D. Griffay

IPHC 5-year Research Program

IPHC-2017-093-11

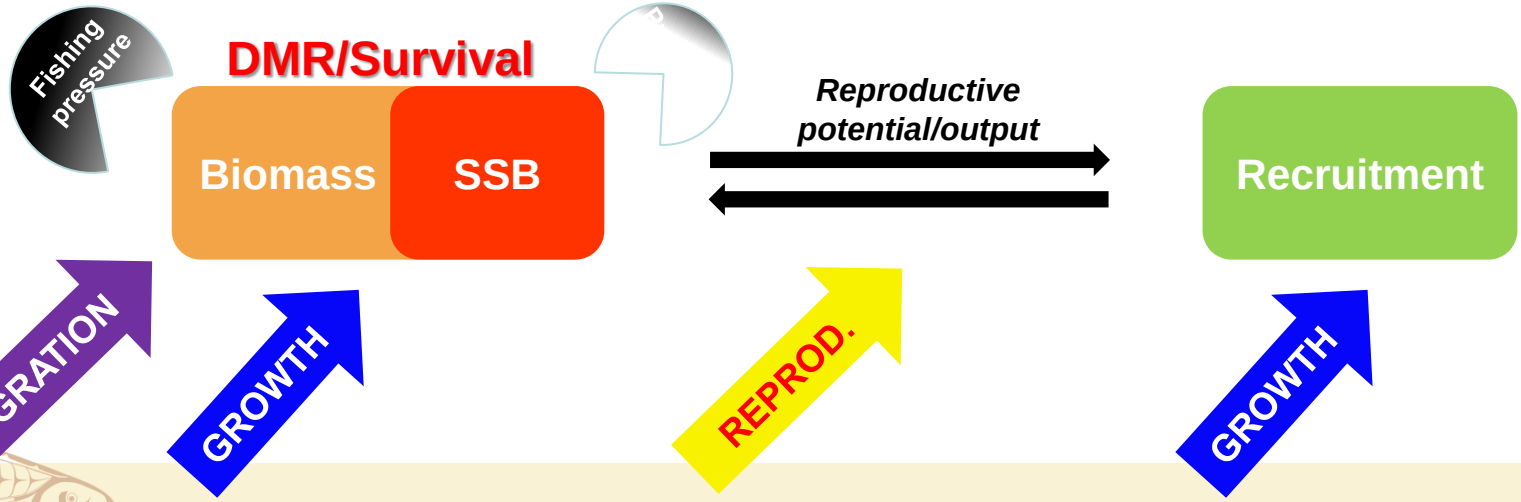
Josep V. Planas
Biological and Ecosystem Science
Program Manager

January 23-27, 2017 IPHC Annual Meeting

Primary research activities at IPHC

Primary objectives

- Identify and address *critical knowledge gaps* in the biology of the Pacific halibut
- Understand the influence of *environmental conditions* on halibut biology
- Apply resulting knowledge to reduce *uncertainty* in current stock assessment models



Primary research activities at IPHC

1. **Reproduction**
   - SEX RATIO OF CATCH
   - IMPROVED MATURATION ESTIMATES OF SPAWNING BIOMASS
2. **Growth**
   - CHANGES IN SIZE AT AGE/BIOMASS
   - TOOLS TO ASSESS FISH CONDITION
3. **DMRs and post-release survival assessment**
   - BYCATCH SURVIVAL ESTIMATES
4. **Migration**
   - LARVAL DISPERSAL
   - ADULT FEEDING AND REPRODUCTIVE MIGRATION
5. **Genetics and genomics**
   - GENETIC STRUCTURE OF THE POPULATION
   - GENOMIC TOOLS (e.g. GENOME)

1. Reproduction: proposed studies

There are important knowledge gaps on the reproductive biology of the species

- **SEX RATIO OF CATCH**
- **IMPROVED MATURATION ESTIMATES OF SPAWNING BIOMASS**

What is needed?

- **Knowledge on reproductive development, maturation, fecundity, sex determination mechanisms (sex identification), environmental and hormonal control of reproduction.**
- **Scientific-based criteria to identify reproductive status and potential.**
- **Updated estimates of age and size at maturation.**
- **Information on skipped spawning.**

New proposed studies:

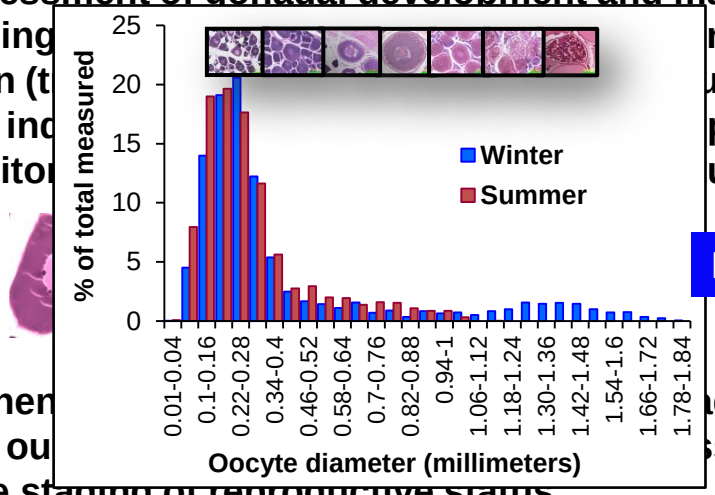
- ***Full characterization of the annual reproductive cycle***
- ***Identification of sex determination mechanism(s) and influencing factors***

1. Reproduction: proposed studies

- ***Full characterization of the annual reproductive cycle***

Objective: **Understand temporal changes in reproductive development throughout an entire annual reproductive cycle in male and female Pacific halibut**

- **Histological assessment of gonadal development and maturation.**
- **Endocrine profiling reproductive cycle.**
- **Gene expression (t uctive axis.**
- **Gonadosomatic inc productive cycle.**
- **Ultrasound monitor uration.**



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RARA 2016 (Ch. 5.6)

Deliverables:

- **Comprehen dult population in order to improve ou ss**
- **Accurate staging of reproductive status**
- **Updated maturity-at-age estimates.**
- **Estimates of skipped-spawning**

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Staff: Josep Planas and Tim Loher

1. Reproduction: proposed studies

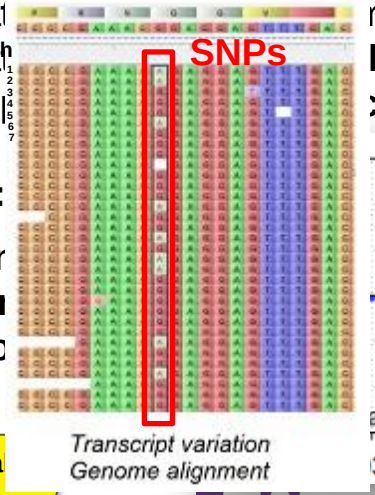
- ***Identification of sex determination mechanisms***

Objective: Understand how sex is established in Pacific halibut

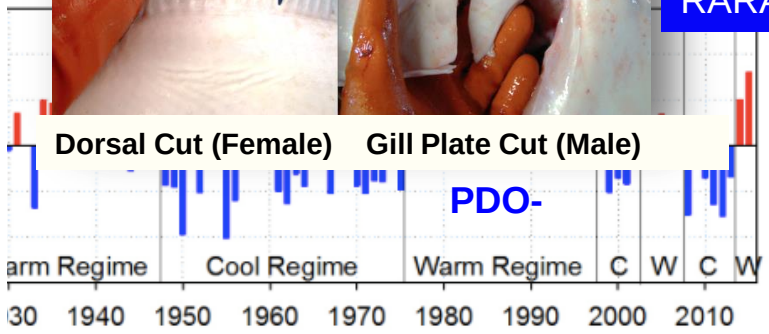
- **Identification of genetic sex markers: Validation of the coast-wide sex-marking project**
- **Identificat … uring early development.**
- **Identifica … determination.**
- **Evaluate … vel.**

Deliverables:

- **Ger …**
- **Ide …**
- **Info …**



Dorsal Cut (Female) Gill Plate Cut (Male)



RARA 2016 (Ch. 2.9, 5.5)

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2. Growth: proposed studies

Little is known regarding what factors influence growth in this species

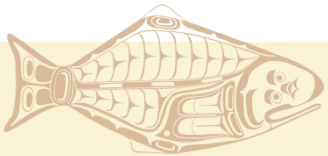
- **CHANGES IN SIZE AT AGE/BIOMASS**
- **TOOLS TO ASSESS FISH CONDITION**

What is needed?

- **Knowledge on growth patterns and environmental influences.**
- **Improved understanding in the possible role of growth alterations in the observed decrease in size at age.**

New proposed studies:

- ***Extensive catalogue of physiological markers to monitor growth***
- ***Evaluation of growth patterns and effects of environmental influences***



2. Growth: proposed studies

- ***Extensive catalo***

Objective: Identify **th studies.**

- **Identification of ex** **ver.**
- **Develop molecula** **ant tissues.**

3. De novo Transcripton

Sample ID	Total trinity 'genes'
R116-pool1	37,161
R116-pool2	38,143
R116-pool5	70,693

5.1 Mapping statistics

Sample ID	Danio rer
R116-pool1	13,8
R116-pool2	13,2
R116-pool5	25,3

Annotation	Gene symbol	Length (nt)	Identity (%)	Function
Androgen receptor	*ar*	4426	81.48	Protein synthesis
Calcium/calmodulin-dependent protein kinase II alpha	*camk2a*	2342	87.27	Force transmission
Creatine kinase, muscle a	*ckma*	2256	89.76	Energy metabolism
Carnitine palmitoyltransferase 1B	*cpt1b*	762	81.82	Lipid metabolism
Dystrophin	*dmd*	1282	75.13	Force transmission
Eukaryotic translation initiation factor 4eb	*eif4eb*	1168	85.19	Protein synthesis
F-box protein 32	*fbxo32*	695	86.25	Protein atrophy
Glycogen synthase 1	*gys1*	3328	89.47	Energy metabolism
Histone deacetylase 1	*hdac1*	2190	96.35	Muscle repressor
Insulin-like growth factor 2 receptor	*igf2r*	5411	70.06	Growth regulator
Insulin-like growth factor binding protein 5b	*igfbp5b*	1372	92.56	Growth regulator
Lipoprotein lipase	*lpl*	1789	80.48	Lipid metabolism
Myocyte enhancer factor 2cb	*mef2cb*	5841	79.8	Muscle growth
Myostatin b	*mstnb*	780	95.74	Growth regulator
Mechanistic target of rapamycin	*mtor*	153	97.92	Protein synthesis
Myogenic factor 6	*myf6*	819	76.19	Muscle growth
Myosin, heavy polypeptide 1.3, skeletal muscle	*myhz1.3*	246	80.42	Muscle growth
Myoblast determination protein 1 homolog	*myod*	2497	72.67	Muscle development
Myozenin 1a	*myoz1a*	795	74.6	Force transmission
Nuclear factor of activated T-cells, cytoplasmic 3	*nfatc3*	1	62.96	Muscle activity
Paired box 3a	*pax3a*	269	75	Muscle development
Paired box 7b	*pax7b*	297	85.71	Muscle development
Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	*ppargc1a*	519	88.7	Energy metabolism
Protein phosphatase 3, catalytic subunit, alpha isozyme	*ppp3ca*	3407	83.69	Muscle activity
Protein kinase, AMP-activated, alpha 1 catalytic subunit	*prkaa1*	1925	70.96	Energy metabolism
Phosphorylase, glycogen, muscle	*pygma*	5514		
Serum response factor	*srf*	4393		
Transforming growth factor, beta 1a	*tgfb1a*	561	77.04	Growth regulator
Tripartite motif containing 63b	*trim63b*	2117	81.16	Protein atrophy

Growth regulation
Energy metabolism
Muscle activity

RARA 2016 (Ch. 5.7)

Deliverables:

- **Establishment of a growth-related gene sequence dataset**
- **Molecular assays to monitor growth patterns**

2. Growth: proposed studies

- ***Evaluation of growth patterns and effects of environmental influences***

  Objective: Identify molecular, biochemical and isotopic profiles characteristic of specific growth patterns and evaluate potential effects of environmental influences.

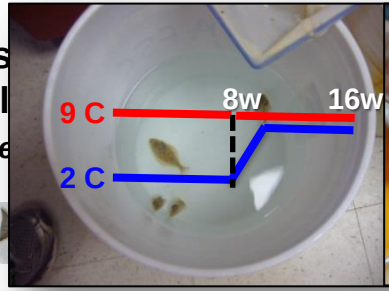
  - **Evaluation of different growth trajectories in the wild.**

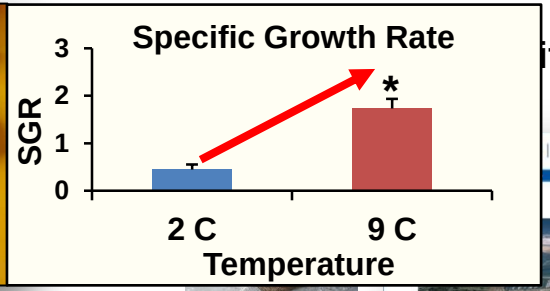
In BS NMFS trawl survey in 2016:

- **- 75 fish <40 cm length**
- **- 75 fish 40-60 cm length**
- **- 75 fish 60-80 cm length**

Characterization of molecular and biochemical growth markers in liver and muscle samples from age-matched individuals

- **Establis… molecul…** ***- Fee…*** **…ify**





- **Isotopic tissue turnover to trace dietary and/or habitat shifts**

^{13}C, ^{15}N

ALASKA PACIFIC UNIVERSITY

2. Growth: proposed studies

- **Investigate the effects of environmental factors on growth performance.**
  - ***Effects of temperature, salinity, dissolved oxygen and water pH on growth.***
  - ***Identify the optimal environmental conditions for growth.***

- **Understand the basis of the sexual dimorphic growth in the Pacific halibut.**

Deliverables:

- **Identification and validation of growth markers for field studies**
- **Characterization of molecular and biochemical growth signatures**
- **Environmental effects on somatic growth**
- **Improved biological inputs on biomass estimates**

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INTERNATIONAL PACIFIC HALIBUT COMMISSION

Staff: Josep Planas and Dana Rudy

3. DMRs and survival: proposed studies

Little is known regarding the factors that influence bycatch survival

- **BYCATCH SURVIVAL ESTIMATES**

What is needed?

- **To introduce quantitative measurable factors that are linked to fish handling practices and to fish physiological condition and ultimately to survival in order to improve current DMR estimations**

New proposed studies:

- ***Evaluation of the effects of fish handling practices on injury levels and the physiological condition of captured Pacific halibut***
- ***Investigate the relationship between physiological condition post-capture and survival as assessed by the use of accelerometer tags.***
- ***Improving estimates of survival of Pacific halibut caught in the trawl fishery***

3. DMRs and survival: proposed studies

- ***Evaluation of the effects of fish handling practices on injury levels and the physiological condition of captured Pacific halibut***

Objective: Understand relationship between handling practices and physiological condition of captured Pacific halibut in the longline fishery

- **Assess injuries associated with release techniques (gangion cut, careful shake, hook straigthening).**
- **Determine the physiological condition of all captured fish with associated injury levels after different deck exposure times: condition factor index (Kn), energy (fat) levels, morphometric analyses.**
- **Measure the levels of stress and physiological disturbance indicators in the blood of all captured fish (cortisol, lactate, glucose, potassium, hematocrit).**

Deliverables:

- **Injury profile for different release techniques in the longline fishery**
- **Physiological assessment of fish handling practices: fish condition index post-capture**

3. DMRs and survival: proposed studies

- ***Investigate the relationship between physiological condition post-capture and survival as assessed by tagging.***

Objective: **Measure survival post-release in Pacific halibut and link this with the physiological condition and capture-related events**

- **Tag fish that have been exposed to different handling practices in the longline fishery with accelerometer tags in addition to conventional tags (wire).**
- **Assess survival of fish according to size and physiological conditon.**

Deliverables:

- **Information on post-release survival in relation to handling practices and physiological condition.**
- **Information on post-release survival in relation to size.**
- **Estimating DMRs by EM.**

INTERNATIONAL PACIFIC HALIBUT COMMISSION
Staff: Josep Planas, Claude Dykstra,Tim Loher, Ian Stewart, Allan Hicks

3. DMRs and survival: proposed studies

- ***Improving estimates of survival of Pacific halibut in the trawl fishery***

Objective: Assess the conditi  discarded Pacific halibut in the non-directed trawl fishery an nates of discard mortality rates

- Continue and capitalize c .g. Amendment 80 fleet) to plan collaborative research on
- Apply methods to assess captured halibut.
- Determine survival rates of discarded halibut after tagging.
- Relate physiological condition with survival rates of discarded halibut

Deliverables:

- Improved knowledge of survival of discarded halibut and, consequently, improved estimates of discard mortality rates in the trawl fishery.

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Staff: Josep Planas, Claude Dykstra,Tim Loher, Ian Stewart, Allan Hicks

4. Migration: proposed studies

- **LARVAL DISPERSAL**
- **ADULT FEEDING AND REPRODUCTIVE MIGRATION**

What is needed?

- **Improve our understanding on larval, juvenile and reproductive migration.**
- **Incorporate additional sources of biological information on migration.**

New proposed studies:

- ***Towards a more integrative view on migration***
- ***Larval migration and connectivity***
- ***Swimming and migratory performance***

4. Migration: proposed studies

- ***Towards a more integrative view on migration***

Objective: **Combine current tagging efforts with genetic and otolith and tissue composition analyses.**

- **Genetic analyses of tagged fish to shed light on migration patterns and geographic origin.**
- **Otolith microchemical and stable isotope analyses and tissue stable isotope analyses.**
- **Reproductive monitoring of PAT-tagged adult females: blood endocrine reproductive parameters, ovarian tissue biopsies and ultrasound for ovarian staging.**

Deliverables:

- **Genetic and elemental and isotopic information on migratory adult fish**
- **Improved knowledge on reproductive migrations and identification of spawning areas**

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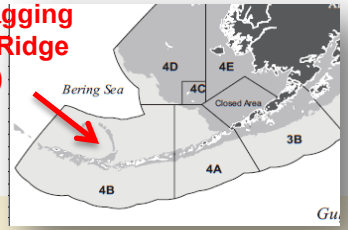
- **PAT tagging Bower's Ridge (4B)**



- **Tail pattern recognition**

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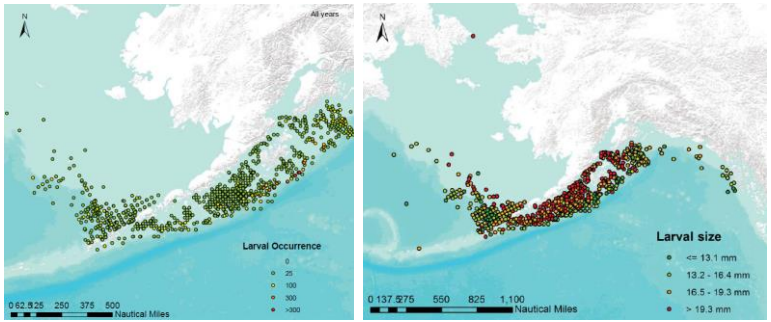
Staff: Tim Loher, Josep Planas, Claude Dykstra, Tracee Geernaert

4. Migration: proposed studies

- ***Larval migration and connectivity***

Objective: Understand the mechanisms of larval connectivity between GOA and BS.

- **Collect data from the NMFS icthyoplankton survey and map larval distribution over time and space.**
- **Collect larval samples from the survey to conduct genetic analyses.**



Collaboration with Janet Duffy-Anderson, Esther Goldstein, William Stockhausen (NOAA-AFSC)

Deliverables:

- **Improved knowledge on larval distribution, migration and genetic structure within the population**

Staff: Lauri Sadorus, Josep Planas

5. Genetics and genomics: proposed studies

- **GENETIC STRUCTURE OF THE POPULATION**
- **GENOMIC TOOLS (e.g. GENOME)**

What is needed?

- **Improved knowledge on the genetic composition of the population**
- **Establish genomic resources for the species**
- **Genome-wide association studies to evaluate genetic effects of fishery-dependent and fishery-independent influences on growth, reproduction, nutrition, etc.**

New proposed studies:

- ***Population genetic studies***
- ***Sequencing of the Pacific halibut genome***

5. Genetics and genomics: proposed studies

- ***Population genetic studies***

Objective: **Genetic characterization of Pacific halibut throughout its distribution range**

- **Characterization of population structure by RAD sequencing and SNP analysis.**
- **Identification of genetic signatures of geographical population groups**

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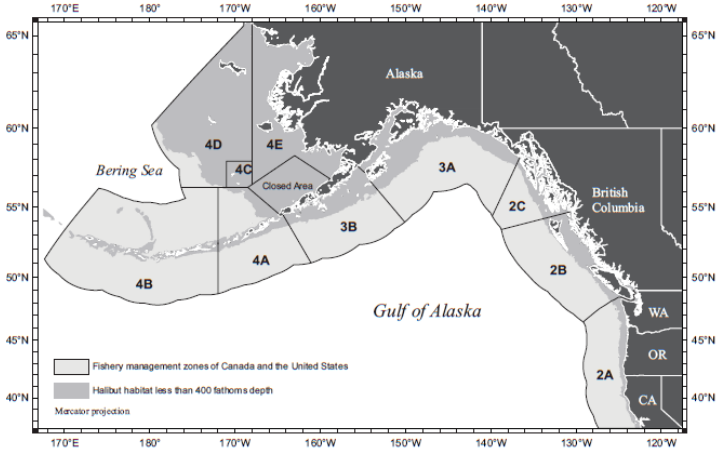

INTERNATIONAL PACIFIC HALIBUT COMMISSION

Staff: Josep Planas, Tim Loher

5. Genetics and genomics: proposed studies

- ***Pacific halibut genome***

Objective: Obtain a first draft sequence of the Pacific halibut genome

- **Provide genomic resolution to genetic markers (SNPs or transcripts).**
- **Identify genomic regions and genes responsible for temporal and spatial adaptive characteristics.**
- **Genome-wide association studies to try to understand the genetic basis of growth, reproductive performance, migratory behavior and performance, etc.**
- **Link genotype and phenotype.**

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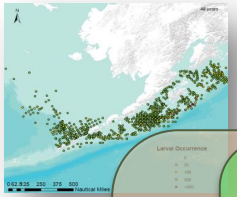
Temporal chart of activities

	2016	2017	2018	2019	2020	2021
Reproduction		Annual reproductive cycle				
			Sex determination mechanisms			
	Sex identification					

Research projects proposed for 2017

Project #	Project Name	Priority	Budget (US$)	Principal Investigator	Management implications
New Projects					
2017-01	**Full characterization of the annual reproductive cycle**	High	91,098	Planas	Maturity assessment
2017-02	**Investigation of Pacific halibut dispersal on Bowers Ridge**	High-Medium	124,527	Loher	Spawning areas
2017-03	**Tail pattern recognition analysis in Pacific halibut**	High	2,370	Dykstra	Adult distribution
2017-04	**Condition Factors for Tagged U32 Fish**	High	13,000	Dykstra	DMR estimates
2017-05	**Identification and validation of markers for growth**	High	27,900	Planas	Changes in biomass/ size-at-age
2017-06	**Discard mortality rates and injury classification profile by release method**	High-Medium	16,123	Dykstra	DMR estimates
2017-07	**Sequencing the Pacific halibut genome**	High	22,500	Planas	Population estimate
Continuing Projects					
621.15	**Voluntary at-sea sex marking**	High	18,120	Loher	Stock spawning biomass
621.16	**Development of genetic sexing techniques**	High	146,107	Loher	Sex composition of catch
642.00	**Assessment of Mercury and other contaminants**	Medium	8,400	Dykstra	Environmental effects
650.18	**Archival tags: tag attachment protocols**	High	2,800	Loher	Adult distribution
650.20	**Investigation of Pacific halibut dispersal on the 4D Edge**	High	5,500	Loher	Spawning areas
661.11	**Ichthyophonus Incidence Monitoring**	Medium	8,055	Dykstra	Environmental effects
669.11	**At-sea Collection of Pacific Halibut Weight to Reevaluate Conversion Factors**	High	1,500	Soderlund	Length-weight relationship
670.11	**Wire tagging of Pacific halibut on NMFS trawl and setline surveys**	High	12,000	Forsberg	Juvenile and adult distribution
	Total - New Projects		**297,518**		
	Total - Continuing Projects		**202,482**		
	Overall Total (all projects)		**500,000**		

Research projects for 2017



- Contaminants
- *Ichthyophonus*

ENVIRONMENT

- Larval connectivity
- Tail pattern recognition
- Archival tags
- Wire tagging of U32 fish

MIGRATION

- PAT tags in 4B and 4D Edge
- Annual Reproductive Cycle

REPRODUCTION

- Genetic sex markers
- Sex marking at sea

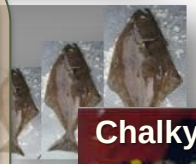
GENOMICS

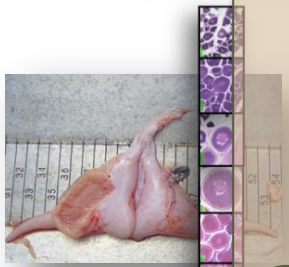
- Genome sequencing

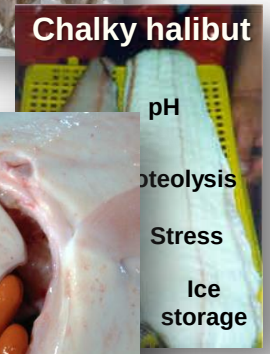
- Temperature
- Size-at-age

GROWTH

- Growth marker identification







- Weights at

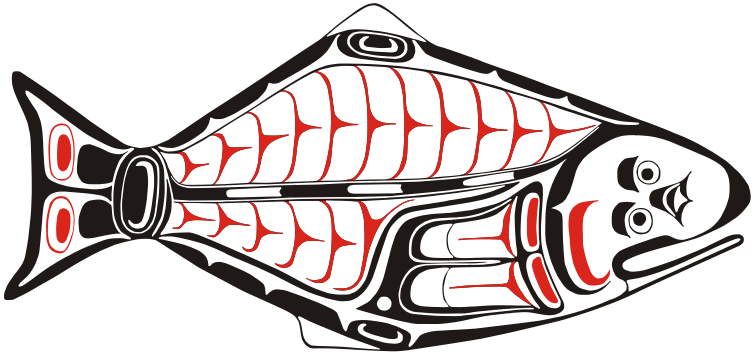
Dorsal Cut (Female) Gill Plate Cut (Male)

Research proposals submitted for external funding

Project #	Grant agency	Project name	Partners	IPHC Budget (US$)	PI	Management implications	Submission status
1	**Saltonstall-Kennedy NOAA**	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival	Alaska Pacific University	223,220	Planas (lead PI) Dykstra Loher Stewart Hicks	Bycatch estimates	Submitted in December 2016
2	**NPRB**	Somatic growth processes in the Pacific halibut (Hippoglossus stenolepis) and their response to temperature, density and stress manipulation effects	AFSC-NOAA-Newport	122,264	Planas (lead PI)	Changes in biomass/size-at-age	Submitted in December 2016
3	**NPRB**	Larval transport, supply, and connectivity of Pacific halibut between the Gulf of Alaska and the Bering Sea	AFSC-NOAA-Seattle UAF	8,000	Sadorus Planas Stewart	Biomass distribution	Submitted in December 2016
4	**Essential Fish Habitat NOAA**	Validating biochemical markers of growth for habitat assessment in flatfishes	AFSC-NOAA-Newport	35,000	Planas	Changes in biomass/ recruitment	Submitted in November 2016
			Total requested ($)	**388,884**			

New special research projects with outside agencies for 2017

Project #	Outside agency	Project name	Regulatory area	Status	Management implications
1	**NOAA**	Testing the efficacy of stereoscopic electronic monitoring for species identification, enumeration and length measurement	2B, 2C, 3A, 3B	New	Integrating EM into observer program
2	**PBS-DFO**	Opportunistic shark sampling	2B	New	Understanding shark biology and distribution
3	**WDWF**	Yelloweye rockfish tagging on rockfish index stations	2A	New	Distribution
4	**NOAA**	Longnose skate age and maturity	2A, 2B, 2C, 3A, 3B, 4A	New	Age, growth and maturity
5	**PBS-DFO**	Rockfish sampling in area 2B	2B	Continuing	Age, size and maturity
6	**WDFW**	Rockfish sampling in area 2A	2A	Continuing	Species composition, relative abundance
7	**ADFG**	Yelloweye rockfish enumeration in Alaska	2C, 3A	Continuing	Distribution and assessment
8	**ADEC**	Environmental contaminant sampling	2C, 3A, 3B	Continuing	Environmental monitoring
9	**NOAA**	Pacific cod length frequency sampling	4A, 4B and 4D	Continuing	Stock assessment
10	**NMFS-Auke Bay**	Spiny dogfish sampling	2, 3 and 4	Continuing	Length, sex and distribution
11	**Seattle Aquarium**	Sixgill shark genetics	All areas	Continuing	Population genetics and distribution



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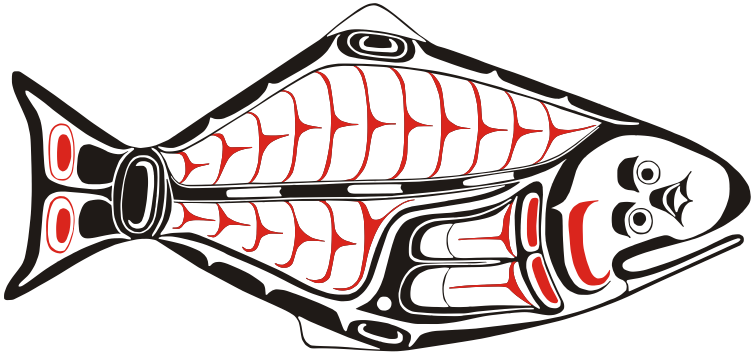


Pacific halibut connectivity research

- Cooperative project with NOAA/FOCI
- Grant submitted to NPRB in December 2016
- First step: synthesize data from 1972-2015 NOAA larval collection cruises (underway)
- Next step: model the synthesized data (dependent on grant)
- Focusing on the western Gulf of Alaska and Bering Sea

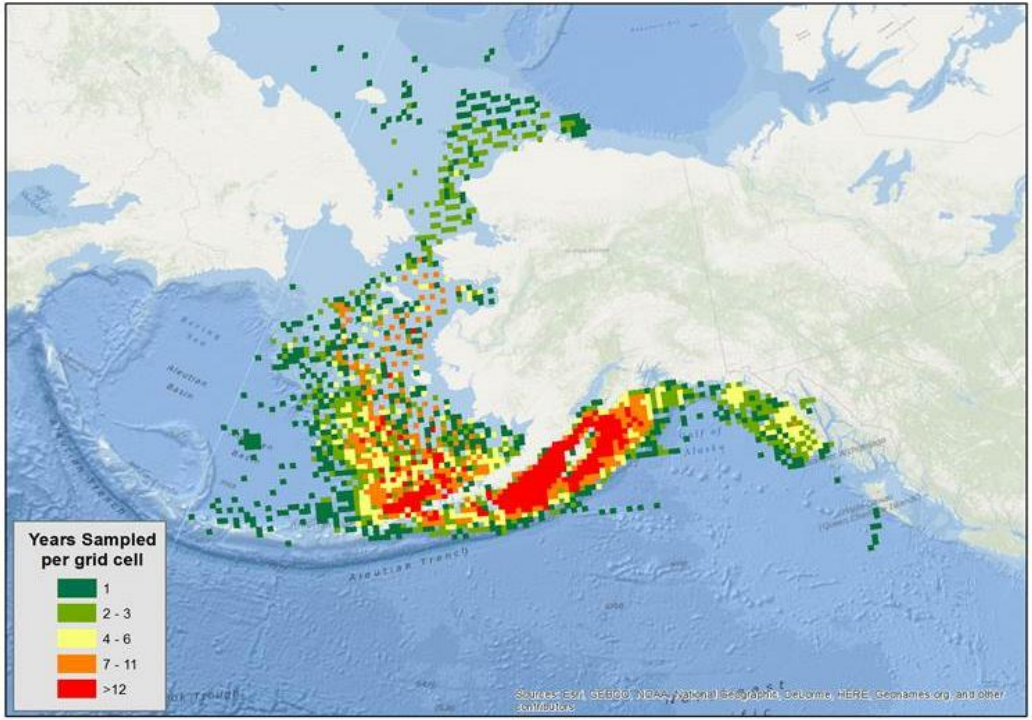
Justification

- Provide additional inputs to the MSE process – i.e. spatial ranges and ecosystem use for early life stages.
- Provide insight into the potential effects of how management choices in each basin may affect the other.
- Help to complete the loop of Pacific halibut life history.

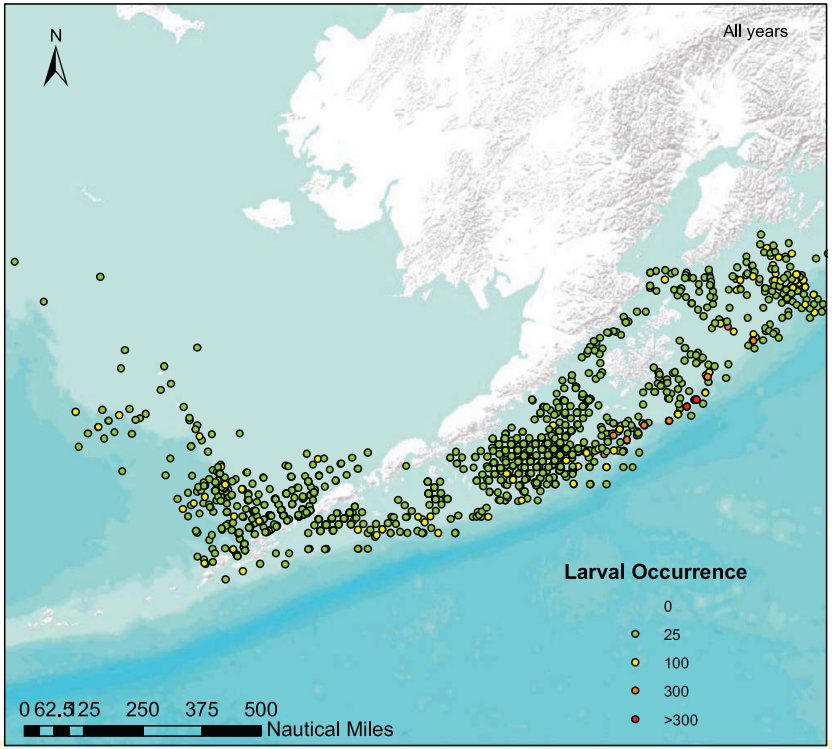
The Project

- Hypothesis: Eastern Bering Sea is a repository for both Bering Sea and Gulf of Alaska eggs and larvae.
- Until recently, could only speculate (which has taken place often in IPHC reports).
- Transport models have now become adequately sophisticated to achieve.
- Will be looking at connectivity between basins, as well as differences over time and environmental conditions.
- Future work may include genetic component

NOAA larval sampling stations over all years



Halibut larval occurrence for all years



Larval occurrence by stage