L. Boitor

INTERNATIONAL PACIFIC HALIBUT COMMISSION

Biological and Ecosystem Science Research Updates

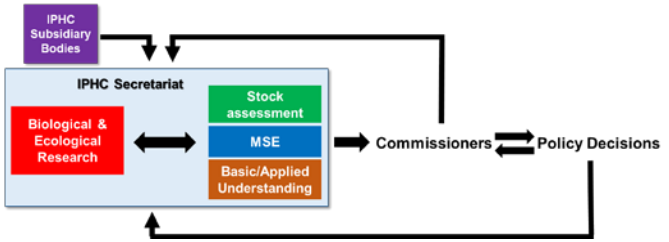
Agenda Item 6

IPHC-2020-SRB017-08

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

SRB016–Req.17 (para. 44)	***Research integration*** The SRB **REQUESTED** an updated presentation on the plan and timelines for integrating research and results from biological and ecosystem science research plan into specific functions and parameters of the assessment and MSE.	**In Progress**: The IPHC Secretariat is updating the plan and timelines of the integration between research activities and stock assessment and MSE needs. This information will be presented at the SRB017

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

Integration of research, SA and MSE

Research areas	Research activities	Research outcomes	Relevance for stock assessment	Relevance for MSE	Specific analysis input
Migration	Larval and juvenile connectivity studies	Improved understanding of larval and juvenile distribution	Improve estimates of productivity	Improve parametization of the Operating Model	Will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region.
Reproduction	Histological maturity assessment	Updated maturity schedule	Scale biomass and reference point estimates	Improve simulation of spawning biomass in the Operating Model	Will be included in the stock assessment, replacing the current schedule last updated in 2006.
	Examination of potential skip spawning	Incidence of skip spawning			Will be used to adjust the asymptote of the maturity schedule, if/when a time-series is available this will be used as a direct input to the stock assessment.
	Fecundity assessment	Fecundity-at-age and -size information			Will be used to move from spawning biomass to egg-output as the metric of reproductive capability in the stock assessment and management reference points.
	Examination of accuracy of current field macroscopic maturity classification	Revised field maturity classification			Revised time-series of historical (and future) maturity for input to the stock assessment.
	Sex ratio of current commercial landings	Sex ratio-at-age	Scale biomass and fishing intensity		Annual sex-ratio at age for the commercial fishery fit by the stock assessment.
	Historical sex ratios based on archived otolith DNA analyses	Historical sex ratio-at-age			Annual sex-ratio at age for the commercial fishery fit by the stock assessment
	Recruitment strength and variability	Establishment of temporal and spatial maturity and spawning patterns	Improve stock-recruitment curve for more precise assessment	Improve simulation of recruitment variability and parametization of recruitment distribution in the Operating Model	May be used to provide a weighted spawning biomass calculation and or inform targets for minimum spawning biomass by Biological Region
Growth	Evaluation of somatic growth variation as a driver for changes in size-at-age	Identification and application of markers for growth pattern evaluation	Scale stock productivity and reference point estimates	Improve simulation of variability and allow for scenarios investigating climate change	May inform yield-per-recruit and other spatial evaluations of productivity that support mortality limit-setting.
		Environmental influences on growth patterns			May provide covariates for projecting short-term size-at-age. May help to delineate between effects due to fishing and those due to environment, thereby informing appropriate management response.
		Dietary influences on growth patterns and physiological condition			May provide covariates for projecting short-term size-at-age. May help to deleineate between effects due to fishing and those due to environment, thereby informing appropriate management response.

Integration of research, SA and MSE

Research areas	Research activities	Research outcomes	Relevance for stock assessment	Relevance for MSE	Specific analysis input
Mortality and survival assessment	Discard mortality rate estimate: longline fishery	Experimentally-derived DMR	Improve trends in unobserved mortality	Improve estimates of stock productivity	Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits.
	Discard mortality rate estimate: recreational fishery	Experimentally-derived DMR			Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits.
	Best handling practices: longline fishery	Guidelines for reducing discard mortality			May reduce discard mortality, thereby increasing available yield for directed fisheries.
	Best handling practices: recreational fishery	Guidelines for reducing discard mortality			May reduce discard mortality, thereby increasing available yield for directed fisheries.
	Whale depredation accounting and tools for avoidance	New tools for fishery avoidance/deterence; improved estimation of depredation mortality	Improve mortality accounting		May reduce depredation mortality, thereby increasing available yield for directed fisheries. May also be included as another explicit source of mortality in the stock assessment and mortality limit setting process depending on the estimated magnitude.
Genetics and genomics	Population structure	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area	Altered structure of future stock assessments		If 4B is found to be functionally isolated, a separate assessment may be constructed for that IPHC Regulatory Area.
	Distribution	Assignment of individuals to source populations and assessment of distribution changes	Improve estimates of productivity	Improve parametization of the Operating Model	Will be used to define management targets for minimum spawning biomass by Biological Region.
	Close-kin mark-recapture studies	Genomic analysis of population size and connectivity			Population size estimates to fit in the stock assessment.
	Landscape genomics	Identification of adaptive loci, decipher genomic basis of adaptation and detect genomic responses to climate change			Will be used to define management targets for minimum spawning biomass by Biological Region.
	Genome-wide association analyses	Understand the genetic basis of phenotypic variation, including size-at-age, age-at-maturity, spawning timing, etc.			May help to delineate between effects due to fishing and those due to environment, thereby informing appropriate management response.

Integration of research, SA and MSE: temporal chart

Research areas	Research activities	Research outcomes	2020				2021				2022				2023				2024			
Migration	Larval and juvenile connectivity studies	Improved understanding of larval and juvenile distribution																				
Reproduction	Histological maturity assessment	Updated maturity schedule								X												
	Examination of potential skip spawning	Incidence of skip spawning								X												
	Fecundity assessment	Fecundity-at-age and -size information														X						
	Examination of accuracy of current field macroscopic maturity classification	Revised field maturity classification								X												
	Sex ratio of current commercial landings	Sex ratio-at-age			X				X				X				X				X	
	Historical sex ratios based on archived otolith DNA analyses	Historical sex ratio-at-age												X								
	Recruitment strength and variability	Establishment of temporal and spatial maturity and spawning patterns																X				
Growth	Evaluation of somatic growth variation as a driver for changes in size-at-age	Identification and application of markers for growth pattern evaluation								X												
		Environmental influences on growth patterns															X					
		Dietary influences on growth patterns and physiological condition															X					

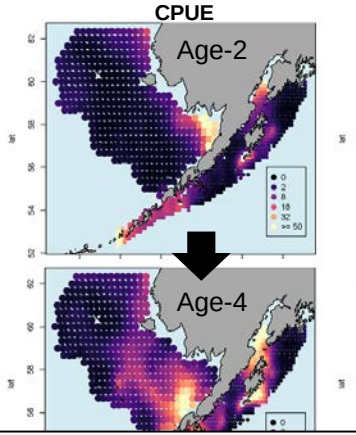
Integration of research, SA and MSE: temporal chart

Research areas	Research activities	Research outcomes	2020				2021				2022				2023				2024			
Discard mortality and survival assessment	Discard mortality rate estimate: longline fishery	Experimentally-derived DMR				X																
	Discard mortality rate estimate: recreational fishery	Experimentally-derived DMR										X										
	Best handling practices: longline fishery	Guidelines for reducing discard mortality						X														
	Best handling practices: recreational fishery	Guidelines for reducing discard mortality												X								
	Whale depredation accounting and tools for avoidance	New tools for fishery avoidance/deterence; improved estimation of depredation mortality												X								
Genetics and genomics	Population structure	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area								X												
	Distribution	Assignment of individuals to source populations and assessment of distribution changes														X						
	Close-kin mark-recapture studies	Genomic analysis of population size and connectivity																				X
	Landscape genomics	Identification of adaptive loci, decipher genomic basis of adaptation and detect genomic responses to climate change																				X
	Genome-wide association analyses	Understand the genetic basis of phenotypic variation, including size-at-age, age-at-maturity, spawning timing, etc.																X				

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



Manuscript currently in (2nd) 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

RB016–Req.11 (para. 36)	***Migration and distribution*** **NOTING** that the genetic data may be complimentary to data collected using other methods, for example, stock structure at the genetic level could be reflected in individual differences in otolith chemistry (if primary otolith annuli are interrogated), the SRB **REQUESTED** that a portion of individuals that are selected for otolith chemistry also be used for whole genome sequencing.	**Pending**: Future planning of studies involving otolith chemistry will incorporate the collection of tissue (fin clip) samples for whole genome sequencing

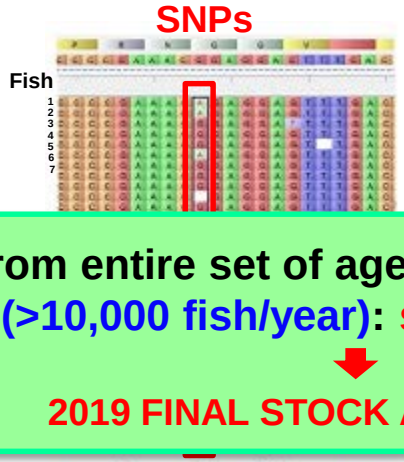
Research projects to investigate larval and early life stage source locations and pelagic duration through otolith geochemical analyses combined with genomic analyses are under consideration

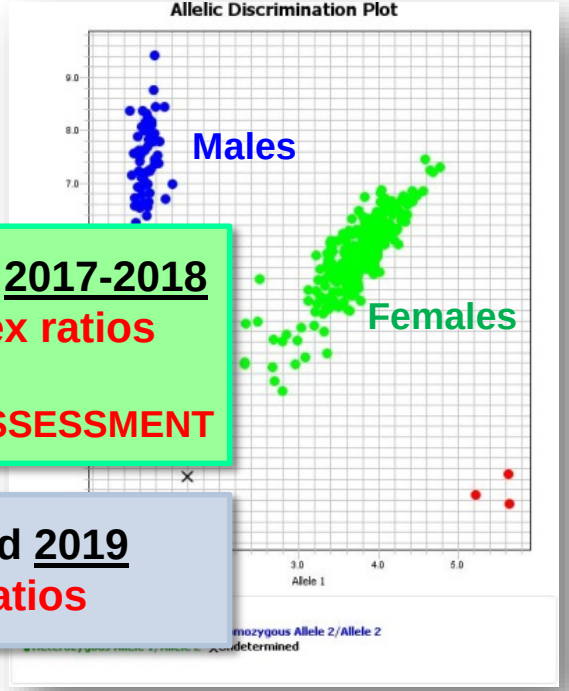
2. Reproduction

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

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

2. Reproduction

1. Identification of sex in the commercial landings

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

SRB016–Req.16 (para. 42)	**NOTING** the importance of genetically determined sex information to stock assessment, the SRB **REQUESTED** that the IPHC Secretariat conduct a pilot study to determine whether DNA and PCR amplification of sex-linked SNP loci can be obtained from archived otoliths of different collection periods to demonstrate feasibility to develop a more comprehensive spatial and temporal sex ratio data base.	**In Progress**: The IPHC Secretariat is conducting studies to determine whether DNA can be extracted from otoliths and whether sex information can be generated. This information will be presented at the SRB017.

2. Reproduction

1. Identification of sex in the commercial landings

DNA Extraction from Archived Otoliths: Current Progress

Storage Type	**n**	**# Successful Genotypes**
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

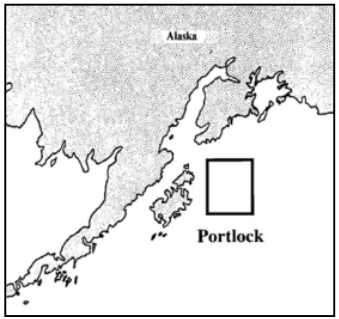
Other potential issues:

- 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



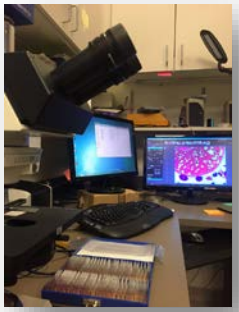
Sept Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug

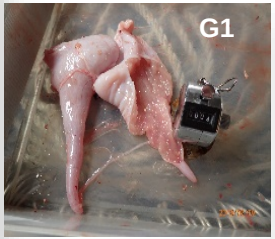
2017 2018

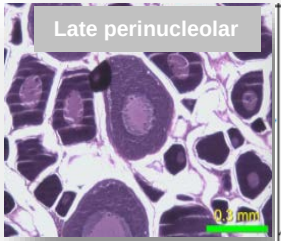
30♀ / 30 ♂

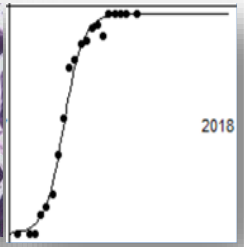
Reproductive cycle

Gonadal growth | Maturation | Spawning









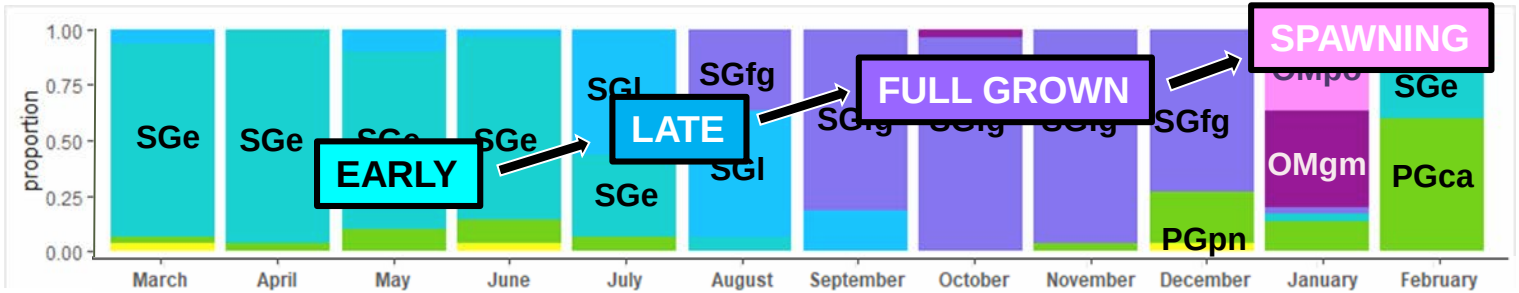
Deliverables:

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

2. Reproduction

RB016–Req.13 (para. 38)	***Reproductive assessment*** The SRB **REQUESTED** a preliminary analysis of existing data on 'skipped spawning'.	**In Progress**: Representative histological characteristics of skipped spawning are being investigated. This information will be presented at the SRB017.

Microscopic maturity staging: based on histological oocyte stages



PGpn Primary Growth Perinucleolar Stage

PGca Primary Growth Cortical alveoli Stage

SGe Secondary Growth Early Stage

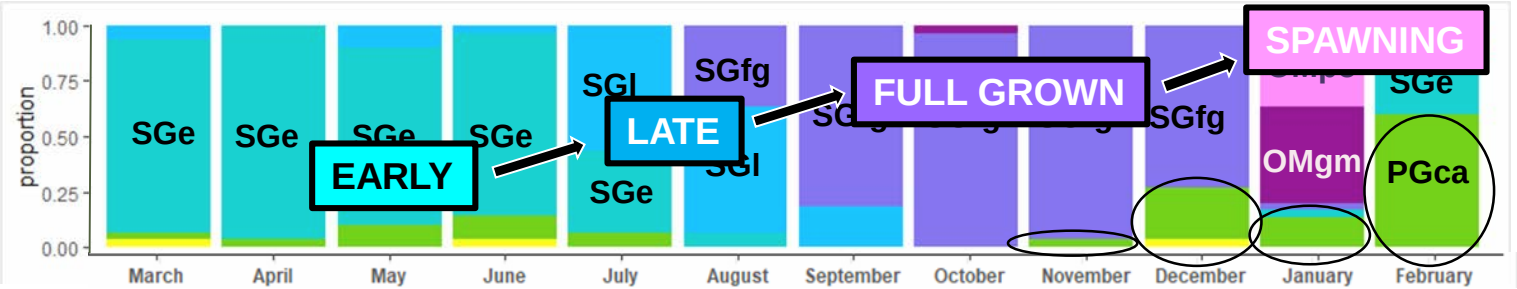
SGl Secondary Growth LateStage

SGfg Secondary Growth Full Grown Stage

OMgm Oocyte maturation Stage

OMpo Postovulatory Stage

2. Reproduction

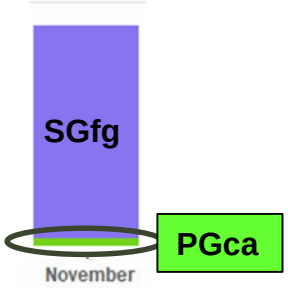


Identification of potential skip-spawners:

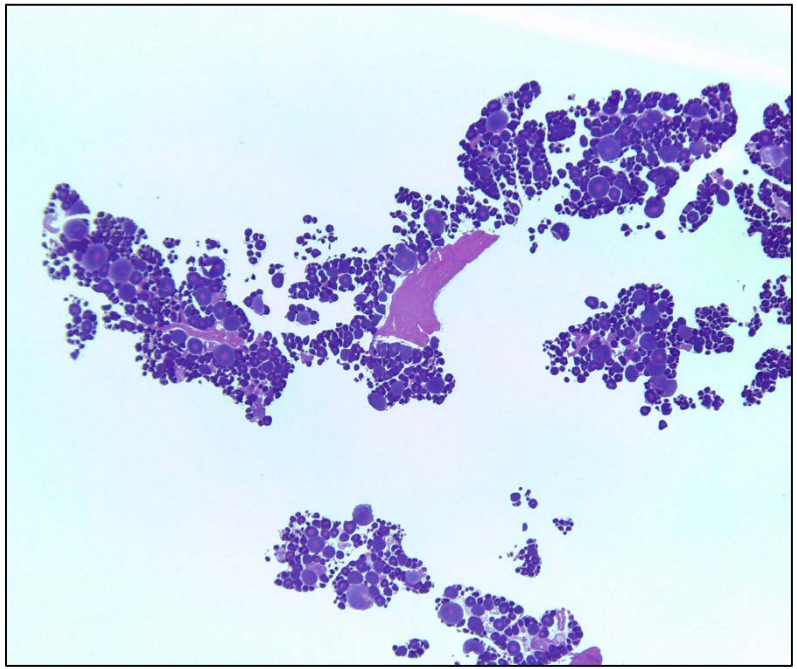
1. Maturity classification prior and during spawning (Nov. – Feb.)
2. Histological examination of aged females at primary growth stages:
   - Presence or absence of post-ovulatory follicles
   - Presence of absence of degenerating follicles
   - General structure of ovarian tissue (compacted versus loose)
3. Examination of additional ovarian parameters:
   - Gonadosomatic index, condition factor, fat content.
   - Endocrine markers in pituitary (luteinizing hormone gene expression) and blood (17β-estradiol and 17α, 20β-dihydroxyprogesterone)

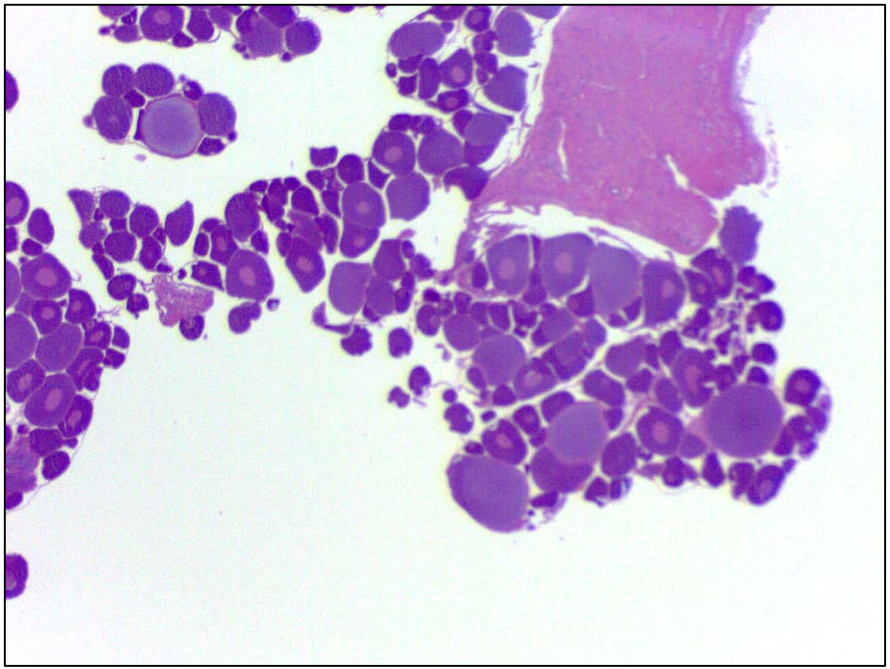
2. Reproduction

Example 1: Potential skip-spawner



Month of collection: November (only female not with full growth vitellogenic oocytes)
Age: 15
Maturity classification: Primary Growth - Cortical Alveoli Stage

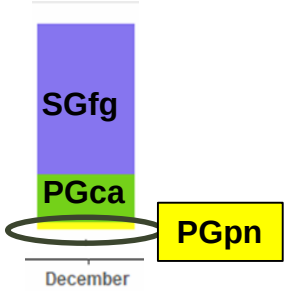


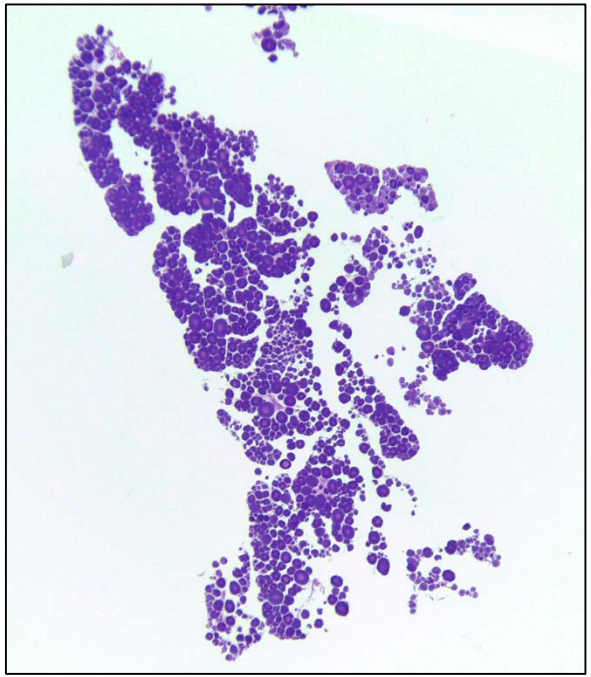


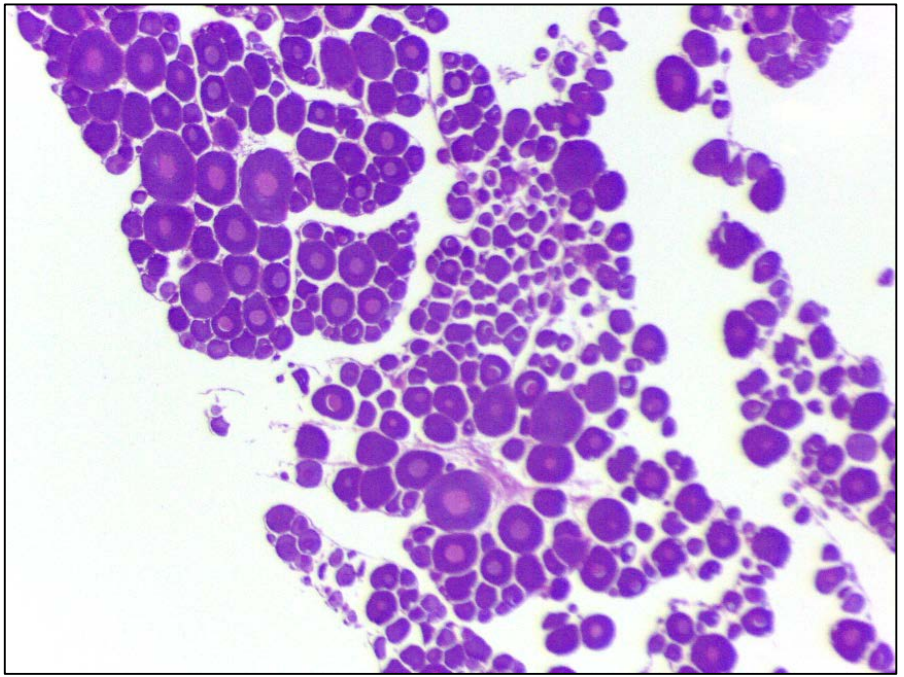
2. Reproduction

Example 2: Immature female during pre-spawning

Month of collection: December
Age: 9
Maturity classification: Primary Growth - Perinuclear Stage







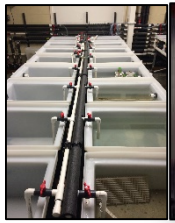
2. Reproduction

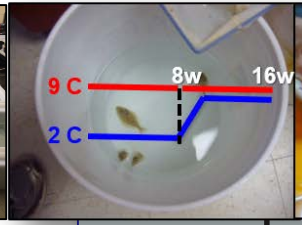
SRB016–Req.14 (para. 39)	The SRB **REQUESTED** that work on size- and age-specific fecundity be incorporated in the next 5-year research plan.	**In Progress**: Studies on size- and age-specific fecundity are being planned for execution in 2021. This information will be presented at the SRB017.

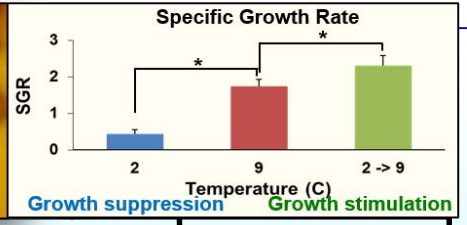
- Objective: establish a fecundity –size (length/weight/age) relationship
- Measure: potential annual fecundity as a measure of annual egg production.
- Important considerations:
  a) Time of sampling. Important to complete annual maturation cycle to select time when individuals are in pre-spawning conditions.
  b) Location of sampling and sample size.
  c) Method: gavimetric versus auto-diametric methods.
- Method testing with ovarian samples collected planned for FISS 2021
- Planned implementation of ovarian collection starting in 2022.

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

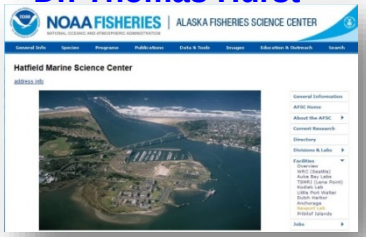
BIOCHEMICAL AND MOLECULAR GROWTH RESPONSES

Application to field studies

IPHC / AFSC-NOAA (Newport, OR)

Dr. Josep Planas (PI)

Dr. Thomas Hurst



NPRB Grant 1704 (2017-2020)

NRPB 1704 Final Report

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

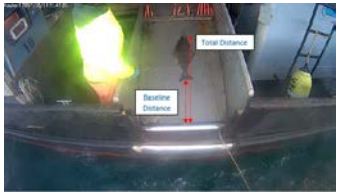
2. Estimate DMRs in the guided recreational fishery

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

- **sPAT tagging produced an estimate of 4-8.7% DMR which is consistent with current estimates.**
- **Ongoing investigations into relationships between individual physiological, environmental, and handling practices with respect to final release viability classifications (Masters prgm).**
- **Electronic monitoring (EM) was effective at accurately capturing hook release method**
- **Ongoing investigations into the ability to estimate individual fish lengths from EM video footage (both with post-hoc camera angle/distance calibration, and with pre-calibrated camera angle/distance calibration).**



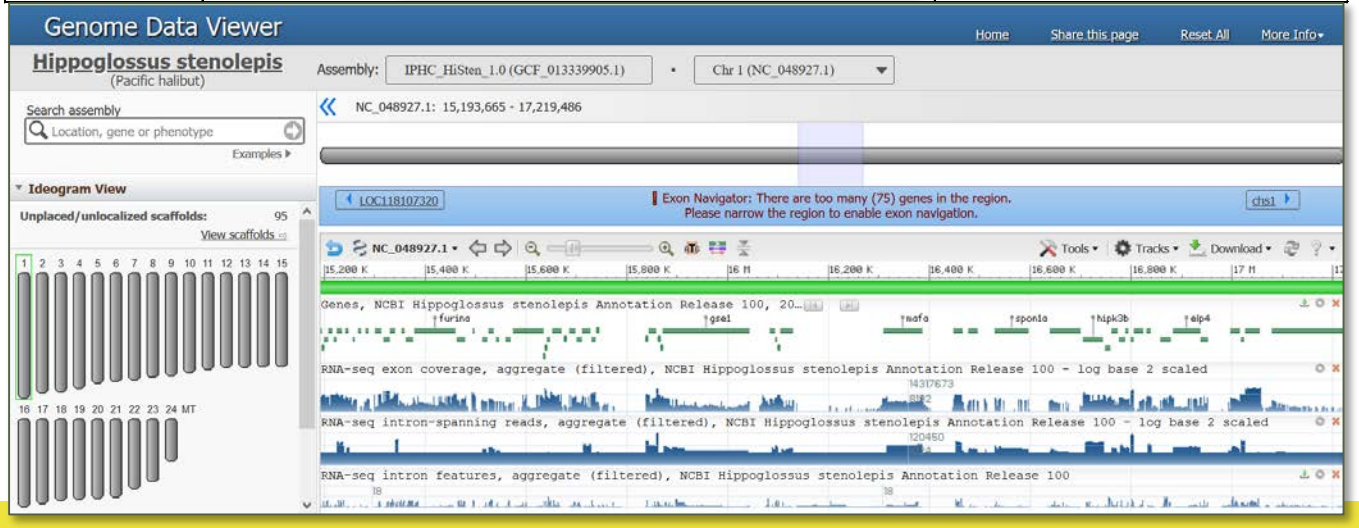
4. DMRs and survival assessment

2. *Guided recreational fishery: Estimation of DMRs*

- **Currently focused on experimental design with experimental field work to occur in Spring 2021. Contemplating two options:**
  - **A. Replicate field treatments based on questionnaire results. This would allow for the generation of an overall DMR for the charter sector, but with lower replicates and confidence for some treatments.**
  - **B. Focus on one set of conditions of predominant interest (circle hook, release vs reversal/twist) to develop a less broad DMR, which would be more transferable to best practices.**
- **Ten variables for testing, several are non-controllable (*Reg Area, Port, Fish Size, Hook Type, Hook Size, Capture conditions, Landing method, Time on Deck, Fish Condition,* and *Release Method*).**
- **This work continues to be the subject of ongoing efforts to secure sufficient external funding for a meaningful number of sPATs.**

5. Genetics and Genomics

SRB016–Req.18 (para. 49)	The SRB **REQUESTED** that the IPHC Secretariat contact the National Center for Biological Information to annotate the genome. Subsequently, existing and newly discovered SNPs be mapped onto the existing Pacific halibut genome. • **Size: 594 million base pairs** • **24 chromosomes** • **27,422 genes** • **91x coverage**	**Completed**: The IPHC Secretariat requested genome annotation from NCBI and the annotation has now been completed and available as NCBI Hippoglossus stenolepis Annotation Release 100.



5. Genetics and Genomics

SRB016–Req.12 (para. 37)	**NOTING** the issues of Gulf of Alaska (GOA) and Bering Sea (BS) connectivity relative to juvenile dispersal, the SRB **REQUESTED** that the IPHC Secretariat include individuals of different ages and locations in the GOA and BS in their whole genome sequencing analysis, including individuals from different places in GOA and BS.	**In Progress**: Tissue (fin clip) samples from juvenile Pacific halibut collected in the GOA and BS are currently being selected for age and capture location for whole genome sequencing analysis. A sample summary will be presented at the SRB017.

5. Genetics and Genomics

SRB016-Req. 12

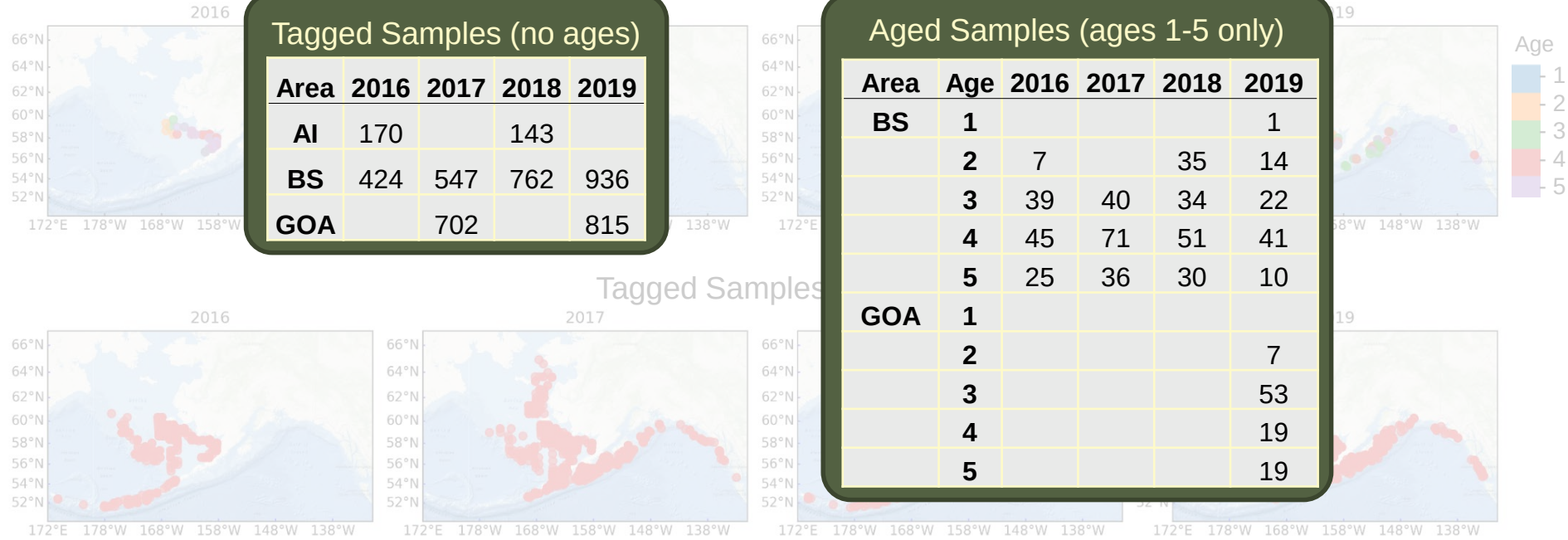
Tissue samples available for genetic analysis

Tagged Samples (no ages)

Area	2016	2017	2018	2019
AI	170		143	
BS	424	547	762	936
GOA		702		815

Aged Samples (ages 1-5 only)

Area	Age	2016	2017	2018	2019
BS	1				1
	2	7		35	14
	3	39	40	34	22
	4	45	71	51	41
	5	25	36	30	10
GOA	1				
	2				7
	3				53
	4				19
	5				19

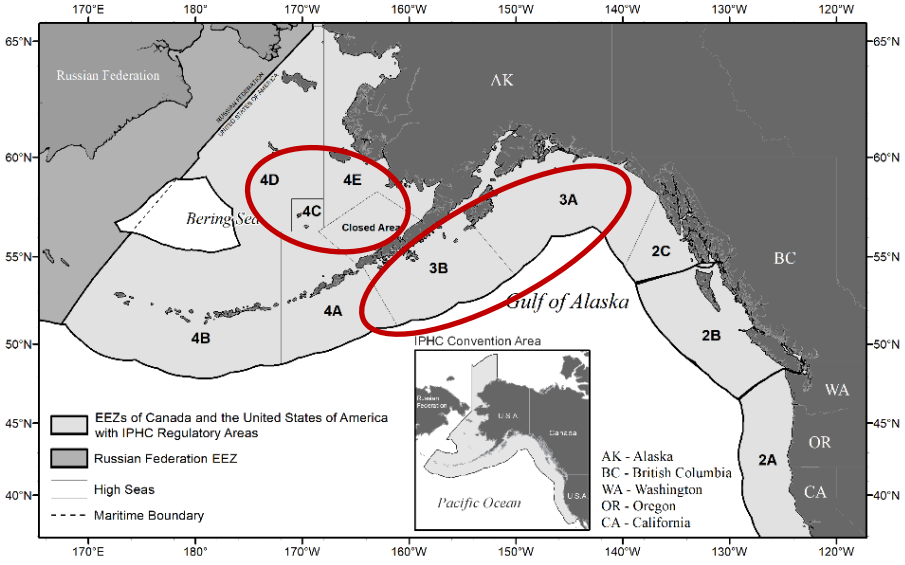


5. Genetics and Genomics

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
  - GOA (2017, 2019)
  - BS (2016-2019)
- Compare genetic diversity metrics between GOA & BS
- Estimate admixture proportions

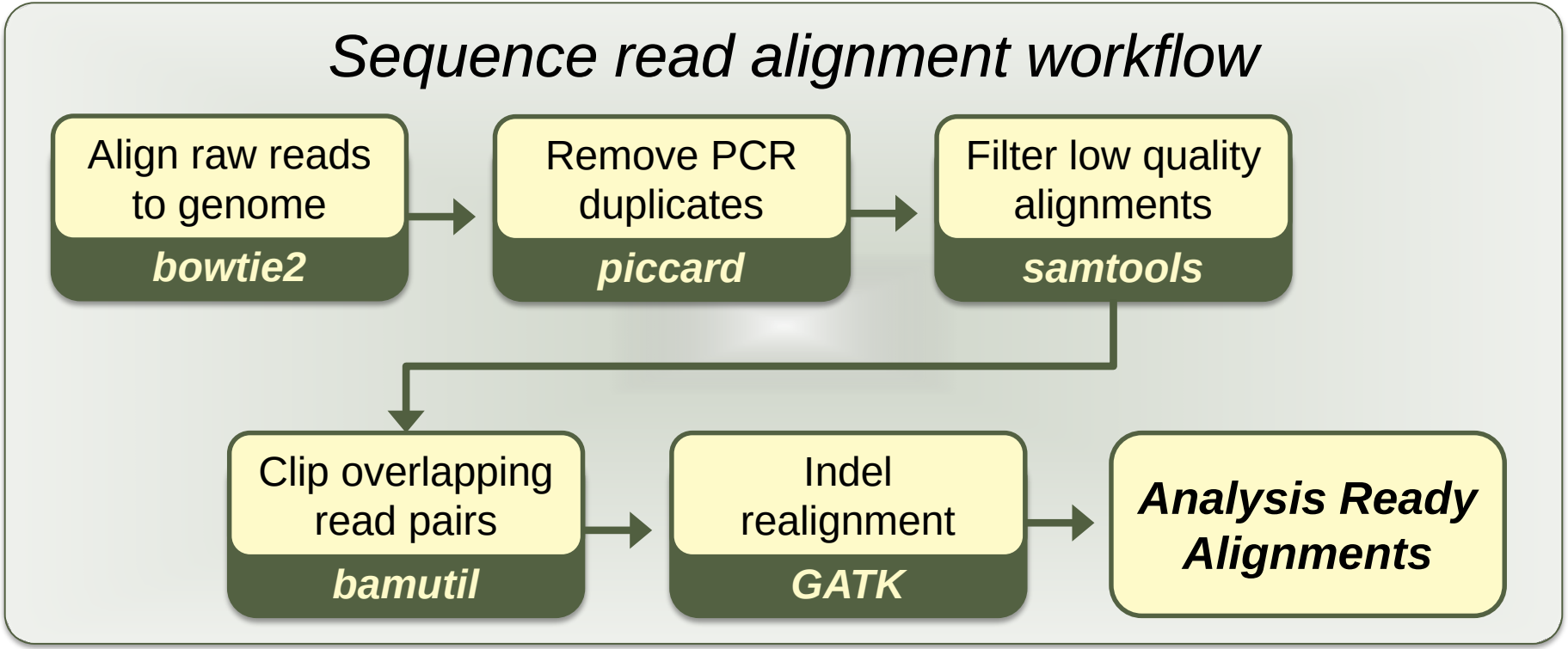


5. Genetics and Genomics

SRB016–Req.15 (para. 41)	***Genetics and genomics*** The SRB **NOTED** that the text in this section of paper IPHC-2020-SRB016-09 was not consistent. A high level of detail was provided in some areas and much less detail was provided in others. At one level, the SRB requires more information on (a) objectives and (b) methods to evaluate study design and the quality of data, however this was not possible given the information provided. For example in the first section on whole genome sequencing there was a major gap in methods. The SRB **REQUESTED** specific information on how the sequence data would be mapped to the reference genome.	**In Progress**: Methods similar to those used by Clucas et al. (2019) will be used to align raw sequence reads to the Pacific halibut reference genome. This information will be presented at the SRB017.

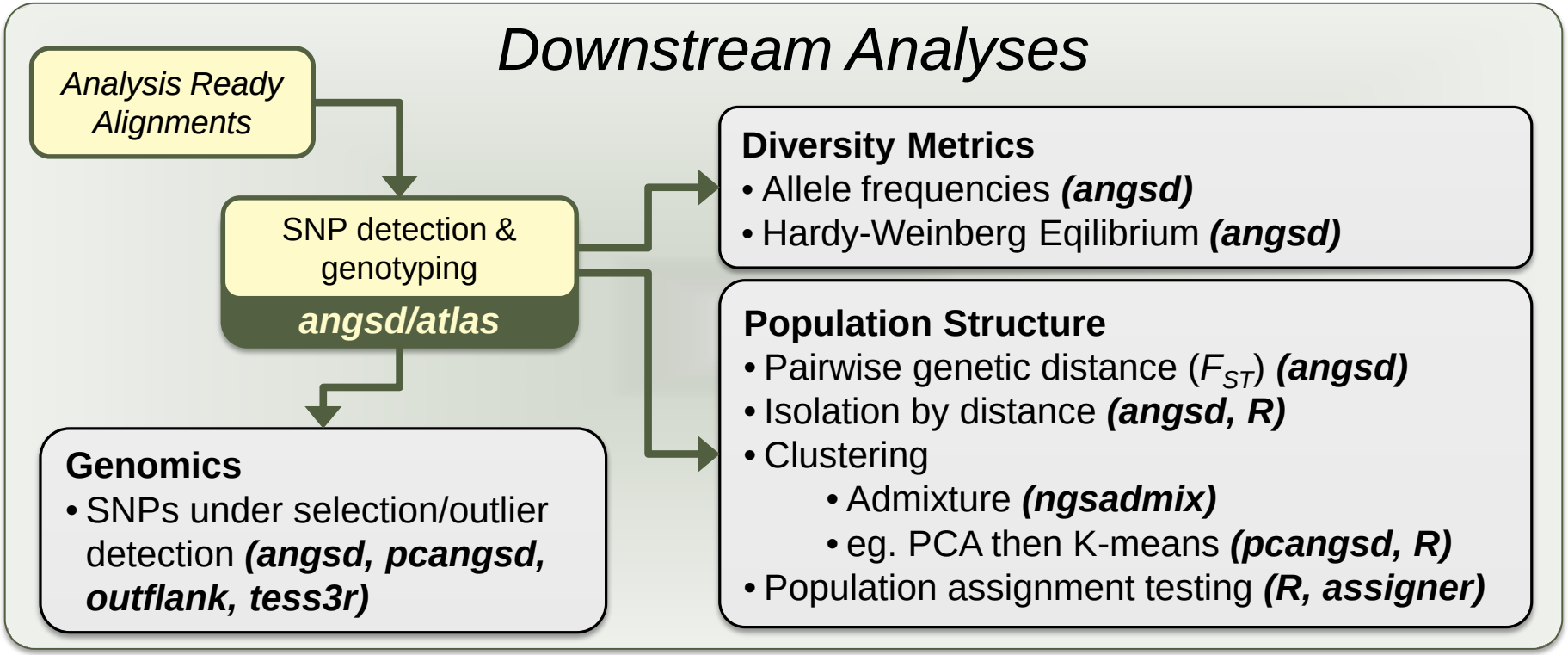
5. Genetics and Genomics

SRB016-Req. 15



5. Genetics and Genomics

SRB016-Req. 15



5. Genetics and Genomics

Progress

- Submitted a trial library for sequencing 9/8/2020
  - 36 samples (Illumina HiSeq 4000)
- Objectives:
  - Validate library construction methods
  - Assess genomic coverage
  - Genotype accuracy
    - RADseq data for 30 individuals from Drinan *et al.* 2018
  - Test software

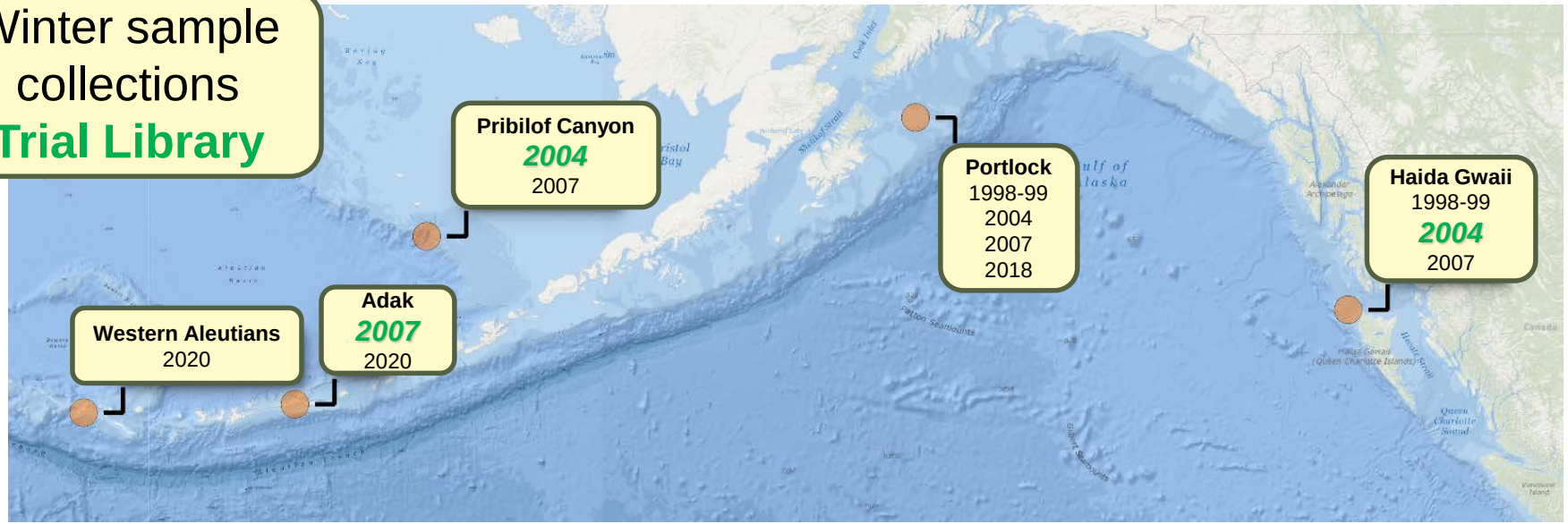
Drinan, D. P., T. Loher, and L. Hauser. 2018. Identification of Genomic Regions Associated With Sex in Pacific Halibut. Journal of Heredity 109(3):326–332.

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

Winter sample collections
Trial Library



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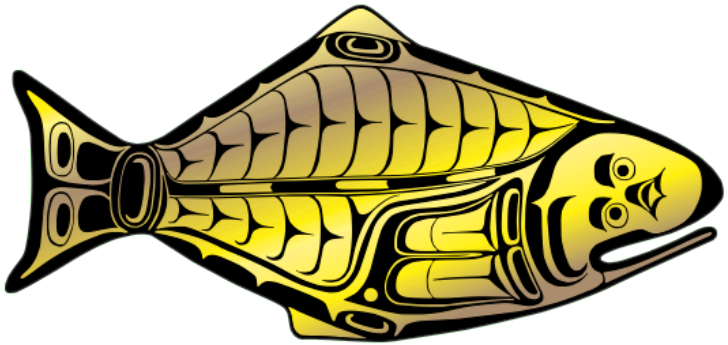

HALIBUT COMMISSION