INTERNATIONAL PACIFIC
HALIBUT COMMISSION

Biological and Ecosystem Science Research Update

Agenda Item 7

IPHC-2020-SRB016-09

Outline

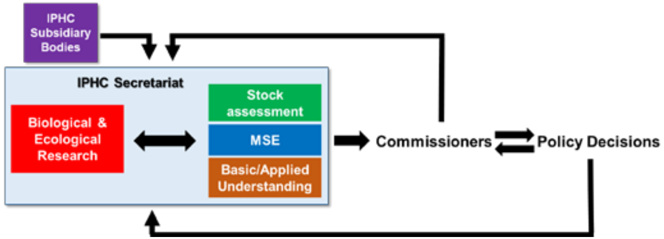
- **Five-year research program and management implications**
- **Progress on ongoing research projects**

Five-year research program and management implications

5-Year Biological and Ecosystem Science Research Plan

Primary Research Areas	*Main Objectives*	*Management implications*
Migration	Improve understanding of migration throughout all life stages (larval, juvenile, adult feeding and reproductive migrations)	Stock distribution, regional management
Reproduction	Information on sex ratios of commercial landings and improved maturity estimates	Female stock spawning biomass
Growth	Improve understanding of factors responsible for changes in size-at-age and development of tools for monitoring growth and physiological condition	Biomass estimates
DMRs and discard survival	Improve estimates of DMRs in the directed longline and guided recreational fisheries	Discard mortality estimates
Genetics and genomics	Improve understanding of the genetic structure of the population and create genomic tools (genome)	Stock distribution, local adaptation

Integration of biological research, stock assessment, and policy



Biological research		Stock assessment	Stock assessment MSE
Research areas	*Research outcomes*	*Relevance for stock assessment*	*Inputs to stock assessment and MSE development*
Migration	Larval distribution Juvenile and adult migratory behavior and distribution	Geographical selectivity Stock distribution	Information for structural choices Recruitment indices Migration pathways and rates Timing of migration
Reproduction	Sex ratio Spawning output Age at maturity	Spawning biomass scale and trend Stock productivity Recruitment variability	Sex ratio Maturity schedule Fecundity
Growth	Identification of growth patterns Environmental effects on growth Growth influence in size-at-age variation	Temporal and spatial variation in growth Yield calculations Effects of ecosystem conditions Effects of fishing	Predicted weight-at-age Mechanisms for changes in weight-at-age
Discard Survival	Bycatch survival estimates Discard mortality rate estimates	Scale and trend in mortality Scale and trend in productivity	Bycatch and discard mortality estimates Variability in bycatch and uncertainty in discard mortality estimates
Genetics and Genomics	Genetic structure of the population Sequencing of the Pacific halibut genome	Spatial dynamics Management units	Information for structural choices

Progress on ongoing research projects

1. Migration and distribution

Research Priorities

Category	Rank within category	Product	Justification	Biological Research Area (from 5-year Research Plan)	Timing	Progress
	3	Estimates of connectivity with Western Pacific waters (i.e. Russia)	Seasonal movement across Convention lines and/or harvest of young Pacific halibut in transit to Convention waters could bias estimates of productivity	Genetics and genomics, Movement	Medium-long term	Not yet planned
MSE biological understanding	1	Estimates of movement between Biological Regions	Improved estimates of movement and annual variation in movement will help parameterize the MSE operating model	Migration		

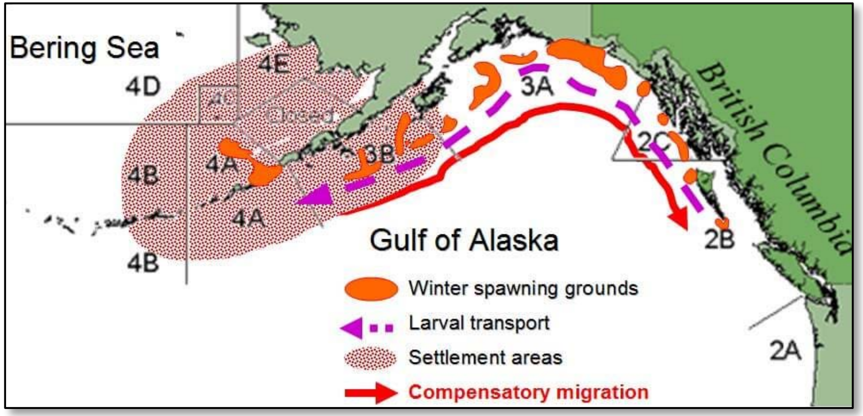
Progress on ongoing research projects

1. Migration and distribution

Projects:

1. Larval and early juvenile dispersal

2. Late juvenile and adult migration



Progress on ongoing research projects

1. Migration and distribution

Projects:

1. Larval and early juvenile dispersal

Manuscript currently in revision in *Fisheries Oceanography*

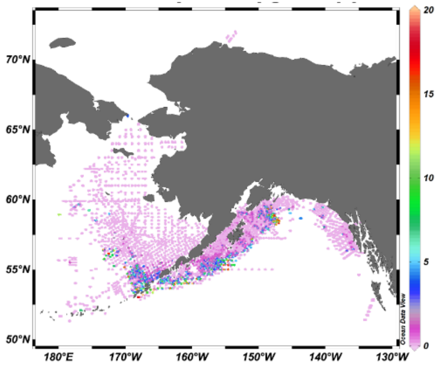
Multiple life-stage connectivity of Pacific halibut (*Hippoglossus stenolepis*) across the Bering Sea and Gulf of Alaska

Sadorus, L. L.[1], Goldstein, E.[2], Webster, R. A.[1], Stockhausen, W. T.[2], Planas, J. V.[1], and Duffy-Anderson, J.[2]

[1] International Pacific Halibut Commission, Seattle, Washington, U.S.A.
[2] National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, Seattle, Washington, U.S.A.

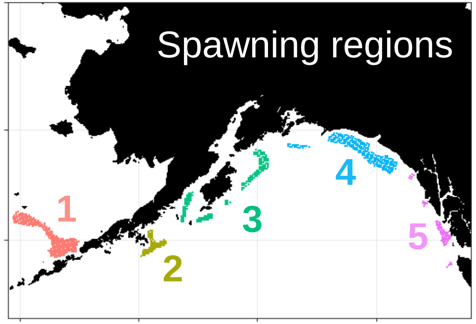
1. Migration and Distribution

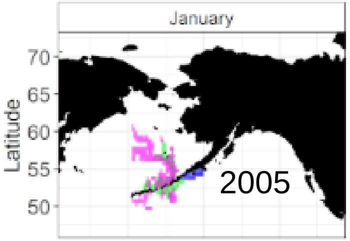
1. Larval and early juvenile dispersal

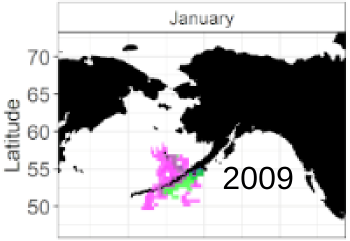


- **Key findings:**
  - Aleutian Islands constrain connectivity, but large island passes act as conduits between the GOA and Bering Sea
  - Degree of inter-basin larval connectivity is influenced by spawning location. % arriving in Bering Sea based on IBM:

Spawn region	Year					
	Warm			Cold		
	2003	2004	2005	2009	2010	2011
1	100	100	100	100	100	100
2	58.0	51.1	58.1	52.7	51.5	47.0
3	17.6	19.3	15.2	17.2	17.2	20.5
4	8.6	4.5	8.2	4.5	7.0	6.5
5	0.2	0.04	0.6	0.08	1.6	0.04



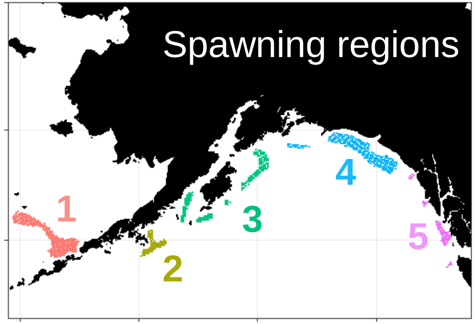


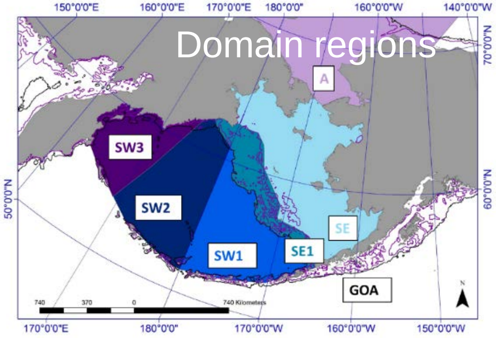


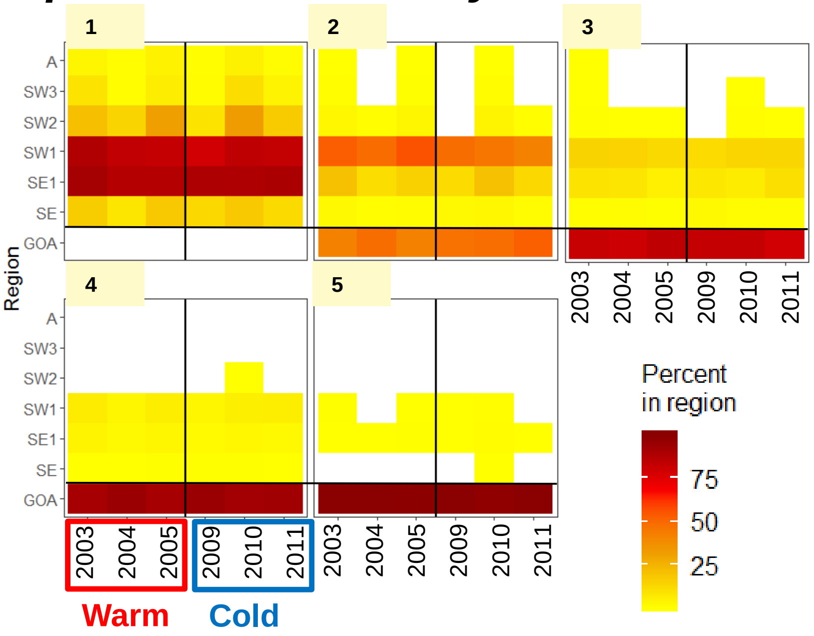
Example: origin - spawning region 2

1. Migration and Distribution

1. Larval and early juvenile dispersal: connectivity between GOA and BS







1. Migration and Distribution

1. Larval and early juvenile dispersal

- **Key findings:**
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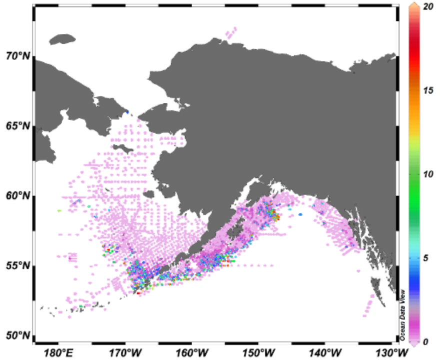
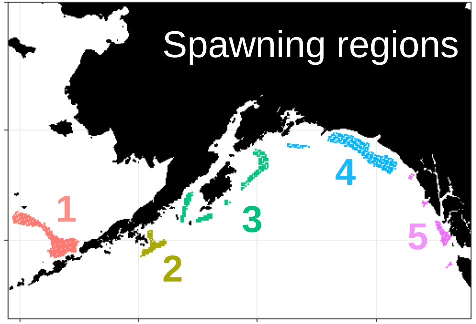
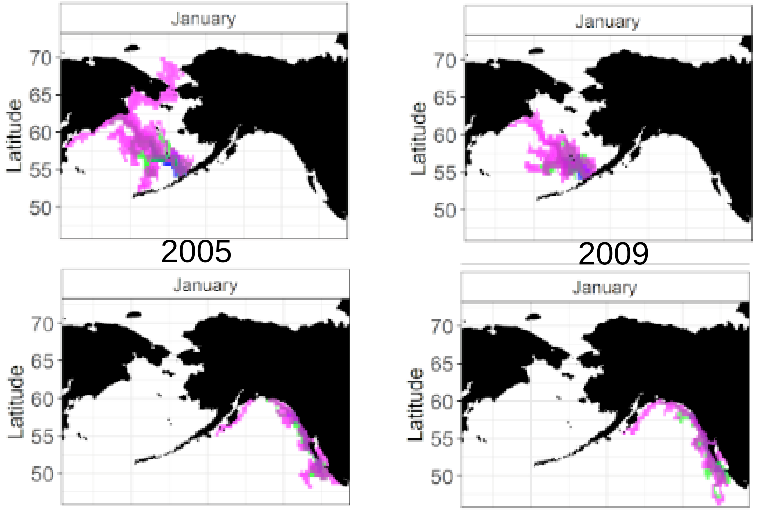
  - Degree of inter-basin larval connectivity is influenced by spawning location
  - Large degree of intra-basin connectivity







Origin – spawning region 1

Origin – spawning region 5

1. Migration and Distribution

1. Larval and early juvenile dispersal

- **Key findings:**
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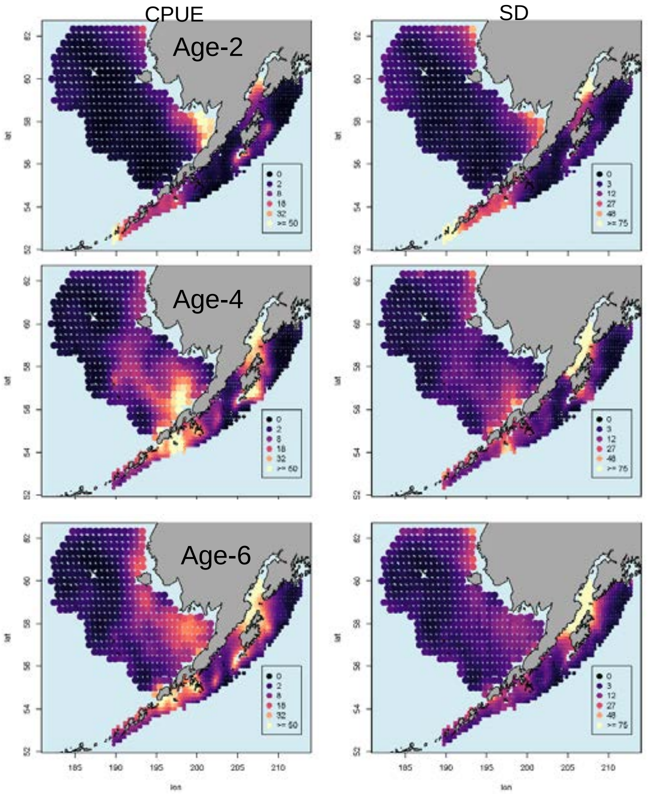
  - Degree of inter-basin larval connectivity is influenced by spawning location.
  - Large degree of within-basin connectivity
  - Demersal stage fish in the Bering Sea migrate outward from Bristol Bay and reach Unimak Pass by age-4, widely dispersed by age-6



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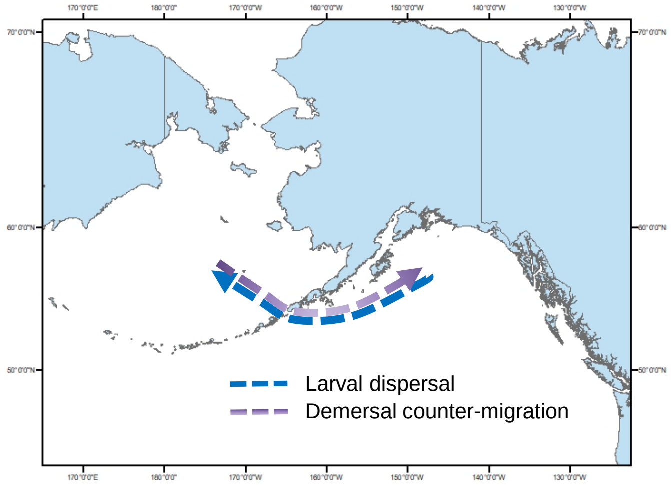
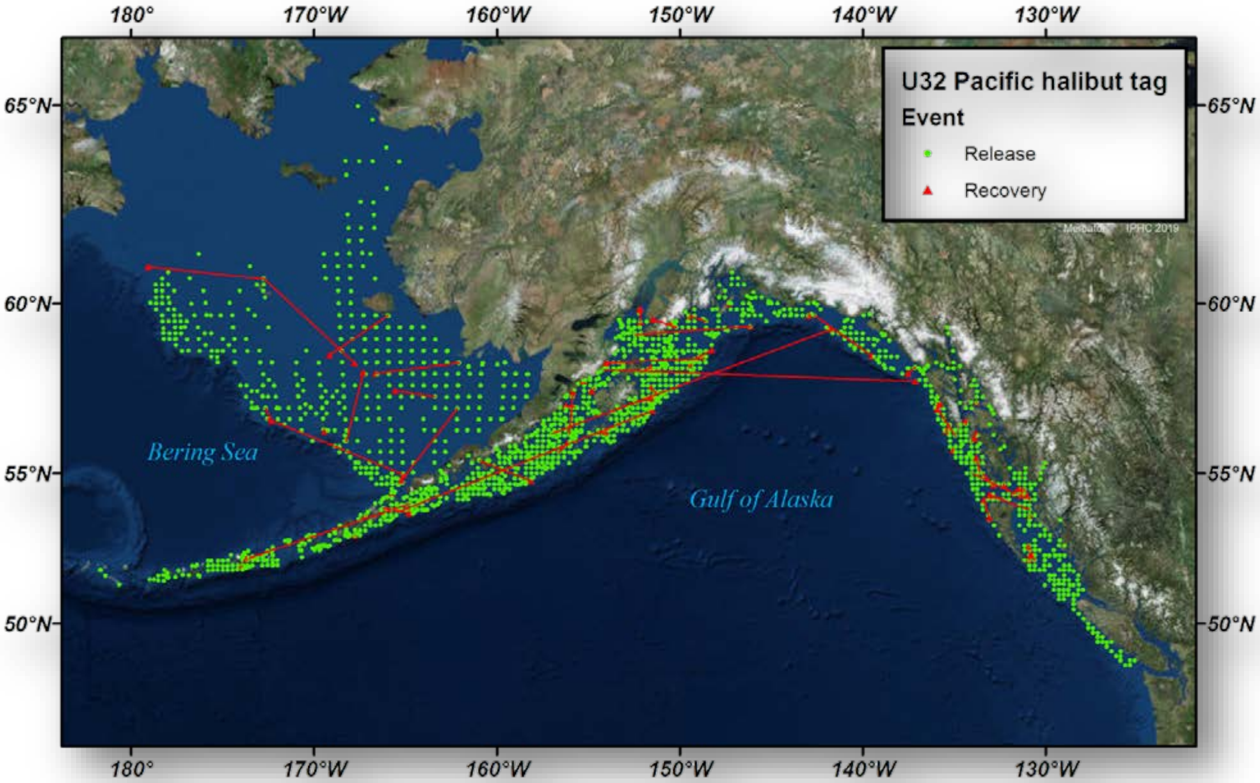
  - Large degree of within-basin connectivity
  - Demersal stage fish in the Bering Sea migrate outward from Bristol Bay and reach Unimak Pass by age-4, widely dispersed by age-6
  - Modeling results show circular, multiple life-stage, connectivity within and between ocean basins



1. Migration and Distribution

2. Late juvenile and adult dispersal: wire tagging of U32 fish



Since 2015:

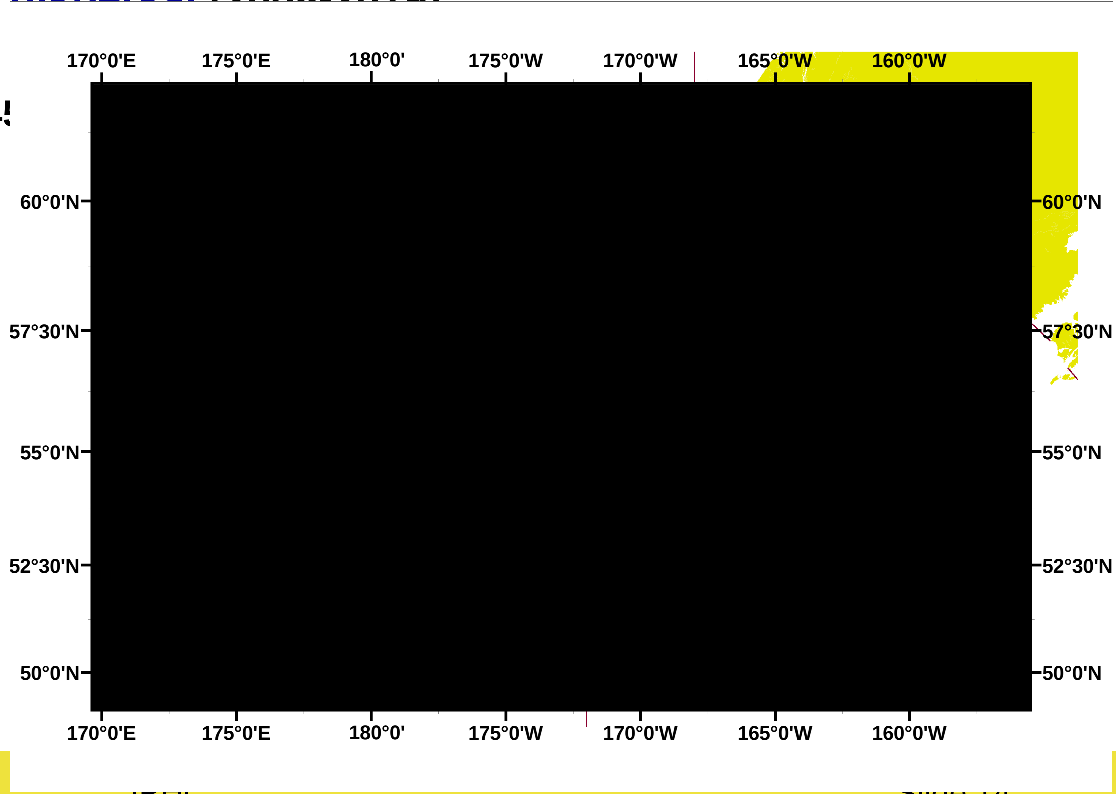
- **10,770 U32 fish wire tagged in FISS and NMFS Trawl Survey**
- **110 recoveries**

1. Migration and Distribution

2. Late juvenile and adult dispersal: electronic tagging of U32 and O32 fish

Bering Sea & Aleutian Island dispersal (2008-2019)

– **Summer-to-summer PAT tags (n=145**



1. Migration and Distribution

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Bering Sea & Aleutian Island dispersal (2008-2019)

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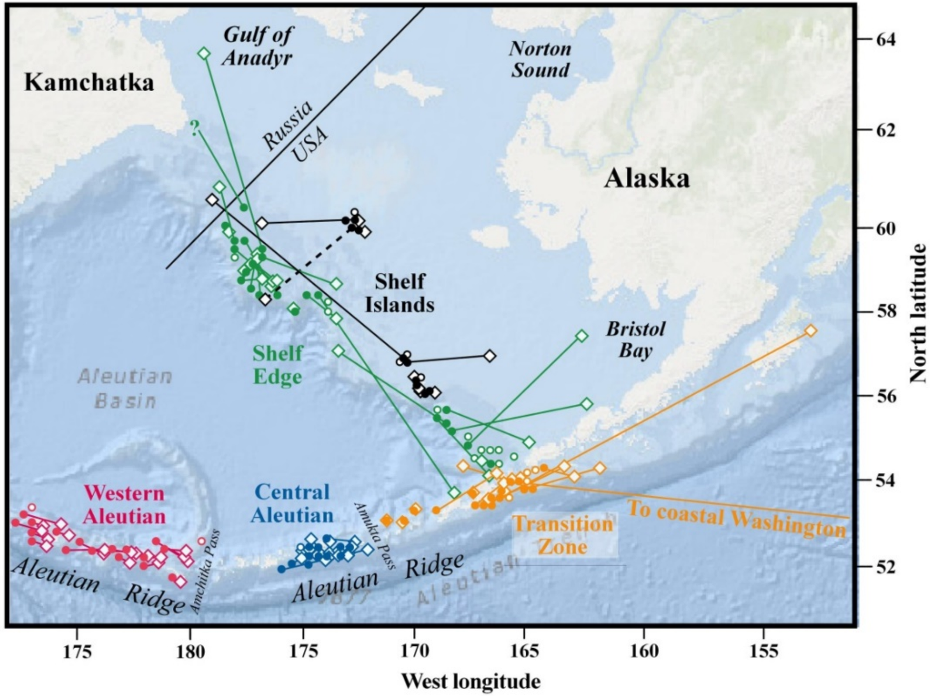

1. Migration and Distribution

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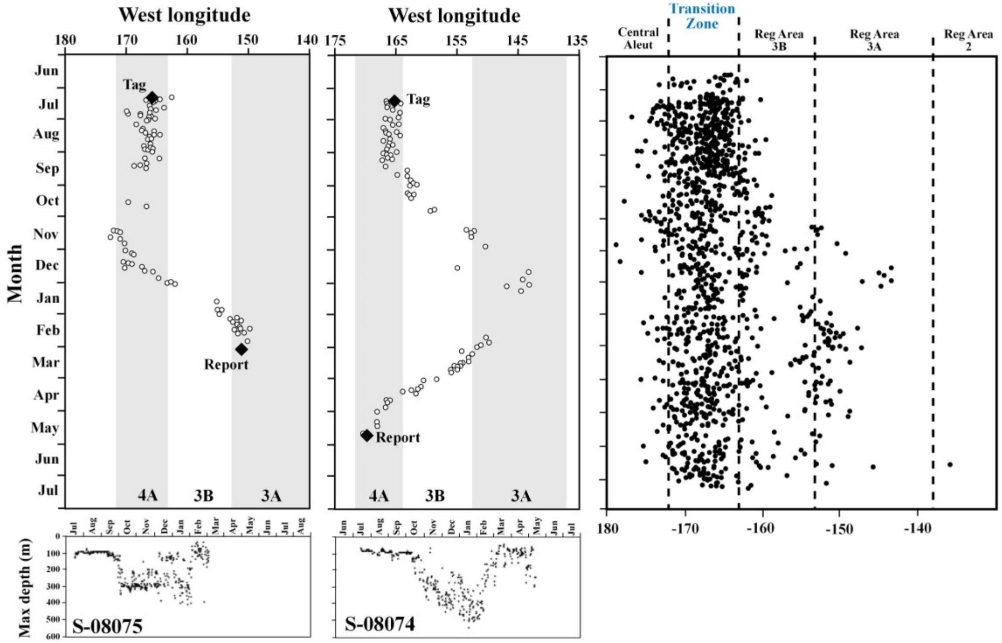
- **Individual migration trajectories: from light-based position estimation**

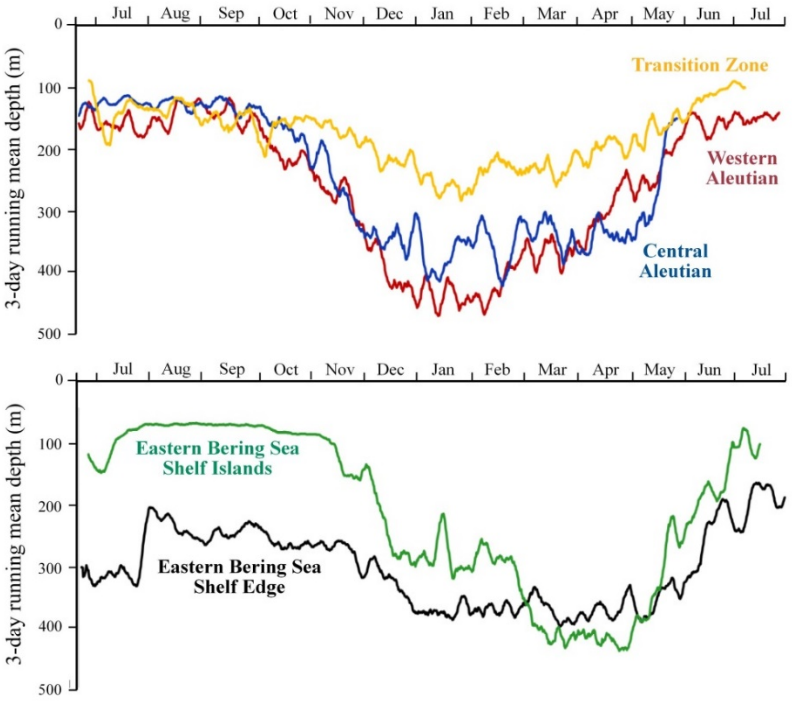


1. Migration and Distribution

2. Late juvenile and adult dispersal: electronic tagging of U32 and O32 fish

Bering Sea & Aleutian Island dispersal (2008-2019)

- **Summer-to-summer PAT tags (n=145)**
- **Interannual dispersal: from mark-report endpoint locations**
- **Individual migration trajectories: from light-based position estimation**
- **Onshore-offshore seasonal migration: from daily depth data**



1. Migration and Distribution

2. Late juvenile and adult dispersal: electronic tagging of U32 and O32 fish

Northeast Bering Sea project (initiated 2019)

- Collaboration with Norton Sound Economic Development Corporation (NSEDC) and the University of Alaska, Fairbanks (UAF) to tag Pacific halibut with pop-up (PAT) tags in Norton Sound and at St. Lawrence Island
  - Logistics and funding through NSEDC
  - Graduate student (Mr. Austin Flanigan, MsC) support through a UAF Rasmuson RFRC Fellowship

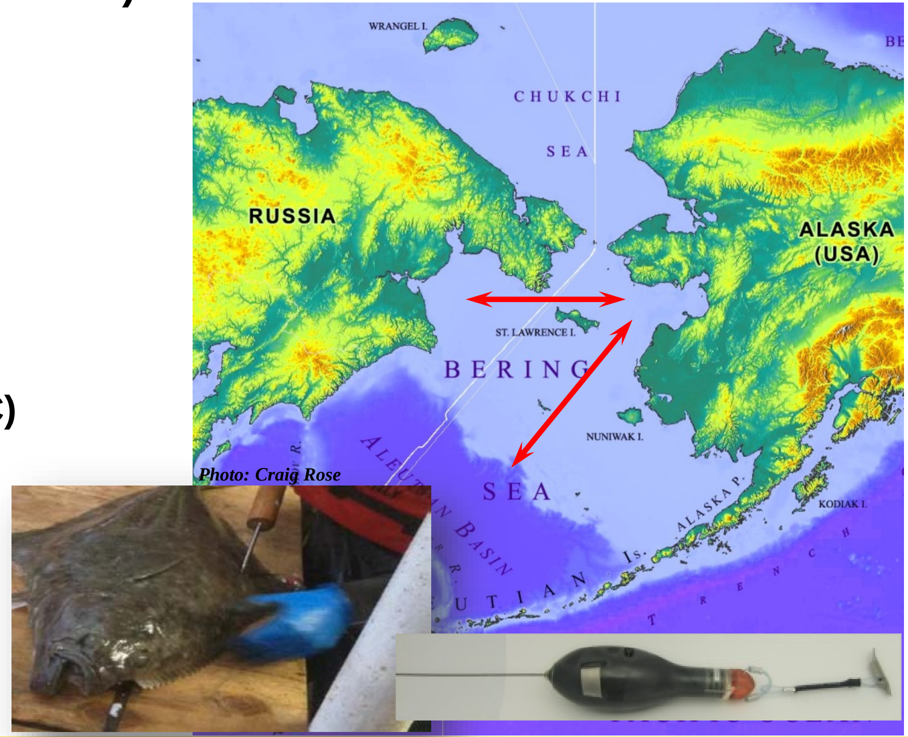


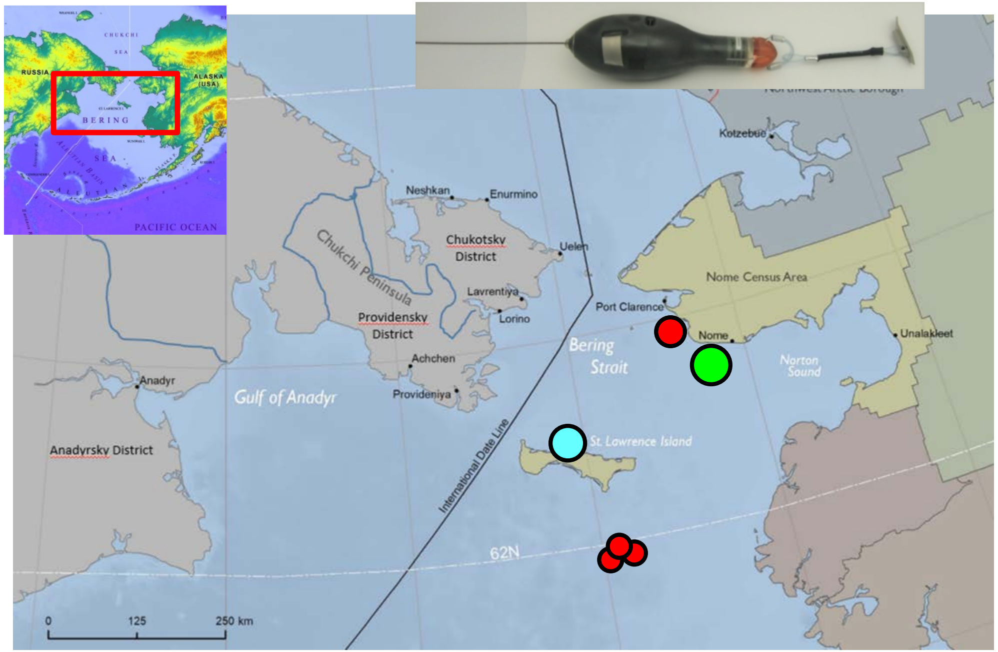
Photo: Craig Rose

1. Migration and Distribution

2. Late juvenile and adult dispersal: electronic tagging of U32 and O32 fish

Northeast Bering Sea project (initiated 2019)

- 44 fish tagged in 2019
- Tags programmed to report in three batches: January 2019, Summer 2020, and Summer 2021



Shelf (n=5) *(NMFS trawl)*

Norton Sound (n=24) *(Nome)*

St. Lawrence (n=15) *(Savoonga)*

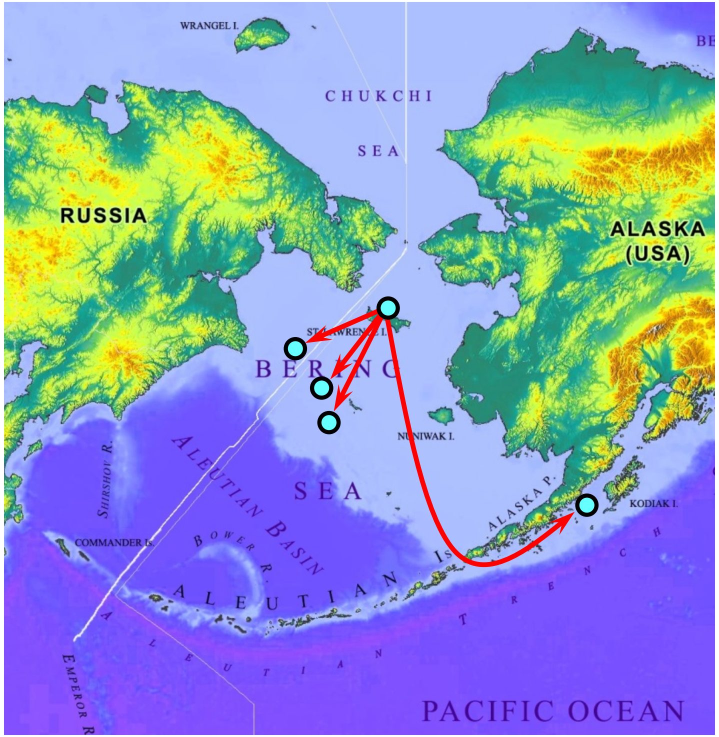
Map image from: *Knapp and Kryukov (2020) Economies of the Bering Strait Region (Springer Verlag)*

1. Migration and Distribution

2. Late juvenile and adult dispersal: electronic tagging of U32 and O32 fish

Northeast Bering Sea project (initiated 2019)

- **44 fish tagged in 2019**
- **Tags programmed to report in three batches: January 2019, Summer 2020, and Summer 2021**
  - **Only 4 of 14 tags reported this January…**

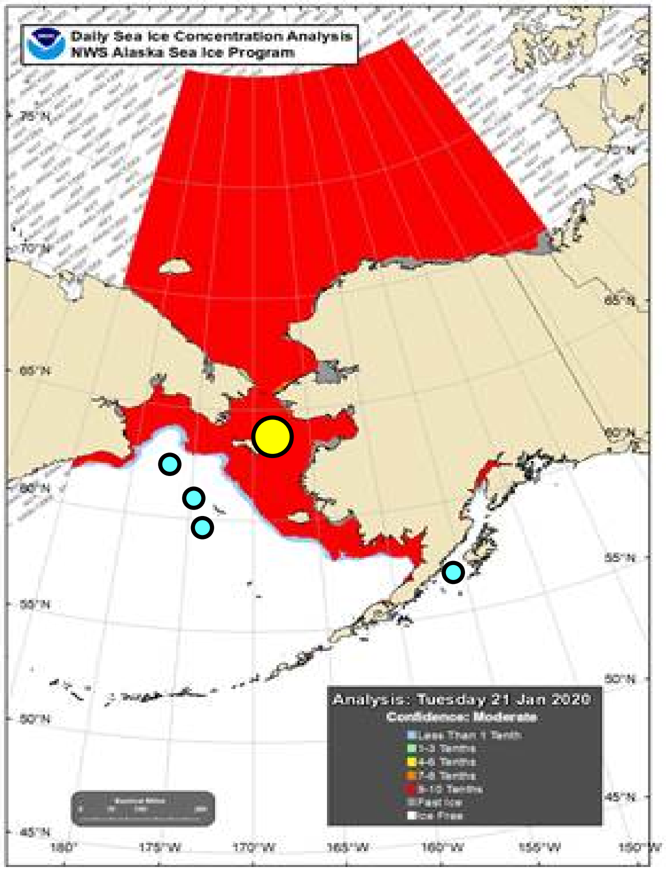


1. Migration and Distribution

2. Late juvenile and adult dispersal: electro

Northeast Bering Sea project (initiated 2019)

- 44 fish tagged in 2019
- Tags programmed to report in three batches: January 2019, Summer 2020, and Summer 2021
  - Only 4 of 14 tags reported this January, likely due to sea-ice coverage



1. Migration and Distribution

2. Late juvenile and adult dispersal: electronic tagging of U32 and O32 fish

Northeast Bering Sea project (initiated 2019)

- **Limited deployments will occur in 2020: Nome is subject to a 2-week COVID entry quarantine and Savoonga is off-limits to non-residents**
- **Project is scheduled to continue into 2022, pending additional funding from NSEDC**

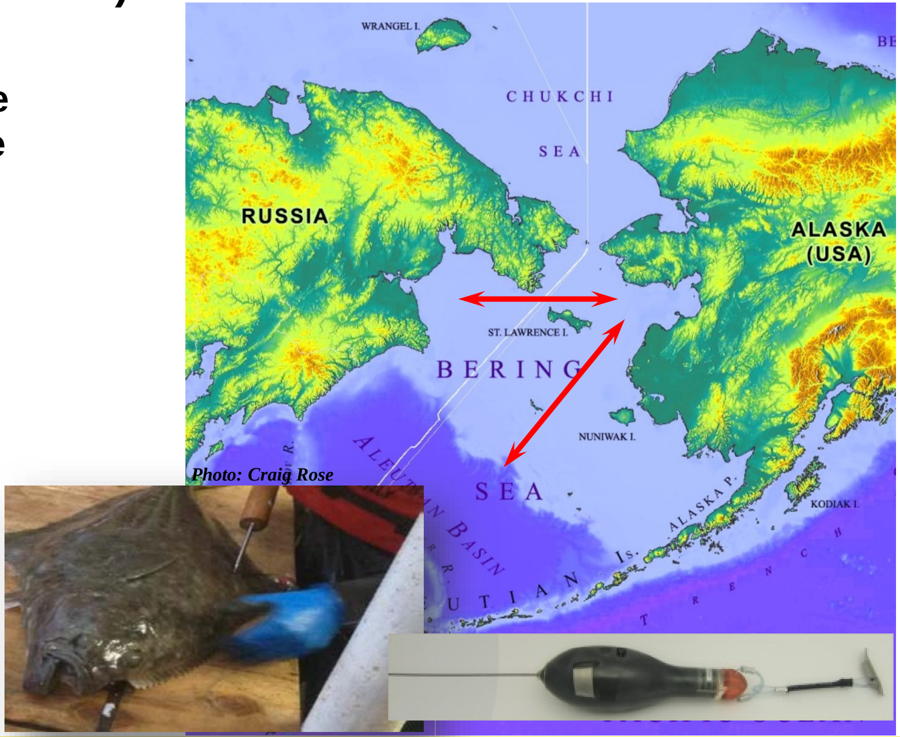
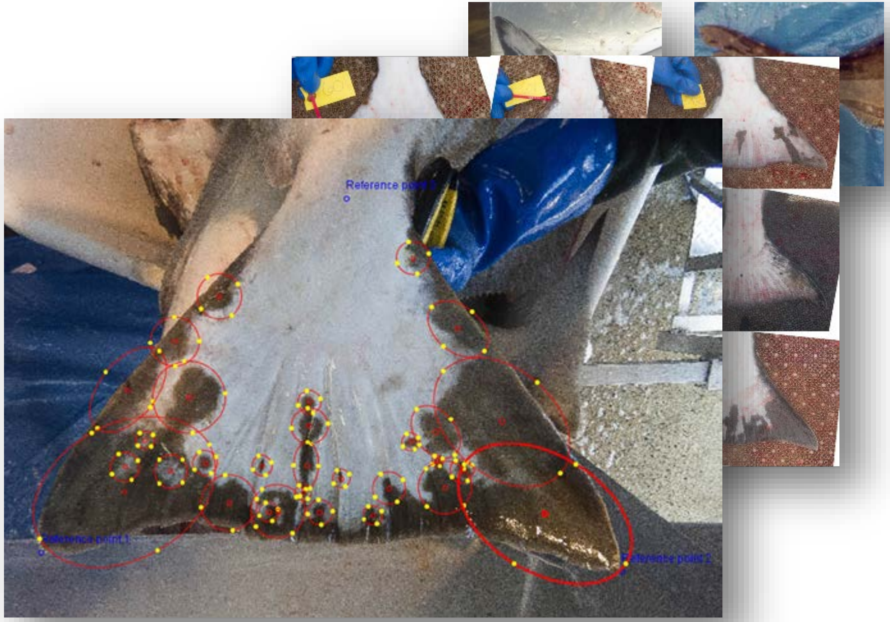


Photo: Craig Rose

1. Migration and Distribution

2. Late juvenile and adult migration: tail pattern recognition

Objective: Use ***natural markings*** to identify individuals over time and inform on movement patterns and growth



- **Blind side of tail is preferable for imaging**
- **Spots and patterns appear to be unique**
- **Markings could be used to identify individuals with image recognition software**
- **Future could integrate into vessel/shoreside electronic monitoring (EM) or recreational fisher applications**
- **To date, 882 U32 Pacific halibut photographed and wire tagged as part of this project**
- **To date, 7 tags recovered with tail pictures**

Progress on ongoing research projects

1. Migration and distribution

Research Priorities

Category¤	Rank·within·category¤	Product¤	Justification¤	Biological·Research·Area·(from·5-year·Research·Plan)¤	Timing¤	Progress¤
	3¤	Estimates·of·connectivity·with·Western·Pacific·waters·(i.e.·Russia)¤	Seasonal·movement·across·Convention·lines·and/or·harvest·of·young·Pacific·halibut·in·transit·to·Convention·waters·could·bias·estimates·of·productivity¤	Genetics·and·genomics,¶ Movement¤	Medium-long·term¤	Not·yet·planned¤

2. Reproduction

Research Priorities:

Category	Rank within category	Product	Justification	Biological Research Area (from 5-year Research Plan)	Timing	Progress
	1	Sex-ratio of the 2018 (and subsequent) commercial landings	Sex-ratio of the commercial landings (and annual variability) is an important scalar of biomass and fishing intensity in the stock assessment, until several complete years are available monitoring needs are unknown	Reproduction	**For 2019**, and medium term	Ongoing, planned
	1	Updated maturity schedule (also fecundity, skip spawning)	Maturity and skip spawning will scale biomass and reference point estimates	Reproduction	Medium term	Ongoing

2. Reproduction

Projects:

1. ***Identification of sex in the commercial landings***

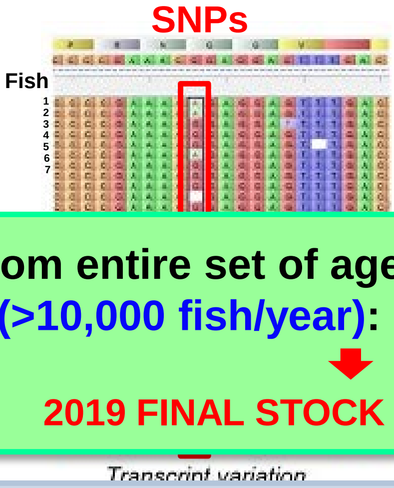
2. ***Full characterization of the annual reproductive cycle to improve current estimates of maturity***

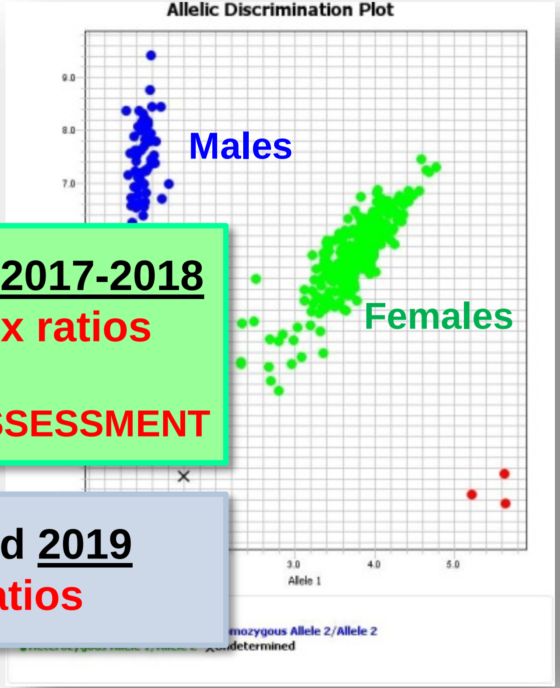
2. Reproduction

1. Identification of sex in the commercial landings

To generate sex-ratio data for use in assessment and policy analysis

Application of genetic techniques (SNPs)





- Completed: Fin clips from entire set of aged 2017-2018 commercial samples (>10,000 fish/year): sex ratios

2019 FINAL STOCK ASSESSMENT

- In Progress: Fin clips from entire set of aged 2019 commercial samples (>10,000 fish) : sex ratios

2. Reproduction

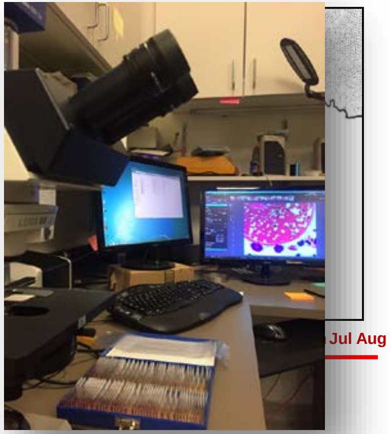
2. Full characterization of the annual reproductive cycle to improve current estimates of maturity

Objective: Revise maturity estimates for male and female Pacific halibut

Annual reproductive cycle

Gonadal growth	Maturation	Spawning

- Histological assessment of gonadal development
- Reproductive hormones in the blood
- Activation of the endocrine reproductive axis (pituitary and gonads)
- Energy levels (fat content/hepatosomatic index)
- Revised scoring criteria of maturity stages by macroscopic observations in the field



Deliverables:

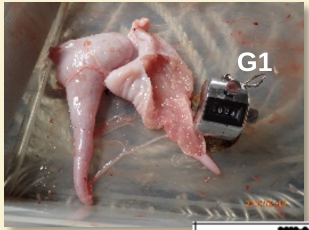
- Accurate staging of reproductive status
- Updated maturity-at-age estimates
- Estimates of skipped-spawning

2. Reproduction

2. Full characterization of the annual reproductive cycle

Objective: **Revise maturity estimates for male and female Pacific halibut**

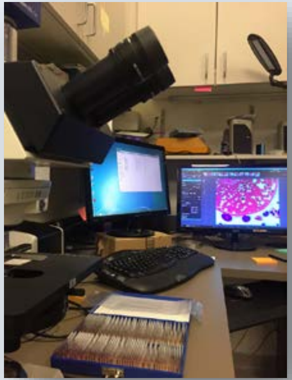
Macroscopic maturity staging (*visual assessment*)



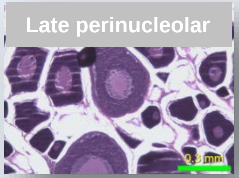
Maturity Stage
1 immature
2 maturing
3 ripe
4 resting

vs

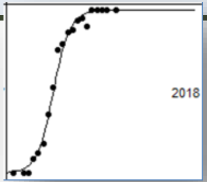
Microscopic maturity staging (*histological assessment*)



Ovarian histology
⬇
Oocyte stage classification
⬇
Maturity staging

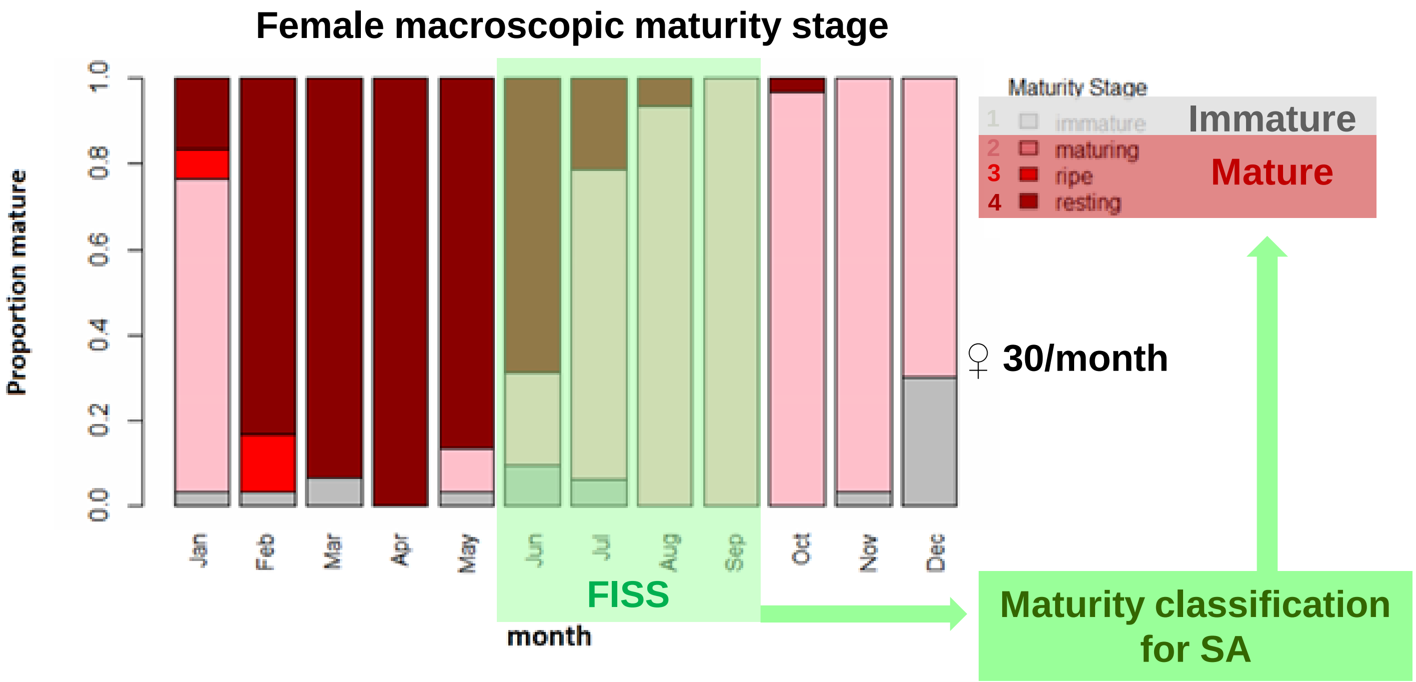


- Maturity ogives
- Maturity estimates



2. Reproduction

Macroscopic maturity staging

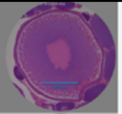
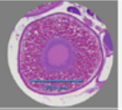
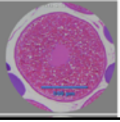


2. Reproduction

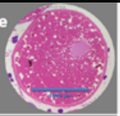
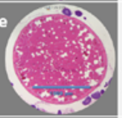
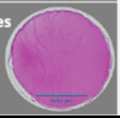
Microscopic maturity staging: Oocyte stage classification by histology

Primary Growth

Secondary Growth

Secondary Growth (SG)	early (SGe)		Yolk granules first appear at the periphery, stain pink, and fill inwards occupying up to 1/3 of the cytoplasm.	428–761 567
	late (SG*l*)		Yolk granules transition from only the periphery of the ooplasm and fill inwards to the nucleus.	544–892 737
	full grown (SGfg)		Yolk granules completely fill the ooplasm to the central nucleus and coalesce to form larger yolk globules.	757–1670 1214

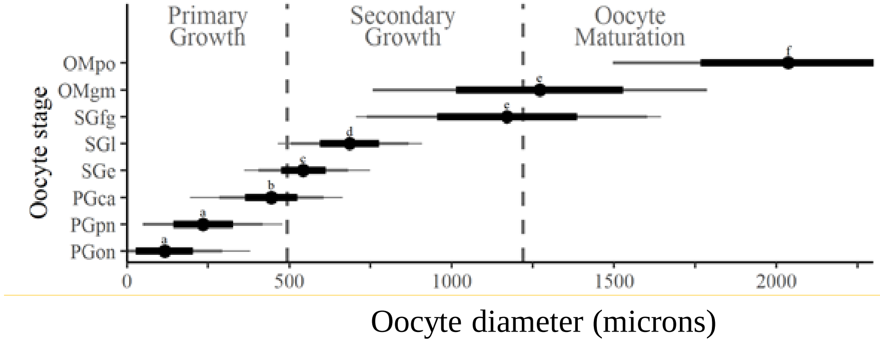
Oocyte Maturation

Oocyte Maturation (OM)	germinal vesicle migration (OMgvm)		The nucleus begins to migrate through a cytoplasm fully filled with yolk globules.	1065–1738 2067
	germinal vesicle breakdown (OMgvbd)		Nucleus is no longer visible and dark pink yolk globules coalesce into light pink stained yolk masses occupying less than ½ the oocyte area.	na
	meiosis resumes hydration (OMmr)		Light pink stained yolk coalesces into a central mass, occupying over ½ the area. Oocyte is still within the follicle wall.	1729–2516 2067

2. Reproduction

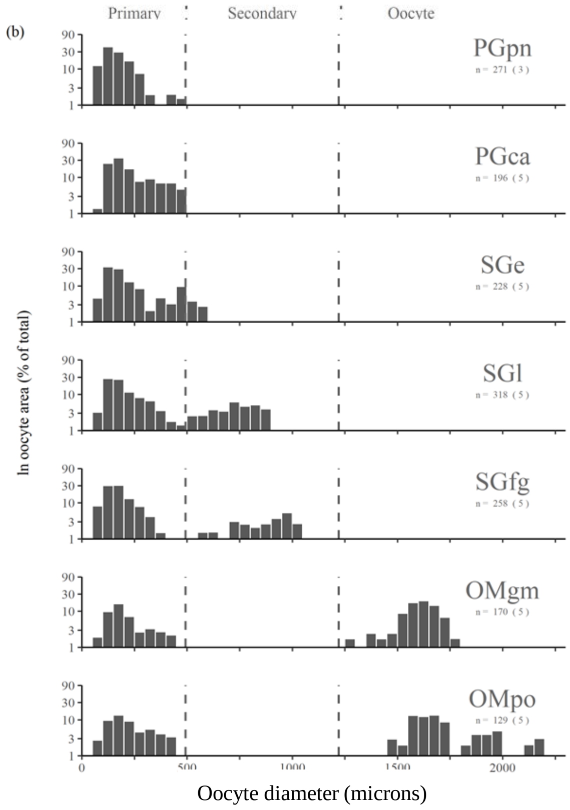
Microscopic maturity staging: Oocyte stage classification by histology

Growth phase (acronym)	Developmental stage (acronym)	Description	Photo
Primary Growth (PG)	One nucleolus (PGon)	Oocytes are small, angular, and compact with a single large nucleolus. Cytoplasm stains dark purple.	
	Perinucleolar (PGpn)	Oocytes are larger and rounder than PGon and nuclei develop and flatten around the nucleus. Cytoplasm stains light purple.	
	Cortical alveolar (PGca)	First cortical alveoli appear as white stain in the periphery of the oocyte.	
Secondary Growth (SG)	Early (SGe)	Yolk globules first appear at the periphery, stain pink, and fill inwards occupying up to 1/3 of the cytoplasm.	
	Late (SG*l*)	Yolk globules transition from only the periphery of the ooplasm and fill inwards to the nucleus.	
	Full Grown (SGfg)	Yolk globules completely fill the ooplasm to the central nucleus and coalesce into larger yolk globules.	
Oocyte Maturation (OM)	Germinal vesicle migration (OMgm)	The nucleus begins to migrate through a cytoplasm fully filled with large yolk globules.	
	Periovulatory (OMpo)	Nucleus no longer visible and the yolk globules coalesce into a central yolk mass. Oocyte is still within the follicle wall.	



2. Reproduction

Microscopic maturity staging: Oocyte stage classification by histology

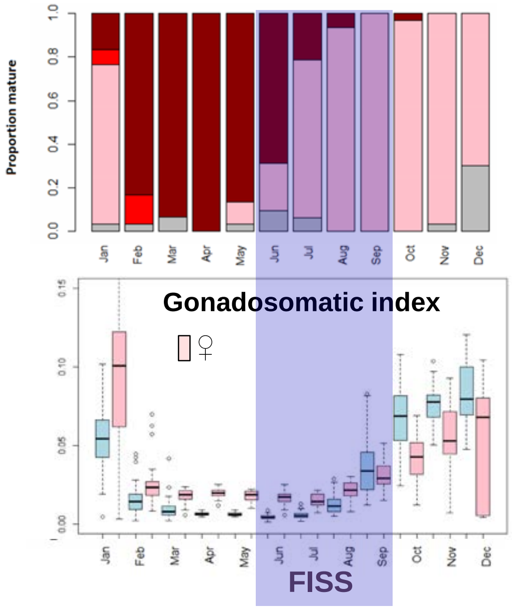


2. Reproduction

Female maturity information available from one region: Portlock

- **Full annual collection (2018)**

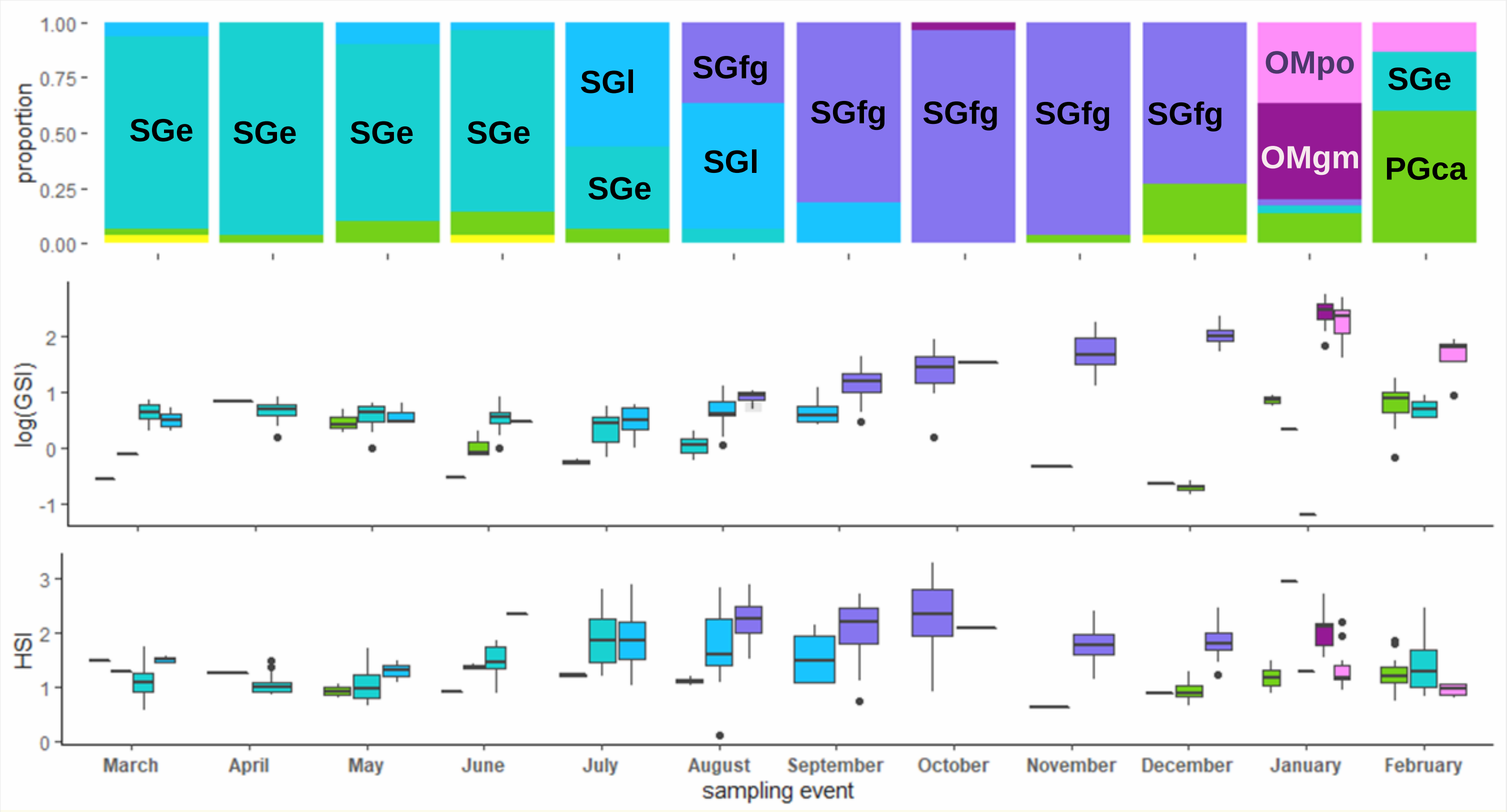
Macroscopic maturity staging (♀)



- **Interannual collection**
  June 2017, 2018, 2019, 2020

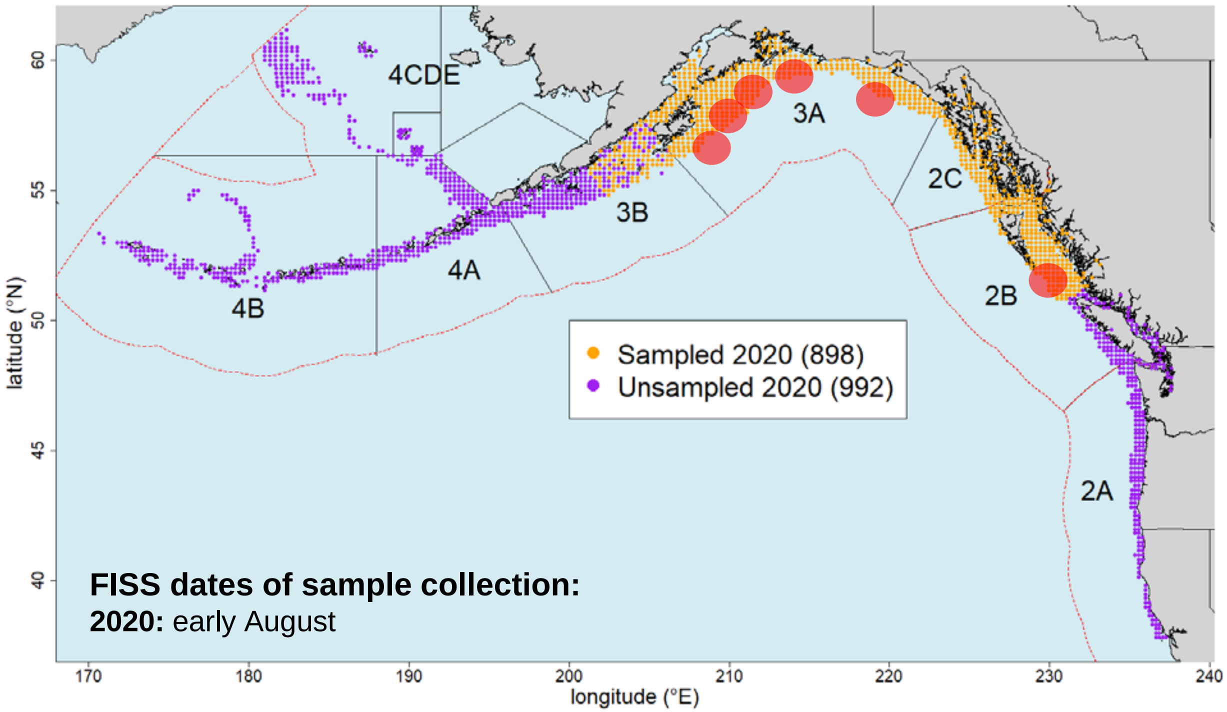
2. Reproduction

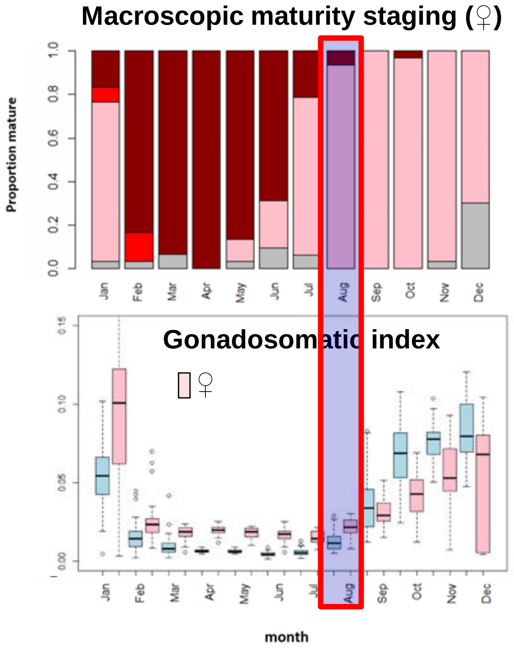
Microscopic maturity staging: based on histological oocyte stages



2. Reproduction

Research proposal: Spatial analysis of maturity



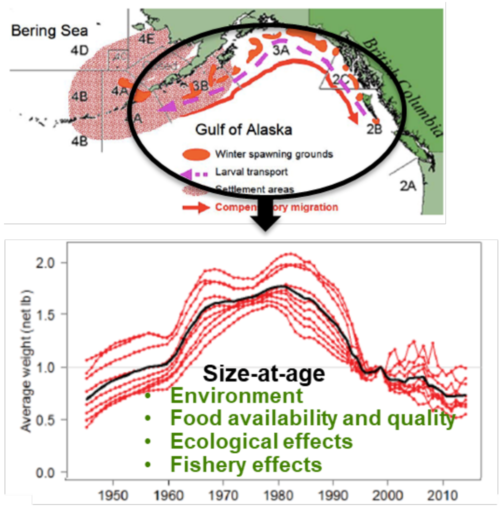


- **July-August collection in FISS?**

3. Growth

Research Priorities

Category¤	Rank· within· category¤	Product¤	Justification¤	Biological· Research·Area· (from·5-year· Research·Plan)¤	Timing¤	Progress¤
¶ ¶ Assessment· Biological·inputs¤	Unranked¤	Size-at-age¤	Size-at-age·is·a·primary· scalar·of·productivity· for·the·stock.·Potential· fishery·effects·are·not· accounted·for·in·status· and·reference·point· calculations.¤	Growth¤	Medium-long·term¤	Ongoing¤



• Changes in growth?

Projects:

1. *Identification and validation of physiological markers for somatic growth*
2. *Evaluation of growth patterns in the Pacific halibut population and possible effects of environmental variability*

3. Growth

1. Identification and validation of physiological markers for growth

White muscle:
- **Transcriptomics**
- **Proteomics**

Temperature | **Population Density** | **Dominance** | **Capture Stress**

Effects on transcriptome and proteome

Identification of physiological growth markers

MUSCLE

LIVER

BIOCHEMICAL AND MOLECULAR GROWTH RESPONSES

Application to field studies

IPHC / AFSC-NOAA (Newport, OR)

Dr. Josep Planas (PI)

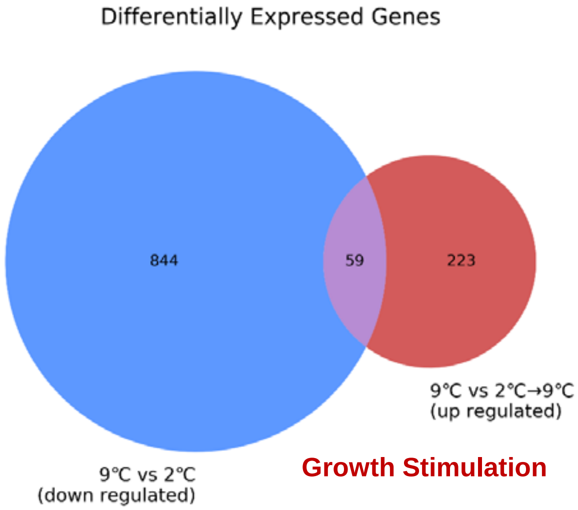
Dr. Thomas Hurst

NPRB Grant 1704 (2017-2020)

3. Growth

1. Identification and validation of physiological markers for growth

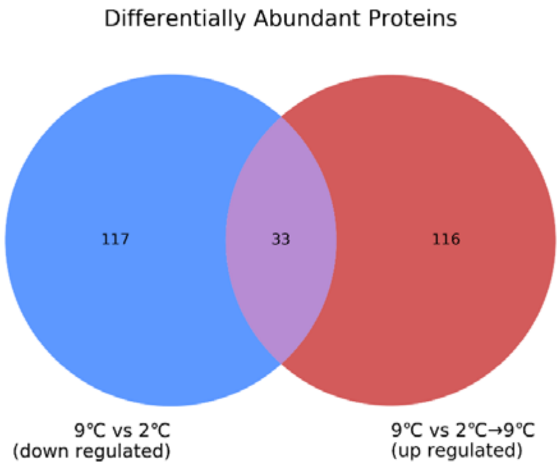
- **Transcriptomics**



Growth Stimulation

Growth Suppression

- **Proteomics**

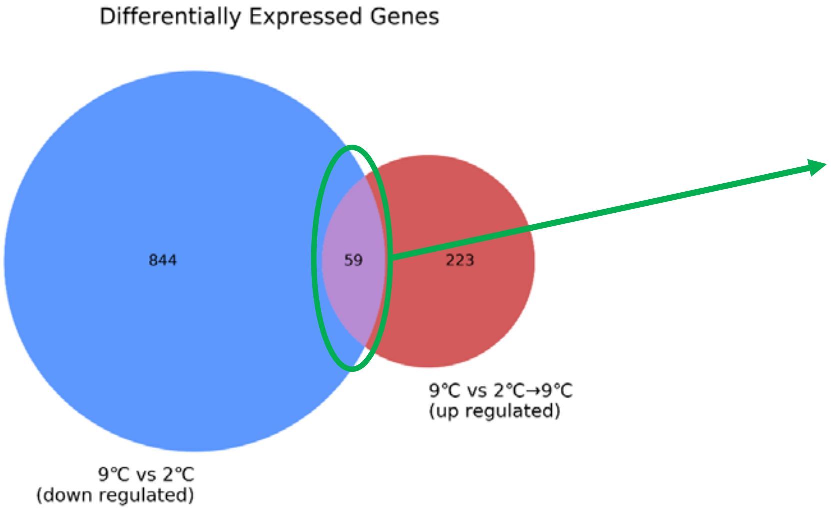


Growth Suppression **Growth Stimulation**

3. Growth

1. Identification and validation of physiological markers for growth

- **Transcriptomics: common genes**

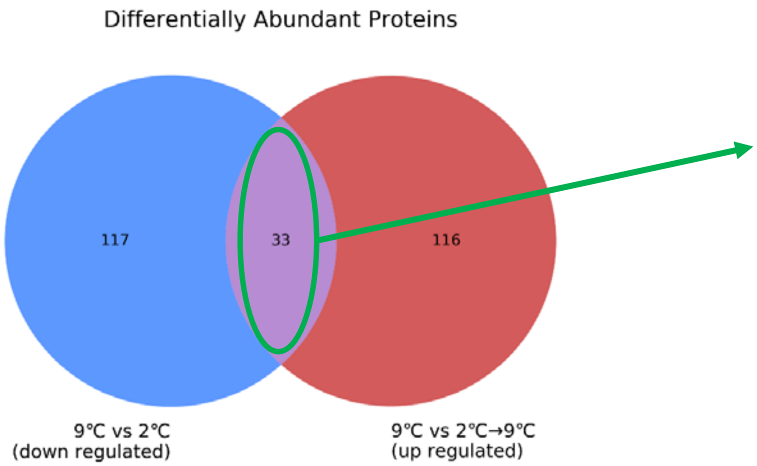


Actin (Fragment) OS=Spodoptera littoralis PE=2 SV=1
Actin, alpha skeletal muscle OS=Oreochromis mossambicus GN=a
Actin, aortic smooth muscle OS=Gallus gallus GN=ACTA2 PE=3 SV=
Ammonium transporter Rh type C 2 OS=Takifugu rubripes GN=rhc
Asparagine synthetase [glutamine-hydrolyzing] OS=Gallus gallus
Cdc42 effector protein 3 OS=Homo sapiens GN=CDC42EP3 PE=1 SV
Cytochrome c OS=Danio rerio GN=cyc PE=3 SV=3
Glucose-6-phosphatase 3 OS=Rattus norvegicus GN=G6pc3 PE=2 S
Glutathione-specific gamma-glutamylcyclotransferase 1 OS=Dani
Glycine--tRNA ligase (Fragment) OS=Rattus norvegicus GN=Gars P
Hemoglobin subunit alpha-A OS=Seriola quinqueradiata GN=hbaa
Hemoglobin subunit beta-A OS=Seriola quinqueradiata GN=hbb1
Kelch domain-containing protein 1 OS=Homo sapiens GN=KLHDC1
Musculoskeletal embryonic nuclear protein 1 OS=Xenopus laevis
Myosin heavy chain, fast skeletal muscle OS=Cyprinus carpio PE=2
Myosin phosphatase Rho-interacting protein OS=Mus musculus G
Myosin regulatory light chain 2, skeletal muscle isoform OS=Gallu
Myosin-15 OS=Homo sapiens GN=MYH15 PE=1 SV=5
Oxysterol-binding protein-related protein 3 OS=Homo sapiens GN
PREDICTED: Cottoperca gobio sodium- and chloride-dependent cr
PREDICTED: Paralichthys olivaceus tripartite motif containing 9
PREDICTED: Paralichthys olivaceus tubulin-specific chaperone
PREDICTED: Paralichthys olivaceus vacuole membrane protein 1-l
PREDICTED: Paralichthys olivaceus zinc finger protein 638-like
PREDICTED: Paralichthys olivaceus zinc finger protein 638-like
Protein FAM166A OS=Rattus norvegicus GN=Fam166a PE=2 SV=1
Putative protein MSS51 homolog, mitochondrial OS=Homo sapier
Reverse transcriptase domain-containing protein
Titin OS=Homo sapiens GN=TTN PE=1 SV=4
Troponin I, slow skeletal muscle OS=Oryctolagus cuniculus GN=TN

3. Growth

1. Identification and validation of physiological markers for growth

- **Proteomics: common proteins**



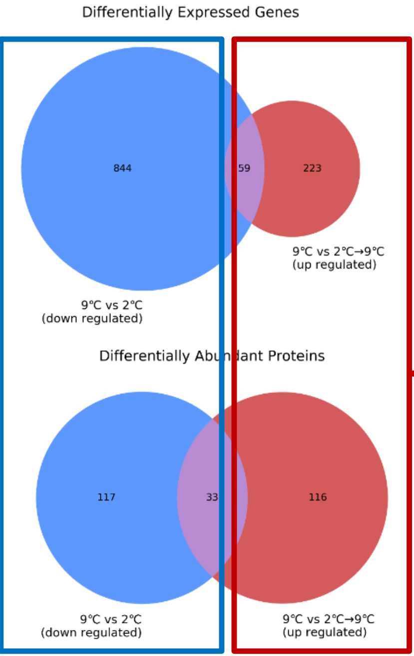
Isoform 1 of Fibronectin	cell adhesion and motility
Protein BCCIP homolog	Cell cycle
SERPIN domain-containing protein	Extracellular space
Ras GTPase-activating protein-binding protein 1	Immune response
Cytoplasmic dynein 1 heavy chain 1	Intracellular transport
Importin N-terminal domain-containing protein	Intracellular transport
Importin N-terminal domain-containing protein	Intracellular transport
Calponin-homology (CH) domain-containing protein	Neurogenesis
Transmembrane protein 161A	Protection against oxidative stress
Ubiquitin carboxyl-terminal hydrolase 13	Protein catabolism
Probable N-acetyltransferase san	Protein modification
Asparagine synthetase domain-containing protein (Fragment)	Protein synthesis
tRNA-synt_1e domain-containing protein	Protein synthesis
D-3-phosphoglycerate dehydrogenase	Protein synthesis
Methionine aminopeptidase 2	Protein synthesis
Methionine--tRNA ligase, cytoplasmic	Protein synthesis
Phosphoserine aminotransferase	Protein synthesis
Reticulocalbin-3	Protein synthesis
S-adenosylmethionine synthase isoform type-2	Protein synthesis
Serine hydroxymethyltransferase	Protein synthesis
Coatomer subunit gamma-1	Protein transport
N-alpha-acetyltransferase 16, NatA auxiliary subunit	Transcription regulation
Protein arginine N-methyltransferase 5	Transcription regulation
ATP-binding cassette sub-family F member 2	Translation
eIF2B_5 domain-containing protein	Translation
Eukaryotic peptide chain release factor subunit 1	Translation
Heat shock protein 14	Translation
Isoform 2 of La-related protein 1B	Translation
Ornithine carbamoyltransferase, mitochondrial	Translation
Eukaryotic translation initiation factor 4 gamma 1	Translation

3. Growth

1. Identification and validation of physiological markers for growth

Arginine--tRNA ligase, cytoplasmic OS=Rattus norvegi
Asparagine synthetase [glutamine-hydrolyzing] OS=Ra
ATP-binding cassette sub-family E member 1 OS=Mus
Carboxypeptidase A5 OS=Mus musculus GN=Cpa5 PE=
Collagen alpha-1(V) chain OS=Mus musculus GN=Col5a
Collagen alpha-2(I) chain (Fragments) OS=Gallus gallus
Collagen alpha-6(VI) chain OS=Homo sapiens GN=COL
Coronin-1A OS=Homo sapiens GN=CORO1A PE=1 SV=4
Elongation factor 1-delta OS=Xenopus laevis GN=eef1
Eukaryotic translation initiation factor 2 subunit 2 OS=
Eukaryotic translation initiation factor 3 subunit J-A OS
Eukaryotic translation initiation factor 4 gamma 2 OS=
Glycine--tRNA ligase OS=Homo sapiens GN=GARS PE=
Heat shock 70 kDa protein 4 OS=Canis lupus familiaris
Heat shock protein beta-11 OS=Danio rerio GN=hspb1
Histone-lysine N-methyltransferase SETD7 OS=Danio
Importin-13 OS=Pongo abelii GN=IPO13 PE=2 SV=1
Influenza virus NS1A-binding protein homolog A OS=D
Kelch-like protein 10 OS=Homo sapiens GN=KLHL10 PE
Myozenin-2 OS=Pongo abelii GN=MYOZ2 PE=2 SV=1
N-alpha-acetyltransferase 38, NatC auxiliary subunit C
Ornithine carbamoyltransferase, mitochondrial OS=H
Peptidyl-prolyl cis-trans isomerase FKBP7 OS=Mus mu
Phenylalanine--tRNA ligase alpha subunit OS=Danio re
Phosphoserine aminotransferase OS=Mus musculus G
Protein BCCIP homolog OS=Danio rerio GN=bccip PE=2
Troponin I, fast skeletal muscle OS=Oryctolagus cunicu
Ubiquitin carboxyl-terminal hydrolase isozyme L1 OS=
Unconventional myosin-VI OS=Homo sapiens GN=MY

23



Growth Suppression

Growth Stimulation

10

- **Common genes and proteins**

60S ribosomal protein L22 OS=Ictalurus punctatus GN=rpl22 PE=2 SV=3
Asparagine synthetase [glutamine-hydrolyzing] OS=Gallus gallus GN=ASNS
Collagen alpha-3(VI) chain OS=Gallus gallus GN=COL6A3 PE=2 SV=2
Immunoglobulin-like and fibronectin type III domain-containing protein 1 O
Leucine-rich repeat-containing protein 2 OS=Homo sapiens GN=LRRC2 PE=2
Methionine aminopeptidase 2 OS=Homo sapiens GN=METAP2 PE=1 SV=1
Ornithine carbamoyltransferase, mitochondrial OS=Homo sapiens GN=OTC
Prolyl 4-hydroxylase subunit alpha-2 OS=Caenorhabditis elegans GN=phy-2
Titin OS=Homo sapiens GN=TTN PE=1 SV=4
Ubiquitin carboxyl-terminal hydrolase 25 OS=Homo sapiens GN=USP25 PE=

3. Growth

Physiological growth markers → Application to field studies

2. Validation of physiological growth markers for adult Pacific halibut

- 44 adult Pacific halibut in captivity in Seward, AK (collaboration with Alaska Pacific University)
- Establishment of different growth rates through dietary manipulation
- Validation of physiological growth markers to infer growth patterns (slow versus fast growth) in adult Pacific halibut

4. Discard mortality rates and survival assessment

Research Priorities

Category	Rank within category	Product	Justification	Biological Research Area (from 5-year Research Plan)	Timing	Progress
¶ Assessment Biological inputs	Unranked	Updated estimates of discard mortality rates	Trends in unobserved (or miss-specified) mortality may lead to bias in scale and trend of assessment results	Sources of mortality	Medium-long term	Ongoing

Projects:

1. ***Improve DMR estimations in the directed longline fishery***

NOAA FISHERIES Saltonstall – Kennedy Grant NA17NMF4270240 ALASKA PACIFIC UNIVERSITY

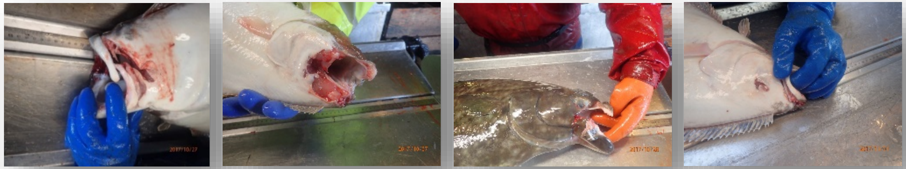
2. ***Estimate DMRs in the guided recreational fishery***

NFWF National Fish and Wildlife Foundation UAF UNIVERSITY OF ALASKA FAIRBANKS ALASKA PACIFIC UNIVERSITY ACA Alaska Charter Association

4. DMRs and survival assessment

1. *<u>Directed longline fishery</u>: A. Relationship between handling practices and injury levels and physiological condition of released Pacific halibut*

– Assessed *injuries* and *release condition* associated with release techniques (careful shake, gangion cut, hook stripping).
  - Injury evaluation



– *Physiological condition* of released fish
  - Condition factor indices

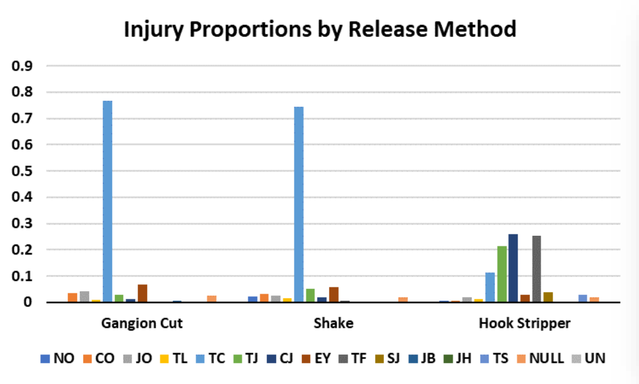
– *Capture conditions*

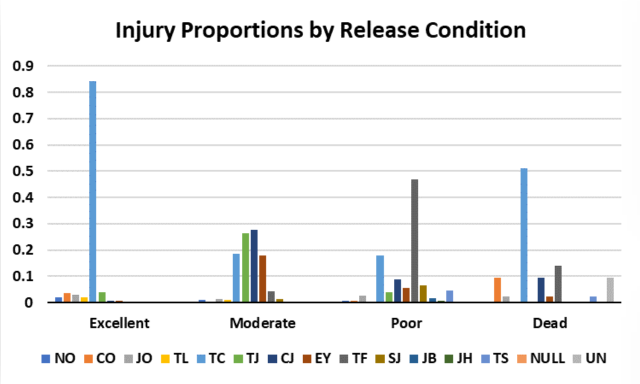
•Time

4. DMRs and survival assessment

1. <u>Directed longline fishery</u>: A. Relationship between handling practices and injury levels and physiological condition of released Pacific halibut

– **Assessed *injuries* and *release condition* associated with release techniques (careful shake, gangion cut, hook stripping).**



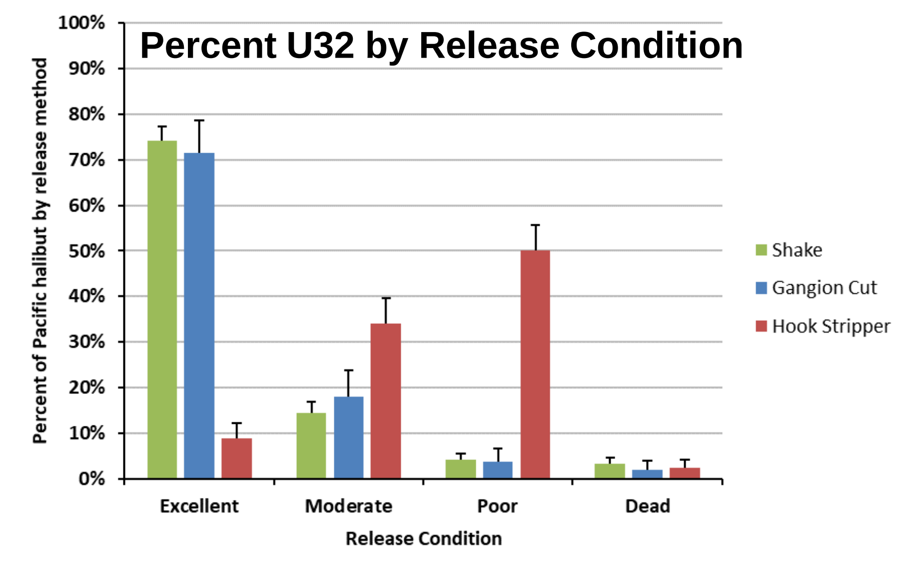


Code	Description
NO	No apparent injury.
CO	Cheek only (not through skin).
JO	Jaw only (but not clear through the jaw).
TL	Torn lip (skin covering external portion of jaw), cheek not punctured.
TC	Torn cheek, small hole through cheek only.
TJ	Torn jaw, either side. Little or no tearing in cheek.
CJ	Cheek and jaw. Tear in cheek extending through jaw.
EY	Hook penetrated eye.
TF	Torn face. Torn though cheek and jaw, like above, but large flap of side of head is ripped/missing.
SJ	Split jaw. Lower jaw is split laterally.
JB	Jig body. Fish snagged by hook somewhere on body other than head.
JH	Jig head. Fish snagged by hook in the head (not through mouth).
TS	Torn snout. Upper jaw is split laterally, usually tearing through the snout as well.
UN	Injury unknown or unrecorded.

4. DMRs and survival assessment

1. <u>Directed longline fishery</u>: A. Relationship between handling practices and injury levels and physiological condition of released Pacific halibut

- **Assessed *injuries* and *release condition* associated with release techniques (careful shake, gangion cut, hook stripping).**

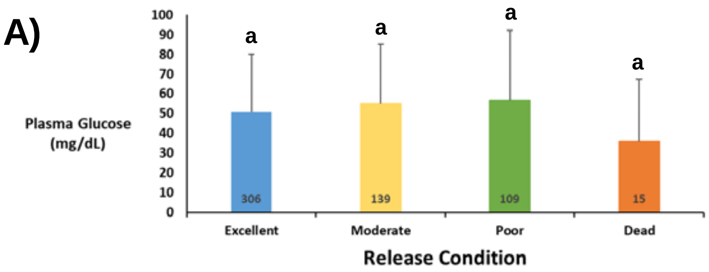


4. DMRs and survival assessment

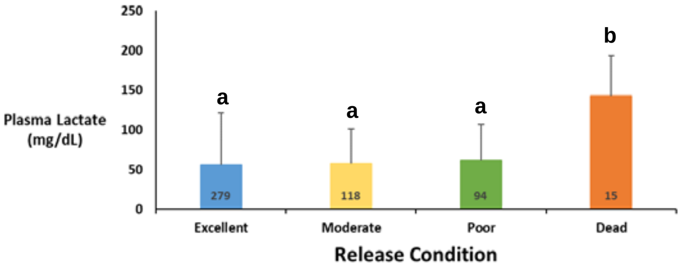
1. <u>Directed longline fishery</u>: *A. Relationship between handling practices and injury levels and physiological condition of released Pacific halibut*

– *Physiological condition* of released fish: Blood stress indicators by <u>release condition</u>

A)

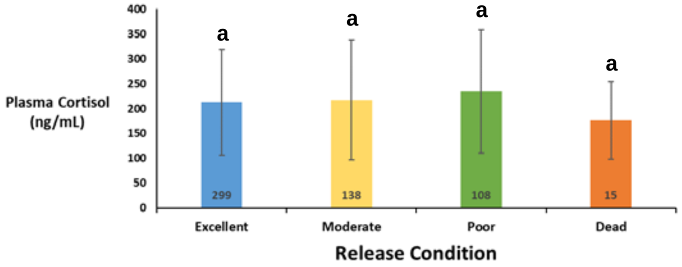


B)



* Significant difference (one way ANOVA, F(3,502)=16.82 p<0.001)

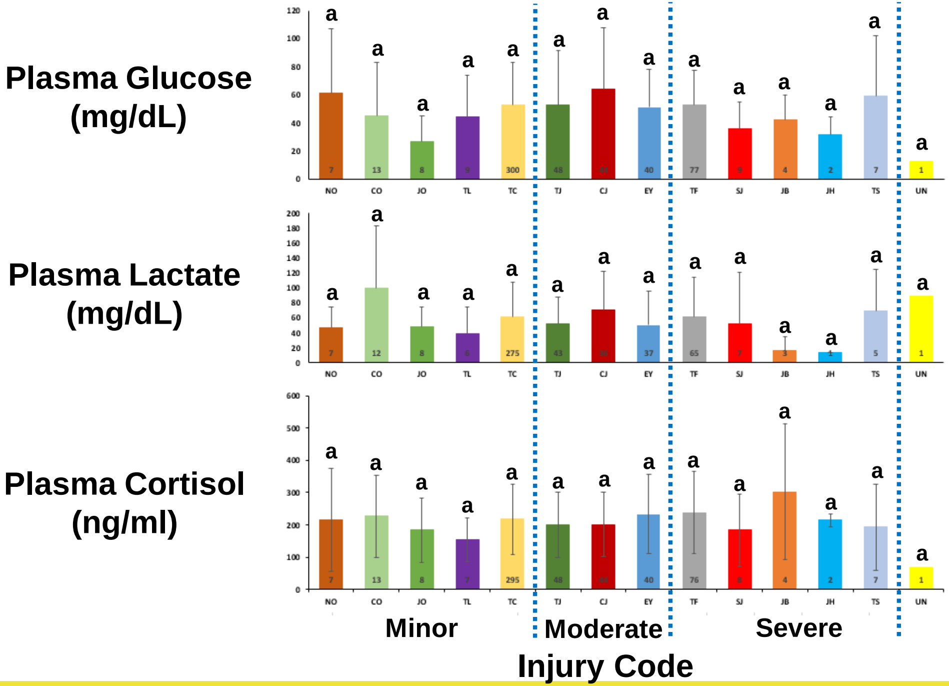
C)



4. DMRs and survival assessment

1. <u>Directed longline fishery</u>: A. Relationship between handling practices and injury levels and physiological condition of released Pacific halibut

– ***Physiological condition*** **of released fish: Blood stress indicators by <u>injury code</u>**

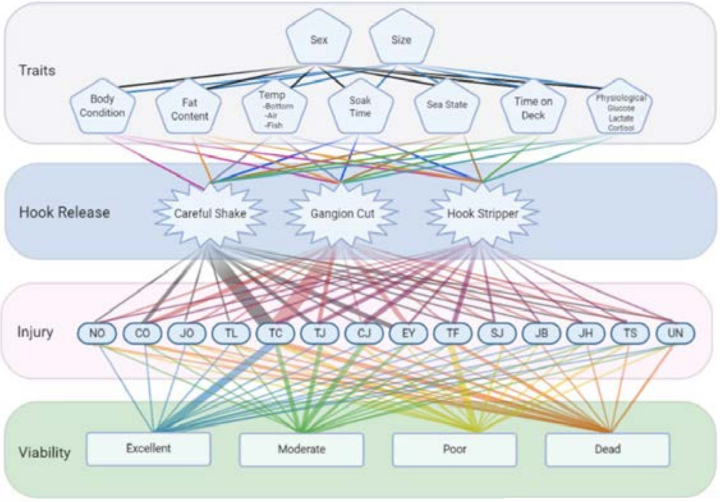


Severity	Code	Description
Minor	NO	No apparent injury.
	CO	Cheek only (not through skin).
Mi	JO	Jaw only (but not clear through the jaw).
	TL	Torn lip (skin covering external portion of jaw), cheek not punctured.
	TC	Torn cheek, small hole through cheek only.
Moderate	TJ	Torn jaw, either side. Little or no tearing in cheek.
	CJ	Cheek and jaw. Tear in cheek extending through jaw.
Mo	EY	Hook penetrated eye.
Severe	TF	Torn face. Torn though cheek and jaw, like above, but large flap of side of head is ripped/missing.
	SJ	Split jaw. Lower jaw is split laterally.
S	JB	Jig body. Fish snagged by hook somewhere on body other than head.
	JH	Jig head. Fish snagged by hook in the head (not through mouth).
	TS	Torn snout. Upper jaw is split laterally, usually tearing through the snout as well.
Unknown	UN	Injury unknown or unrecorded.

4. DMRs and survival assessment

1. <u>Directed longline fishery</u>: A. Relationship between handling practices and injury levels and physiological condition of released Pacific halibut

- *Continuing Analysis:* **Relationships of individual (physiological, fitness) and environmental (time out of water, soak time, temperature differences etc.) traits on release viability**



4. DMRs and survival assessment

2. *Guided recreational fishery: Estimation of DMRs*

Objectives:

2019

1. **Collect information on hook types and sizes and handling practices**

Survey: Dock-side interviews (n=51)
2C: Sitka (n=16), Juneau (n=8)
3A: Homer (n=12), Seward (n=15)

Results:

1. **75% Circle Hooks / 25% Jigs (J-hook)**
2. **Hook removal: 54% reverse the hook**
   40% twist with gaff
3. **Fish Handling upon release:**
   a) **Body and tail supported (65%)**
   b) **Operculum (10%**
   c) **Tail only (10%)**

Guided recreational

Captured Pacific halibut

NFWF National Fish and Wildlife Foundation

UAF University of Alaska Fairbanks; Alaska Pacific University; ACA Alaska Charter Association

4. DMRs and survival assessment

2. *Guided recreational fishery: Estimation of DMRs*

– **Project initiated in 2019**

Objectives (cont'd):

2021
Field Experiment

1. **Investigate the relationship between gear types and capture conditions and size composition of captured fish**
2. **Injury profiles and physiological stress levels of captured fish**
3. **Assessment of mortality of discarded fish**

Hook injury assessment

Tagging with sPATs

NFWF **National Fish and Wildlife Foundation**

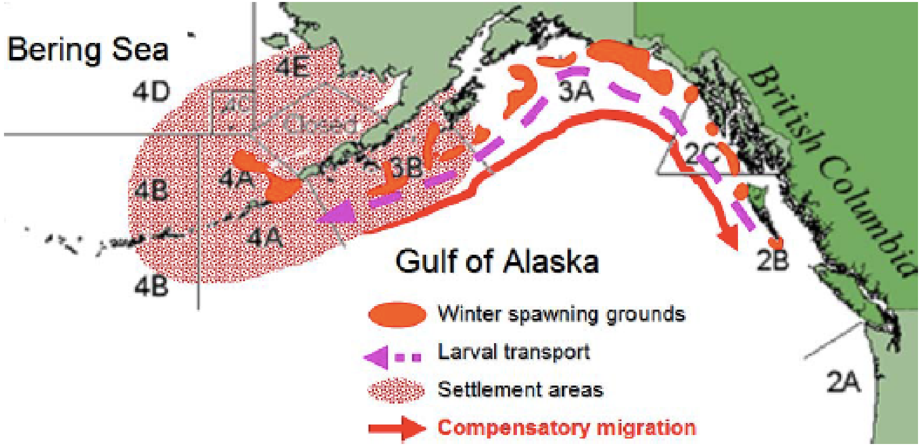
UAF University of Alaska Fairbanks | Alaska Pacific University | ACA Alaska Charter Association

5. Genetics and Genomics

Research priorities

Category	Rank within category	Product	Justification	Biological Research Area (from 5-year Research Plan)	Timing	Progress
Assessment Biological inputs	2	Stock structure of IPHC Regulatory Area 4B relative to the rest of Convention Area	Delineating discrete stock components will structure future assessments. If isolated, IPHC Regulatory Area 4B may require separate management from the remainder of the Convention Area	Genetics and genomics	Medium term	Planned
	3	Estimates of connectivity with Western Pacific waters (i.e. Russia)	Seasonal movement across Convention lines and/or harvest of young Pacific halibut in transit to Convention waters could bias estimates of productivity	Genetics and genomics, Movement	Medium-long term	Not yet planned

5. Genetics and Genomics



5.1.1 - Determine the genetic structure of the Pacific halibut population in the North-eastern Pacific Ocean.

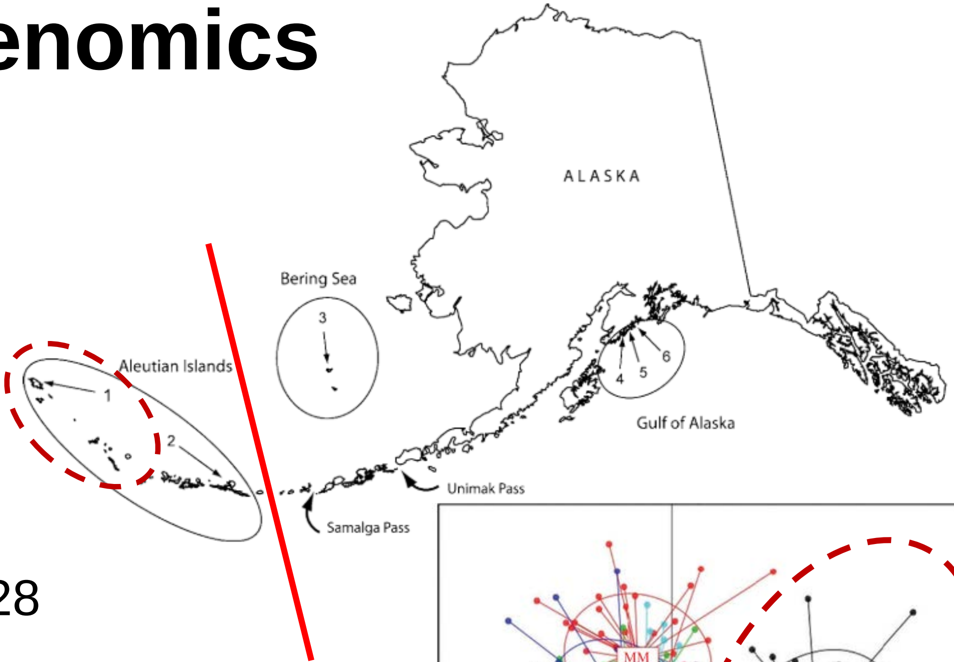
5.1.2 - Analysis of genetic variability among juvenile Pacific halibut in the Bering Sea and the Gulf of Alaska

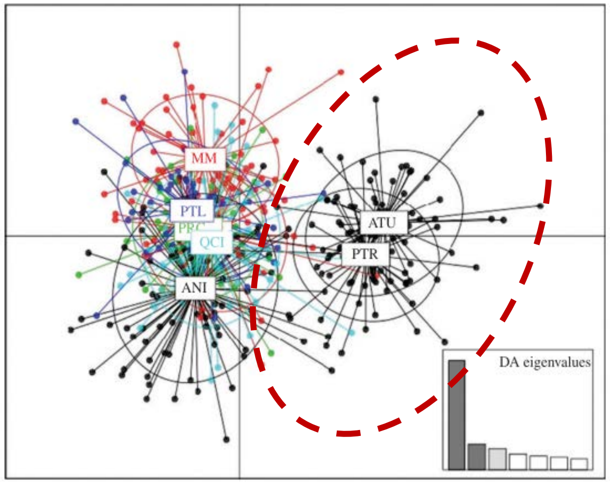
5. Genetics and Genomics

Previous Studies

- Nielsen et al. 2010
  - 9 microsatellite loci, n = 228
  - Summer sample collections

- Drinan et al. 2016
  - Dataset 1:
    - 9 microsatellite loci, n = 828
  - Dataset 2:
    - 16 + 32 microsatellite loci, n = 435
  - Winter & summer sample collections





Nielsen, J. L., S. L. Graziano, and A. C. Seitz. 2010. Fine-scale population genetic structure in Alaskan Pacific halibut (*Hippoglossus stenolepis*). Conservation Genetics 11(3):999–1012.

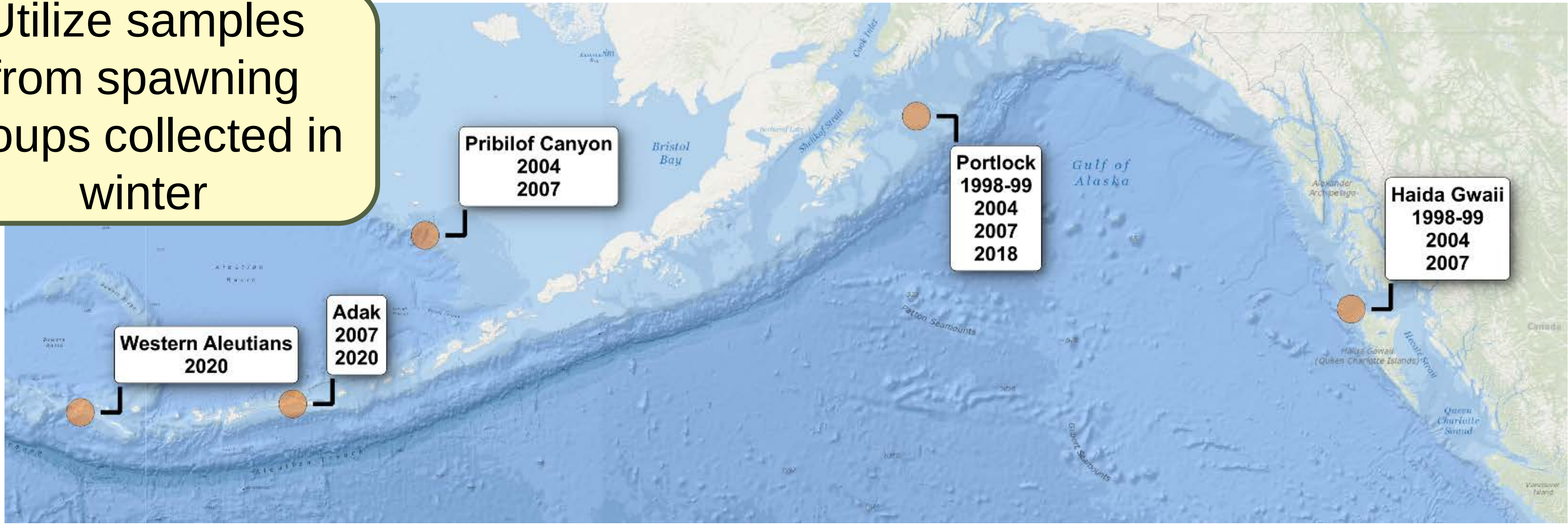
Drinan, D. P., H. M. Galindo, T. Loher, and L. Hauser. 2016. Subtle genetic population structure in Pacific halibut *Hippoglossus stenolepis*. Journal of Fish Biology 89(6):2571–2594.

5. Genetics and Genomics

Revise our understanding of genetic structure of the Pacific halibut population in the North-eastern Pacific Ocean

Analysis of structure in IPHC Regulatory Area 4B

Utilize samples from spawning groups collected in winter



5. Genetics and Genomics

Methods

- Low-coverage whole-genome resequencing
  - Successfully applied to Atlantic cod (Clucas et al. 2019) & Atlantic silverside (Therkildsen et al. 2017) genetic studies
- ~millions of Single Nucleotide Polymorphisms (SNPs)
- ~50 individuals per collection (~600 total)
- 5X sequence coverage target
- Align sequence reads to Pacific halibut genome

Clucas, G. V., R. N. Lou, N. O. Therkildsen, and A. I. Kovach. 2019. Novel signals of adaptive genetic variation in northwestern Atlantic cod revealed by whole-genome sequencing. Evolutionary Applications 12(10):1971–1987.

Therkildsen, N. O., and S. R. Palumbi. 2017. Practical low-coverage genomewide sequencing of hundreds of individually barcoded samples for population and evolutionary genomics in nonmodel species. Molecular Ecology Resources 17(2):194–208.

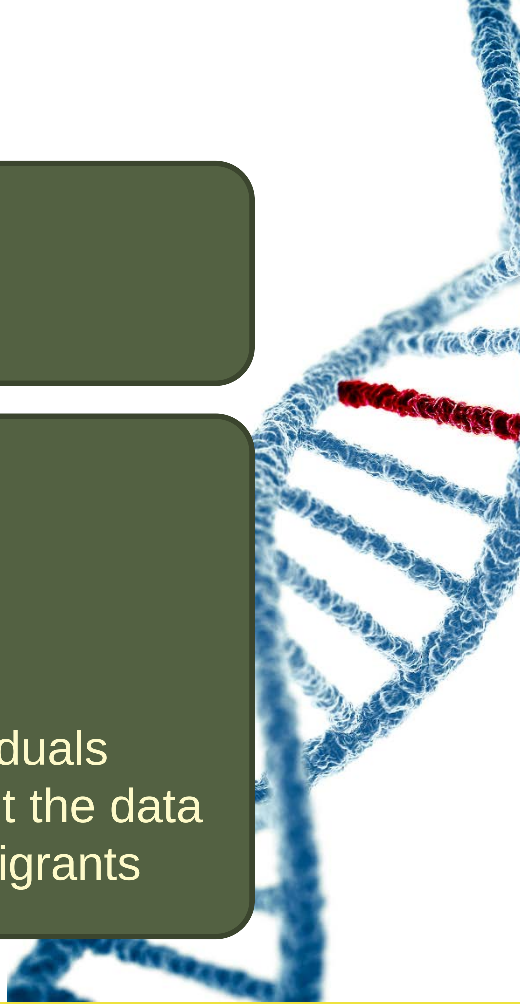
5. Genetics and Genomics

Diversity Metrics

- Allele frequencies
- Hardy-Weinberg equilibrium

Population Structure

- Pairwise genetic distance (F_{ST})
- Isolation by distance
- Clustering
  - Admixture (model based) – estimates ancestry of individuals
  - eg. PCA then K-means – makes few assumptions about the data
- Population assignment testing – Identification of potential migrants



5. Genetics and Genomics

Pacific halibut genome: complete (2020)

- 24 Chromosomes (120 scaffolds total) 586 Mbp
- 98.6 % of assembly in chromosomes
- 23,625 genes identified

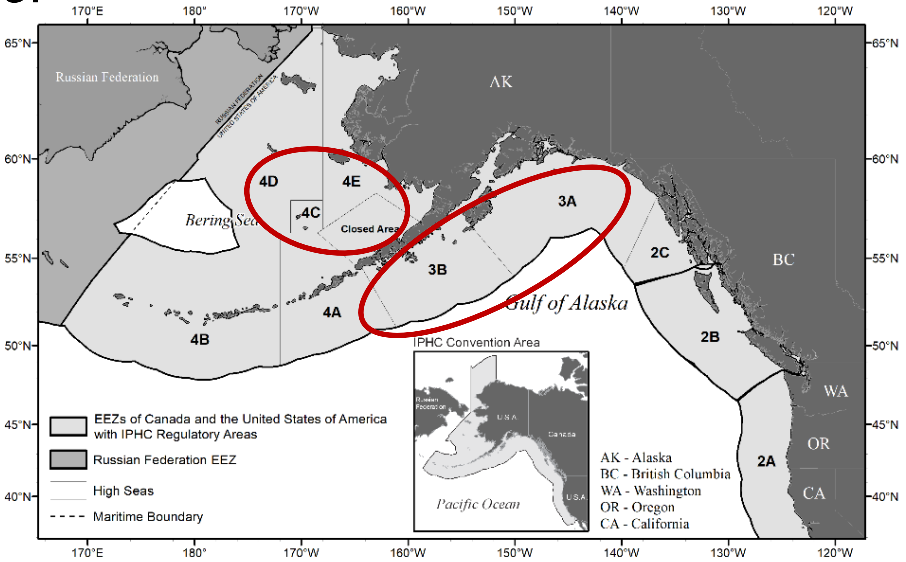
NCBI BioProject: **PRJNA622249**
https://www.ncbi.nlm.nih.gov/bioproject/622249
**annotation has been requested from NCBI*

- Aid the development of SNP panel(s)
  - Population assignment, kinship, etc.

5. Genetics and Genomics

Analysis of genetic variability among juvenile Pacific halibut in the Bering Sea and the Gulf of Alaska

- *Infer the potential contribution of fish spawned in different areas to the Gulf of Alaska (GOA) and Bering Sea (BS)*
- Fin clips collected during NMFS trawl surveys
  - Gulf of Alaska (2015, 2017, 2019)
  - Bering Sea (2015-2019)
- Compare genetic diversity metrics between GOA & BS
- Estimate admixture proportions



Exploratory Analyses

- RAD sequence data from sex marker identification study
  - ~1-5% genome sampled

Table 1 Samples used to identify sex-linked loci in Pacific halibut (*Hippoglossus stenolepis*)

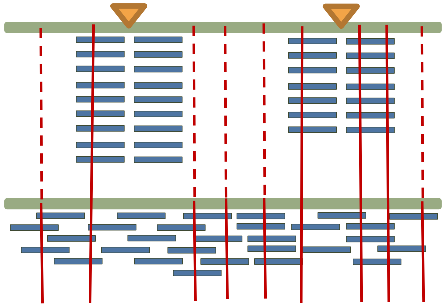
Sampling location	Region	N_f	N_m	Sampling year
Adak Island	Aleutian Islands	9	9	2007
Attu Island	Aleutian Islands	12	7	2003
Petrel Bank	Aleutian Islands	14	5	2003
Pribilof Canyon	Bering Sea	10	10	2004
Haida Gwaii	British Columbia	10	9	2004
Total		**55**	**40**	

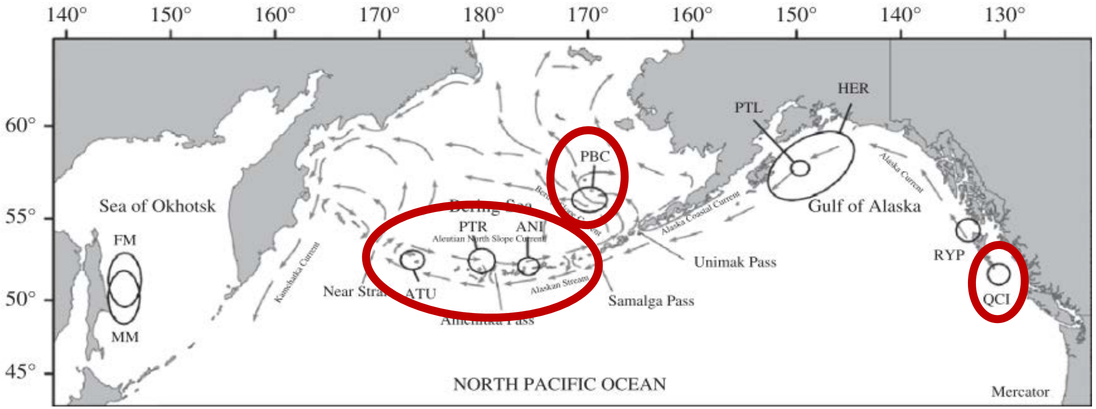
All samples collected during winter except for Attu Island & Petrel Bank

RAD-seq

vs

low-coverage whole-genome



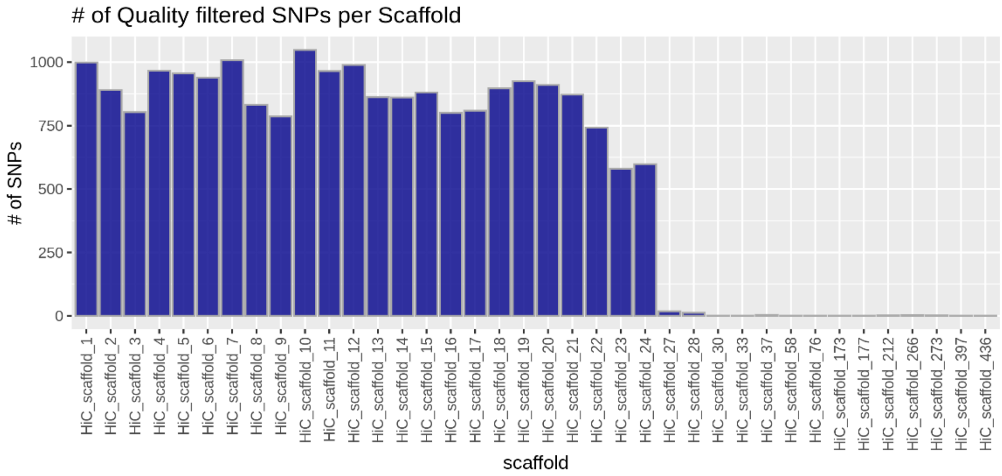


Drinan, D. P., Loher, T., & Hauser, L. (2018). Identification of Genomic Regions Associated With Sex in Pacific Halibut. *Journal of Heredity*, *109*(3), 326–332.

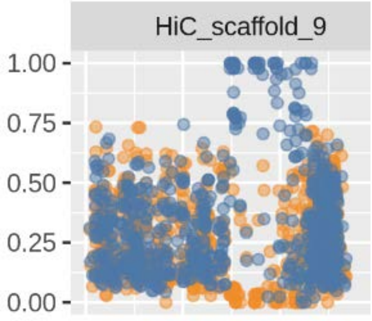
Exploratory Analyses: SNP Dataset

- Quality Filtering:
  - Sequencing depth < 5x
  - Heterozygote excess
  - Minor allele frequency < 0.05
  - SNPs: missing data > 30%
  - Individuals: missing data > 50%
    - Kept 85 out of 96
  - Sex linked SNPs: chromosome 9
  - 20,132 SNPs

  - Thinned data so that SNPs > 10,000 bp apart
    - 7,792 SNPs



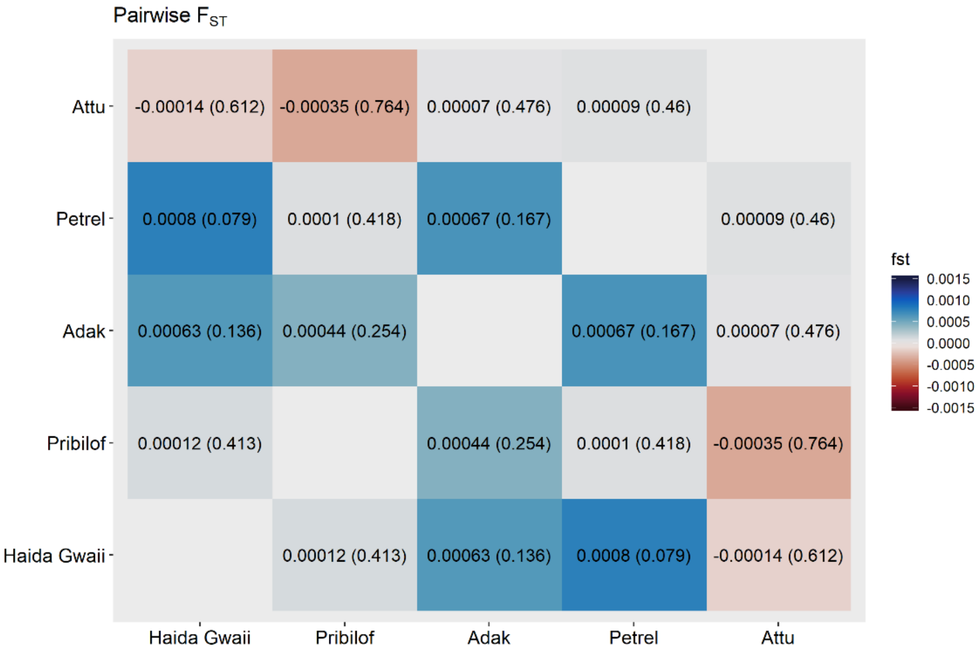
Heterozygosity

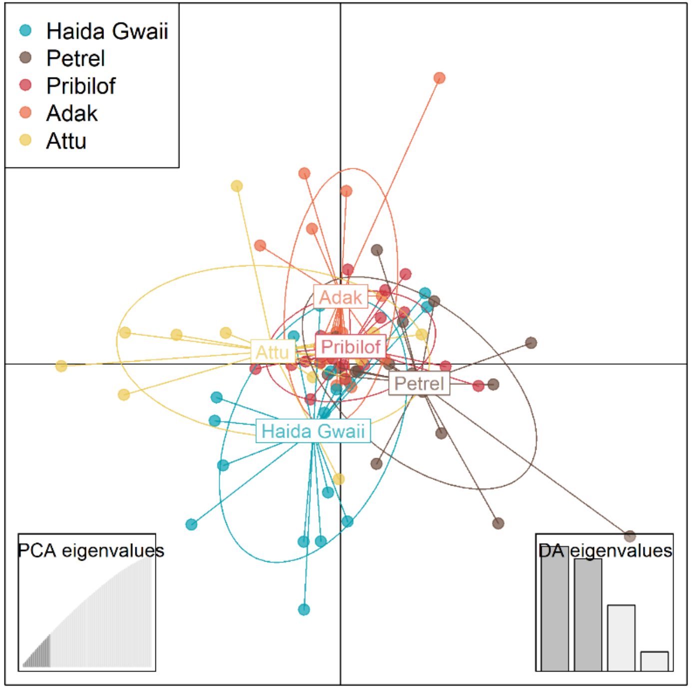


F_{ST} (M vs. F)

Exploratory Analyses: Population Structure

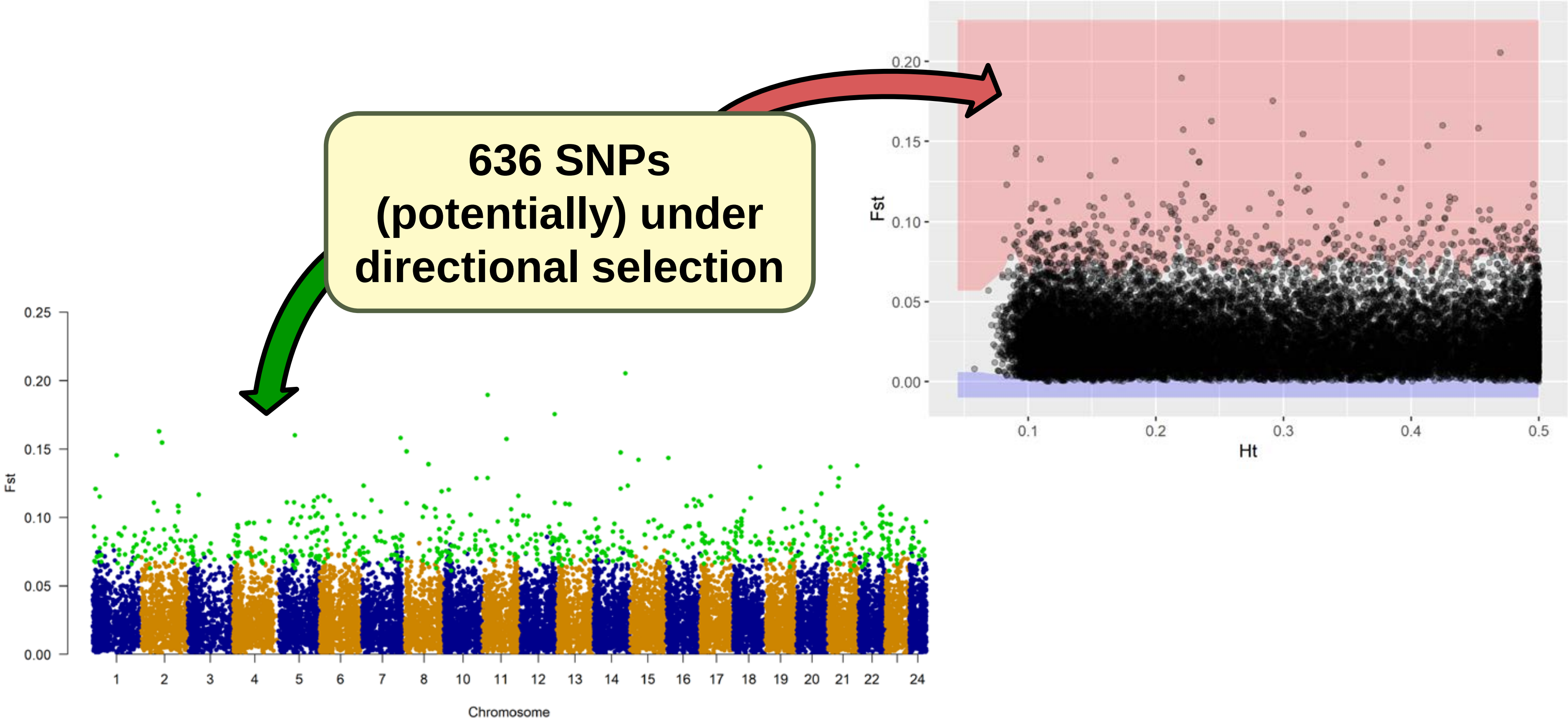
- Thinned dataset: 7,792 SNPs





Exploratory Analyses: SNPs under selection

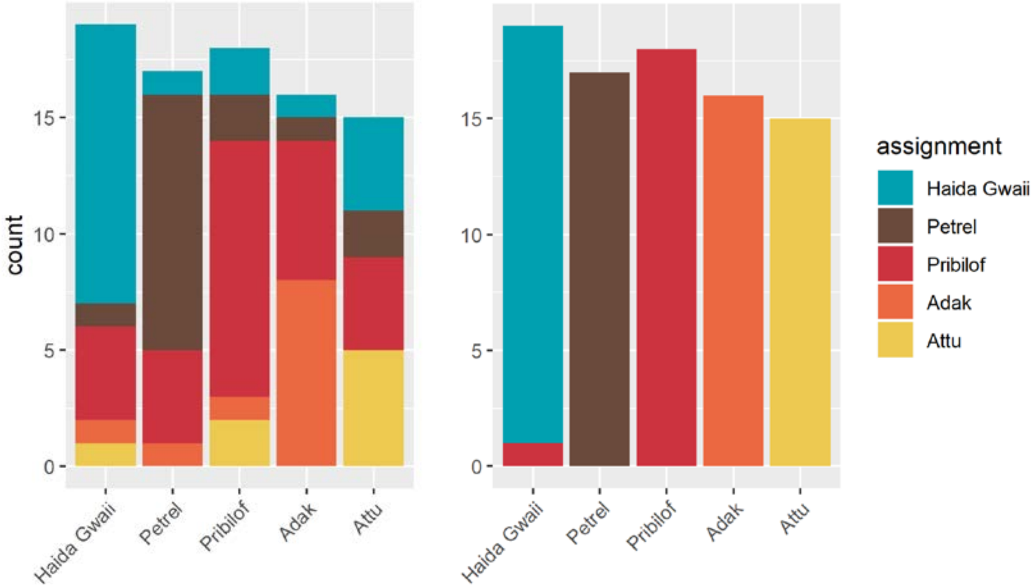
636 SNPs (potentially) under directional selection

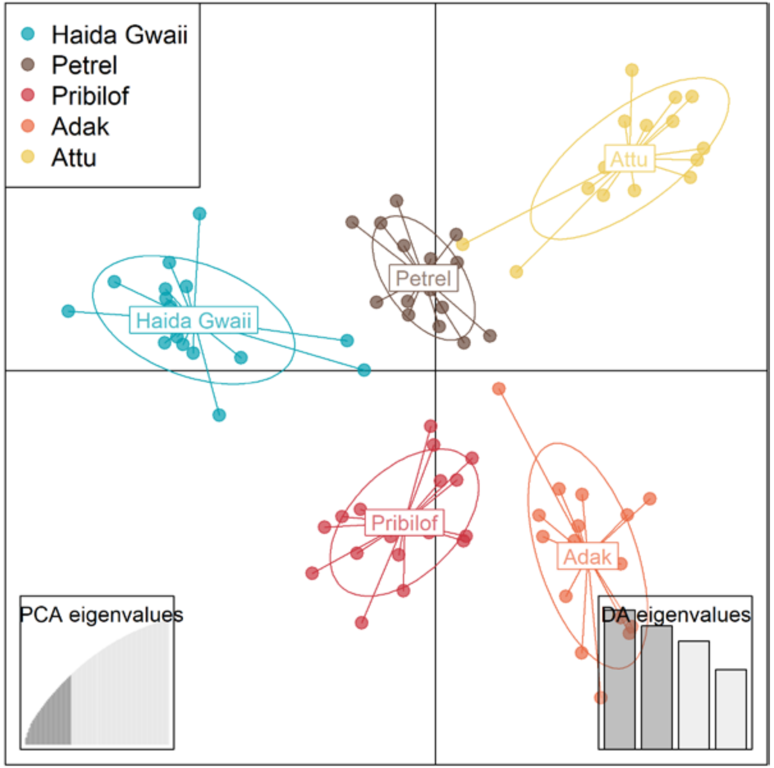


Exploratory Analyses: SNPs under selection

DAPC Population Assignments

7,762 SNPs **SNPs Under Selection**





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