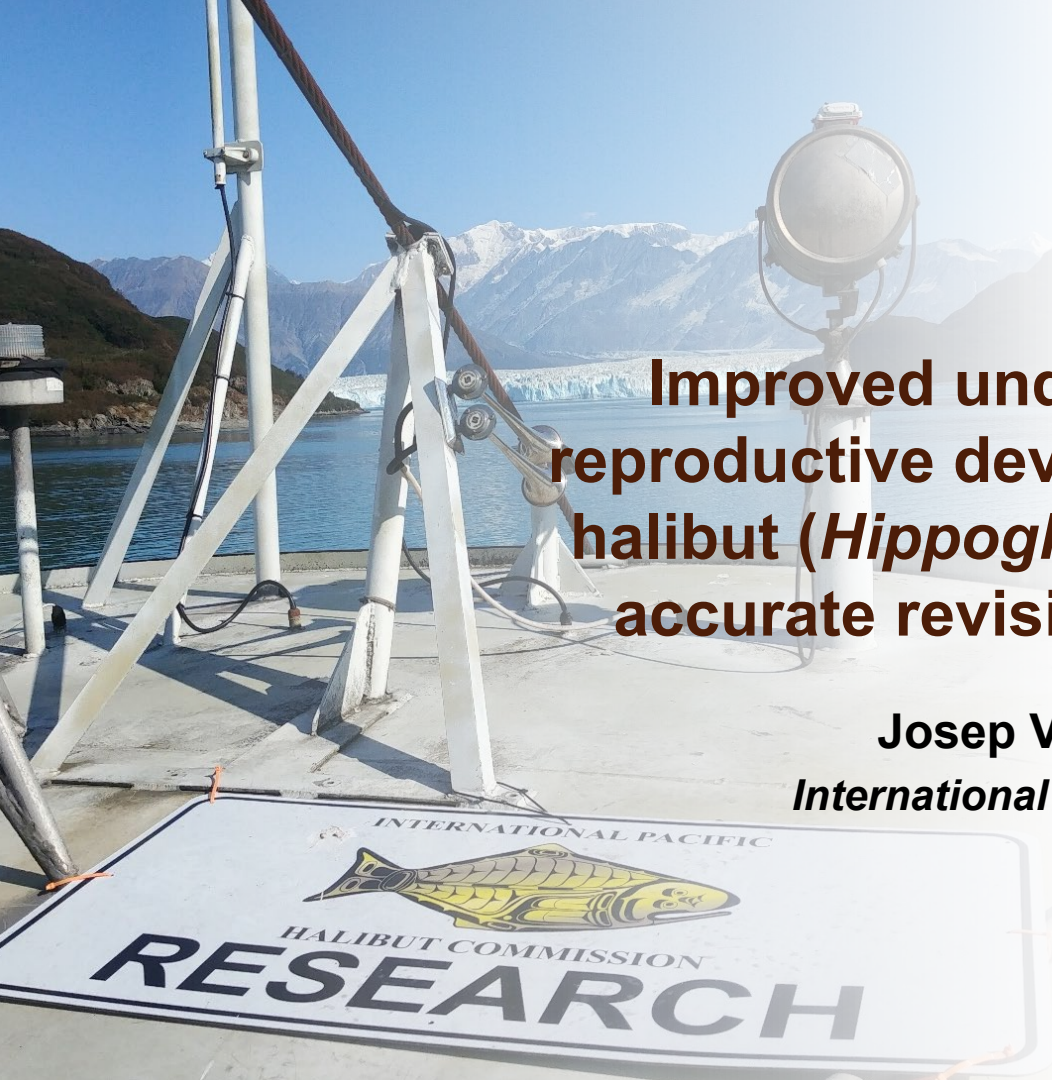


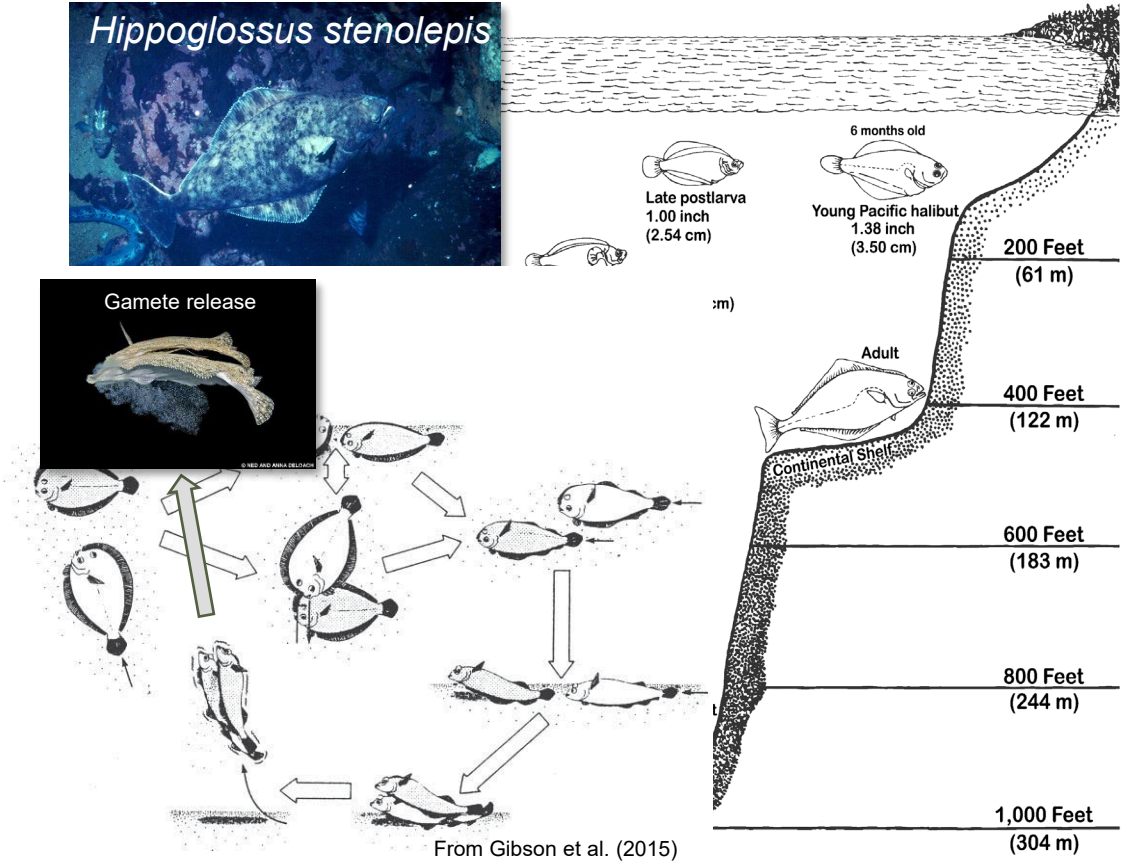
INTERNATIONAL PACIFIC HALIBUT COMMISSION

Improved understanding of seasonal reproductive development in female Pacific halibut (*Hippoglossus stenolepis*) guiding accurate revision of maturity estimates

Josep V. Planas, Colin Jones

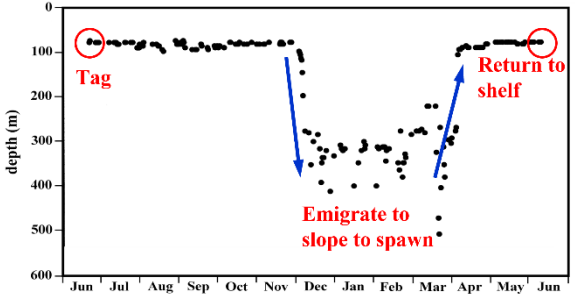
International Pacific Halibut Commission

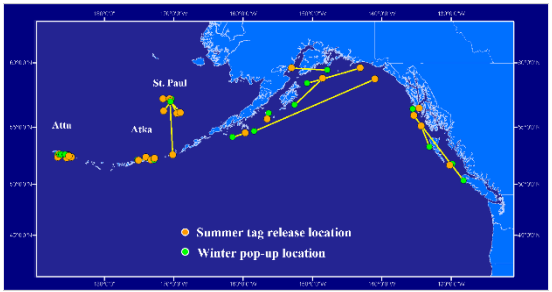
Pacific halibut: reproductive biology



From Gibson et al. (2015)

- Fall offshore migrations from summer feeding grounds to winter spawning grounds



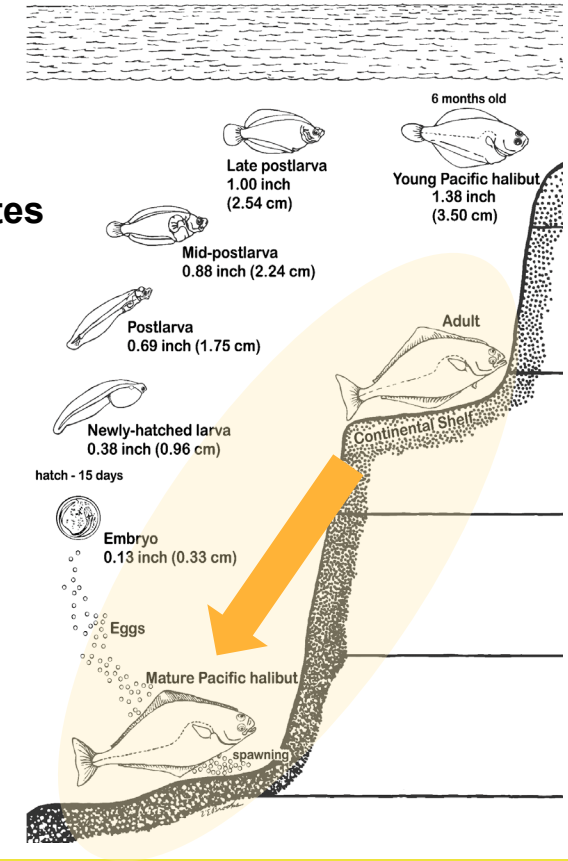


Pacific halibut: reproductive biology

Current maturity estimates

- Visual assessment (macroscopic)

- Summer surveys



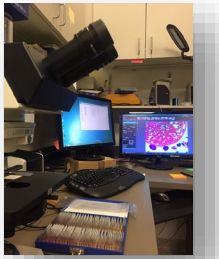
There are still important knowledge gaps:

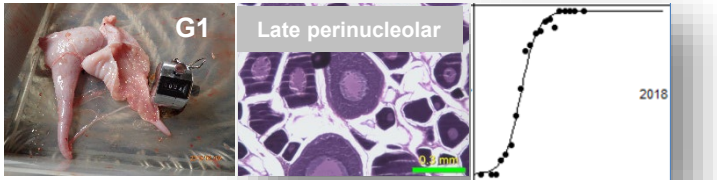
- What is the nature of the signals that trigger adult reproductive development and behaviors leading to successful spawning?
- What are the physiological characteristics of reproductive development?
- What physiological indicators can we use to monitor the progression of reproductive development and determine maturity?

We need this information to improve the accuracy of maturity and fecundity estimation methods

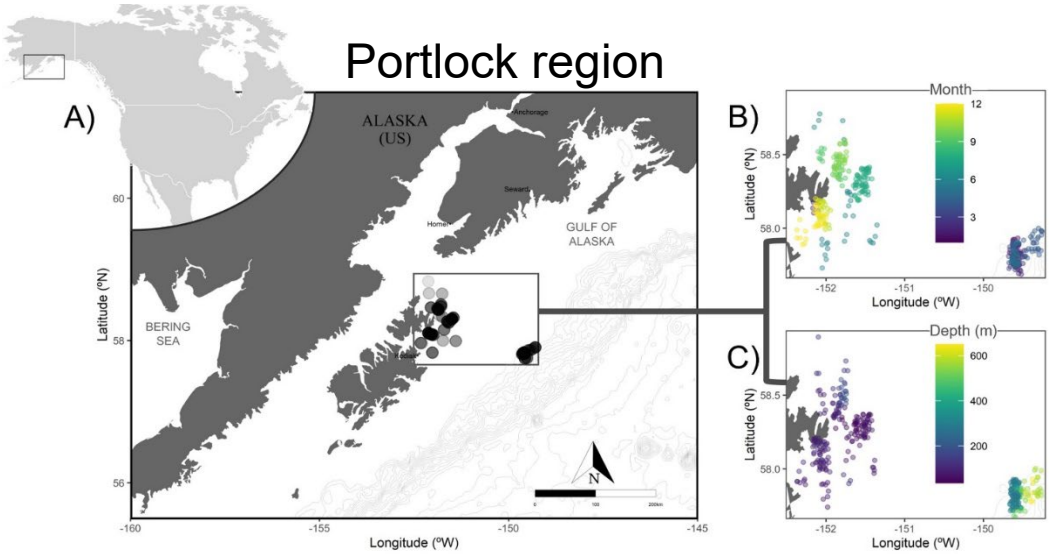
Objectives

1. ***Characterize reproductive development in mature female Pacific halibut by gonadal histology.***
2. ***Identify physiological features of reproductive development.***
3. ***Update maturity estimates based on histological and physiological criteria.***
4. ***Revise current field maturity classification criteria.***





Methods



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

2017 2018

30♀ / month

♀ > 90 cm FL

Samples collected:

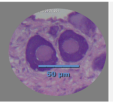
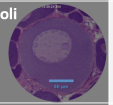
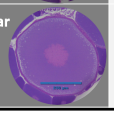
- Gonads in 10% buffered formalin for histology
- Gonad and liver weights
- Blood samples for reproductive parameters
- Pituitary and gonads in RNAlater for gene expression

Methods:

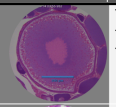
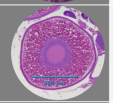
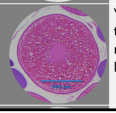
- Staining of sections of paraphing-embedded tissues with H&E
- Plasma determination of sex steroids and vitellogenin levels by ELISA
- Pituitary and gonad gene expression by qPCR
- Somatic fat content by Fatmeter

Results: oocyte developmental stages

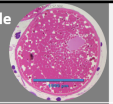
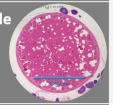
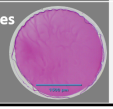
Primary Growth

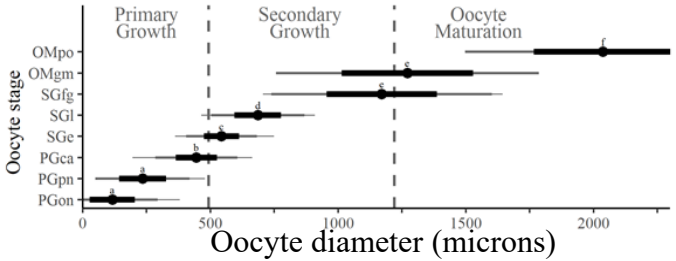
	Female Pacific halibut histology stages	Oocyte description	Oocyte diameter (µm)
Primary Growth (PG)	one nucleolus (PGon) 	Oocytes are small, angular and compact with a single large nucleolus. Cytoplasm stains dark purple.	na
	multiple nucleoli (PGmn) 	Oocytes are larger and rounder than PGon, cytoplasm stains lighter purple and numerous nucleoli develop.	222–457 329
	cortical alveolar (SGca) 	First cortical alveoli appear as white stain in the periphery of the oocyte.	317–638 465

Secondary Growth

	Female Pacific halibut histology stages	Oocyte description	Oocyte diameter (µm)
Secondary Growth (SG)	early (SGe) 	Yolk granules first appear at the periphery, stain pink, and fill inwards occupying up to 1/3 of the cytoplasm.	428–761 567
	late (SGl) 	Yolk granules transition from only the periphery of the ooplasm and fill inwards to the nucleus.	544–892 737
	full grown (SGfg) 	Yolk granules completely fill the ooplasm to the central nucleus and coalesce to form larger yolk globules.	757–1670 1214

Oocyte Maturation

	Female Pacific halibut histology stages	Oocyte description	Oocyte diameter (µm)
Oocyte Maturation (OM)	germinal vesicle migration (OMgvm) 	The nucleus begins to migrate through a cytoplasm fully filled with yolk globules.	1065–1738 2067
	germinal vesicle breakdown (OMgvbd) 	Nucleus is no longer visible and dark pink yolk globules coalesce into light pink stained yolk masses occupying less than ½ the oocyte area.	na
	meiosis resumes hydration (OMmr) 	Light pink stained yolk coalesces into a central mass, occupying over ½ the area. Oocyte is still within the follicle wall.	1729–2516 2067



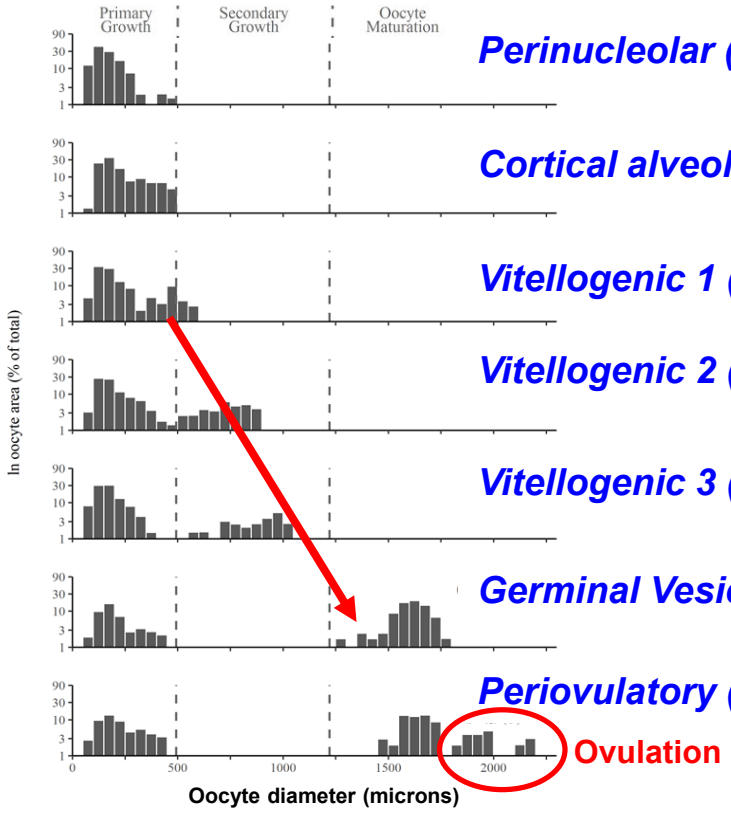
Female developmental phase determined based on the stage of the most advanced oocytes

Fish et al. (2020) *J. Fish Biol.* 97: 1880

Results: oocyte size distribution

Least advanced ♀

Most advanced ♀



Perinucleolar (Pgca) stage

Cortical alveoli (Pgca) stage

Vitellogenic 1 (Vtg1) stage

Vitellogenic 2 (Vtg2) stage

Vitellogenic 3 (Vtg3) stage

Germinal Vesicle Migration (GVM) stage

Periovulatory (PO)stage

Ovulation

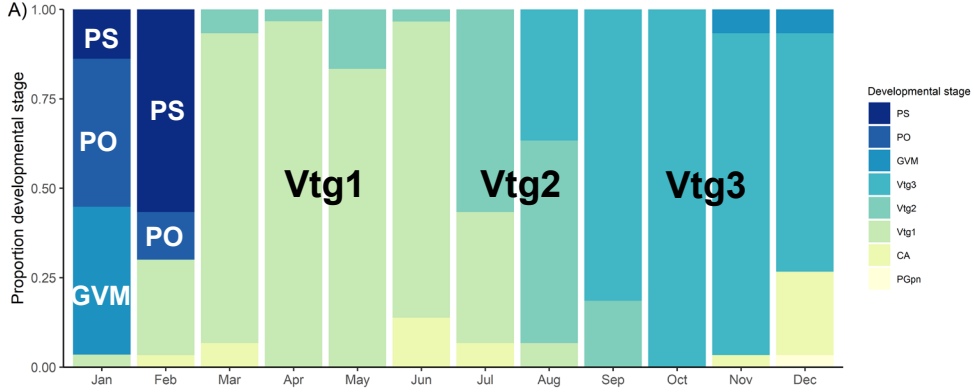
Female Pacific halibut are:

- Batch spawners
- Group synchronous
- Determinate fecundity

Fish et al. (2020). *J. Fish Biol*. 97: 1880

Results: seasonal changes

Female developmental stages



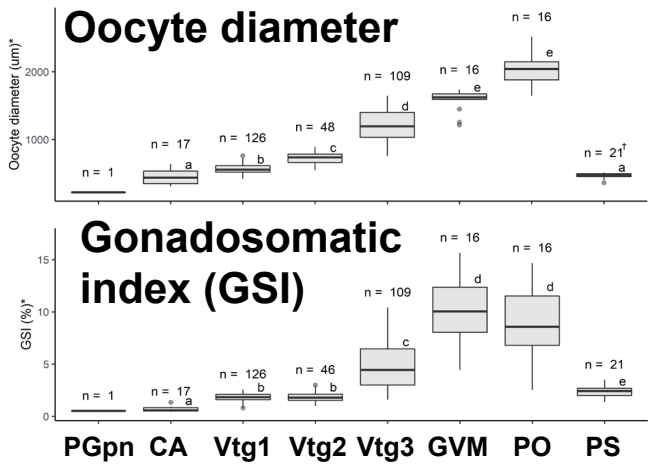
Oocyte secondary growth (Vitellogenesis) | **Oocyte maturation** | **Spawning**

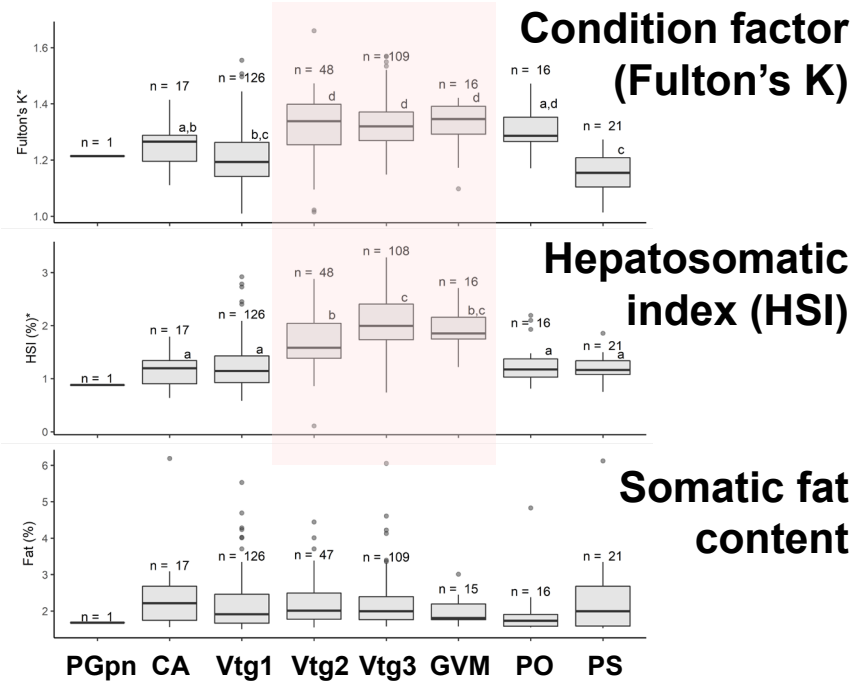
Vtg1 → Vtg2 → Vtg3 → GVM → PO → PS

Female Pacific halibut follow an annual reproductive cycle

Fish et al. (2022). *Front. Mar. Sci. 9:801759*

Results: developmental stage characteristics





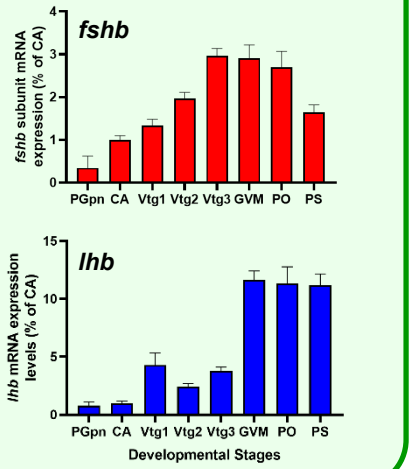
These indices can assist in predicting the female developmental stage

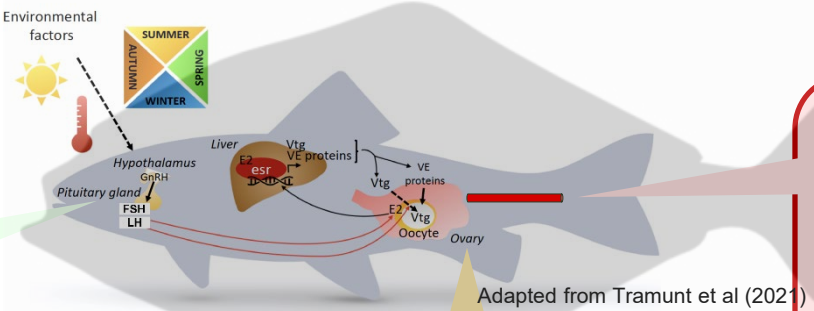
Better reproductive markers are needed to support histological staging and identify potential deviations from expected maturity progression

Fish et al. (2022). *Front. Mar. Sci. 9:801759*

Results: endocrine reproductive markers

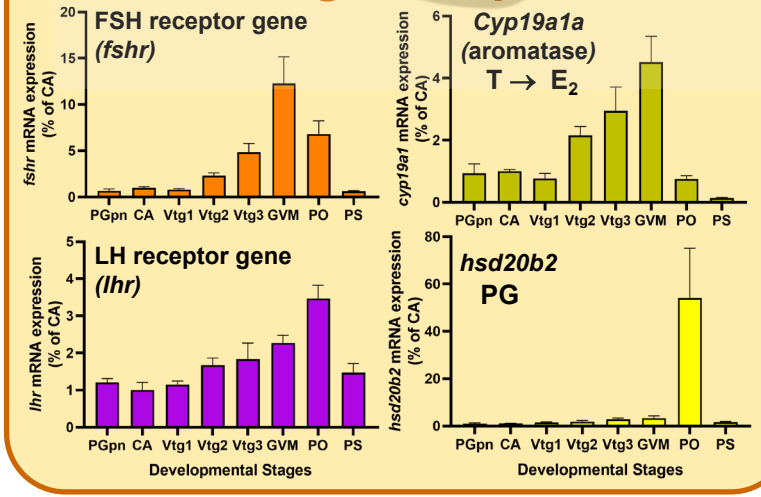
Pituitary gonadotropin gene expression



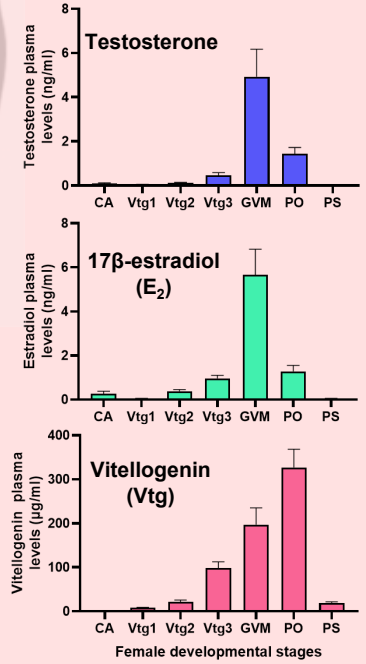


Adapted from Tramunt et al (2021)

Ovarian gene expression

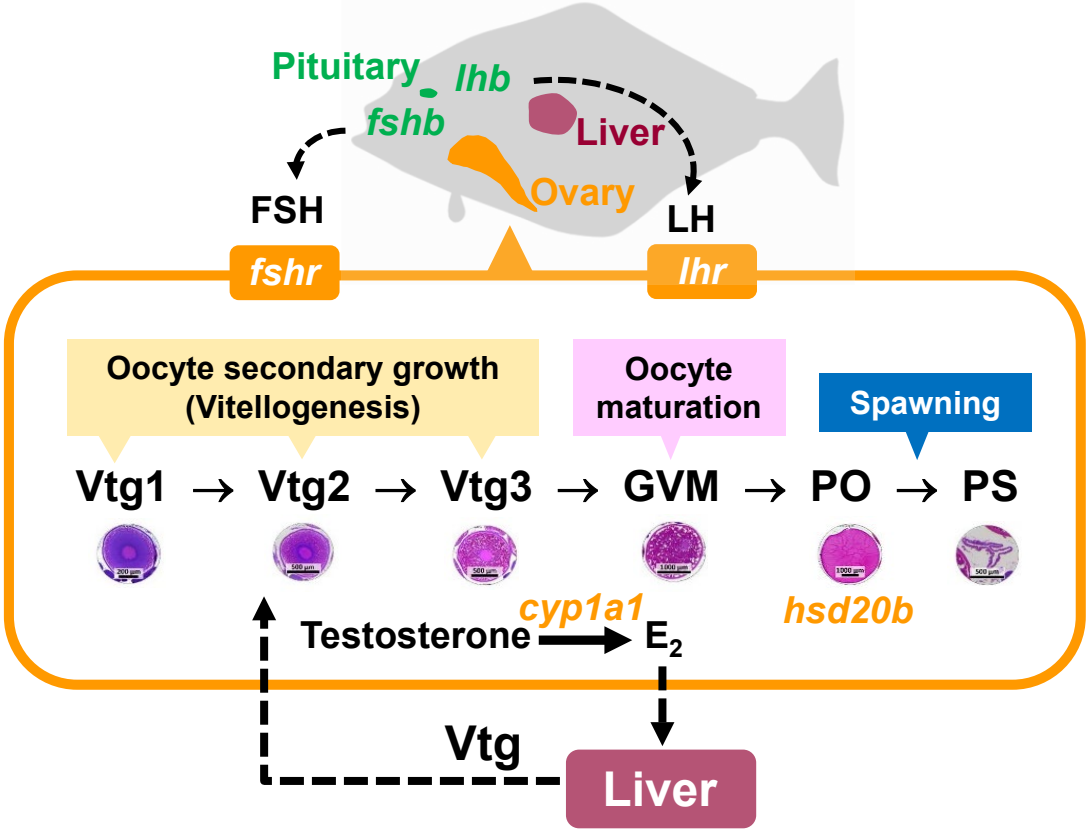


Blood levels



Planas et al. (unpublished data)

Results: endocrine reproductive markers



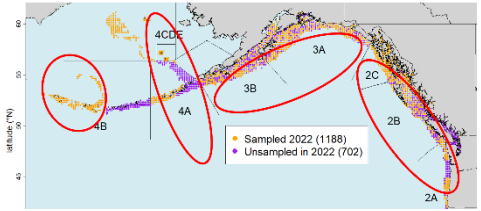
- **Pituitary markers**
  fshb, lhb
- **Ovarian markers**
  fshr, lhr, cyp1a1, hsd20b
- **Blood markers**
  Testosterone, E_2, Vtg

Planas et al. (unpublished data)

Endocrine markers will help characterize and define developmental stages and maturity

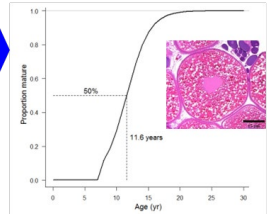
Next steps

- **Revise maturity schedules**: ovarian samples collected during IPHC's fishery-independent setline survey (FISS) in 2022.



Biological Region	Collected samples
2	437
3	348
4	180
4B	51

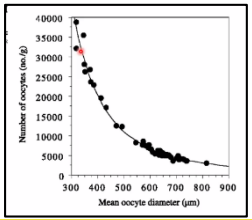
- **Maturity estimates per biological region by histological staging**



In progress

- **Compare microscopic and macroscopic maturity staging**: using samples with parallel macroscopic (visual) staging collected in 2022.
- **Conduct fecundity estimations by age and size:** sample collections planned for the summer of 2023.

Autodiametric method



Thorsen and Kjesbu 2001

Acknowledgements

International Pacific Halibut Commission

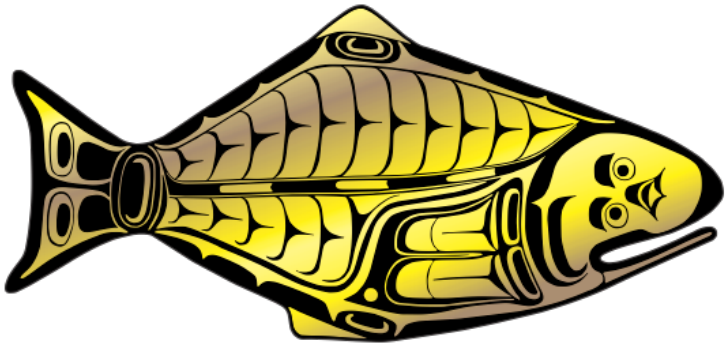
Crystal Simchick
Anna Simeon
Andy Jasonowicz
F/V skippers and IPHC sea samplers

Alaska Pacific University

Teresa Fish
Anita Kroska
Dr. Nathan Wolf
Dr. Brad Harris

Thank you for your attention!

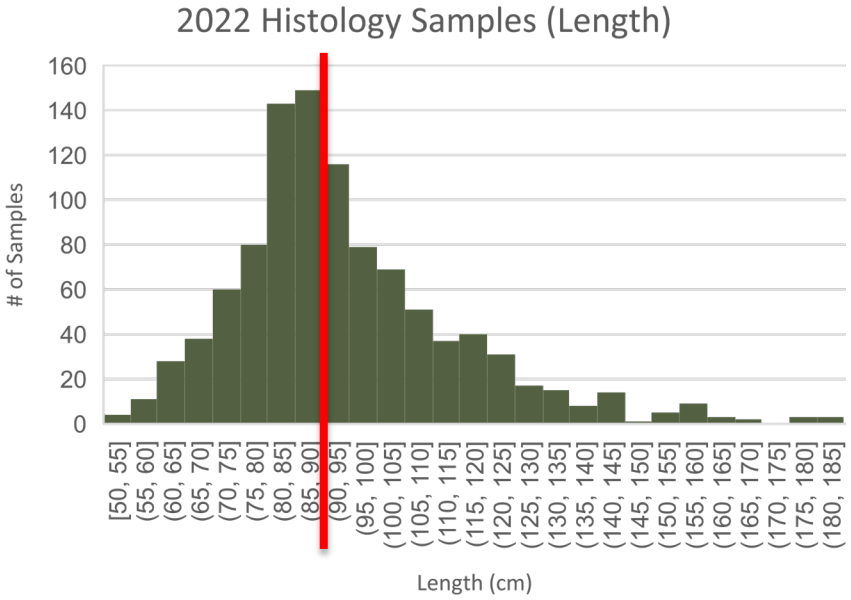
INTERNATIONAL PACIFIC

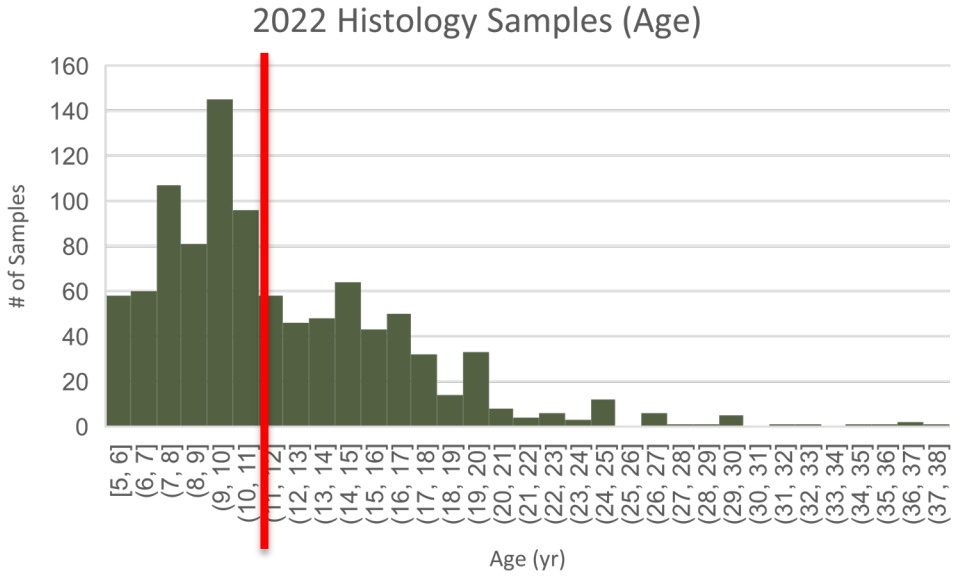


HALIBUT COMMISSION

1. Maturity

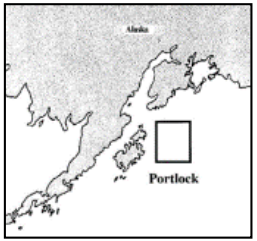
FISS 2022: ovarian sample collection for histology-based maturity

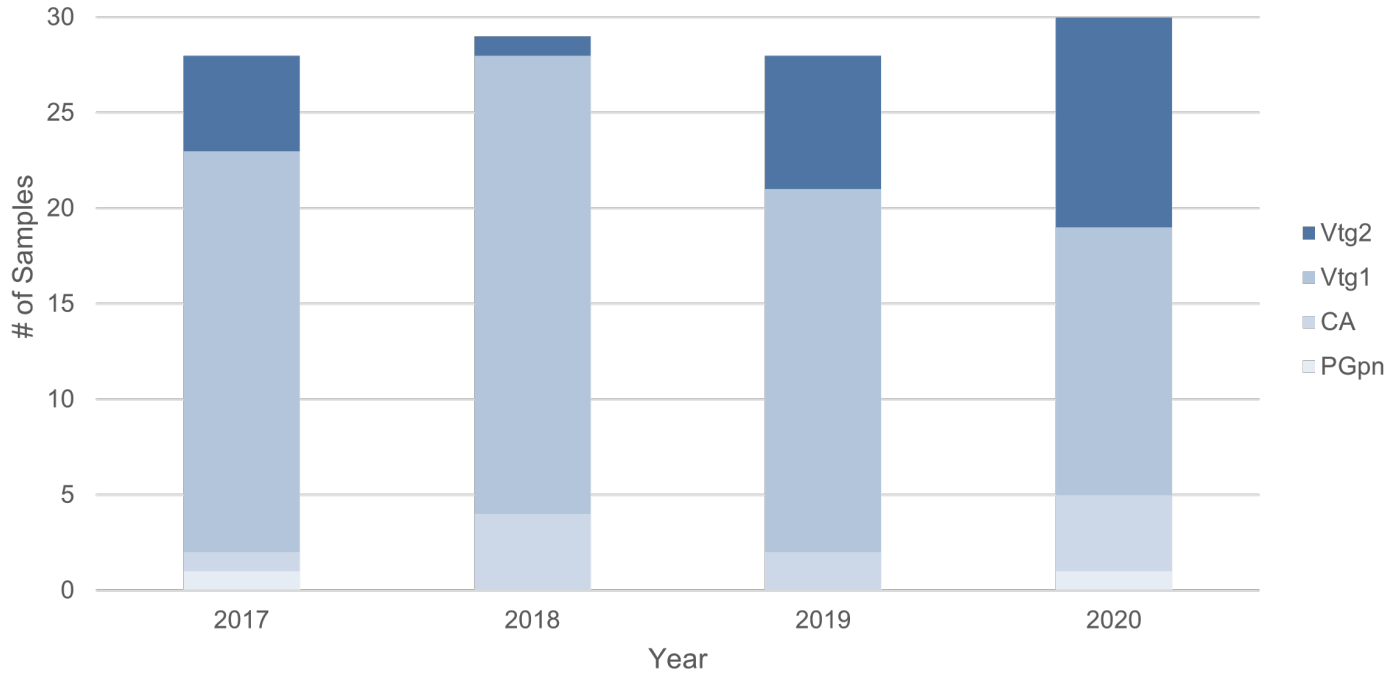




Maturity: temporal assessment

Microscopic maturity staging: based on histological oocyte stages





June-July: > 85% at Vtg1-Vtg2