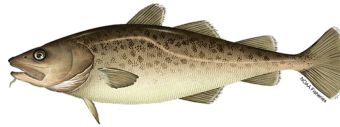


Molecular indicators of performance in a marine fish exposed to temperature stress










Laura Spencer, NOAA AFSC Affiliate
University of Washington SAFS & CICOES



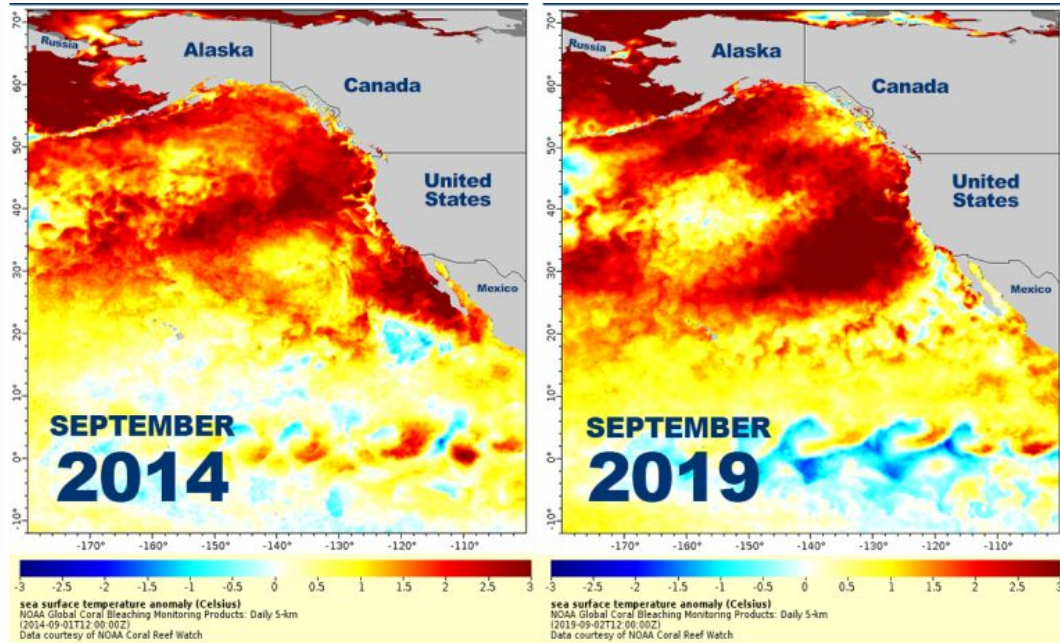
NOAA surveys & manages Alaskan fisheries (AFSC)



VALUE & VOLUME OF KEY SPECIES, 2019

Salmon  \$715 <small>EX-VESSEL VALUE \$MILLIONS</small> \$1,733 <small>FIRST WHOLESALE (FW) VALUE \$MILLIONS</small> 865 <small>HARVEST MILLION LBS</small> \$2.00 <small>FW VALUE PER ROUND LB.</small>	Pollock  \$484 <small>EX-VESSEL VALUE \$MILLIONS</small> \$1,636 <small>FIRST WHOLESALE (FW) VALUE \$MILLIONS</small> 3,353 <small>HARVEST MILLION LBS</small> \$0.49 <small>FW VALUE PER ROUND LB.</small>	Crab  \$226 <small>EX-VESSEL VALUE \$MILLIONS</small> \$294 <small>FIRST WHOLESALE (FW) VALUE \$MILLIONS</small> 47 <small>HARVEST MILLION LBS</small> \$6.30 <small>FW VALUE PER ROUND LB.</small>	Pacific Cod  \$203 <small>EX-VESSEL VALUE \$MILLIONS</small> \$382 <small>FIRST WHOLESALE (FW) VALUE \$MILLIONS</small> 464 <small>HARVEST MILLION LBS</small> \$0.82 <small>FW VALUE PER ROUND LB.</small>
Halibut & Sablefish  \$166 <small>EX-VESSEL VALUE \$MILLIONS</small> \$187 <small>FIRST WHOLESALE (FW) VALUE \$MILLIONS</small> 46 <small>HARVEST MILLION LBS</small> \$4.05 <small>FW VALUE PER ROUND LB.</small>	Flatfish, Rockfish, & Atka Mackerel  \$168 <small>EX-VESSEL VALUE \$MILLIONS</small> \$391 <small>FIRST WHOLESALE (FW) VALUE \$MILLIONS</small> 804 <small>HARVEST MILLION LBS</small> \$0.49 <small>FW VALUE PER ROUND LB.</small>	TOTAL IMPACTS 2021/2022 AVG (INCLUDING SECONDARY IMPACTS) <div> Fisheries closures </div> <div> 48,000 WORKERS </div> <div> \$2.3B LABOR INCOME </div> <div>  \$6.0B ECONOMIC OUTPUT </div>	

Heatwaves!





[NOAA feature story](#), September 05, 2019



NOAA surveys & manages Alaskan fisheries (AFSC)

VALUE & VOLUME OF KEY SPECIES, 2019



Salmon		Pollock		Crab		Pacific Cod	
							
\$715	\$1,733	\$484	\$1,636	\$226	\$294	\$203	\$382
EX-VESSEL VALUE \$MILLIONS	FIRST WHOLESALE (FW) VALUE \$MILLIONS	EX-VESSEL VALUE \$MILLIONS	FIRST WHOLESALE (FW) VALUE \$MILLIONS	EX-VESSEL VALUE \$MILLIONS	FIRST WHOLESALE (FW) VALUE \$MILLIONS	EX-VESSEL VALUE \$MILLIONS	FIRST WHOLESALE (FW) VALUE \$MILLIONS
865	\$2.00	3,353	\$0.49	47	\$6.30	464	\$0.82
HARVEST MILLION LBS	FW VALUE PER ROUND LB.	HARVEST MILLION LBS	FW VALUE PER ROUND LB.	HARVEST MILLION LBS	FW VALUE PER ROUND LB.	HARVEST MILLION LBS	FW VALUE PER ROUND LB.

Halibut & Sablefish



\$166	\$187
EX-VESSEL VALUE \$MILLIONS	FIRST WHOLESALE (FW) VALUE \$MILLIONS
46	\$4.05
HARVEST MILLION LBS	FW VALUE PER ROUND LB.

Flatfish, Rockfish, & Atka Mackerel



\$168	\$391
EX-VESSEL VALUE \$MILLIONS	FIRST WHOLESALE (FW) VALUE \$MILLIONS
804	\$0.49
HARVEST MILLION LBS	FW VALUE PER ROUND LB.

**TOTAL IMPACTS 2021/2022 AVG
(INCLUDING SECONDARY IMPACTS)**



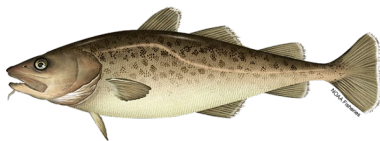
48,000
WORKERS



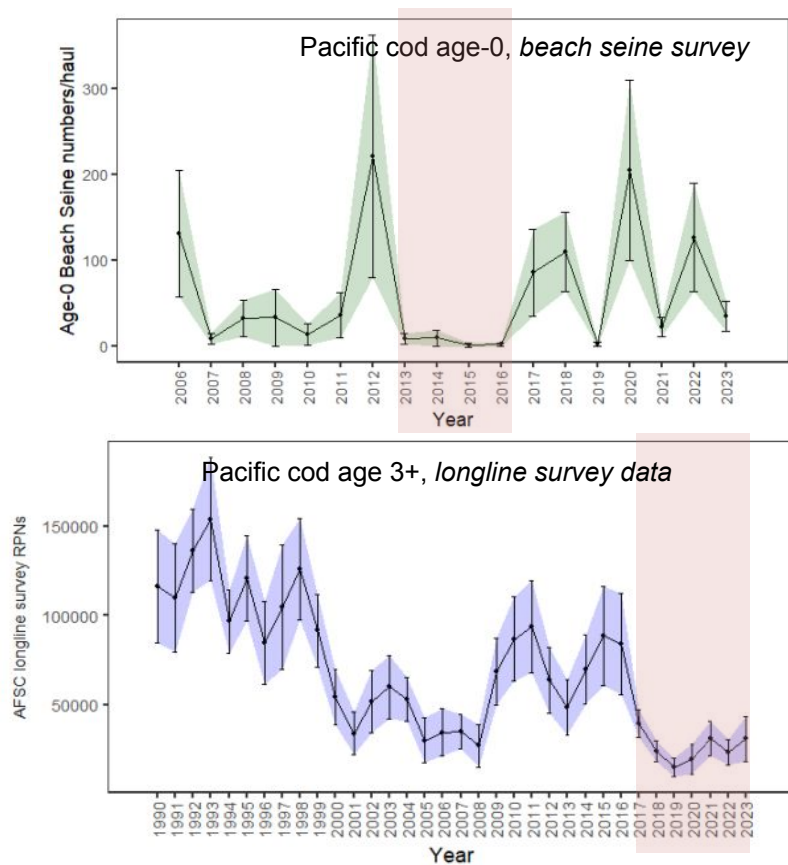
\$2.3B
LABOR INCOME



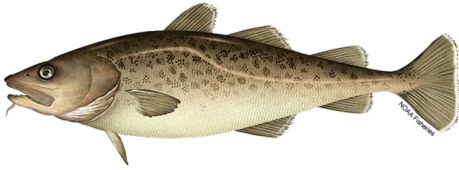
\$6.0B
ECONOMIC OUTPUT



Pacific cod, *Gadus macrocephalus*

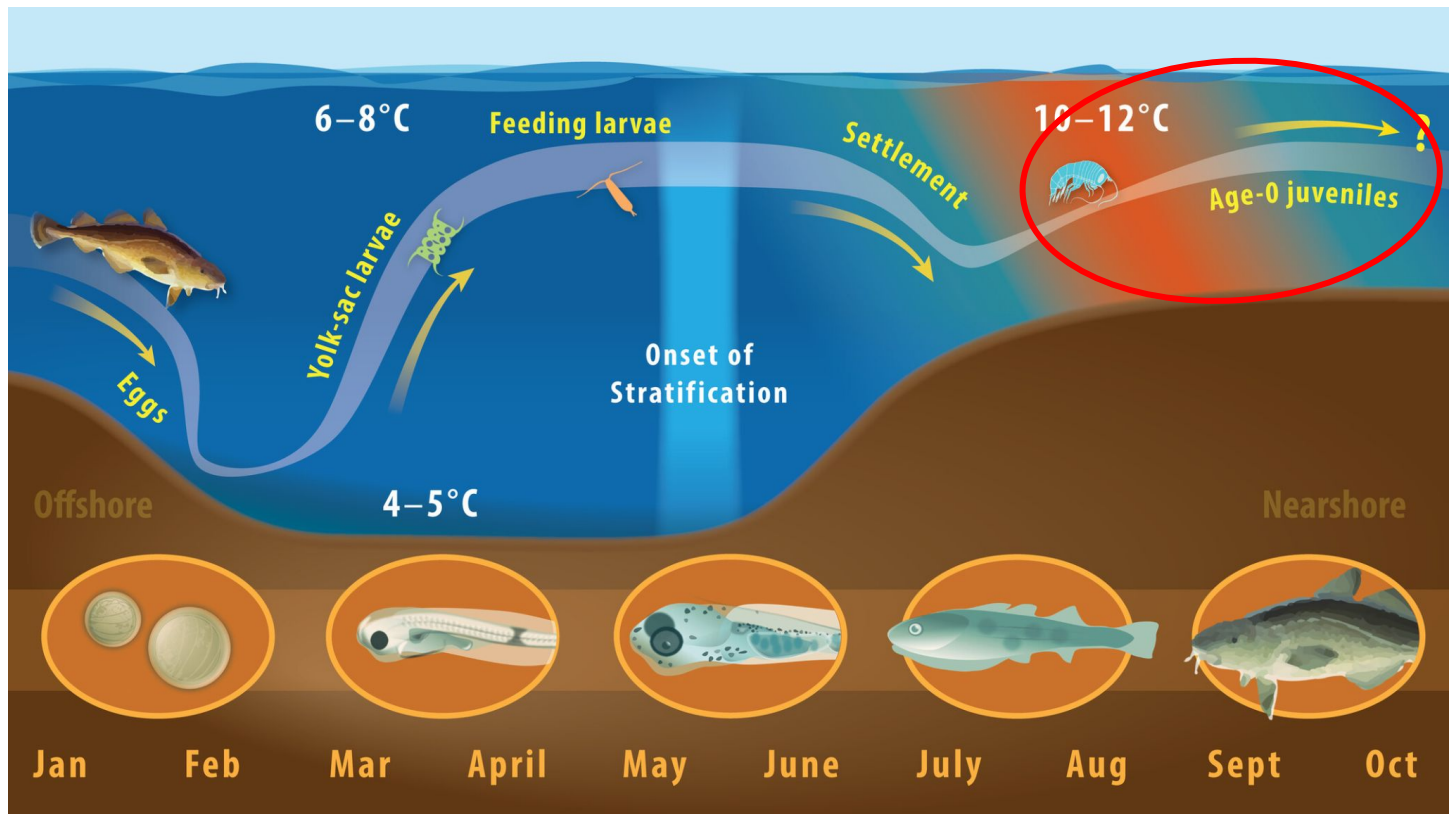


[Stock Assessment Report,
2023, Gulf of Alaska.](#)



Big Questions

- Why & how does warming affect Pacific cod recruitment?
- Do stock assessment reference points need to be updated?
 - *E.g. natural mortality rates*
- How resilient are Pacific cod populations in Alaska to warming?





How does temperature affect **juvenile** Pacific cod **growth, survival, & energy allocation** in the pre-winter period?

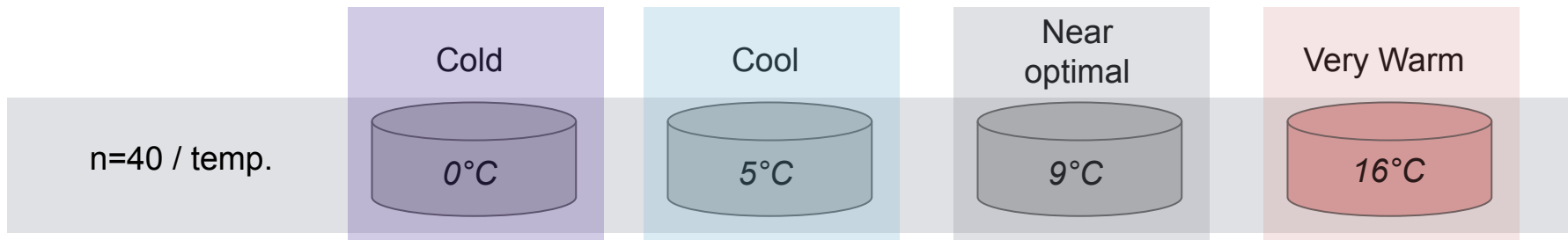
- Wild juveniles (age-0) caught off Kodiak, AK late summer
- Transported to Newport, OR wet lab
- ~6 weeks experiment (age-0)





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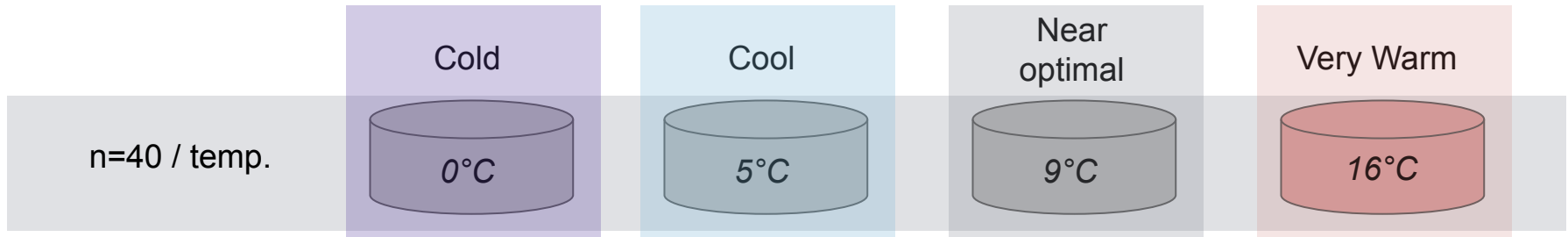
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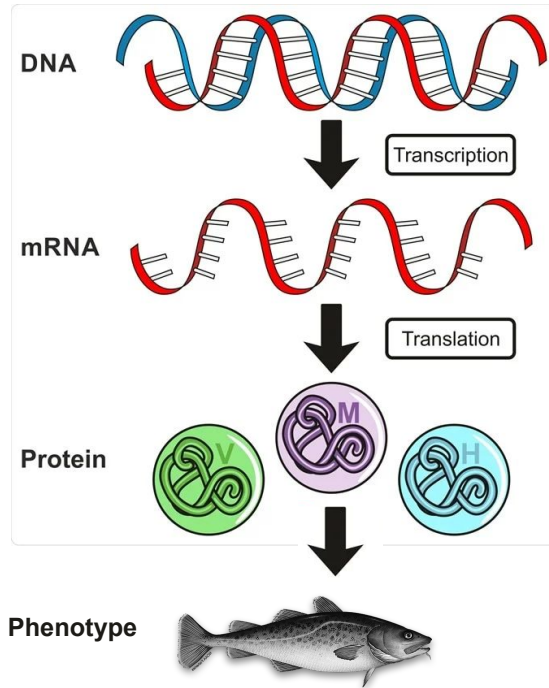


Individuals tagged, collected:

- a. Genetics with fin clips, $n=40/\text{temp}$ (IcWGS)
- b. Growth rates (length & wet weight) during acclimation, treatment
- c. Body condition (Kwet)
- d. Liver condition (HSI)
- e. Survival
- f. Liver lipid components ($n=25/\text{temp}$)
- g. Gene expression with liver, $n=18/\text{temp}$ (RNASeq)



“Genome-to-Phenome” dataset, Pacific cod juvenile temperature response



1. Genetics –
Who are they?
2. Phenotypes –
How does warming affect key traits?
3. Integrate datasets –
Why are some fish less sensitive?

Adapted from: [udaix/Shutterstock.com](https://www.shutterstock.com/user/udaix)

“Genome-to-Phenome” dataset, Pacific cod juvenile temperature response

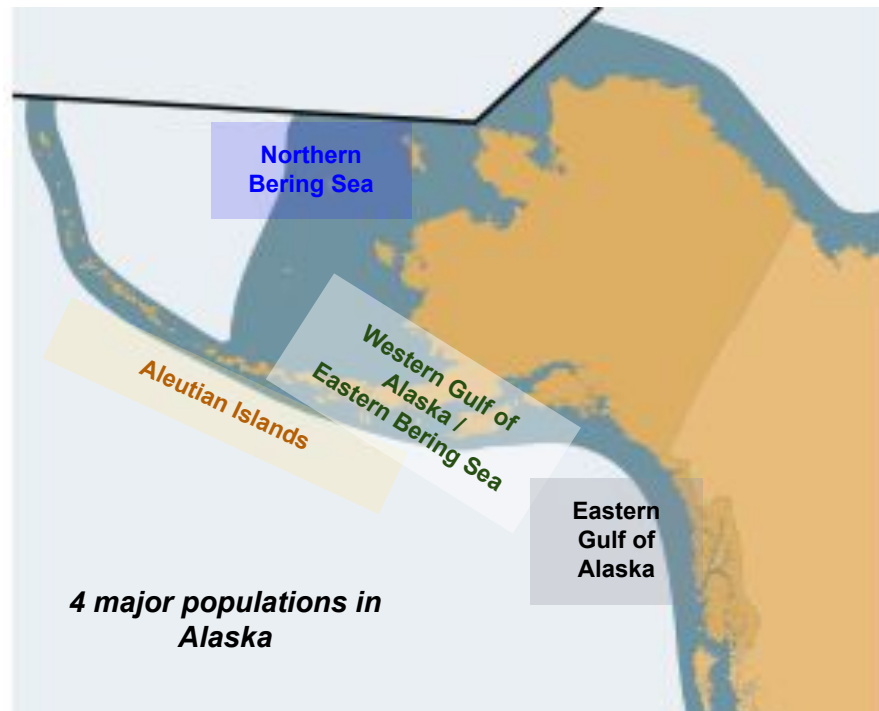
1. Genetics – Who are they (wild caught)?

DNA

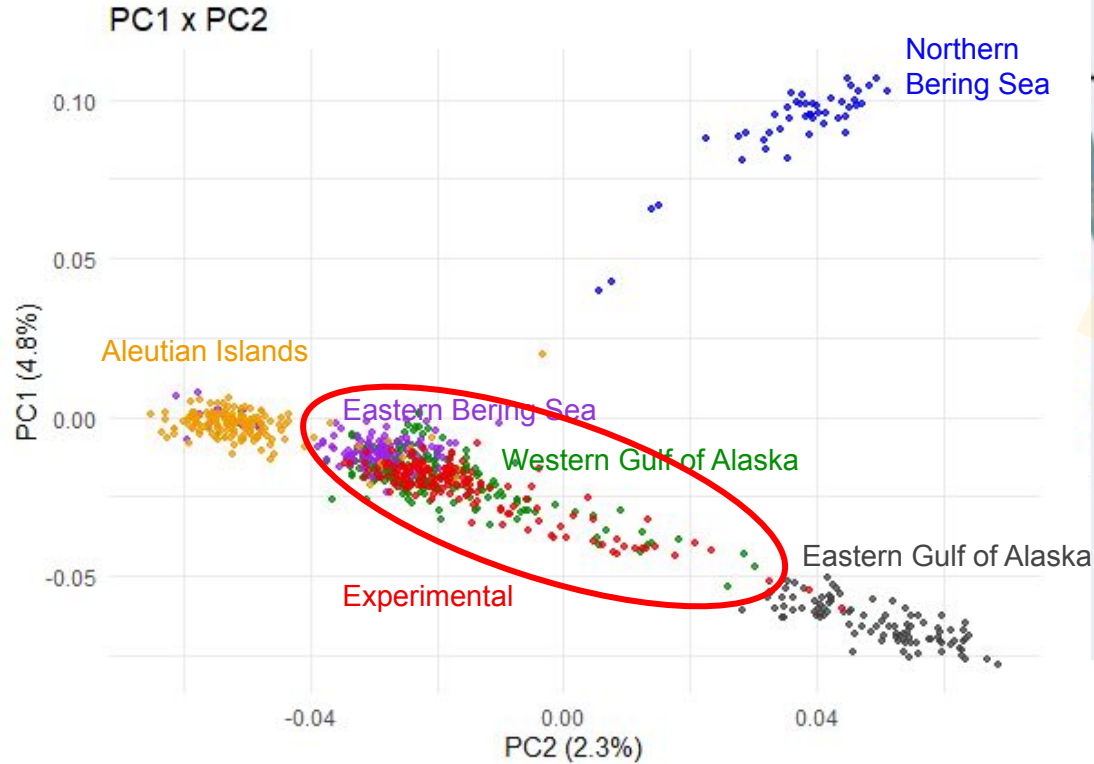


Tools used:

- Sequence whole genome ~3x (i.e. “low-coverage”), n=160
- ~\$100/fish, genome size ~500Mb (similar to pacific oyster)
- High-quality reference genome for alignment & genotype data (bwa mem & angsd)
- More data from ~55 reference fish per population, assign our fish (wgsassign)
- 6,101 sites used to **predict population of origin of experimental fish**, 96% accurate

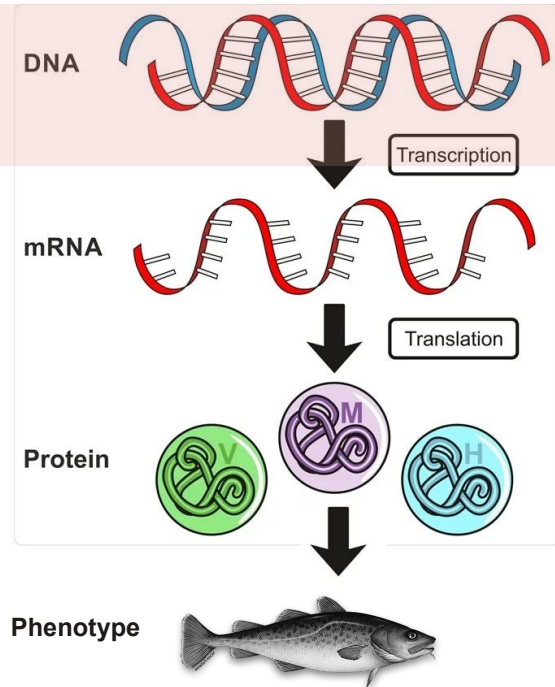


Genetics - They are the Western GOA / Eastern Bering Sea group



“Genome-to-Phenome” dataset, Pacific cod juvenile temperature response

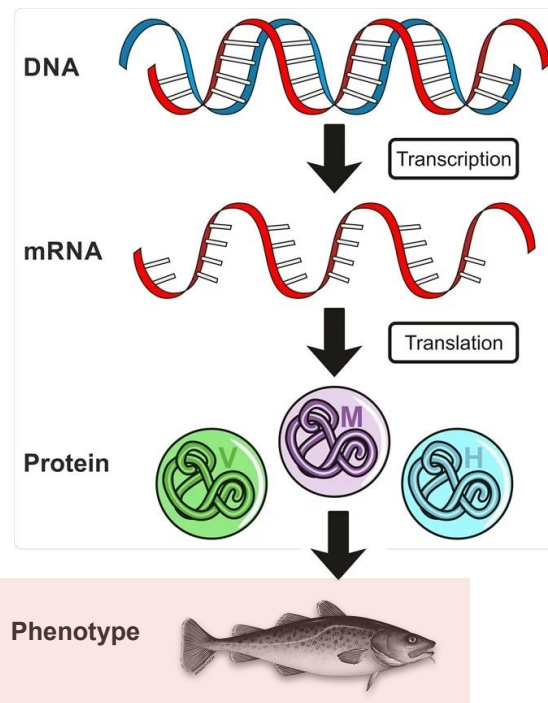
- ✓ Genetics – **they are one population**
western Gulf of Alaska / Eastern Bering Sea



Adapted from: udaix/Shutterstock.com

“Genome-to-Phenome” dataset, Pacific cod juvenile temperature response

- ✓ Genetics – **they are one population**
western Gulf of Alaska / Eastern Bering Sea
- Phenotypes – how are survival-associated biometrics affected?

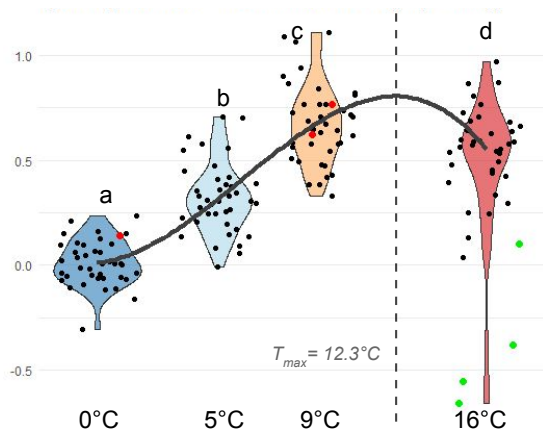


Adapted from: udaix/Shutterstock.com

Warming decreased lipid reserves

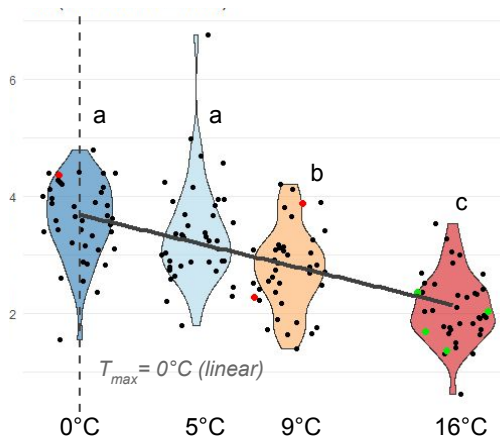
Growth Rate (weight)

Faster growth 👍



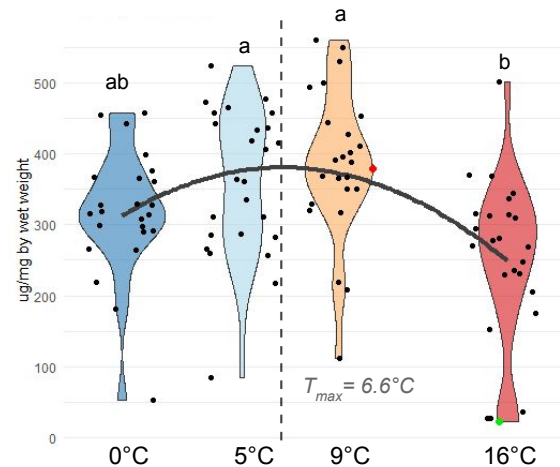
Hepatosomatic index (liver size)

Larger liver 👍



Total Lipid Content in liver

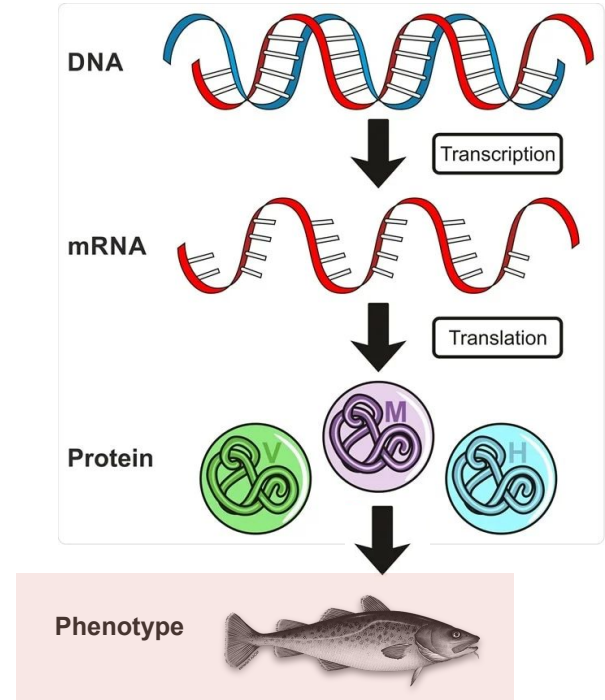
More lipid 👍



- Survived
- Died

“Genome-to-Phenome” dataset for juvenile Pacific cod

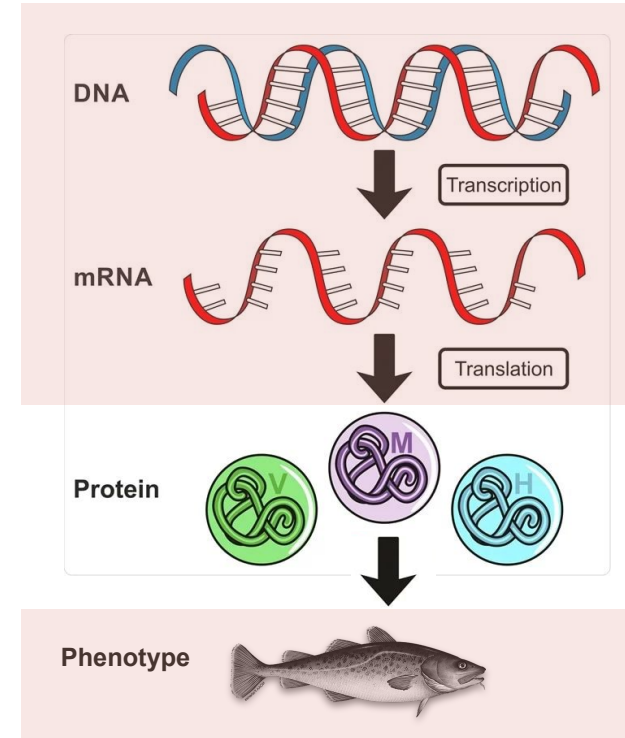
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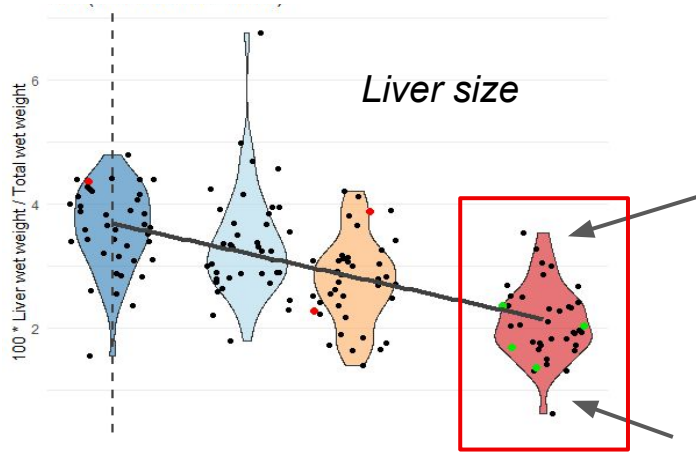
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- Integrate datasets – Performance indicators!
 - a. Genetic variants
 - b. Expression patterns



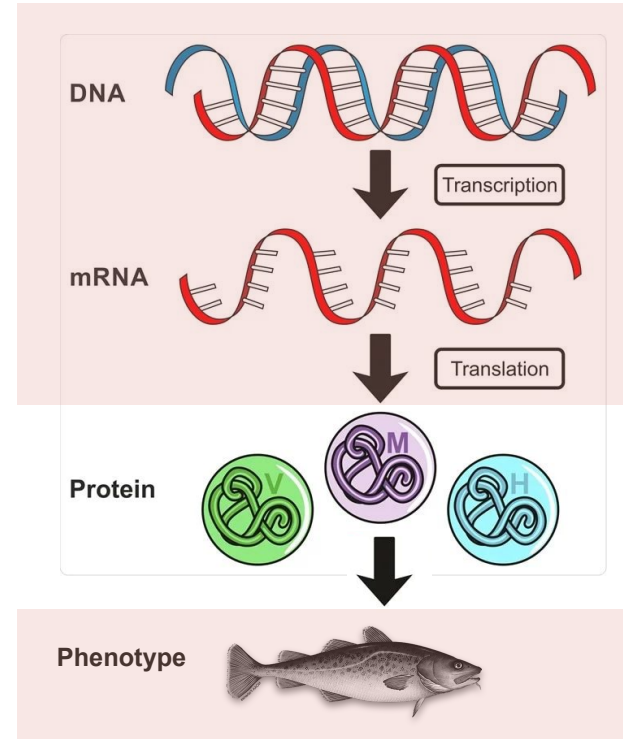
Adapted from: udaix/Shutterstock.com

Variation within each temperature - opportunity!



1. Identified sites on genome associated with liver size, lipid content, & growth in warming
2. Identified best sites/genotypes that **predict performance in warming, are adaptive**

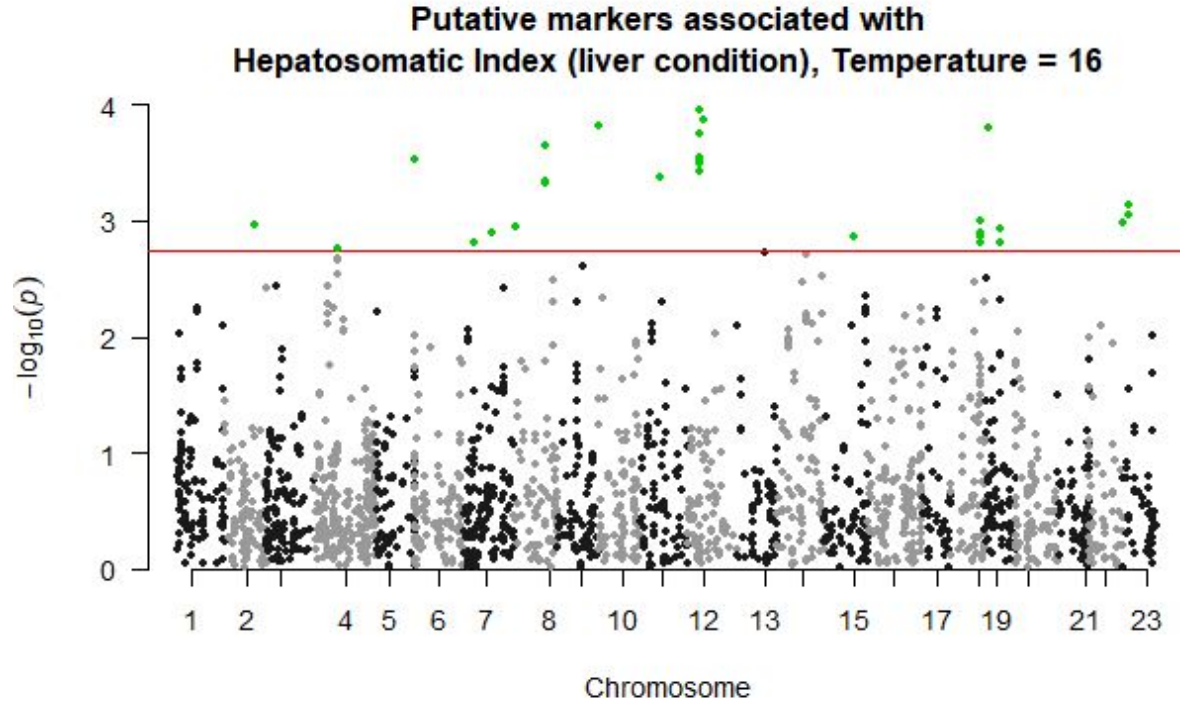
Genome-Wide Association Studies (GWAS) within each treatment



Adapted from: udaix/Shutterstock.com

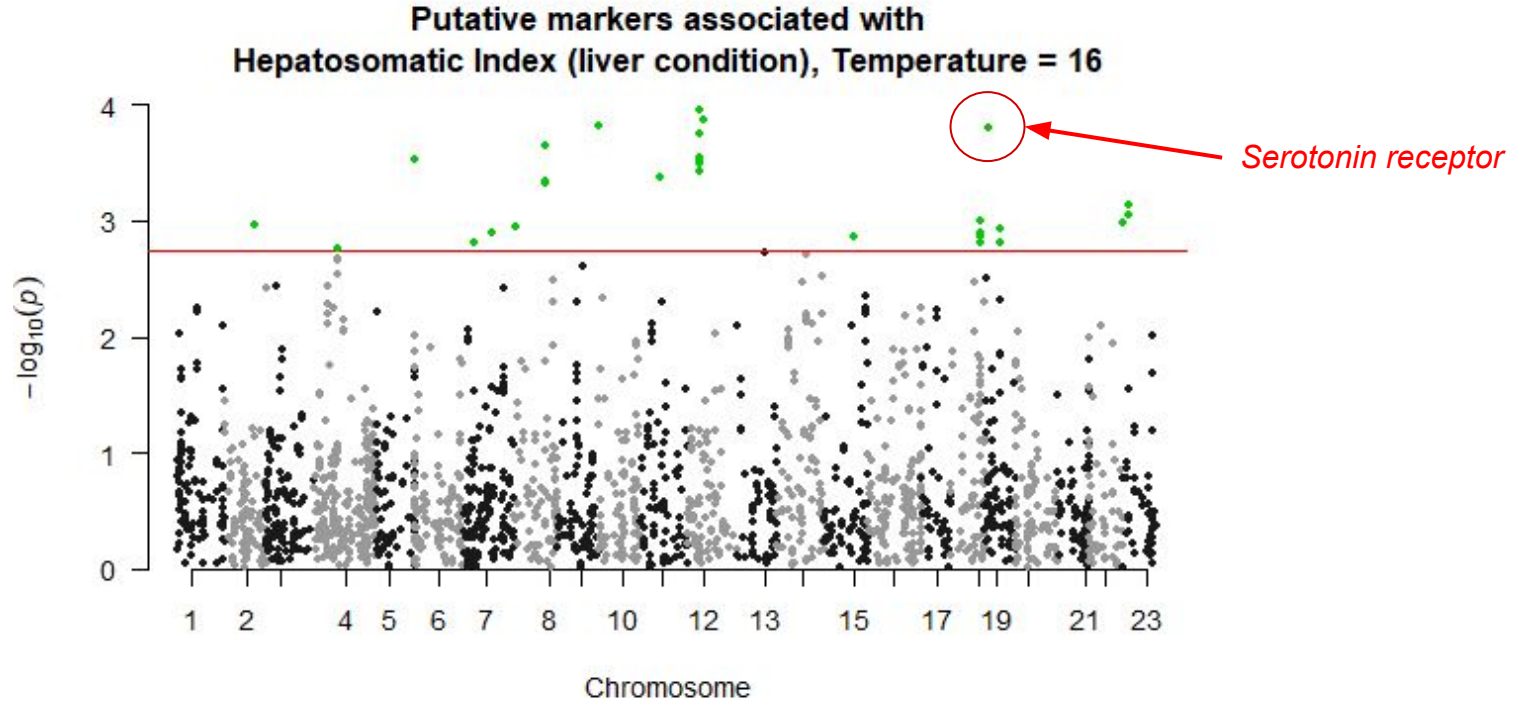


~100 markers putatively associated with liver size in Pacific cod juveniles exposed to warming



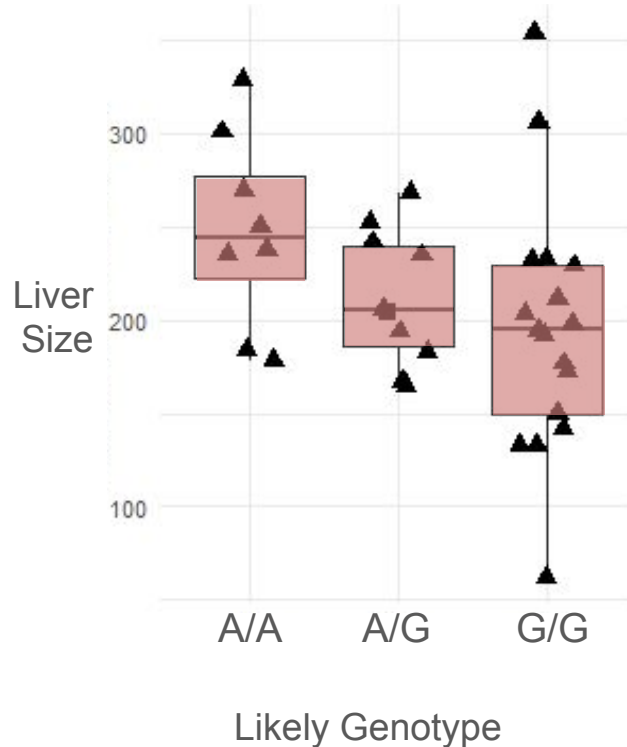


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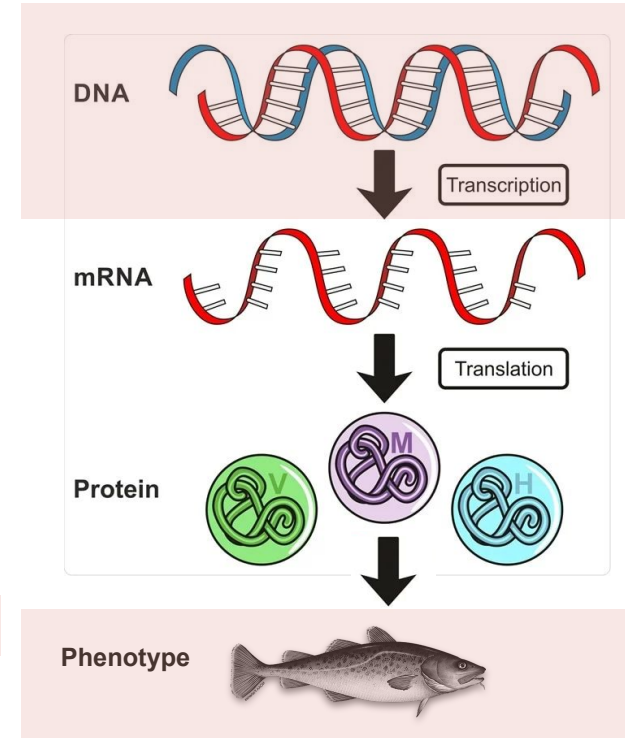
~100 markers putatively associated with liver size in Pacific cod juveniles exposed to warming



*Marker in a gene coding for a **receptor for serotonin** (5-HT4) which regulates appetite*

“Genome-to-Phenome” dataset for juvenile Pacific cod

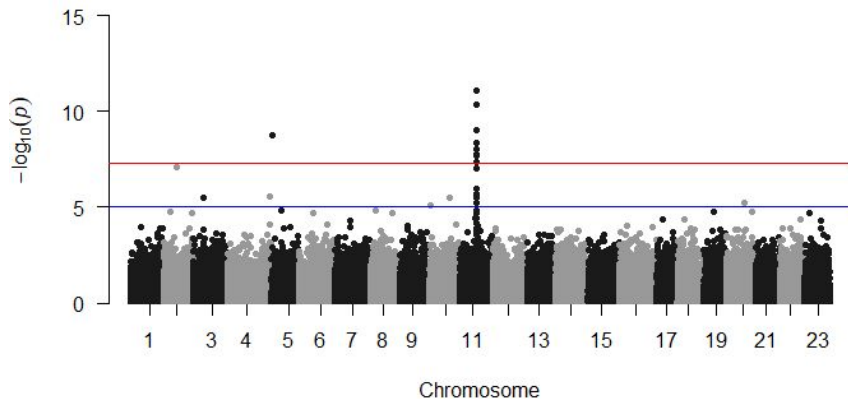
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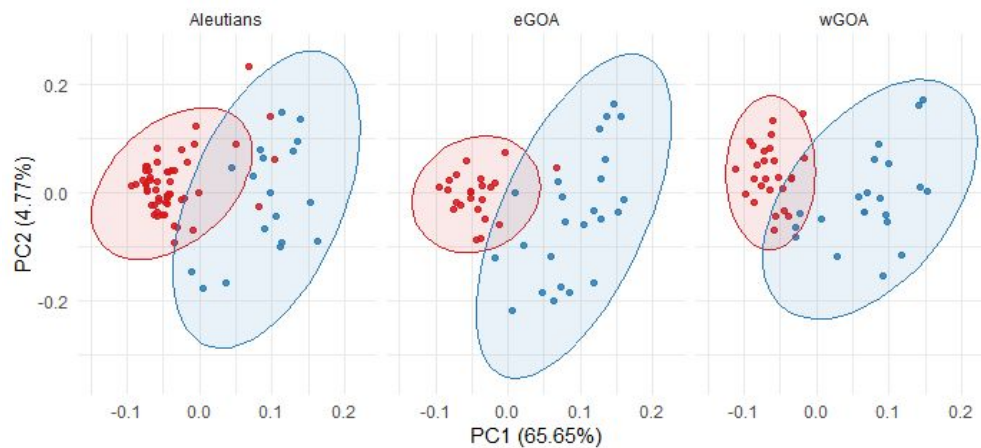
Adapted from: udaix/Shutterstock.com

Example Genome Wide Association Study – Sex markers discovery (preliminary)

Sex-association, GWAS



PCAs with ~50 sex-associated sites



Female
Male

=

Tangent –

Sex marker discovery (preliminary) using Genome Wide Association Study

*Also Chromosome 11
in Atlantic cod*



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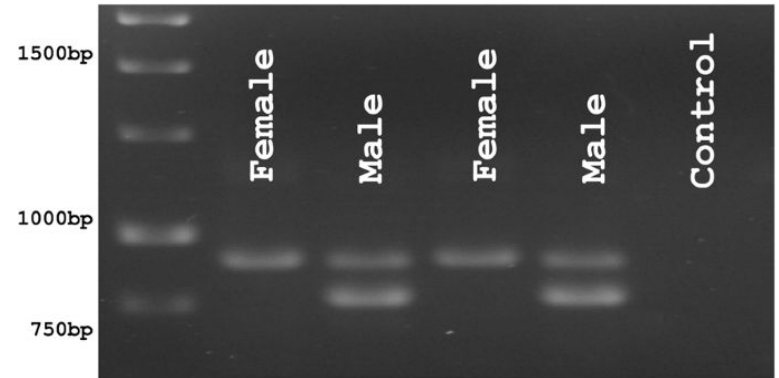
Article | [Open access](#) | Published: 15 January 2019

Characterization of a male specific region containing a candidate sex determining gene in Atlantic cod

[Tina Graceline Kirubakaran](#), [Øivind Andersen](#), [Maria Cristina De Rosa](#), [Terese Andersstuen](#), [Kristina Hallan](#), [Matthew Peter Kent](#)  & [Sigbjørn Lien](#) 

[Scientific Reports](#) **9**, Article number: 116 (2019) | [Cite this article](#)

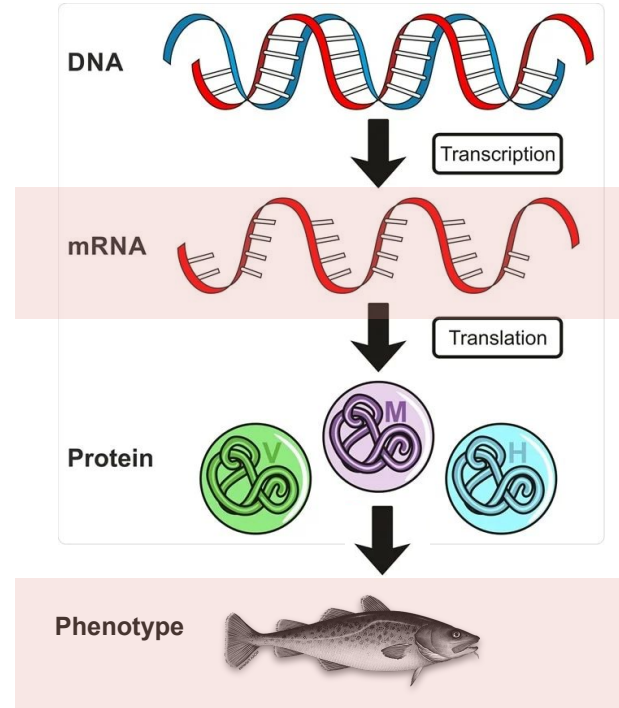
Benchtop sex assay in Atlantic cod



Pacific halibut assessment uses sex data based on DNA sample

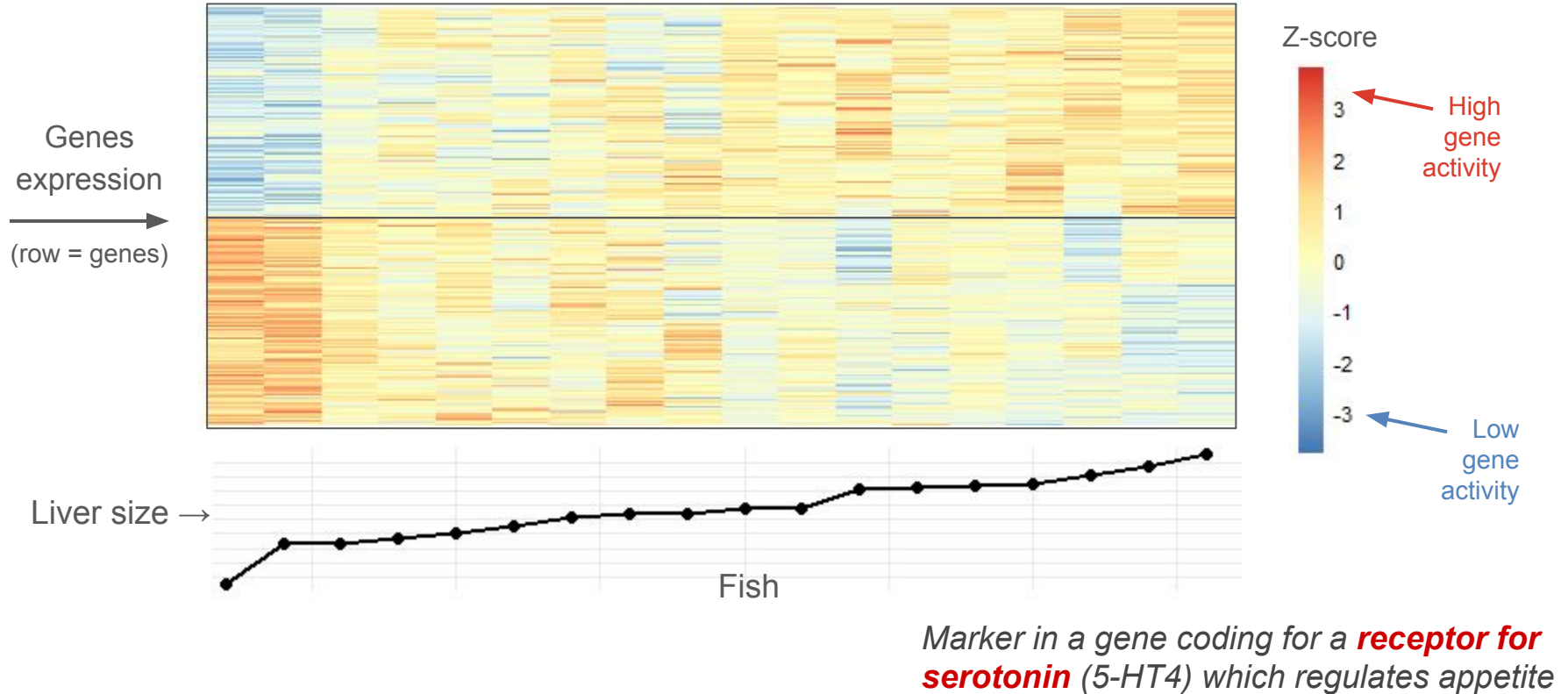
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1. Integrate datasets – Performance indicators!
 - ✓ **~100 genetic markers** of liver size in warming
 - a. Expression patterns



Adapted from: udaix/Shutterstock.com

~ 1,600 genes with expression associated with liver size in warming



Liver size performance indicators in warming, both GENETICS and EXPRESSION

*Cell
adhesion*

*Calcium
transport*

*Immune
system*

Chromosome	# of markers	Gene ID	Protein Name	Function
4	3	LOC132456135	Netrin receptor UNC5D	Cell adhesion, apoptosis in response to DNA damage
12	2	tmco1	Calcium load-activated calcium channel	Calcium transport, endoplasmic reticulum calcium homeostasis
10	1	LOC132466560	TBC1 domain family member 9B	Membrane trafficking, calcium transport
2	1	LOC132453053	Stonustoxin subunit beta	May be related immune system function. From stonefish, toxic/fatal to mammals.
5	1	LOC132457513	Stonustoxin subunit beta	May be related immune system function. From stonefish, toxic/fatal to mammals.
23	1	LOC132452628	NLR family CARD domain-containing protein 3	Negative regulator of the innate immune response
23	1	LOC132452644	HERV-H LTR-associating protein 2	Enhances T-cell proliferation and cytokine production
17	2	LOC132445594	Unknown	Unknown

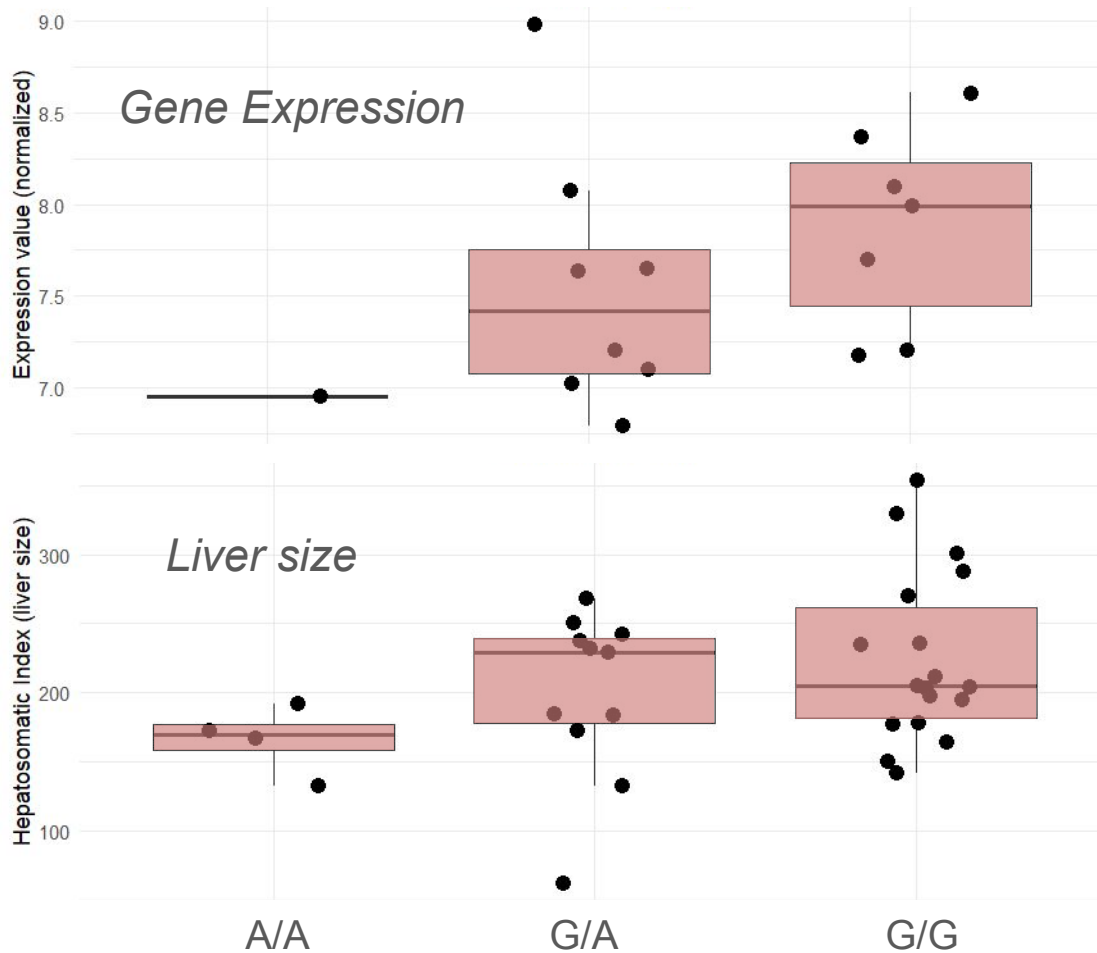
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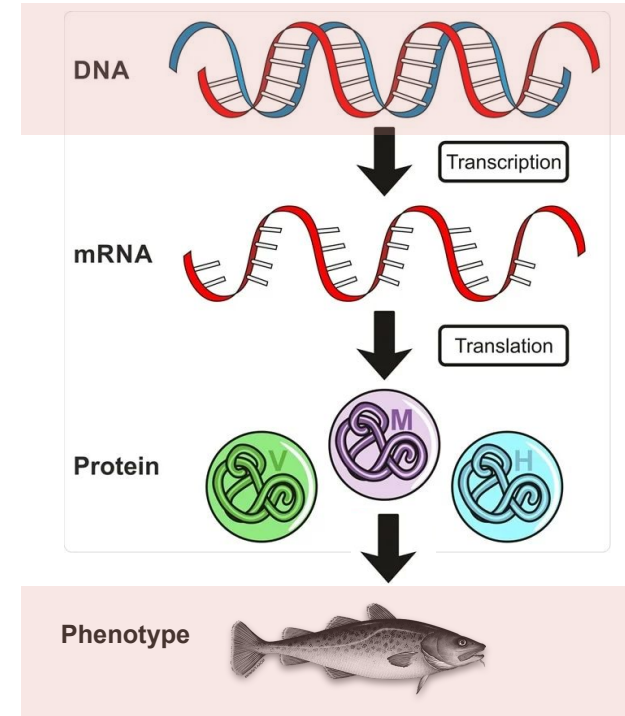


*NLR family CARD
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Negatively
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“Genome-to-Phenome” dataset for juvenile Pacific cod

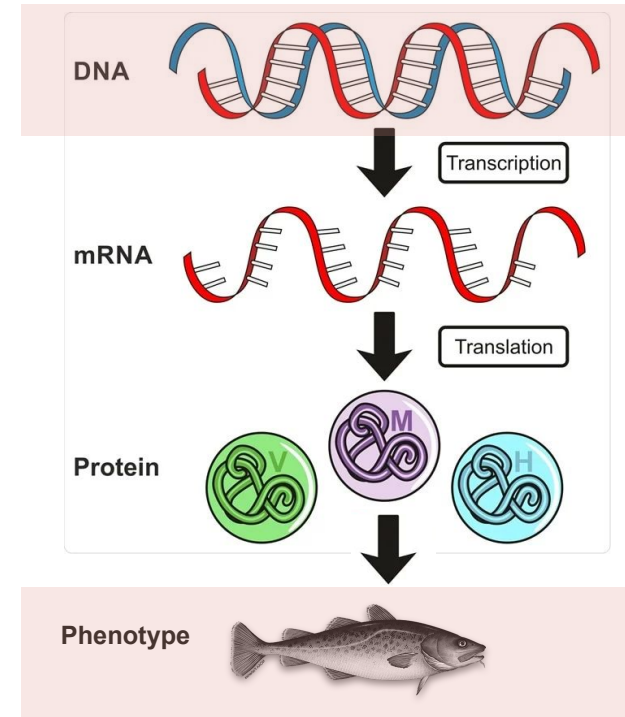
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 - ✓ ~100 genetic markers
 - ✓ ~1,600 gene expression indicators



Adapted from: udaix/Shutterstock.com

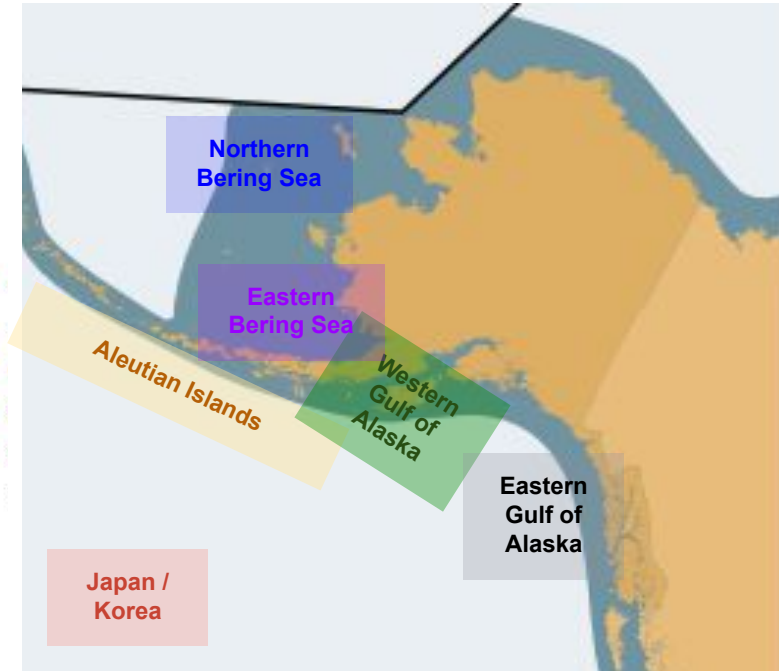
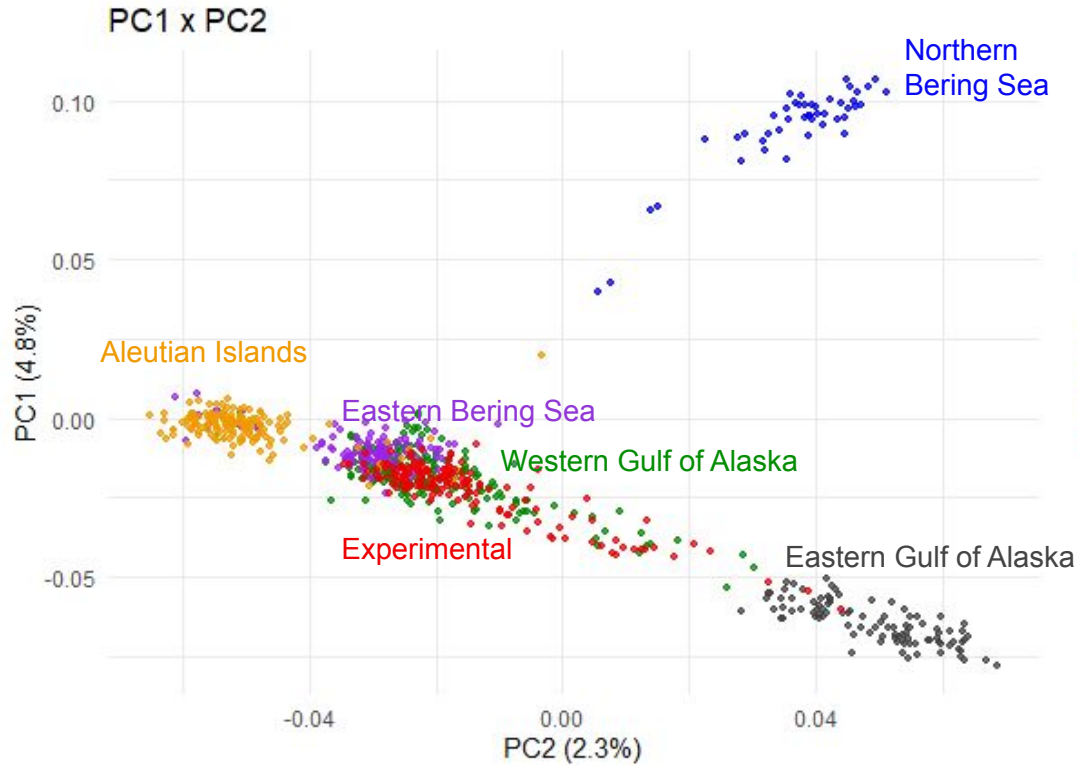
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 - ✓ ~1,600 gene expression indicators
- Can we predict “performance” or “resilience” of other cod groups using our markers?

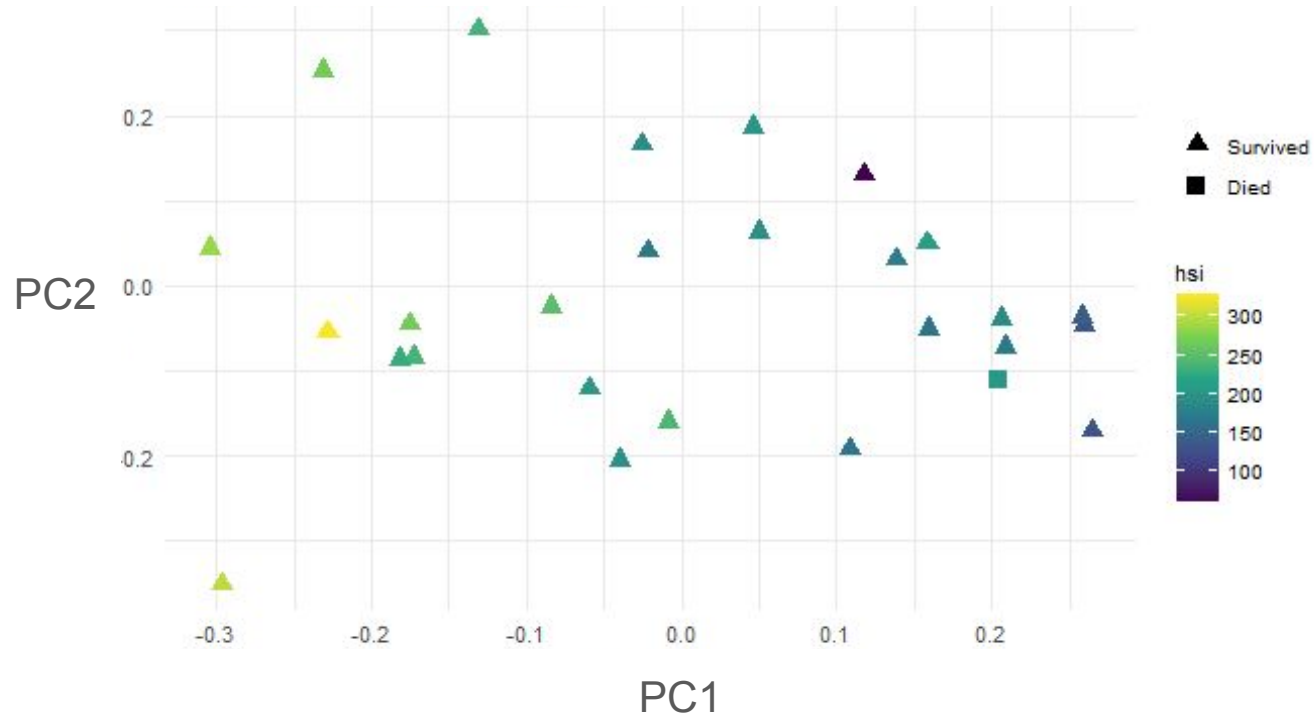


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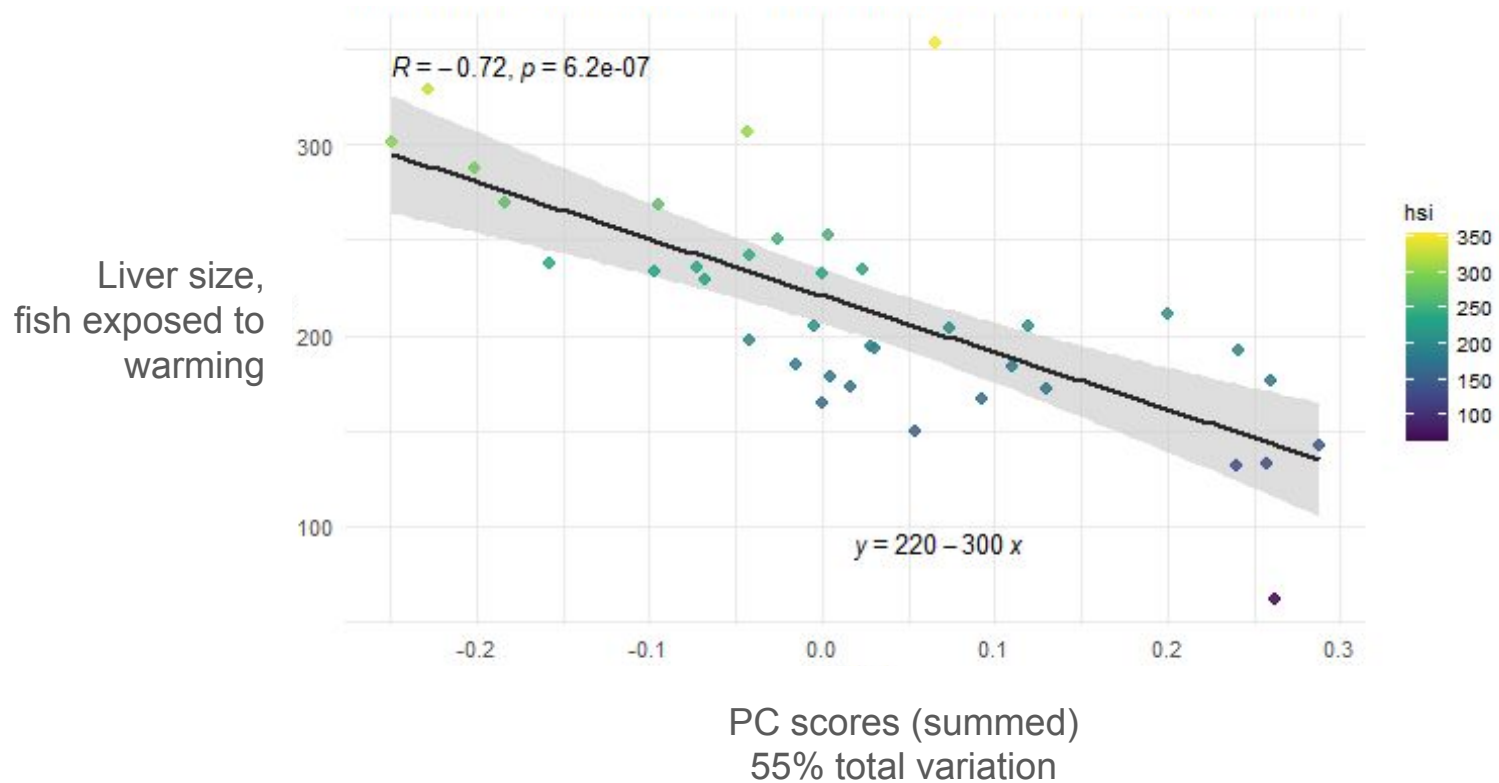
Which populations would we predict to have largest livers in warm conditions?



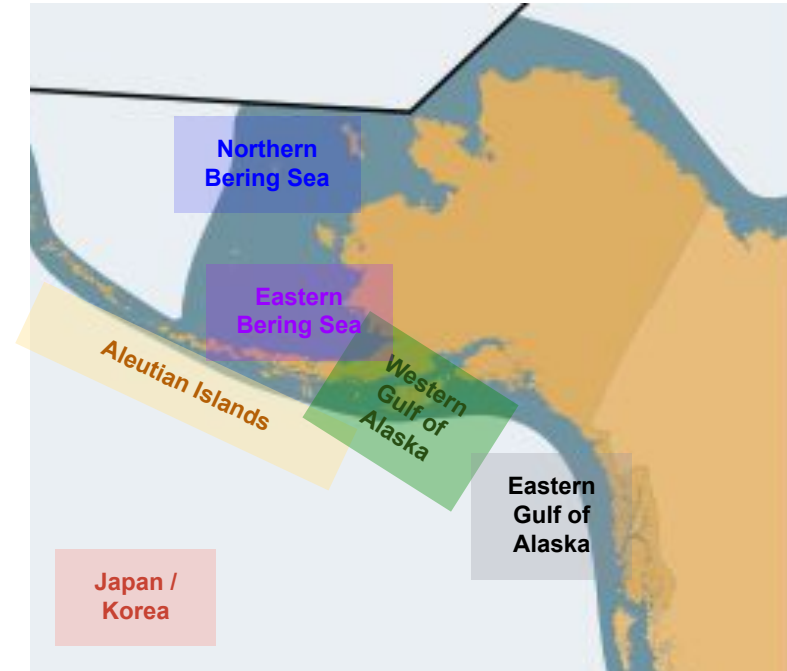
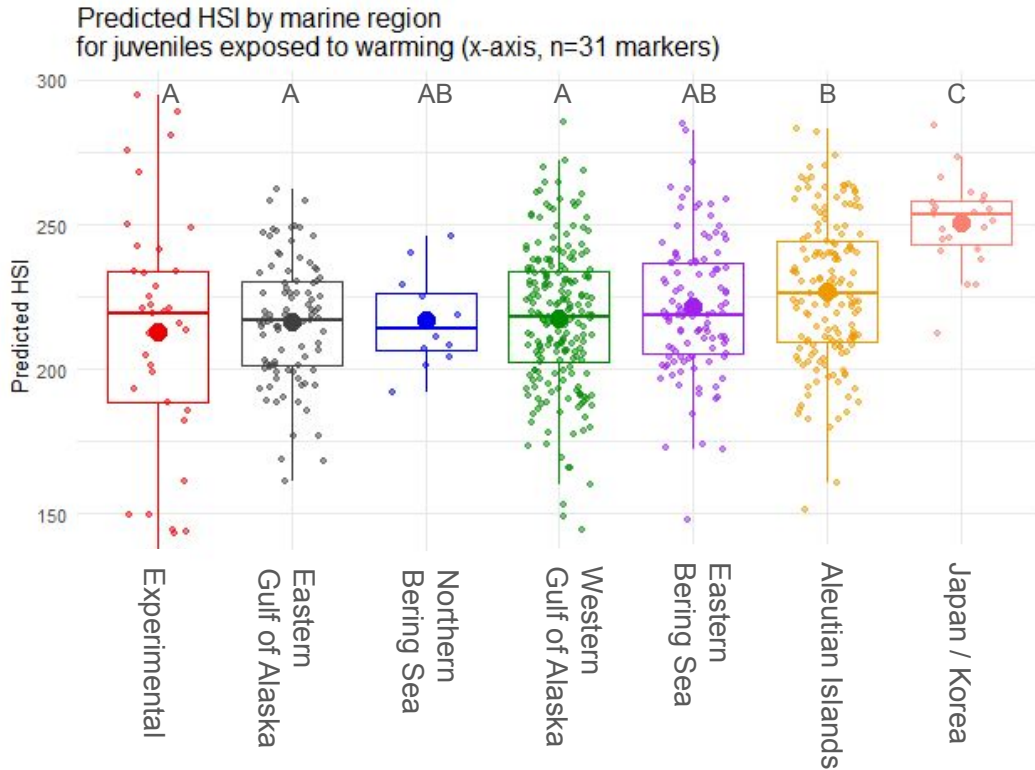
Analyses so far - use genotypes at **32 markers** to **predict liver size** with PC scores



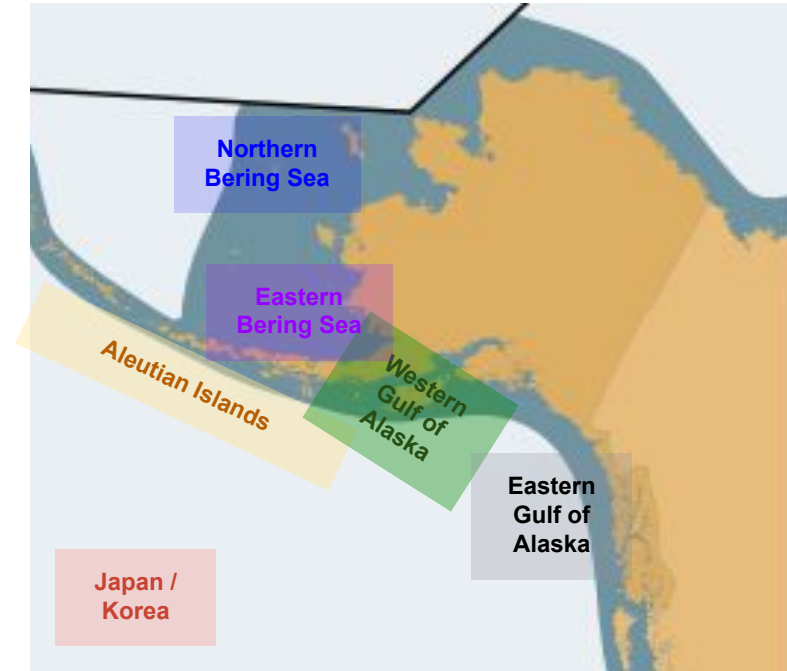
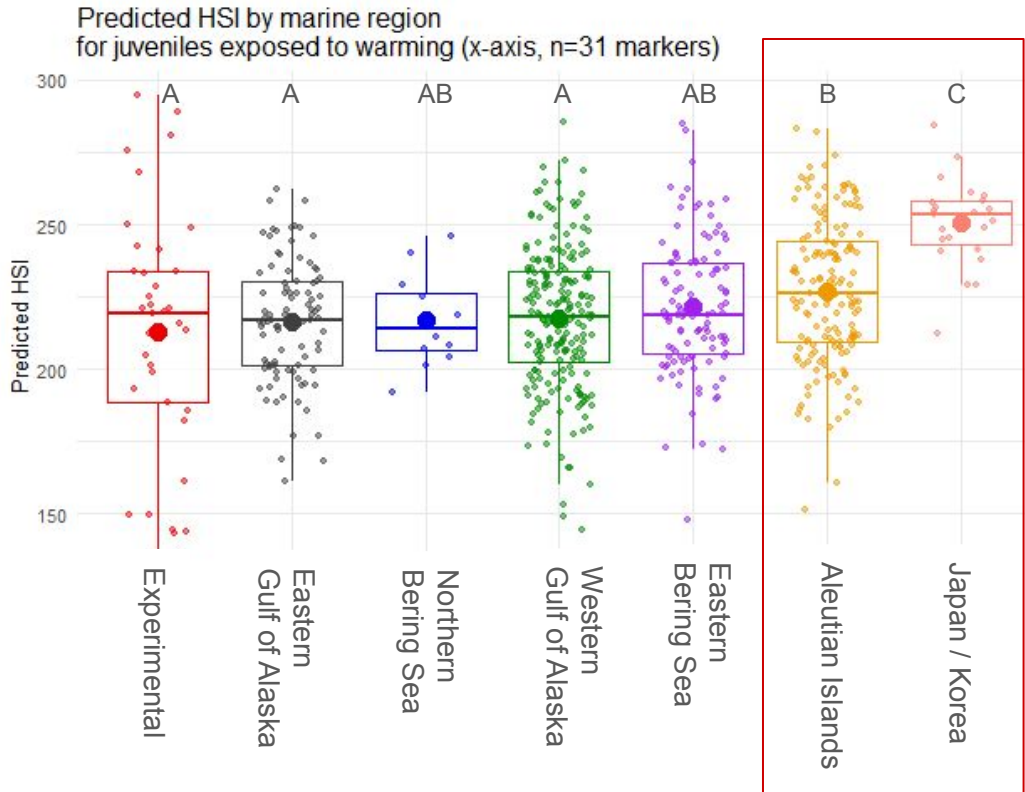
Analyses so far - use genotypes at **32 markers** to **predict liver size** with PC scores



Which populations would we predict to have largest livers in warm conditions?



Which populations would we predict to have largest livers in warm conditions?





Broad Conclusions (preliminary)

- **Low juvenile recruitment in GoA during heatwaves** is likely related to low overwintering survival due to **fewer lipid reserves**.
- Stock assessments may need to **adjust mortality/recruitment rates** in heat wave years
 - Larval study (Slesinger et al. 2024; Spencer et al. *in revision*) - much *higher mortality*
- **Genetic variability related to adaptive traits** may enable selection for individuals **more capable of allocating lipid reserves**
- Other Pacific cod groups could be screened for **putative markers of performance**
 - Through time – before/during/after heat waves (future project!) - is selection happening?
 - Distinct Pacific cod groups – are some groups more resilient than others?
- Building Resources for Pacific cod – lists of genes that are temperature sensitive in larvae, juveniles, indicators of performance, etc.

Collaborators & Funders



Newport (Hatfield)

Ben Laurel
Emily Slesinger
Louise Copeman
MaryBeth Hicks
Tom Hurst

Seattle (Regional office)

Ingrid Spies
Sara Schaal



University of Washington

Steven Roberts



Questions?