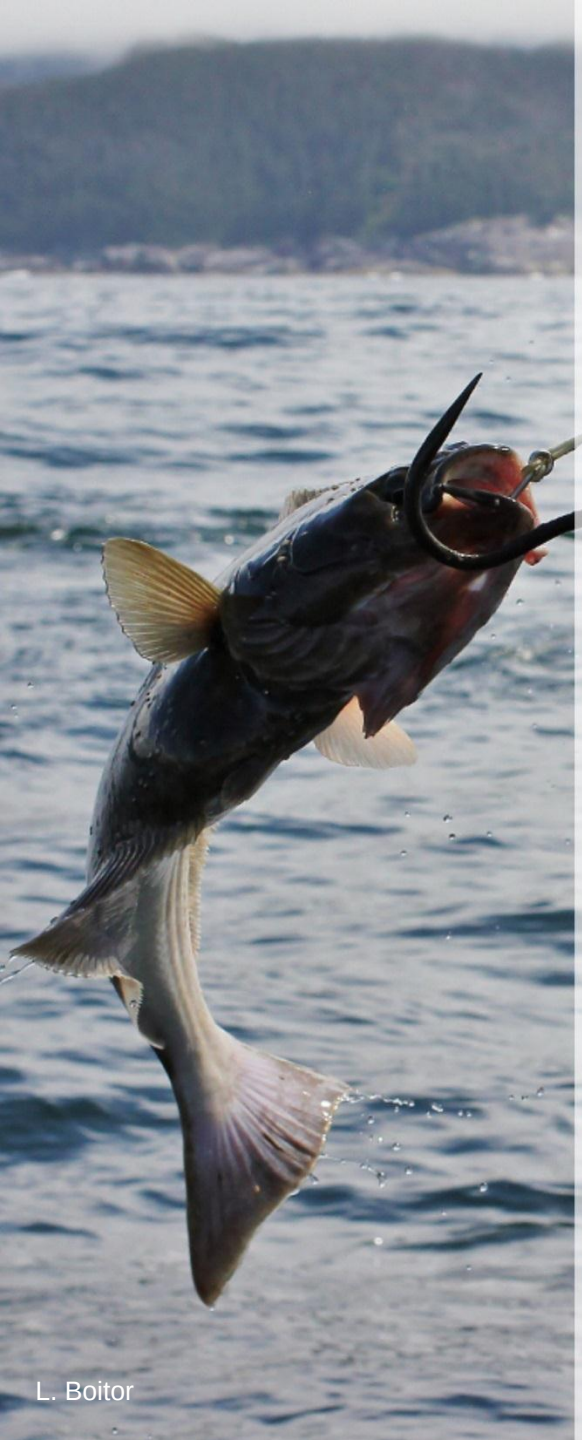


Biological and Ecosystem Science Program :

Current and Future Research Activities

SRB Meeting

June 21, 2016

Josep V. Planas
Program Manager

L. Boitor

Personal background

- **Personal information:**
  - Born in Barcelona, Spain (Spanish citizen)

- **Education:**
  - BSc in Biology. University of Barcelona, Spain (1984)
  - MA in Zoology, University of California, Berkeley (1988)
  - PhD in Biology, University of Barcelona, Spain (1989)
  - PhD in Fisheries, University of Washington, Seattle (1993)

- **Professional experience:**
  - Postdoctoral Fellow, University of Washington, Seattle (1993-1996)
  - Postdoctoral Fellow, University of Barcelona, Spain (1996-1998)
  - Assistant Professor, University of Barcelona, Spain (1998-2001)
  - Associate Professor, University of Barcelona, Spain (2001- 2015)

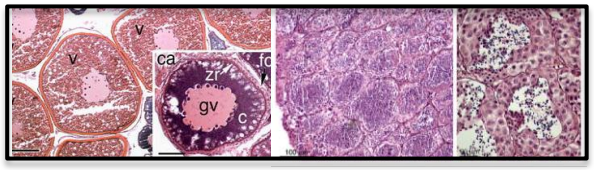
- **Expertise in fish physiology and genomics**



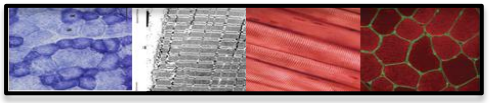

Personal background

Scientific areas of expertise in fish biology:

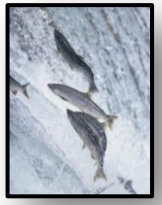
- ***Reproductive physiology***
  - Gametogenesis
  - Final maturation and ovulation



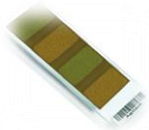
- ***Growth and metabolism***
  - Regulation of carbohydrate and lipid metabolism
  - Muscle growth characteristics



- ***Swimming physiology***
  - Skeletal and cardiac muscle adaptations
  - Swimming performance



- ***Fish genomics***
  - Development of genomic resources for flatfish
  - Application of genomic tools to flatfish biology

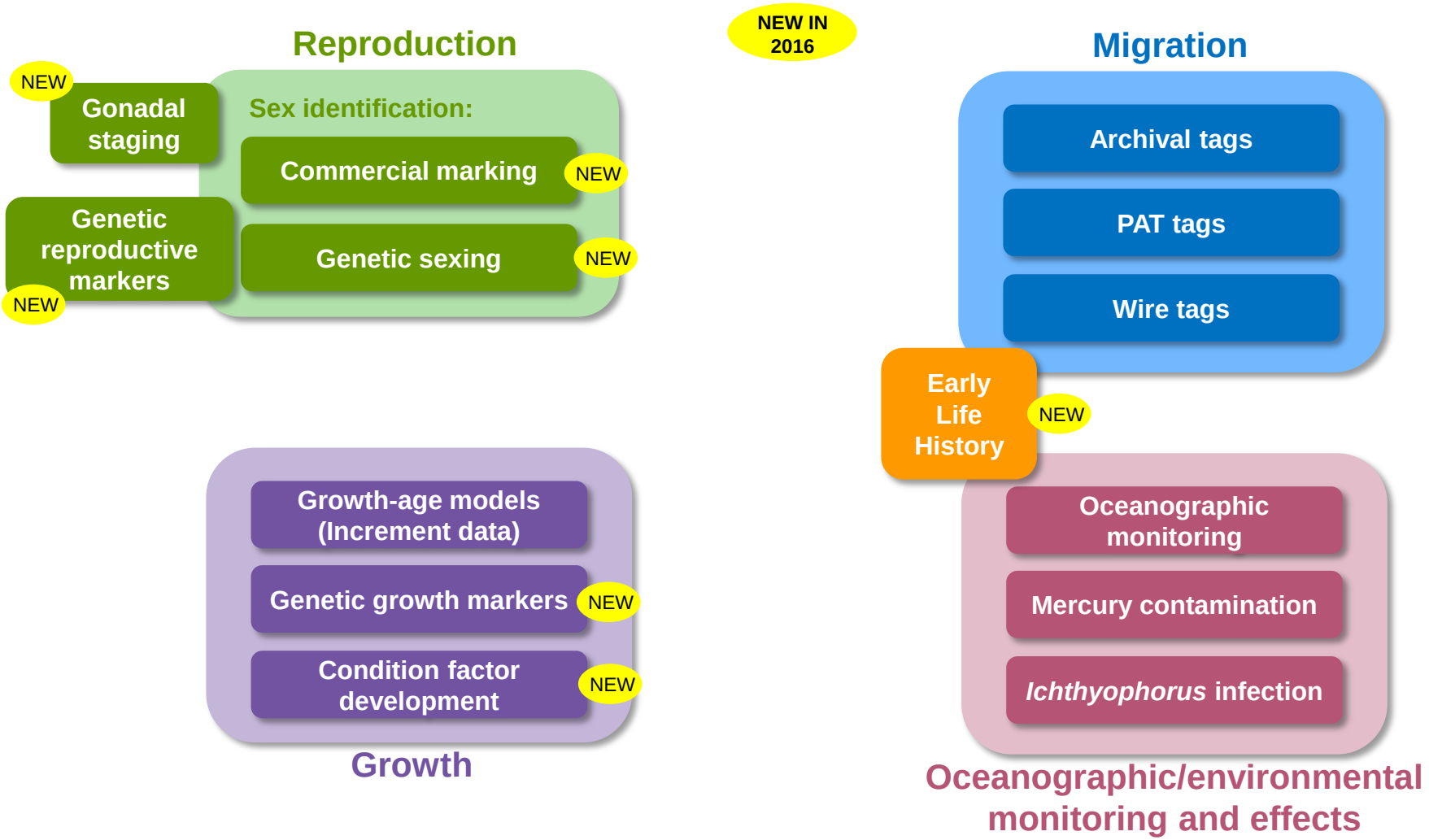


Biological research activities at IPHC

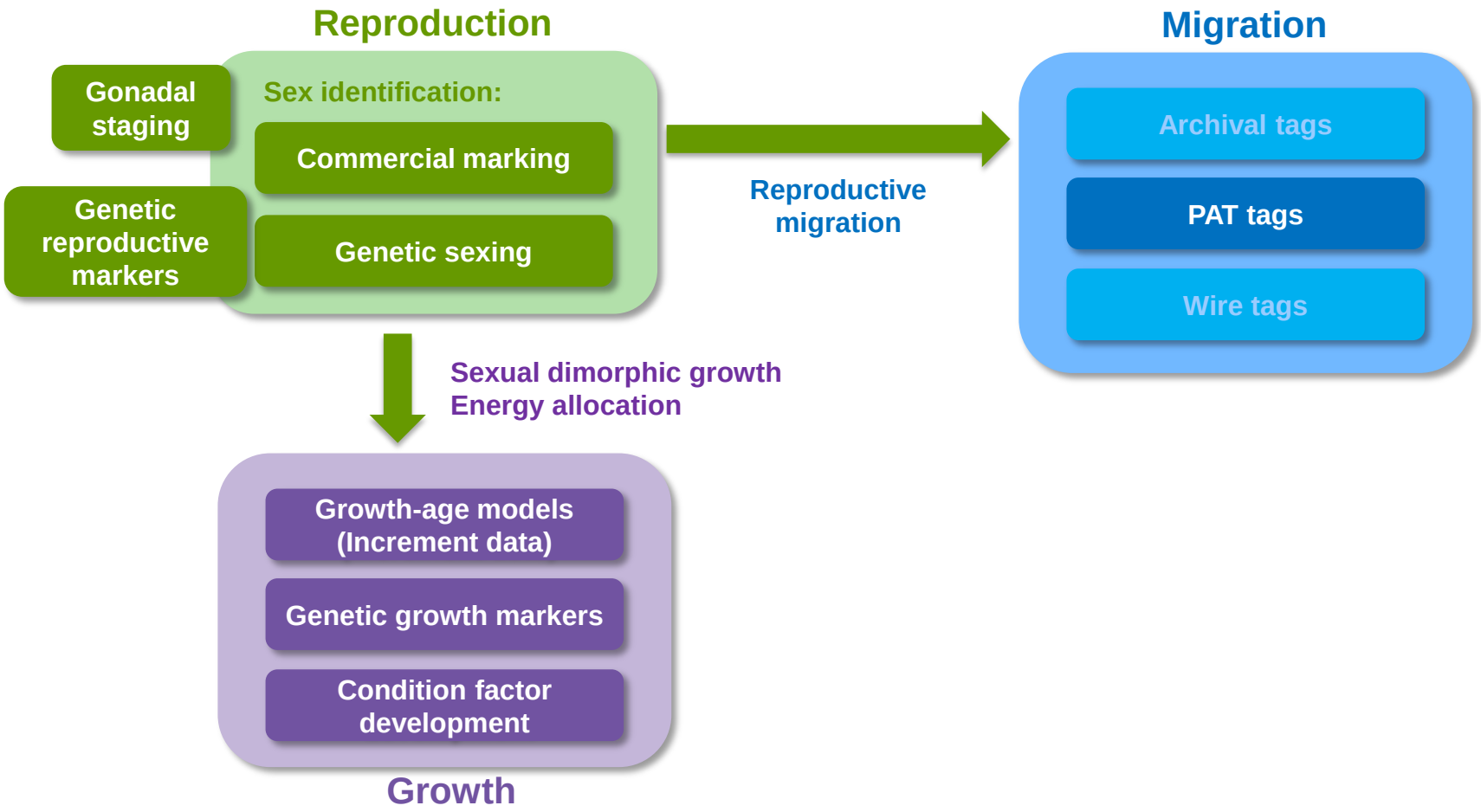
- **What are the current objectives?**
  - **Identify and address critical knowledge gaps in the biology of the Pacific halibut**
  - **Understand the influence of environmental conditions on halibut biology**
  - **Apply resulting knowledge to reduce uncertainty in current stock assessment models**

- **What have been traditionally the main research activities at IPHC?**
  - **Migration. Adult movement across regulatory areas and to identify spawning grounds.**
  - **Size and age structure of the population by body measurements and otholith ageing.**
  - **Oceanographic monitoring.**

Current biological research activities



Reproduction studies



Reproduction: ongoing studies

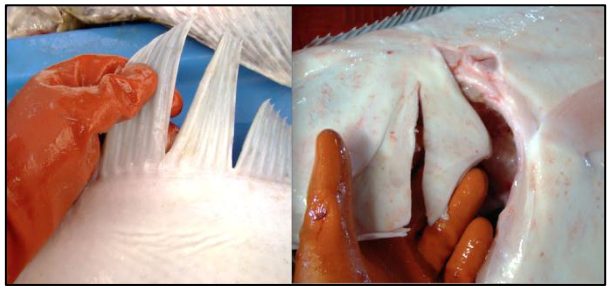
Gonadal staging

Genetic reproductive markers

Sex identification:

- Commercial marking
- Genetic sexing

- **We need to know the sex of extracted fish**
  - **Commercial marking at sea**

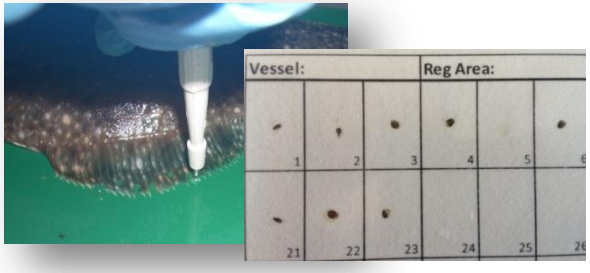


Dorsal Cut (Female) **Gill Plate Cut (Male)**

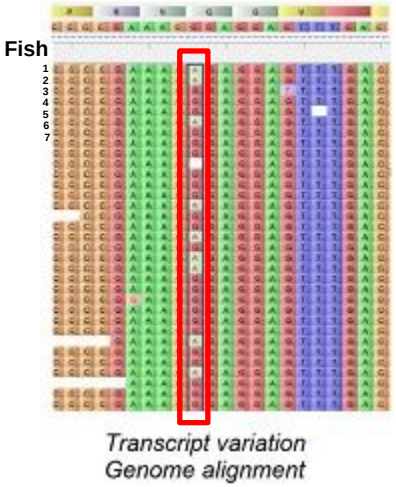
Ongoing

- **Identification of genetic markers for sex: Single Nucleotide Polymorphisms (SNPs)**

Fin clips



→ DNA extraction → DNA sequencing



Transcript variation
Genome alignment

Ongoing

We are mining for SNPs associated with sex (male vs female genetic signatures)

Reproduction: ongoing studies

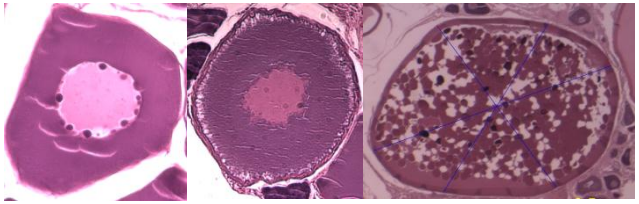
Gonadal staging

Sex identification:

- Commercial marking
- Genetic sexing

Genetic reproductive markers

- Pilot study on ovarian staging: summer and winter samples



Ongoing

- Identification of *molecular markers* of ovarian and testicular function

- **Identification of sex marker genes**
- **Identification of maturation marker genes**

RNA sequencing

Ongoing

3. De novo Transcriptome Assembly Stats

Sample ID	Total trinity 'genes'	Total trinity transcripts	Percent GC	Contig N50	Median contig length	Average contig	Total assembled bases
R116-pool3	48,573	60,084	48.89	2,494	582	1,240.16	74,513,854
R116-pool4	74,363	87,644	47.10	2,004	489	1,014.53	88,917,698

OVARY
TESTIS

5.1 Mapping statistics

Sample ID	Danio rerio	uniprot	est others	total	unmapped	Danio%	uniprot%	est others%	unmapped%
R116-pool3	18,426	4,259	37,267	60,084	132	30.67%	7.09%	62.02%	0.22%
R116-pool4	23,644	5,539	58,303	87,644	158	26.98%	6.32%	66.52%	0.18%

OVARY
TESTIS

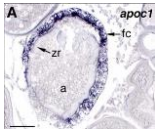
Reproduction: proposed studies

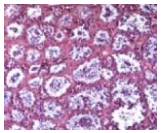
There are important knowledge gaps on the reproductive biology of the species

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

1. Characterization of the annual reproductive cycle

Objective: Understand temporal changes in reproductive development throughout an entire annual reproductive cycle.





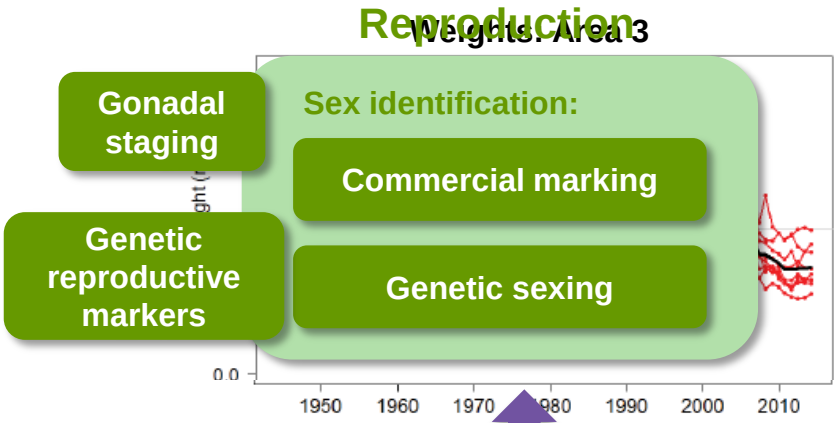
- **Histological assessment of gonadal development and maturation.**
- **Hormonal profiling (blood sex steroids) of the reproductive cycle.**
- **Gene expression (transcriptome) profiling of the reproductive axis.**
- **Gonadosomatic index (GSI) determinations throughout the reproductive cycle.**
- **Ultrasound monitoring of gonadal development and maturation.**

2. Sex determination mechanisms

Objective: Understand the sex determination mechanism(s) in Pacific halibut.

- **Identification of sex determining mechanism(s) and its onset during early development.**
- **Identification of environmental influences (temperature) on sex determination.**
- **Evaluate possible consequences on sex ratios at the population level.**

Growth studies



Reproduction

Gonadal staging

Genetic reproductive markers

Sex identification:

Commercial marking

Genetic sexing

Reproductive performance
Skipped spawning

Migration

Archival tags

PAT tags

Wire tags

Fitness

Early Life History

Genetic growth markers

Condition factor development

Growth-age models (Increment data)

Growth

Environmental factors

Mercury contamination

Ichthyophorus infection

Oceanographic/environmental monitoring and effects

Other factors: Density, resource competition, etc

Growth studies: ongoing studies

- **Identification of *molecular markers* for growth**

RNA sequencing **Ongoing**

3. De novo Transcriptome Assembly Stats

Sample ID	Total trinity 'genes'	Total trinity transcripts	Percent GC	Contig N50	Median contig length	Average contig	Total assembled bases	
R116-pool1	37,161	39,638	47.76	1,198	385	721.49	28,598,382	WHITE MUSCLE
R116-pool2	38,143	40,814	46.02	1,096	398	691.85	28,237,340	LIVER
R116-pool5	70,693	86,561	47.17	2,104	495	1,051.87	91,050,930	RED MUSCLE

5.1 Mapping statistics

Sample ID	Danio rerio	uniprot	est others	total	unmapped	Danio%	uniprot%	est others%	unmapped%	
R116-pool1	13,873	2,661	23,066	39,638	38	35.00%	6.71%	58.19%	0.10%	WHITE MUSCLE
R116-pool2	13,233	2,547	24,998	40,814	36	32.42%	6.24%	61.25%	0.09%	LIVER
R116-pool5	25,341	5,579	55,466	86,561	175	29.28%	6.45%	64.08%	0.20%	RED MUSCLE

- ***Condition factor* development: comprehensive set of measures on the physiological condition of fish**
  - **Length/weight relationships (Fulton's k, etc.).**
  - **Morphometic/shape analyses.**
  - **Energy levels (fat content, metabolite levels, energy-sensing molecules).**
  - **Assessment of muscle quality characteristics (muscle fiber composition).**

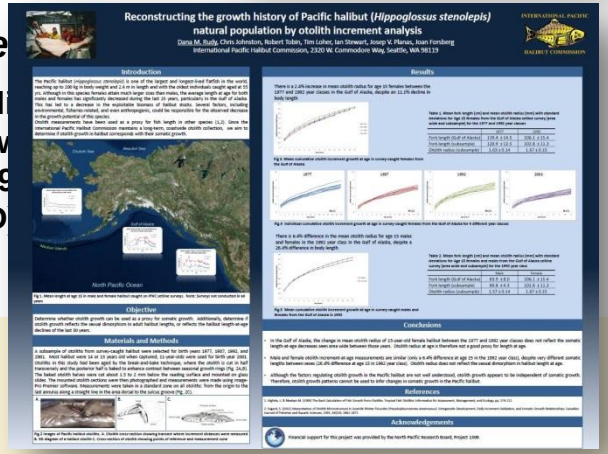
Genetic growth markers

Condition factor development

Growth-age models (Increment data)

Growth

- ***Otolith growth increme***
  - **Assessment of the vali proxy for somatic grov classes (1977, 1987, 19 areas (2B, 3A and 4CD**



Growth: proposed studies

Little is known regarding what factors influence growth in this species

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

1. Extensive catalogue of molecular markers for growth

Objective: Identify and validate molecular growth-related markers for growth studies.

- **Identification of expressed sequences from skeletal muscle (white and red) and liver.**
- **Develop molecular assays to quantify gene expression of growth markers in relevant tissues.**

2. Evaluation of growth patterns and effects of environmental influences

Objective: Identify molecular and biochemical 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

- **Establishment of different growth trajectories in juvenile fish in captivity.**

- Feeding/fasting

Fed

Fasted

- Manipulating growth rates (ration, density, th compensation, etc.)

Low rate

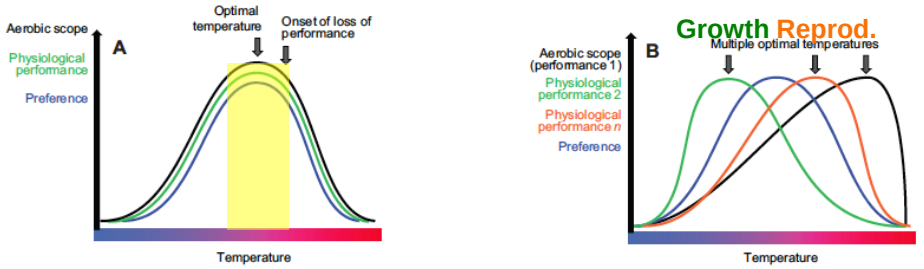
Normal rate

Growth: proposed studies

2. Evaluation of growth patterns and effects of environmental influences (cont.)

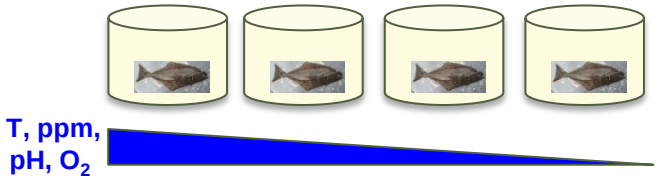
- **Understand the basis of the sexual dimorphic growth in the Pacific halibut.**
- **Investigate the effects of environmental factors on growth performance.**
  - ***Effects of temperature, salinity, dissolved oxygen and water pH on growth.***
  - ***Identify the optimal environmental conditions for growth.***

e.g. Temperature: **- Is there a relationship between growth rates and T changes?**
- What is the optimal T range for growth?
- What is the relationship between aerobic scope, temperature and optimal growth?



- Is growth affected by changes in salinity, dissolved O_2 (hypoxia), water pH?

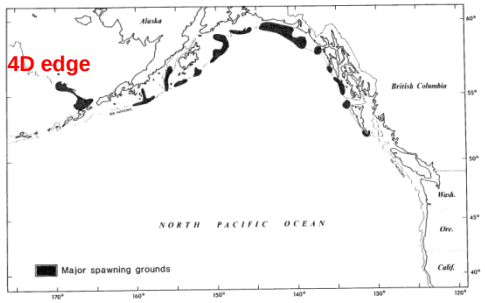
a) Evaluate growth after exposure to different environmental conditions



b) In the wild, correlate size at age with environmental levels and individual history

Migration: ongoing studies

- **Adult migration studied by tagging**
  - **Wire tags**
  - **PAT satellite tags**



June-July 2016: 32 females
(16♀ 1 year release program)
(16♀ 3 month release program)

- **Bycatch survival as assessed by accelerometer tags**

June-July 2016: 100 fish (60 day release program)

Migration

Archival tags

PAT tags

Wire tags

Early Life History

Environmental conditions

Mercury contamination

***Ichthyophorus* infection**

Oceanographic/environmental monitoring and effects

Migration: proposed studies

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

1. Towards a more comprehensive view on migration

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

- **Collect fin clips for genetic analyses on migration patterns and individual origin determination.**
- **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.**

2. Larval migration and connectivity

Objective: Understand the mechanisms of larval connectivity between the GOA and the 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 (NOAA, AFSC)

Migration: proposed studies

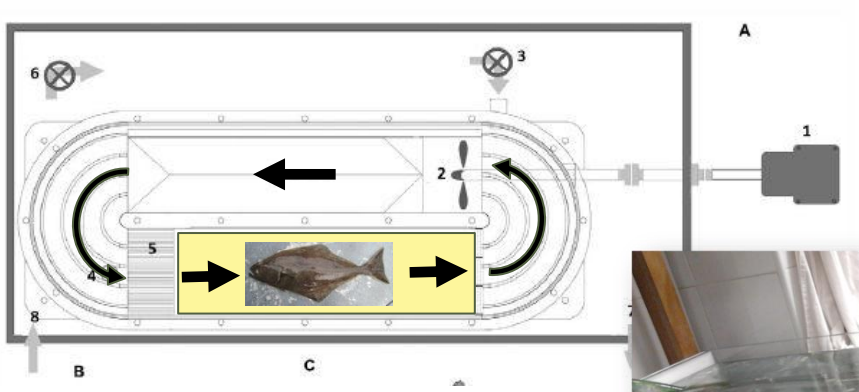
3. Swimming and migratory performance

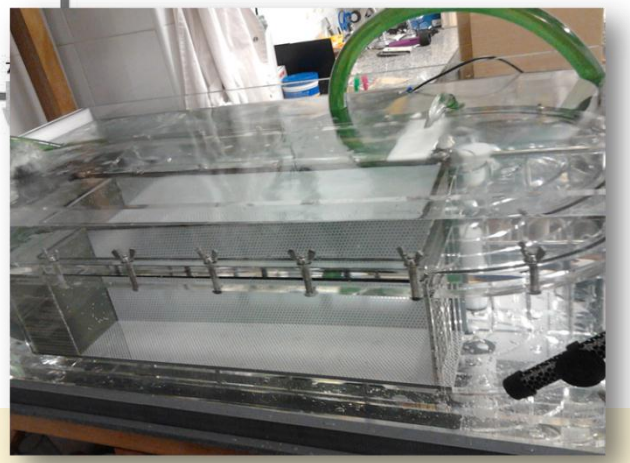
Objective: Evaluate swimming and migratory performance of juvenile Pacific halibut by swim testing.

Measure of fitness

- **Swimming capacity (optimal or maximal sustainable swimming speed; U_{opt}, U_{max}).**
- **Aerobic scope (respirometry/O_2 consumption): measure of aerobic performance.**
- **Biochemical, cellular and molecular indicators of swimming performance in skeletal and cardiac muscle**
- **Drag effects of PATs.**

Swim tunnel:





Genetics and genomics: proposed studies

- **Improve our knowledge on the genetic composition of the population**
- **Establish genomic resources for the species**

1. 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**

2. Sequencing of the Pacific halibut genome

Objective: Genome sequencing at sufficient coverage

- **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 behaviour and performance, etc.**
- **Provide genomic resolution to genetic markers (from RAD tag seq or RNAseq).**
- **Link genotype and phenotype.**
- **Indentify genetic, evolutionary changes in response to environmental and anthropogenic influences.**
- **Eco-evolutionary dynamics. Spatio-temporal population genomics.**

3. Characterization of the Pacific halibut epigenome

Objective: Identify epigenetic effects of environmental and anthropogenic factors on the genome

- **Identification of genomic regions potentially subjected to epigenetic regulation, allowing for rapid phenotypic changes and adaptive responses to environmental and anthropogenic influcences.**
- **Complement transcriptomic information on growth and reproductive performance.**
- **Identification of genetic signatures of geographical population groups**

Temporal chart of activities

2016	2017	2018	2019	2020	2021
	Annual reproductive cycle				
	Sex determination mechanisms				
Population genetic structure					
	Genome sequencing				
		Genome-wide association studies			
	Epigenome characterization				
Growth transcriptome					
Growth-related patterns					
	Regulation of growth by environmental factors				
	Comprehensive studies on migration				
	Larval migration and connectivity				
Tagging sublegal halibut					
Reproductive monitoring of PAT-tagged adults					

Thank you for your attention!