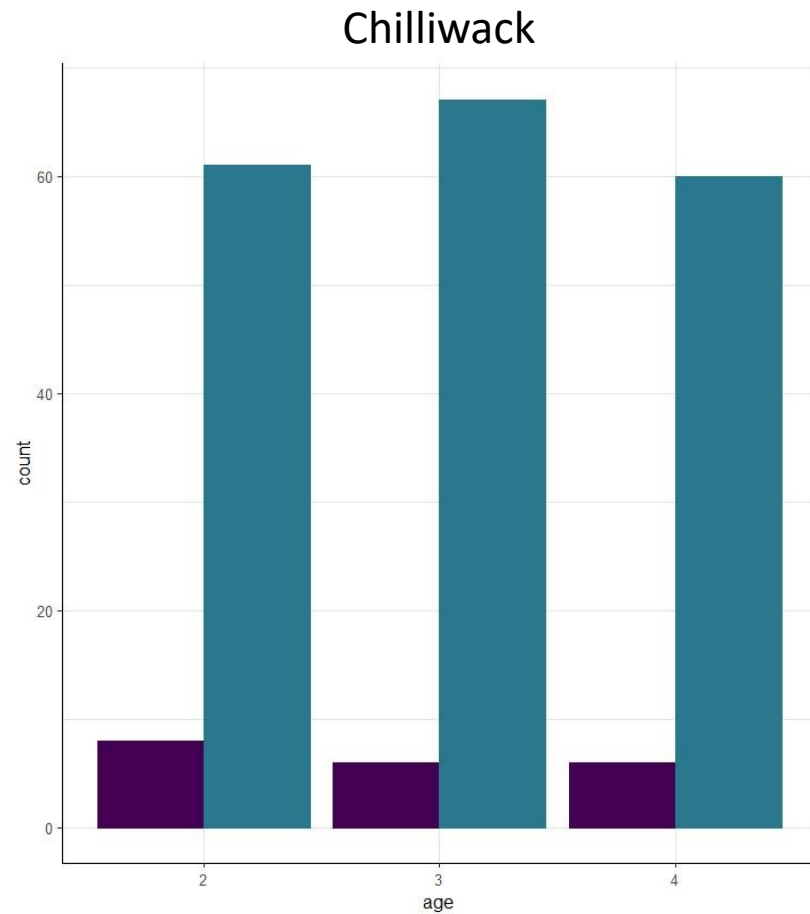
Puntledge/Qualicum male-specific haplotypes



Genotype	Expected in males	Observed in males
XY	337	298
XX	0	39
YY	0	0

- Two male-specific haplotypes or putative y-chromosomes

Haplotypes by age-of-return



- No variation in haplotype frequency associated with age-of-return

Preliminary conclusions

- Impact of putative Y-chromosome haplotypes on age-of-return in males is dependent on population and possibly life history variation (e.g., age-of-return distribution in the population)
- No haplotypes are highly predictive of a male returning as a jack, although environmental effects of hatchery rearing may be obscuring any relationship
- Regardless, this suggests that any increase in the frequency of jacks associated with SEP's hatchery practices are potentially environmental

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Nanaimo River Spawning Stream

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Acknowledgements

- DFO Molecular Genetics:

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