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IPHC Interim Meeting 28 – 29 November, 2017



Outline of the presentation

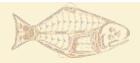






- Update on the research activities of the Biological and Ecosystem Science Program

 Paper IPHC-2017-IM093-11
- Outcome of external funding applications
- Proposed research projects for 2018
- Establishment of the IPHC research laboratory



Outline of the presentation







- Update on the research activities of the Biological and Ecosystem Science Program
- Outcome of external funding applications
- Proposed research projects for 2018
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Primary research activities at IPHC

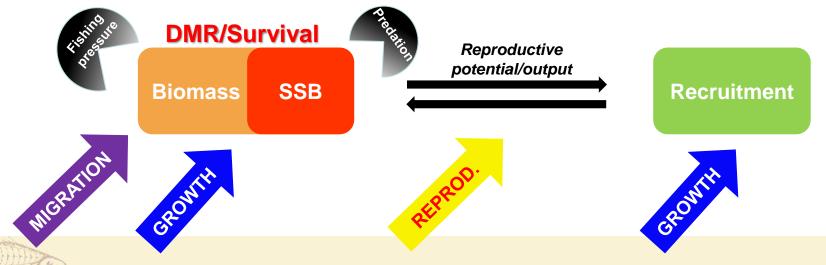






Primary objectives

- 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 areas at IPHC







- 1. Reproduction
- SEX RATIO OF COMMERCIAL 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
- ADULT FEEDING AND REPRODUCTIVE MIGRATION
- LARVAL DISPERSAL
- 5. Genetics and genomics
- GENETIC STRUCTURE OF THE POPULATION
- GENOMIC TOOLS (e.g. GENOME)



Update of research activities at IPHC







- 1. Reproduction
- SEX RATIO OF COMMERCIAL CATCH
- IMPROVED MATURATION ESTIMATES OF SPAWNING BIOMASS

- 2. Growth
- 3. DMRs and post-release survival assessment
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There are important knowledge gaps on the reproductive biology of the species

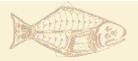
- SEX RATIO OF COMMERCIAL CATCH
- IMPROVED MATURATION ESTIMATES OF SPAWNING BIOMASS

Projects:

- 1. Sex marking and identification of genetic sex (Projects 621.15 and 621.16)
- 2. Full characterization of the annual reproductive cycle (Project 674.11)
- 3. Identification of genetic reproductive markers

Objectives:

- Identification of genetic markers of sex and information on sex ratios.
- Knowledge on reproductive development, maturation, fecundity, 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.



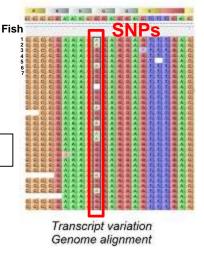
Development of genetic markers for sex identification (Project 621.16)

Objectives: To identify genetic markers for sex identification in the commercial catch

- Restriction site-associated DNA sequencing (RADseq) approach
 - 95 fish (55 female and 40 male) sequenced
 - 40,308 loci identified
 - 56 loci linked to sex (70 SNPs) based on FST values ≥ 0.4
 - Most females heterozygous and most males homozygous (ZW/ZZ)
 - 3 loci found only in females (0 only in males)
 - TaqMan assays developed for 2 sex-linked loci: Hs10183, Hs23885

Genetic assay accuracy (based on 199 morphologically sexed fish): 97.5%





Drinan, Loher and Hauser (2017) J. Heredity. In Press



Sex marking at sea and validation of genetic sex (Project 621.15)

Objectives: To establish a method for physically marking sex by the commercial fleet and validation of marking efficiency by genetic sex identification



Dorsal Cut (Female) Gill Plate Cut (Male)

Reg Area	Sampled trips	Number of fish
2A	36	70
2B	5	84
2C	16	116
3A	10	113
3B	9	292
4A	2	77
4B	2	95
4C	4	86
4D	1	19
TOTAL	84	929

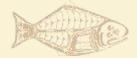
- 2016 (Reg Area 2B; 10 R/V; 288 samples)

79% marking accuracy (validated genetically)

- 2017 (Coastwide; 87 R/V; 929 samples)

??% marking accuracy (validated genetically)

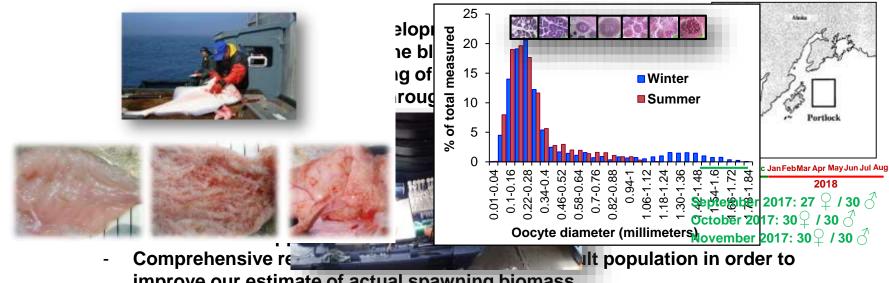
- 71 US vessels
- 16 BC vessels
- Wide participation WA Tribes

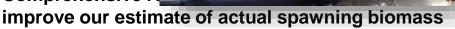


2017

Full characterization of the annual reproductive cycle (Project 674.11)

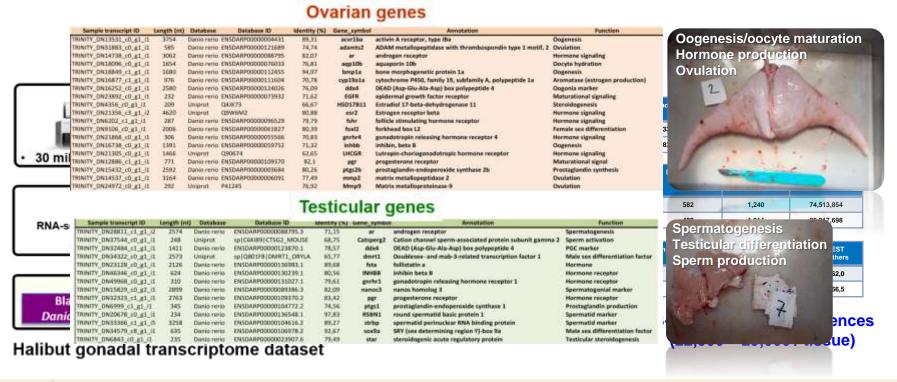
Objective: Understand temporal changes in reproductive development throughout an entire annual reproductive cycle in male and female Pacific halibut







Identification of genetic reproductive markers by RNA sequencing





Primary research areas at IPHC







- 1. Reproduction
- 2. Growth
- CHANGES IN SIZE AT AGE/BIOMASS
- TOOLS TO ASSESS FISH CONDITION
- 3. DMRs and post-release survival assessment
- 4. Migration
- 5. Genetics and genomics



Little is known regarding what factors influence growth in this species

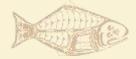
- CHANGES IN SIZE AT AGE/BIOMASS
- TOOLS TO ASSESS FISH CONDITION

Projects:

- 1. Identification and validation of physiological markers for growth (Project 673.14)
- 2. Evaluation of growth patterns and effects of environmental influences (NPRB 1704)

Objectives:

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



1. Mass identificat

Objective: Identify

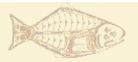
Identification of g

Develop molecula

	Gene	Lengtn			
Annotation	symbol	(nt)	Identity (%)	Function	
Androgen receptor	ar	4426	81.48	Protein synthesis	
Calcium/calmodulin-dependent protein kinase II alpha	camk2a	2342	87.27	Force transmission	1
Creatine kinase, muscle a	ckma	2256	89.76	Energy metabolism	4)
Carnitine palmitoyltransferase 1B	cpt1b	762	81.87	Lipid metabolism	-
Dystrophin	dmd	1282	75.23	Force transmission	th studies.
Eukaryotic translation initiation factor 4eb	eif4eb	1168	55.19	Protein synthesis	tii Studies.
F-box protein 32	fbxo32	695	86.25	Protein atrophy	
Glycogen synthase 1	gys1	303	89.47	Inergy metabolism	
Histone deacetylase 1	hdac1	2490	96.25	Muscle repressor	
Insulin-like growth factor 2 receptor	igf2r	511	0.62	Growth regulator	
Insulin-like growth factor binding protein 5b	igfbp5	1372	81.5	Growth to lawr	ant tissues.
Lipoprotein lipase		1789	60.48	Lipid netabolism	ant tissaes.
Myocyte enhancer factor 2cb	mef2cb	504	79.8	Miscle growth	
Myostatin b	mstnb	1.89	95.74	Growth regulator	
Mechanistic target of rapamycin	mtor	1153	97.92	Protein synthesis	
Myogenic factor 6	my (819	1 19	Muscle growth	
Myosin, heavy polypeptide 1.3, skeletal muscle	n 0 1.3	246	86.42	Muscle growth	
Myoblast determination protein 1 homolog	myod	2427	72.67	Muscle development	
Myozenin 1a	myoz1a	735	74.6	Force transmission	
Nuclear factor of activated T-cells, cytoplasmic 3	nfatc3 🔨 📿	1587	62.96	Muscle activity	
Paired box 3a	pax3	269	75	Muscle development	
Paired box 7b	(lax7)	297	85.71	Muscle development	
Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	, pargc1a	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	90.91	Energy metabolism	
Serum response factor	srf	4393	63.81	Muscle development	
Transforming growth factor, beta 1a	tgfb1a	561	77.04	Growth regulator	
Tripartite motif containing 63b	trim63b	2117	81.16	Protein atrophy	

Deliverables:

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



2. 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.

- Establishment of different growth trajectories in juvenile fish in captivity to identify molecular and biochemical signatures of growth patterns.

- Manipulating growth rates (ration, density, thermal- or fasting-induced compensation, etc.):







- Evaluation of different growth patterns in the wild.

Samples collected in NMFS trawl survey In 2016 and 2017 from 3 size categories:

- <40 cm length
- 40-60 cm length
- 60-80 cm length

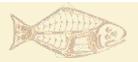


Characterization of molecular and biochemical growth markers in muscle samples from agematched individuals

Isotopic tissue turnover to trace dietary and/or habitat shifts

¹³C, ¹⁵N





- 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.
 - Establishment of different growth trajectories in juvenile fish in captivity to identify molecular and biochemical signatures of growth patterns.

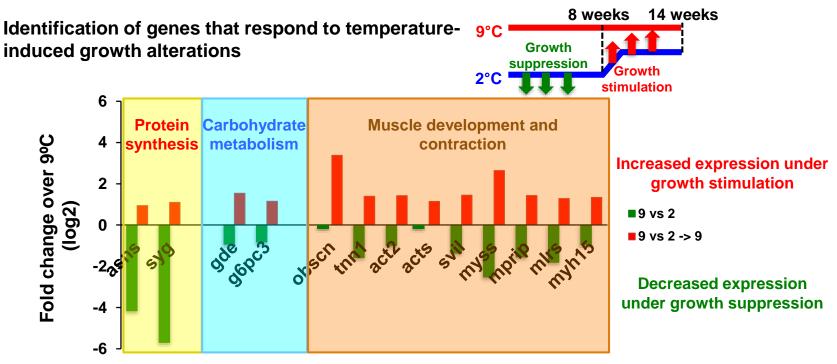


- Isotopic tissue turnover to trace dietary and/or habitat shifts

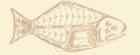
13C, 15N



Evaluation of growth patterns and effects of environmental influences



Potential molecular markers for temperature-regulated growth





NPRB Grant 1704 (2017-2019): "Somatic growth processes in the Pacific halibut (Hippoglossus stenolepis) and their response to temperature, density and stress manipulation effects". IPHC / AFSC – Newport, OR

Dr. Josep Planas (PI)

GROWTH RATES Tmax Temperature Handling/ Density Hierarchical capture stress dominance Effects on transcriptome and proteome **BIOCHEMICAL MUSCLE** AND MOLECULAR LIVER **GROWTH RESPONSES** Identification of molecular **Application to field studies** growth markers

/ ecological conditions (i.e. nursery areas)

Environmental



Dr. Thomas Hurst

NOAA FISHERIES

Discard

survival / fitness

- 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 patterns in the wild.

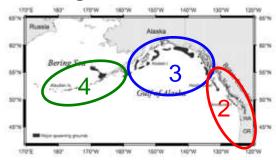
Samples collected in NMFS trawl survey In 2016 and 2017 from 3 size categories:

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Characterization of molecular and biochemical growth markers in muscle samples from agematched individuals

Phase 2: Regional monitorization of growth patterns





- Investigate the effects of other environmental factors on growth performance.
 - Effects of salinity, dissolved oxygen and water pH on growth.



Relate to catch efforts in FISS in time and space model

- Identify the optimal environmental conditions for growth.

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



Primary research areas at IPHC







- 1. Reproduction
- 2. Growth
- 3. DMRs and post-release survival assessment
- BYCATCH SURVIVAL ESTIMATES

- 4. Migration
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Little is known regarding the factors that influence bycatch survival

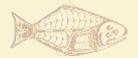
BYCATCH SURVIVAL ESTIMATES

Project components:

- 1. Evaluate the effects of fish handling practices on injury levels and the physiological condition of captured Pacific halibut (Project 672.13, S-K Grant)
- 2. Investigate the relationship between physiological condition post-capture and survival as assessed by the use of accelerometer tags (S-K Grant)
- 3. Explore the applicability of electronic monitoring in DMR estimations (S-K Grant)

Objectives:

 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



• Evaluate 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 straightening, hook stripping).



 Investigate the relationship between physiological condition postcapture and survival as assessed by tagging

Objective: Measure post-release survival in Pacific halibut and relate it to 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 condition.





Explore the applicability of electronic monitoring in DMR estimations

Objective: Test the ability of electronic monitoring to capture fish handling events and fish condition and relate it to survival

- Deploy electronic monitoring (EM) system on a longline vessel.
- Video record fish handling events during capture.
- Determine injury profile by release method.





and associated injury levels

Saltonstall – Kennedy Grant NA17NMF4270240 (2017-2019): "Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival". IPHC / APU – Anchorage, AK

November 2017

- 2 6-day trips (GOA, F/V Kema Sue)
- 38 sets (8 standard skates/set)
- 3 randomized treatments/skate
- 1,048 fish sampled and wire tagged
- 79 fish tagged with accelerometer tags (mini satellite tags; 96 days recording)
- EM on each haul





Dr. Josep Planas (PI)
Claude Dykstra
Dr. Tim Loher
Dr. lan Stewart
Dr. Allan Hicks



Dr. Brad Harris Dr. Nathan Wolf ALASKA PACIFIC



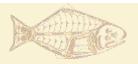
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4. Migration

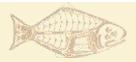
- ADULT FEEDING AND REPRODUCTIVE MIGRATION
- LARVAL DISPERSAL

Projects:

- 1. Juvenile and adult feeding migrations (Project 670.11)
- 2. Tail pattern recognition (Project 675.11)
- 3. Adult dispersal on Bowers Ridge (Reg. Area 4B) (Project 650.21)
- 4. Larval migration and connectivity

Objectives:

To improve our understanding on larval, juvenile and reproductive migration.

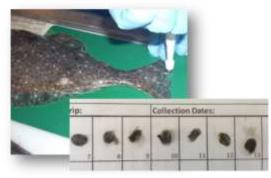


4. Migration

- Juvenile and adult migration studies (Project 670.11)
 - Juvenile wire tagging:
- NMFS trawl tagging project: 1469 fish
 - 713 fish in GOA and 756 fish in BS

- Adult wire tagging:
- IPHC survey tagging project
 - 2016 pilot study in area 4D (U32)
 - 2017 coast-wide study (U32): 1927 fish

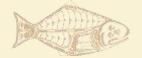
Fin clips are collected: Genetic analyses of tagged fish to shed light on migration patterns and geographic origin.



Tail pattern recognition (Project 675.11)

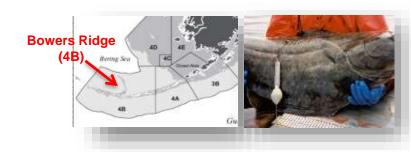


- Blind side of tail is preferable for imaging.
- Spots and patterns appear to be unique.
- Tail markings can be used to identify individuals with image recognition software.
- Promising for implementation in FISS.



4. Migration

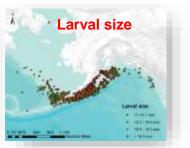
- Reproductive and annual migration (Project 650.21)
 - In 2017: 14 adult fish tagged with sPAT tags (12 females, 2 males). No tag information returned.



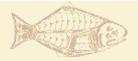
Larval migration and connectivity

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





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



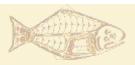
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- GENOMIC TOOLS (e.g. GENOME)



5. Genetics and genomics

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

Projects:

- 1. Sequencing of the Pacific halibut genome (Project 673.13)
- 2. Population genetic studies

Objectives:

- Improve knowledge on the genetic composition of the population
- Establish genomic resources for the species
- Evaluate effects of fishery-dependent and fishery-independent influences on growth, reproduction, nutrition, etc.



5. Genetics and genomics

Pacific halibut genome sequencing (Project 673.13)

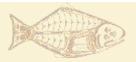


Objective: Generate a first draft sequence of the Pacific halibut genome

- Genomic DNA sequenced from one Pacific halibut female (WZ).
- Conducted first genome assembly:
 - Full genome sequenced. Genome size: 700 Mb
 - Non-continuous genome sequence.
- Additional sequencing is required to complete assembly.





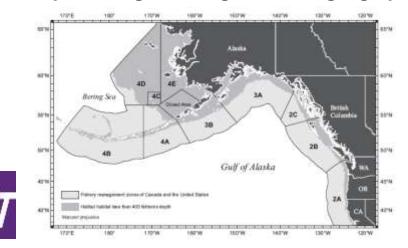


5. Genetics and genomics

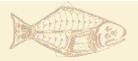
Population genetic studies (PLANNED)

Objective: Genetic characterization of Pacific halibut throughout its distribution range

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



Dr. Lorenz Hauser



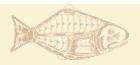
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Research proposals submitted for external funding in 2017

	Project #	Grant agency	Project name	Partners	Budget (US\$)	PI	Management implications	Submission status
NOAA FISHERIES	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	Awarded
	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 Pl)	Changes in biomass/size- at-age	Awarded
	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	Rejected
NOAA FISHERIES	4	Essential Fish Habitat NOAA	Validating biochemical markers of growth for habitat assessment in flatfishes	AFSC- NOAA- Newport	35,000	Hurst (lead PI) Planas	Changes in biomass/ recruitment	Rejected
O NFWF	5	NFWF	Evaluating virtual vitality assessments of discarded Pacific halibut	AFSC- NOAA, APU, NFR	-	Harris (APU), Dykstra	Bycatch estimates	Rejected



Outcome of external funding applications

Project #	Grant agency	Project name	Partners	IPHC Budget (\$US)	PI/IPHC Staff	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	Awarded Started in September 2017
2	NPRB	Somatic growth processes in the Pacific halibut (<i>Hippoglossus stenolepis</i>) and their response to temperature, density and stress manipulation effects	AFSC- NOAA- Newport	\$131,891	Planas (lead PI) Rudy	Changes in biomass/size-at- age	Awarded Started in September 2017
		Total awar	\$355,111				

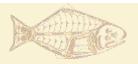


Temporal chart of activities

	2016	2017	2018	2019	2020	2021
		Anı	nual reproductive cy	rcle		
Reproduction			Sex determinati	on mechanisms		
	Sex iden	tification				

NPRB

Saltonstall-Kennedy



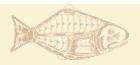
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Research projects proposed for 2018

Project #	Project Name	Priority	Budget (\$US)	External funding for FY2018 (\$US)	Management implications
New Projects					
2018-01	Influence of thermal history on growth	High	\$136,004	-	Changes in biomass / size-at-age
2018-02	Adult captive holding studies	High	\$53,395	-	Changes in biomass / size-at-age / larval distribution
2018-03	Whale detection methods	High	\$37,511	-	Mortality estimation
2018-04	Larval connectivity modeling	High	\$20,000	-	Larval distribution
	Continuing Proje	ects			
621.16	Development of genetic sexing techniques	High	\$33,928	-	Sex composition of the catch
642.00	Assessment of Mercury and other contaminants	Medium	\$8,400	-	Environmental effects
650.18	Archival tags: tag attachment protocols	High	\$800	-	Adult distribution
650.21	Investigation of Pacific halibut dispersal in Regulatory Area 4B	High	\$6,800	-	Spawning areas
661.11	Ichthyophonus Incidence Monitoring	Medium	\$8,755	-	Environmental effects
669.11	At-sea Collection of Pacific Halibut Weight to Reevaluate Conversion Factors	High	\$7,645	-	Length-weight relationship
670.11	Wire tagging of Pacific halibut on NMFS trawl and setline surveys	High	\$12,840	-	Juvenile and adult distribution
672.12	Condition factors for tagged U32 Fish	High	\$9,116	-	DMR estimates
672.13	Discard mortality rates and injury classification profile by release method	High	\$1,037	\$255,402	DMR estimates
673.13	Sequencing the Pacific halibut genome	High	\$32,500	-	Environmental/Fishery effects
673.14	Identification and validation of markers for growth	High	\$25,681	\$57,773	Changes in biomass / size-at-age
674.11	Full characterization of the annual reproductive cycle	High	\$121,488	-	Maturity assessment
675.11	Tail pattern recognition	High	\$3,900	-	Juvenile and adult distribution
	Total - New Projects		\$251,910		
	Total - Continuing Projects		\$273,090		
	Overall Total (all projects for FY2018)		\$525,000		
	External Funding (for FY2018) (\$US)			\$313,175	

New research projects proposed for 2018

Influence of thermal history on growth

- Relate temperature history to individual growth as assessed by archival tagging
- Tag U32 fish with electronic archival tags recording temperature and depth.
- Quantify growth patterns in captured fish and relate them to the thermal history.
- Compare archival data analyses with otolith microchemistry (O¹⁸).

Whale de

- Test aco whale de
- Relate w captures



Adult captive holding studies

- Test permanence of individual tail markings (Tail Pattern Recognition)
- Conduct diet manipulation experiments: fat meter validation, stable isotope studies on growth (N¹5/C¹³)
- Conduct temperature manipulation experiments for growth and O¹⁸ calibration studies
- Perform larval swimming performance tests
- Test transgenerational marking approaches through broodstock labeling

Larval cor

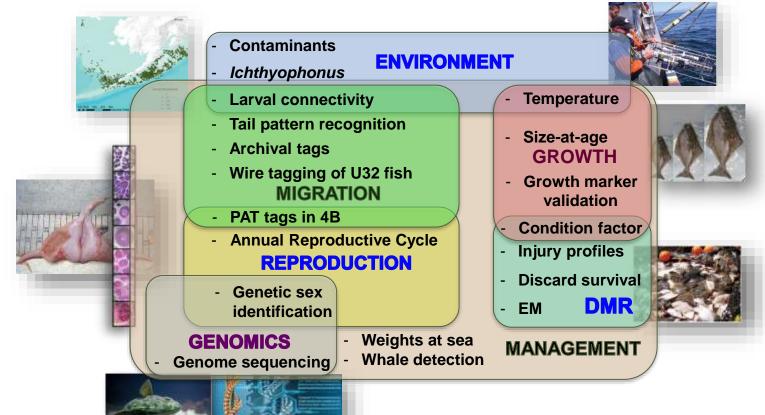
 Model larval abundance and size distribution in the GOA and BS over time and oceanographic and environmental conditions.







Research projects for 2018





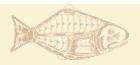
Outline of the presentation







- Update on the research activities of the Biological and Ecosystem Science Program
- Outcome of external funding applications
- Proposed research projects for 2018
- Establishment of the IPHC research laboratory



IPHC Research Laboratory



- Laboratory space has been remodeled
- Laboratory equipment is being purchased
- A laboratory technical position is requested
- Biological samples will be processed and analyzed in house:
 - Genetic sex assays: genotyping
 - Growth and reproductive assessment
 - Blood hormone levels
 - Stress and disturbance indicators

