

INTERNATIONAL PACIFIC



HALIBUT COMMISSION

IPHC 5-year Biological and Ecosystem Science Research Plan: Update

Agenda Item 7.3

IPHC-2020-IM096-10

INTERNATIONAL PACIFIC



HALIBUT COMMISSION

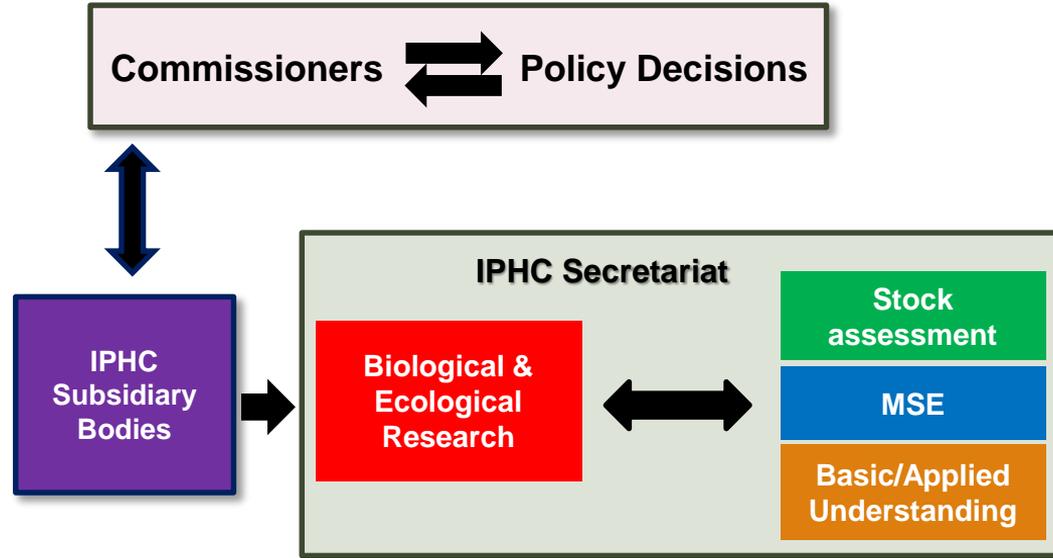
RESEARCH

Outline

- **Five-year biological and ecosystem science research program and management implications**
- **Progress on ongoing research projects**
- **Externally-funded collaborative research projects**



Integration of biological research, stock assessment, and policy



Five-year research program and management implications (2017-2021)

5-Year Biological and Ecosystem Science Research Plan

<i>Primary Research Areas</i>	<i>Main Objectives</i>	<i>Management implications</i>
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

Next 5-Year Research Plan (2021-26) in development



Outline

- Five-year research program and management implications
- **Progress on ongoing research projects**
 1. **Migration and Distribution**
 2. **Reproduction**
 3. **Growth**
 4. **DMRs and Survival Assessment**
 5. **Genetics and Genomics**



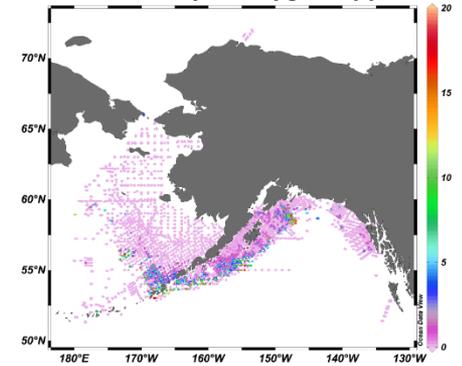
1. Migration and Distribution

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.

Percentage of model larvae reaching the **Bering Sea** based on IBM:

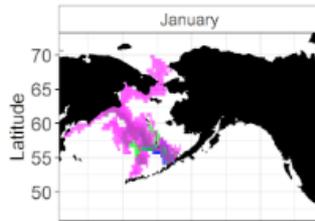
Spawn region	Year					
	Warm			Cold		
	2003	2004	2005	2009	2010	2011
BS 1	100	100	100	100	100	100
GOA 2	58.0	51.1	58.1	52.7	51.5	47.0
GOA 3	17.6	19.3	15.2	17.2	17.2	20.5
GOA 4	8.6	4.5	8.2	4.5	7.0	6.5
GOA 5	0.2	0.04	0.6	0.08	1.6	0.04



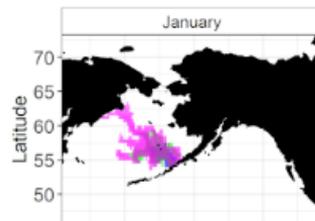
1. Migration and Distribution

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
 - Large degree of intra-basin connectivity



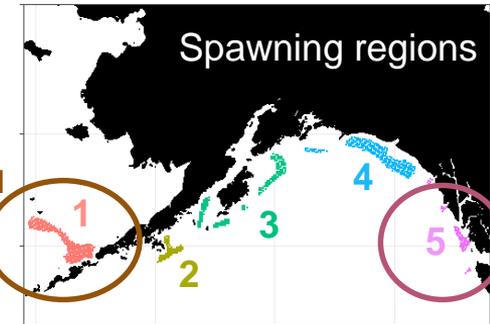
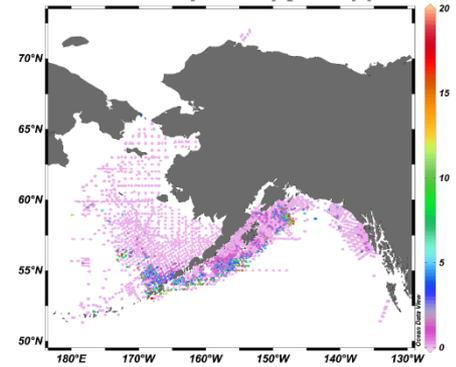
2005



2009

Origin – spawning region 1

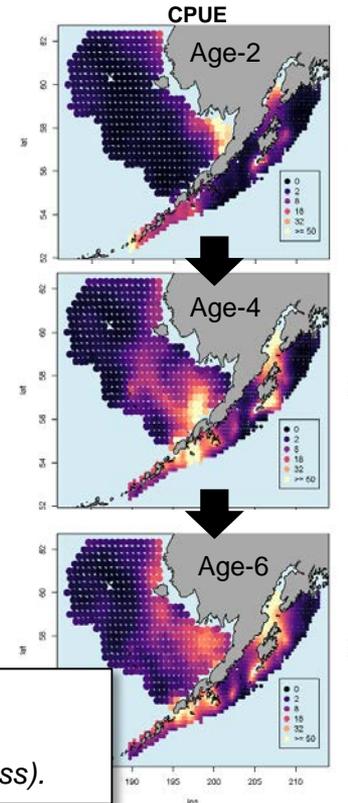
- 1 month post-spawn
- 3 months post-spawn
- 5 months post-spawn



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.
 - 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



Sadorus, L. L., Goldstein, E., Webster, R. A., Stockhausen, W. T., Planas, J. V., and Duffy-Anderson, J. 2020. **Multiple life-stage connectivity of Pacific halibut (*Hippoglossus stenolepis*) across the Bering Sea and Gulf of Alaska.** *Fisheries Oceanography*. (In Press).



2. Reproduction

1. Identification of sex in historical commercial landings

DNA Extraction from Archived Otoliths: Current Progress

<u>Storage Type</u>	<u>n</u>	<u># Successful Genotypes</u>
Dry	7	7
Glycerin	10	0

- Extractions via Qiagen column kits w/ DTT added, low elution volume
- PCR performed w/ BSA, extended cycle number
- No nanodrop signature present for glycerin-stored samples

Other potential issues:

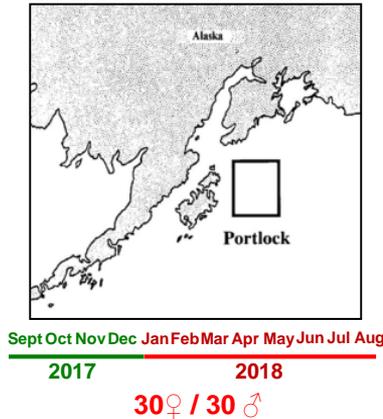
- All otoliths collected prior to 2003 stored in glycerin in batches, not individually
- Glycerin solution sometimes reused
- Some otoliths cleaned in muriatic acid



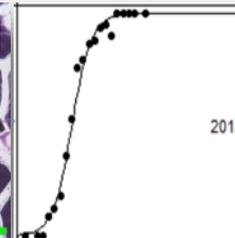
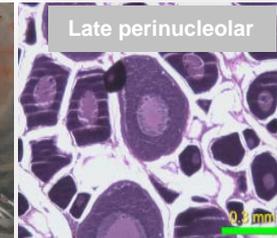
2. Reproduction

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

Objective: Revise maturity estimates for male and female Pacific halibut



Reproductive cycle



Deliverables:

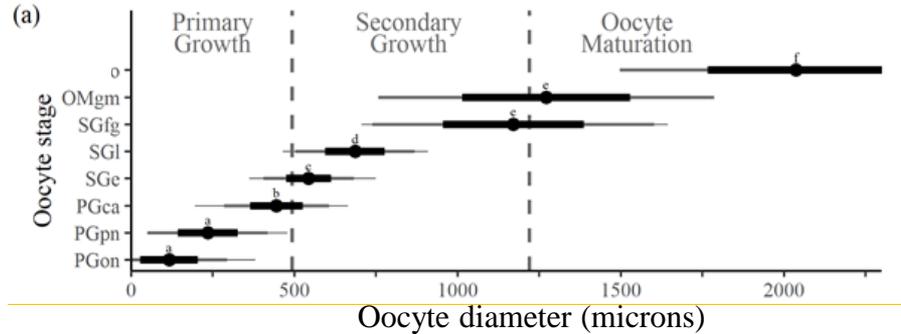
- Accurate staging of reproductive status
- Updated maturity-at-age estimates
- Information on fecundity and skip-spawning



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 (SGl)	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.	
	Periovitulatory (OMpo)	Nucleus no longer visible and the yolk globules coalesce into a central yolk mass. Oocyte is still within the follicle wall.	



Female reproductive phase determined based on the stage of the most advanced oocytes

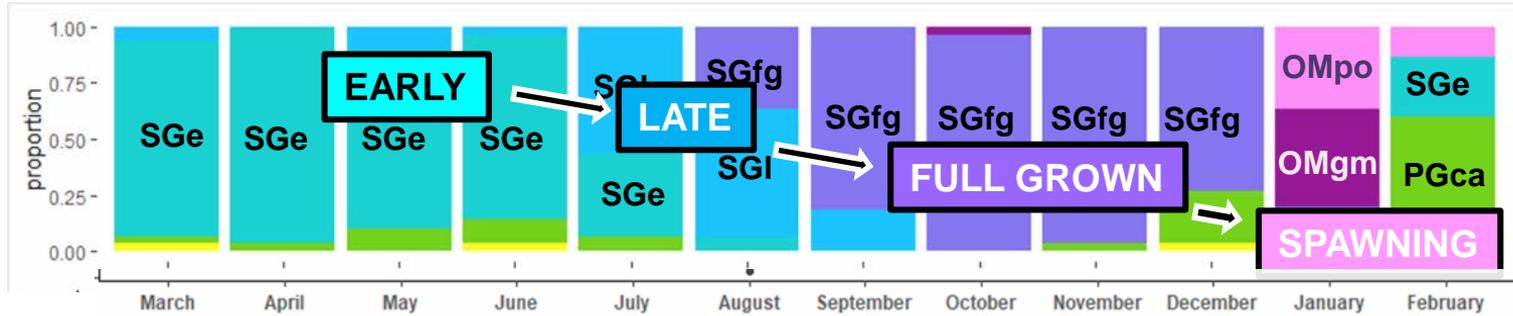
- Pacific halibut is a batch-spawner with a group-synchronous ovarian developmental pattern

Fish, T., Wolf, N., Harris, B. P., and Planas, J. V. 2020. A comprehensive description of oocyte developmental stages in Pacific halibut, *Hippoglossus stenolepis*. *Journal of Fish Biology*. (In Press).

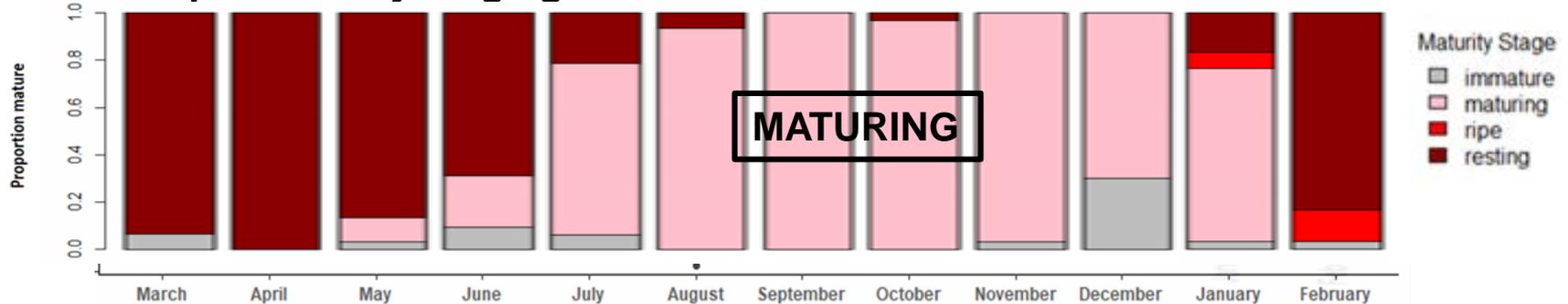


2. Reproduction

Microscopic maturity staging: based on histological oocyte stages

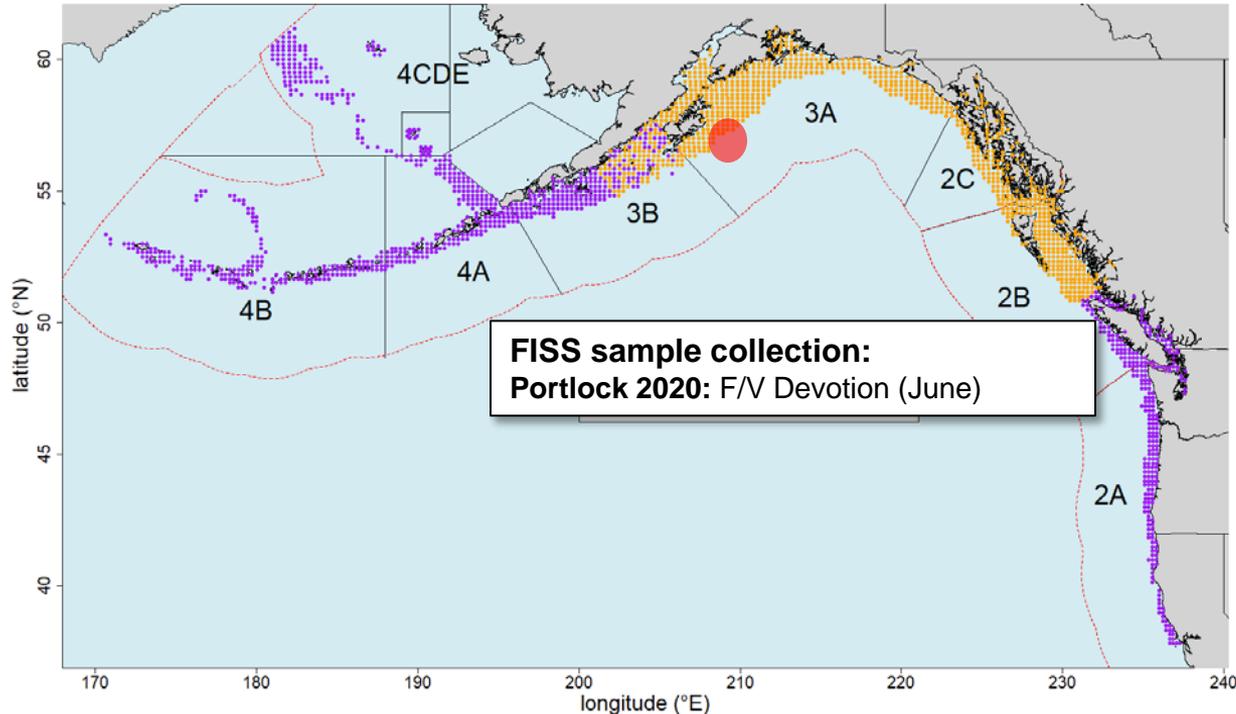


Macroscopic maturity staging: based on field visual observation

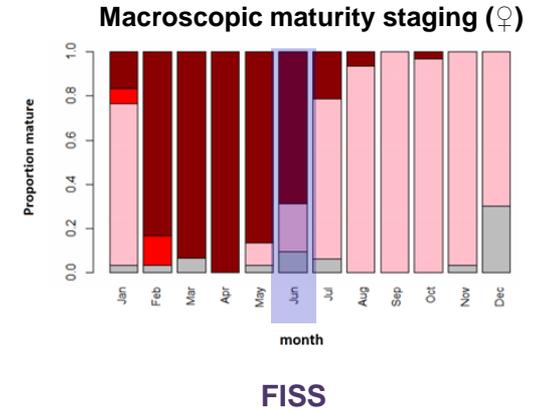


2. Reproduction

2017-2020: Temporal analysis of maturity (Portlock region)



- Full annual collection (2018)

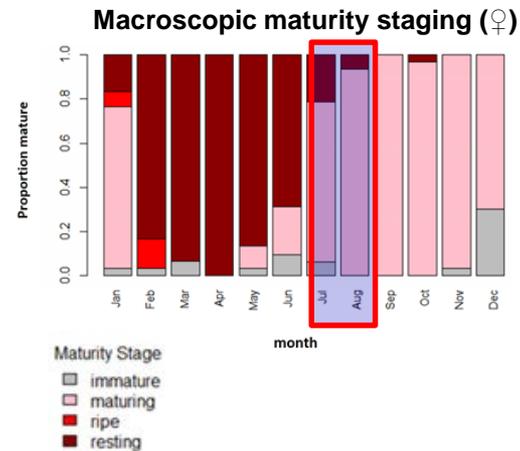
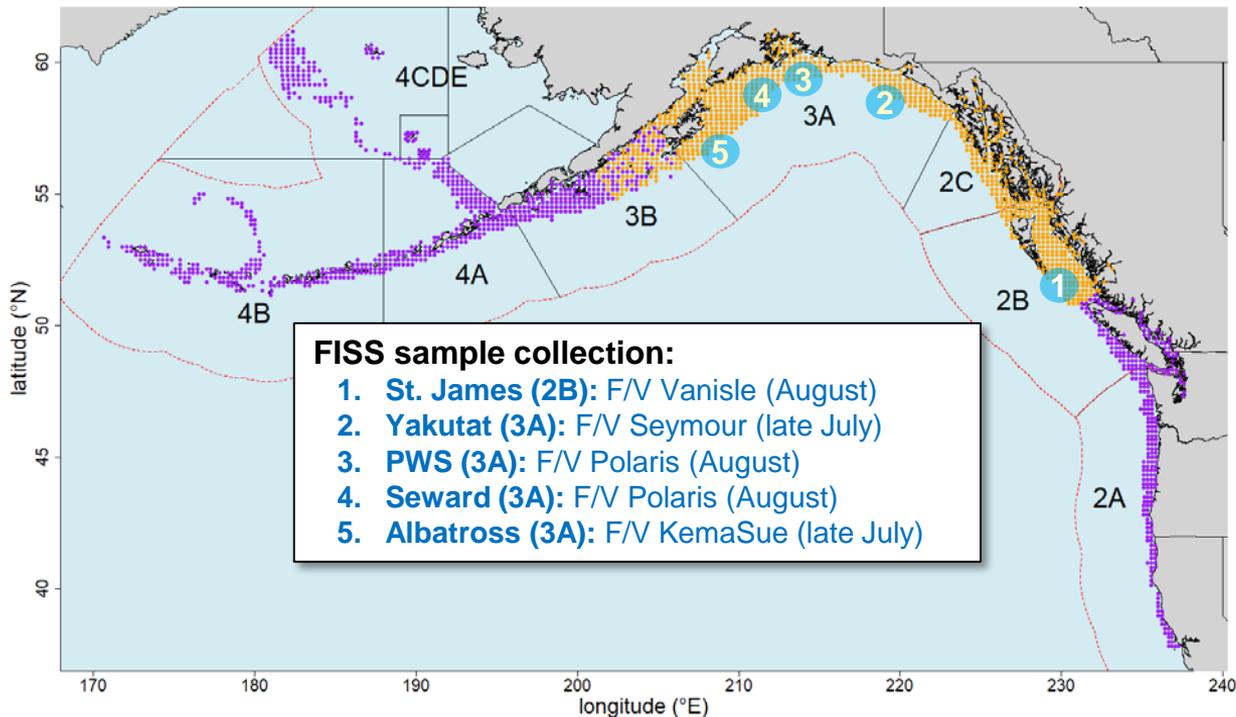


- Interannual collection
June 2017, 2018, 2019, **2020**



2. Reproduction

2020: Spatial analysis of maturity (Gulf of Alaska)

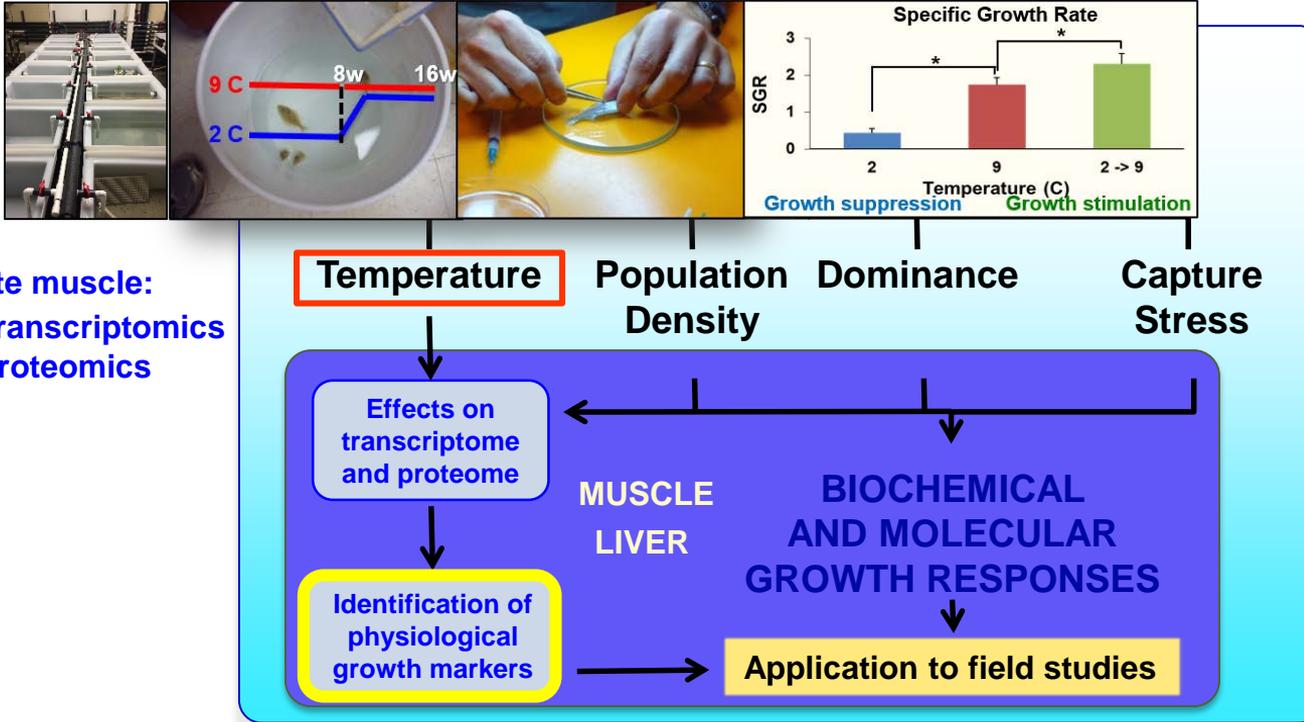


- July-August collection in FISS:
75-90% maturing
(30 females/region)



3. Growth

1. Identification and validation of physiological markers for growth



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

Arginine--tRNA ligase, cytoplasmic OS=Rattus norvegicus
Asparagine synthetase [glutamine-hydrolyzing] OS=Rattus norvegicus
 ATP-binding cassette sub-family E member 1 OS=Mus musculus GN=Cpa5 PE=1 SV=1
 Carboxypeptidase A5 OS=Mus musculus GN=Cpa5 PE=1 SV=1
 Collagen alpha-1(V) chain OS=Mus musculus GN=Col5a1 PE=1 SV=1
 Collagen alpha-2(I) chain (Fragments) OS=Gallus gallus GN=Col1a2 PE=1 SV=1
 Collagen alpha-6(VI) chain OS=Homo sapiens GN=COL6A3 PE=1 SV=1
 Coronin-1A OS=Homo sapiens GN=CORO1A PE=1 SV=4
 Elongation factor 1-delta OS=Xenopus laevis GN=eef1d1 PE=1 SV=1
 Eukaryotic translation initiation factor 2 subunit 2 OS=Mus musculus GN=EIF2B2 PE=1 SV=1
 Eukaryotic translation initiation factor 3 subunit J-A OS=Mus musculus GN=EIF3J PE=1 SV=1
 Eukaryotic translation initiation factor 4 gamma 2 OS=Mus musculus GN=EIF4G2 PE=1 SV=1
 Glycine--tRNA ligase OS=Homo sapiens GN=GARS PE=1 SV=1
 Heat shock 70 kDa protein 4 OS=Canis lupus familiaris GN=HSP70 PE=1 SV=1
 Heat shock protein beta-11 OS=Danio rerio GN=hspb11 PE=1 SV=1
 Histone-lysine N-methyltransferase SETD7 OS=Danio rerio GN=SETD7 PE=1 SV=1
 Importin-13 OS=Pongo abelii GN=IPO13 PE=2 SV=1
 Influenza virus NS1A-binding protein homolog A OS=Danio rerio GN=NS1A PE=1 SV=1
 Kelch-like protein 10 OS=Homo sapiens GN=KLHL10 PE=1 SV=1
 Myozenin-2 OS=Pongo abelii GN=MYOZ2 PE=2 SV=1
 N-alpha-acetyltransferase 38, Nac auxiliary subunit C OS=Homo sapiens GN=NAAC38 PE=1 SV=1
Ornithine carbamoyltransferase, mitochondrial OS=Homo sapiens
 Peptidyl-prolyl cis-trans isomerase FKBP7 OS=Mus musculus GN=FKBP7 PE=1 SV=1
 Phenylalanine--tRNA ligase alpha subunit OS=Danio rerio GN=PAAC PE=1 SV=1
 Phosphoserine aminotransferase OS=Mus musculus GN=PSAT1 PE=1 SV=1
 Protein BCCIP homolog OS=Danio rerio GN=bccip PE=2 SV=1
 Troponin I, fast skeletal muscle OS=Oryctolagus cuniculus GN=TPNI PE=1 SV=1
Ubiquitin carboxyl-terminal hydrolase isozyme L1 OS=Homo sapiens
 Unconventional myosin-VI OS=Homo sapiens GN=MYOVI PE=1 SV=1

↓ Growth Markers

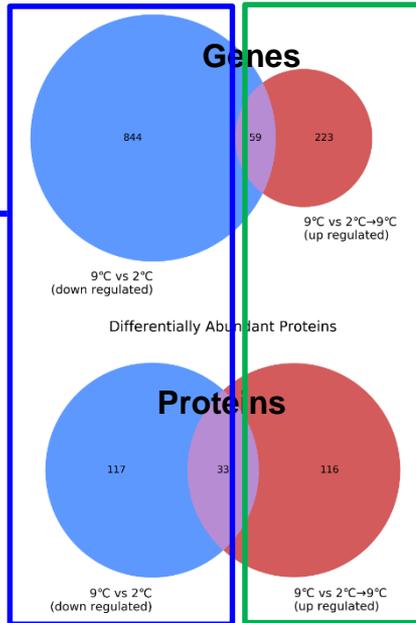
Decreased growth rate



Increased growth rate



Differentially Expressed Genes



- Identify common genes and proteins in muscle that change with changes in growth rate

↑ Growth Markers

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 OS=Homo sapiens GN=IGFBP3 PE=1 SV=1
 Leucine-rich repeat-containing protein 2 OS=Homo sapiens GN=LRRC2 PE=2 SV=1
 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-22 PE=1 SV=1
 Titin OS=Homo sapiens GN=TTN PE=1 SV=4
Ubiquitin carboxyl-terminal hydrolase 25 OS=Homo sapiens GN=USP25 PE=1 SV=1



3. Growth

Physiological
growth markers



Application to field studies

1. Identification and 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



Slow growth rate?

Fast growth rate?



4. DMRs and Survival Assessment

1. Directed longline fishery: 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



- Water temperature loggers



- Fish temperature



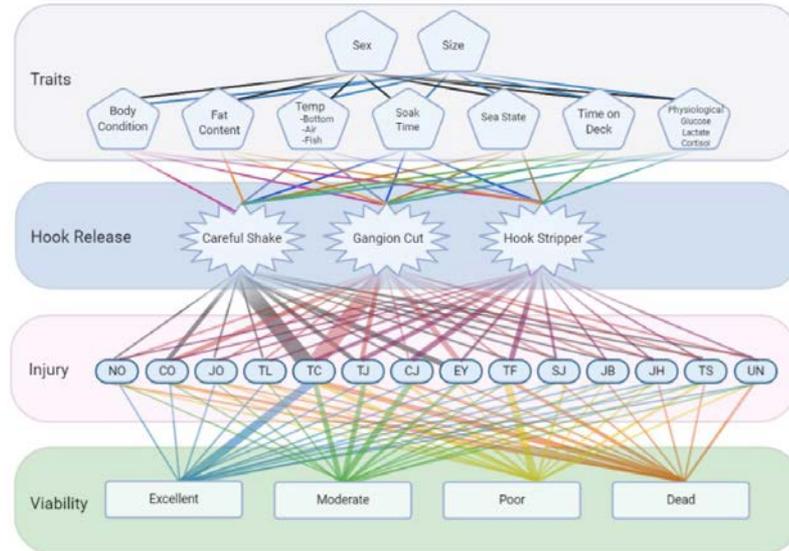
NOAA FISHERIES Saltonstall – Kennedy Grant NA17NMF4270240



4. DMRs and Survival Assessment

1. Directed longline fishery: 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)

- Reg. Area 2C: Sitka (n=16), Juneau (n=8)
- Reg. Area 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

 UNIVERSITY OF ALASKA FAIRBANKS

 ALASKA PACIFIC UNIVERSITY

 ACA Alaska Chapter 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



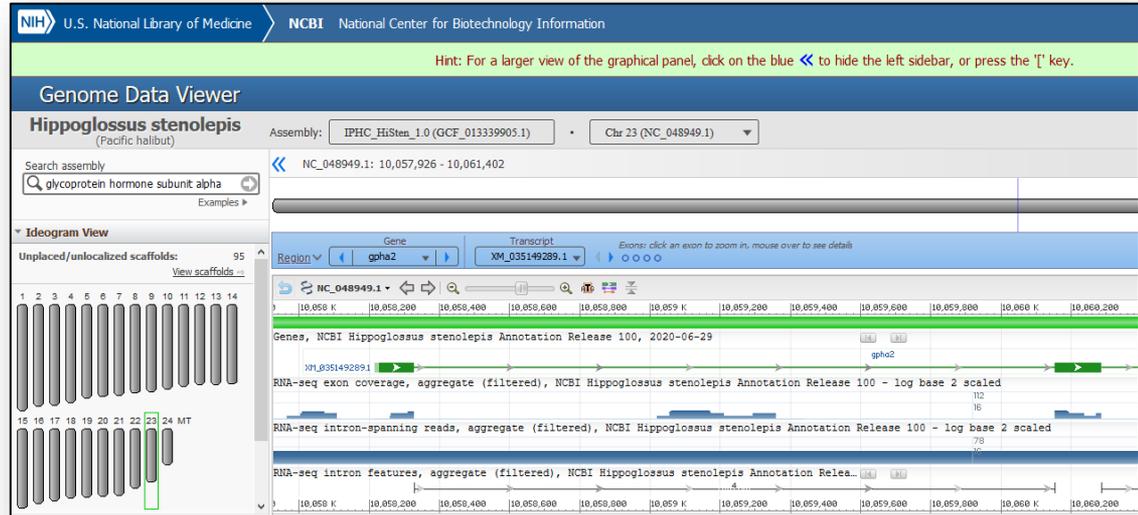
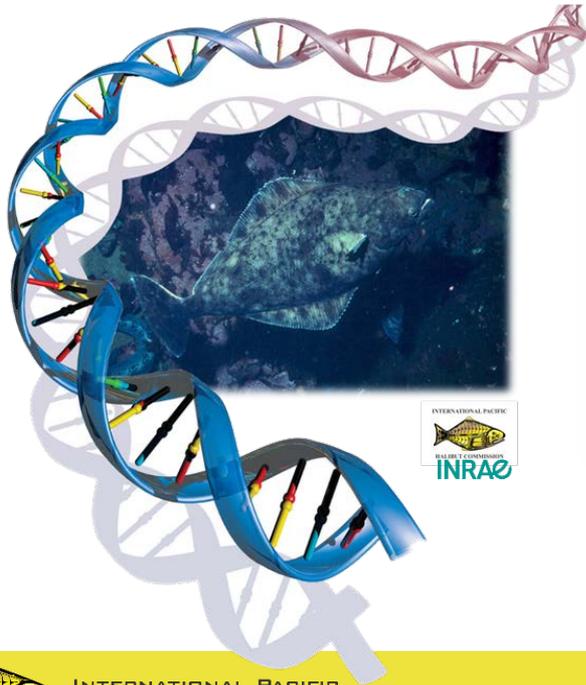
Hook injury assessment



5. Genetics and Genomics

Completed sequence of the Pacific halibut genome

- Size: 594 million base pairs
- 24 chromosomes
- 27,422 genes
- 91 X coverage



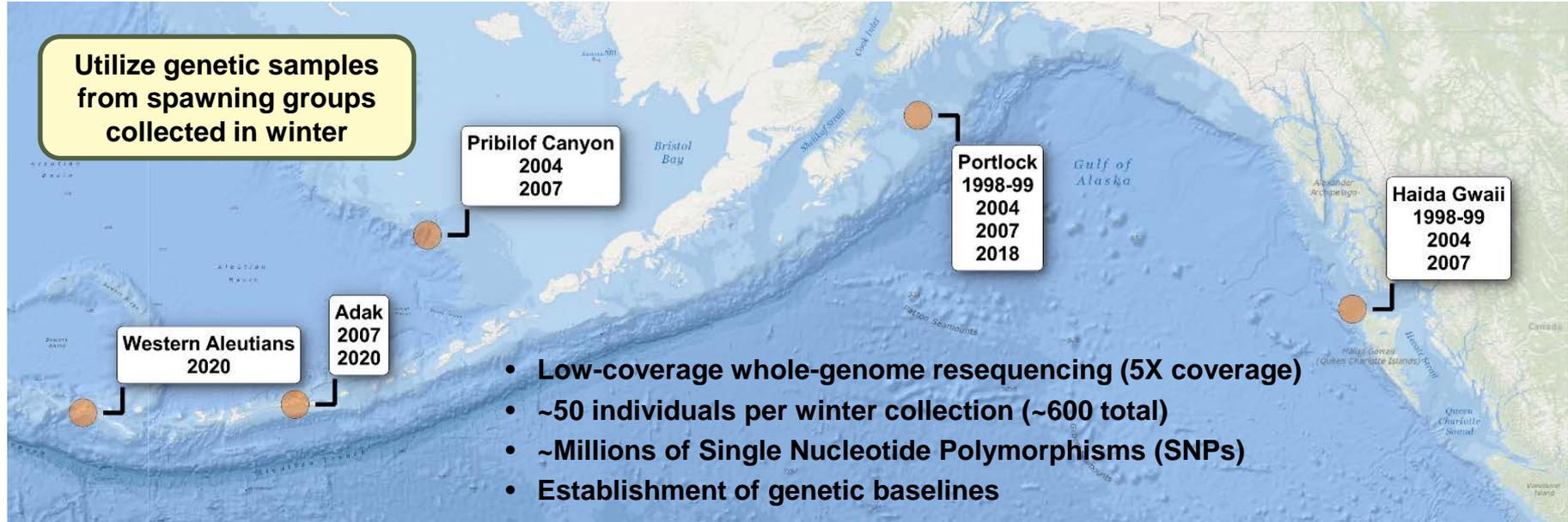
https://www.ncbi.nlm.nih.gov/assembly/GCA_013339905.1



5. Genetics and Genomics

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

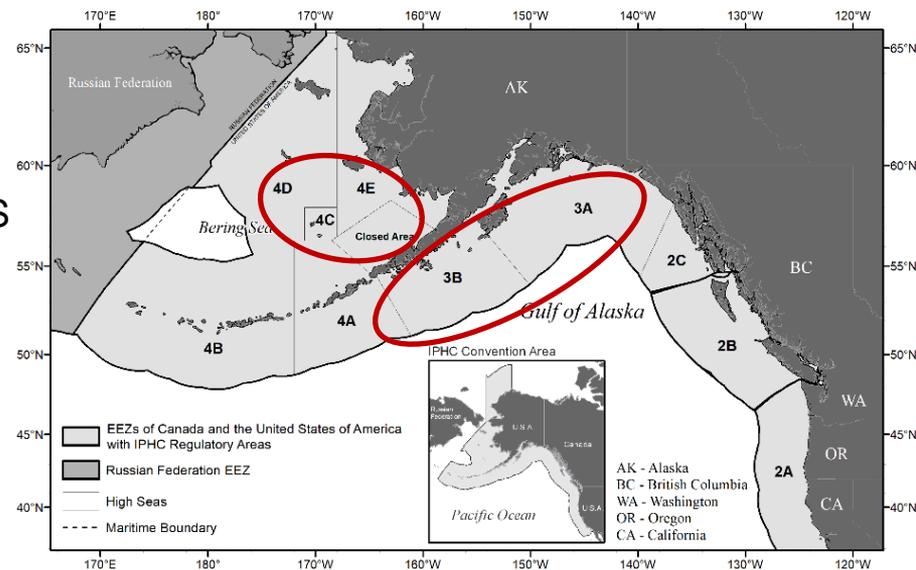
Analysis of structure in IPHC Regulatory Area 4B



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



Outline

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

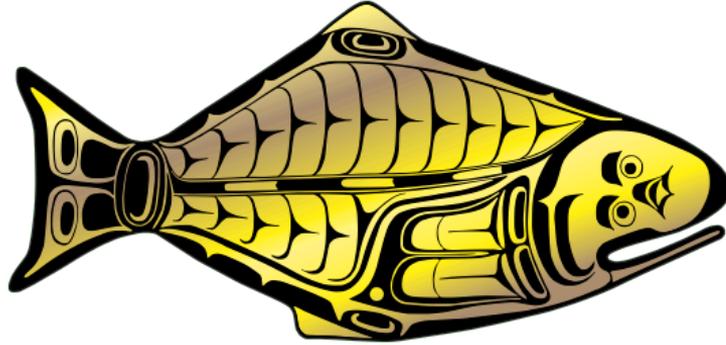


Externally-funded collaborative research

Project #	Grant agency	Project name	PI	Partners	IPHC Budget (\$US)	Management implications	Grant period
1	Saltonstall-Kennedy NOAA	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival (Award No. NA17NMF4270240)	IPHC	Alaska Pacific University	\$286,121	Discard estimates	September 2017 – August 2020
2	North Pacific Research Board	Somatic growth processes in the Pacific halibut (<i>Hippoglossus stenolepis</i>) and their response to temperature, density and stress manipulation effects (NPRB Award No. 1704)	IPHC	AFSC-NOAA-Newport, OR	\$131,891	Changes in biomass/size-at-age	September 2017 – February 2020
3	National Fish and Wildlife Foundation	Discard mortality rate characterization in the Pacific halibut recreational fishery (NFWF Award No. 61484)	IPHC	UA Fairbanks, APU, Grey Light Fisheries, Alaska Charter Association	\$98,901	Discard estimates	April 2019 - June 2021
Total awarded (\$)					\$516,913		



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