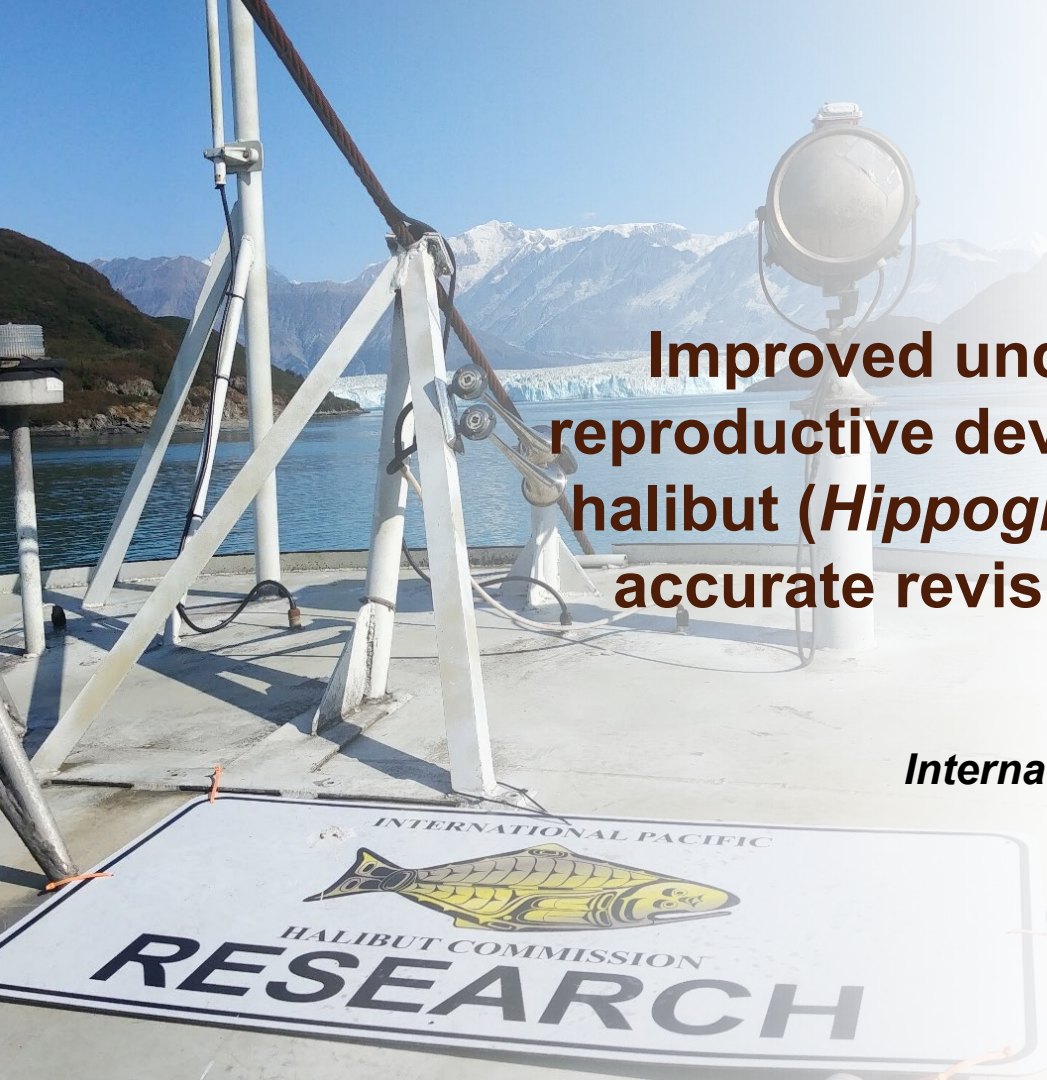

INTERNATIONAL PACIFIC HALIBUT COMMISSION

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

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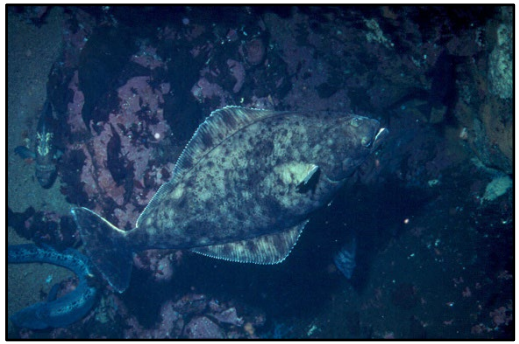
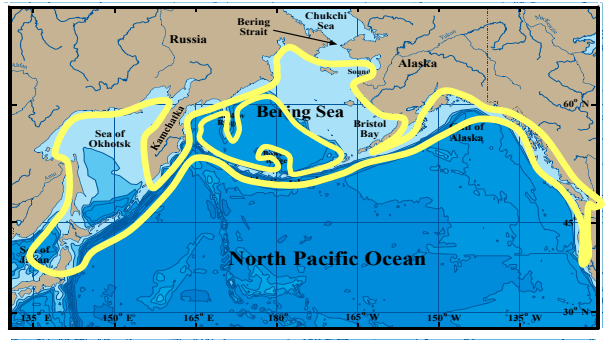
Introduction

- Pacific halibut (*Hippoglossus stenolepis*) is a large (<2.4 m/230 kg) flatfish species of the Pleuronectidae family. Widely distributed in the North Pacific.



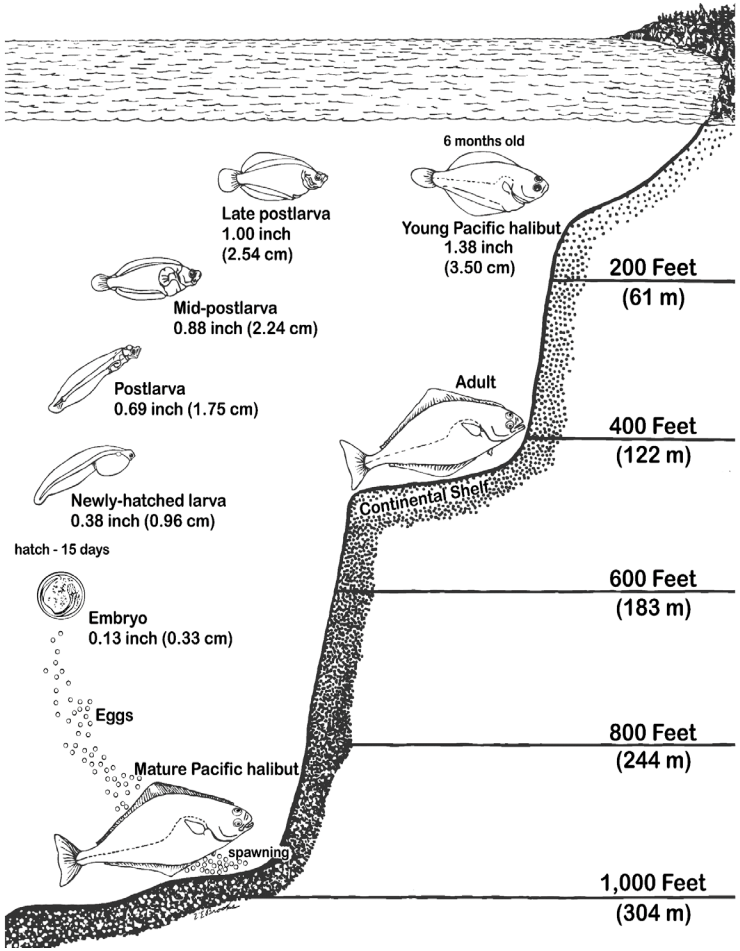


- Important fisheries for the U.S. and Canada.

Introduction

- Spawning occurs offshore
- Pelagic eggs and larvae
- Juveniles use shallow areas on continental shelf
- Move offshore at age ~2 – 3
- Seasonal migrations between spawning grounds (winter) and feeding areas (summer)

Scarce information on the reproductive biology of the female Pacific halibut

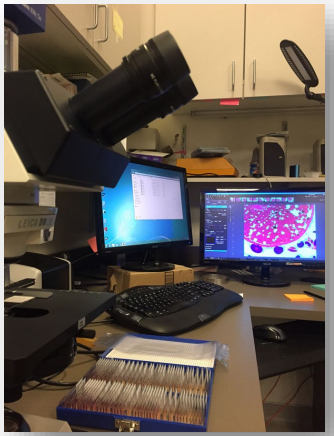


Objectives

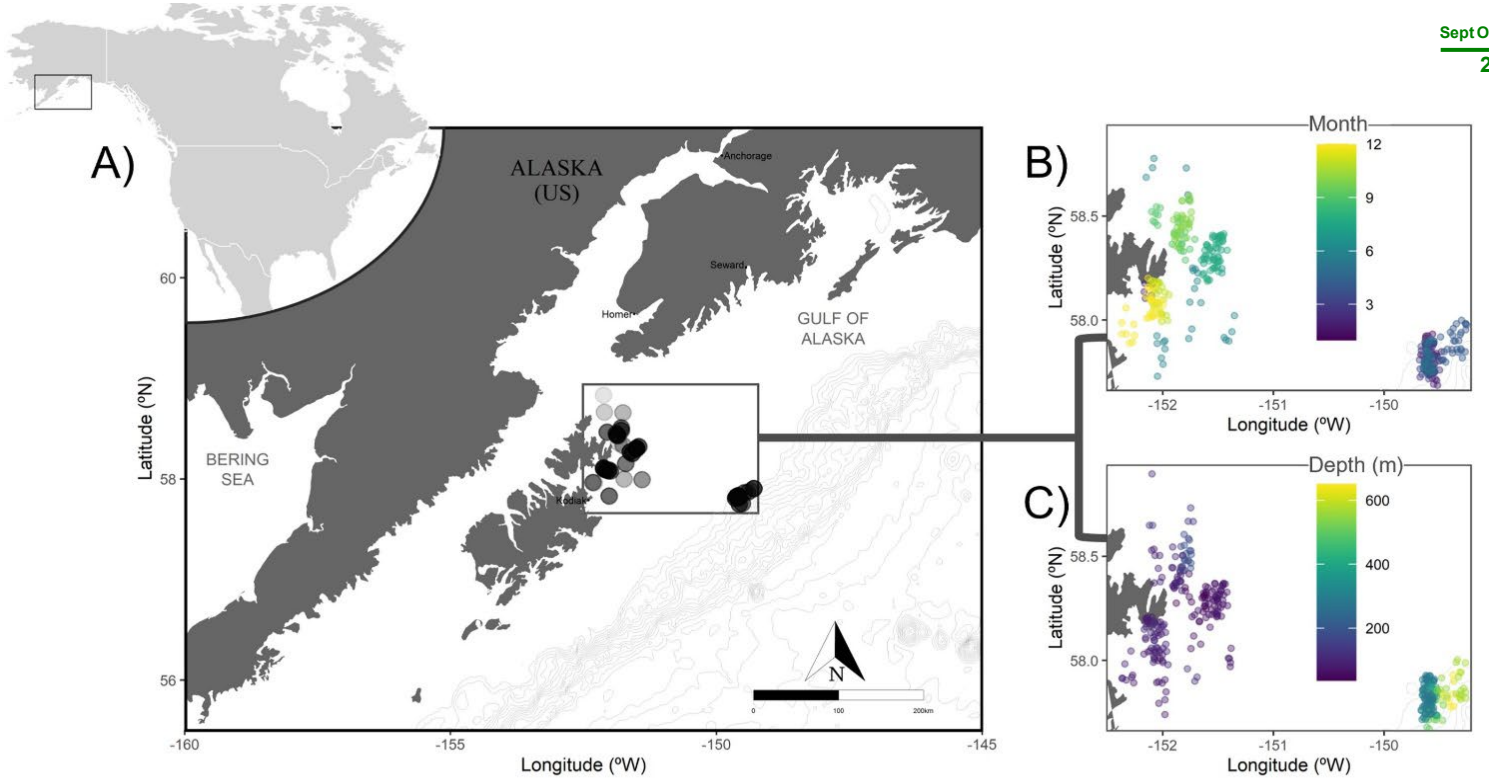
Full characterization of the annual reproductive cycle of female Pacific halibut

Annual reproductive cycle

- Histological assessment of gonadal development
- Energy levels (fat content/hepatosomatic index)
- Activation of the endocrine reproductive axis (pituitary and gonads)
- Reproductive hormones in the blood
- **Revise maturity ogives**
- Revise scoring criteria of maturity stages by macroscopic observations in the field



Methods: Sampling area



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

2017 2018

30♀ / 30 ♂

♀ > 90 cm FL

♂ > 70 cm FL

Results: characterization of oocyte development

- **Histological description of 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

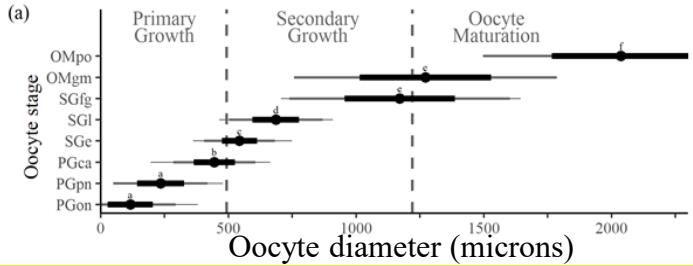
Secondary Growth

	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

- **Oocyte size changes**



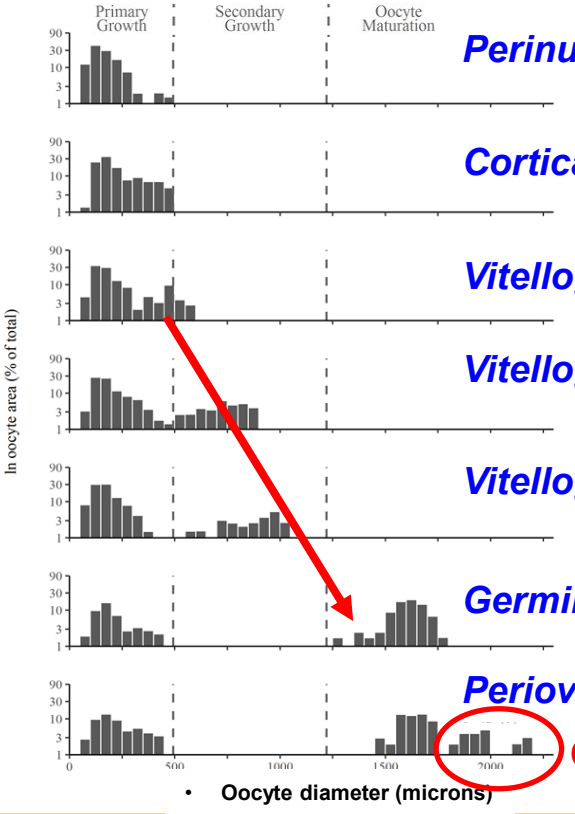
Female developmental phase determined based on the stage of the most advanced oocytes

Fish et al. *J. Fish Biol*. 2020. **97**: 1880-1885

Results: oocyte dynamics

Oocyte size distribution at different female developmental stages

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

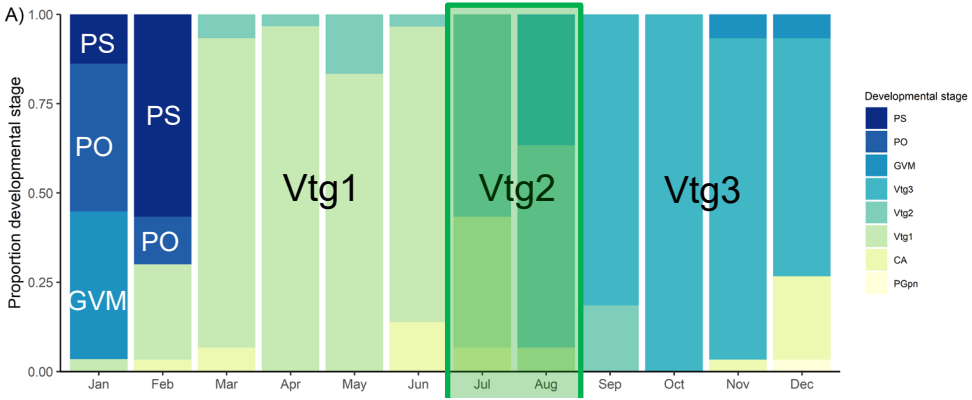
- **Batch spawners**
- **Group synchronous**
- **Determinate fecundity**

Fish et al. *J. Fish Biol*. 2020. **97**: 1880-1885

Results: histological female classification

Microscopic maturity staging: based on histological oocyte stages

Female developmental stages

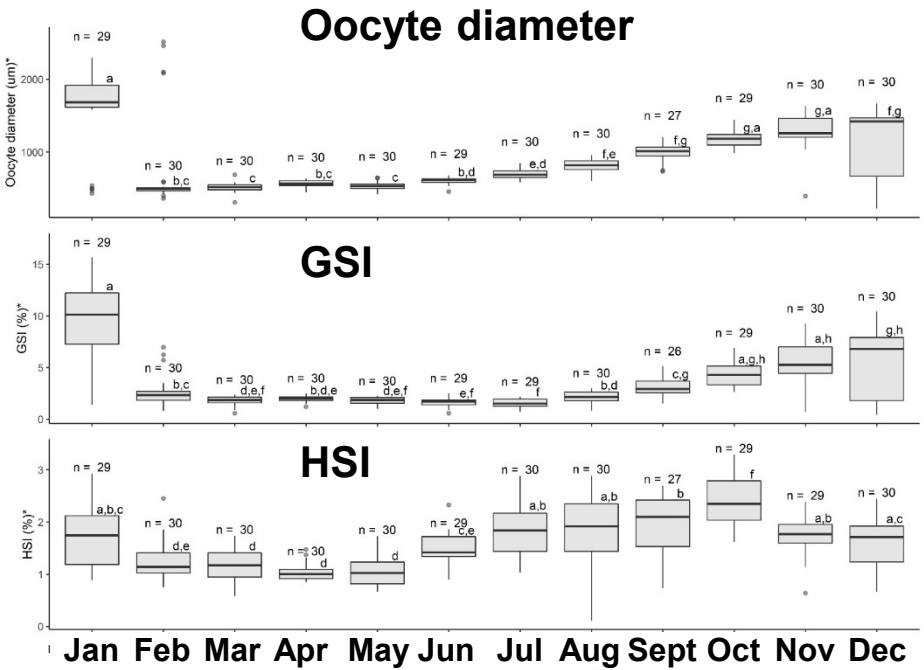


Vtg1 (Vitellogenic 1)
↓
Vtg2
↓
Vtg3
↓
GVM
(Germinal vesicle migration)
↓
PO (Periovulatory)
↓
PS (Post-spawning)

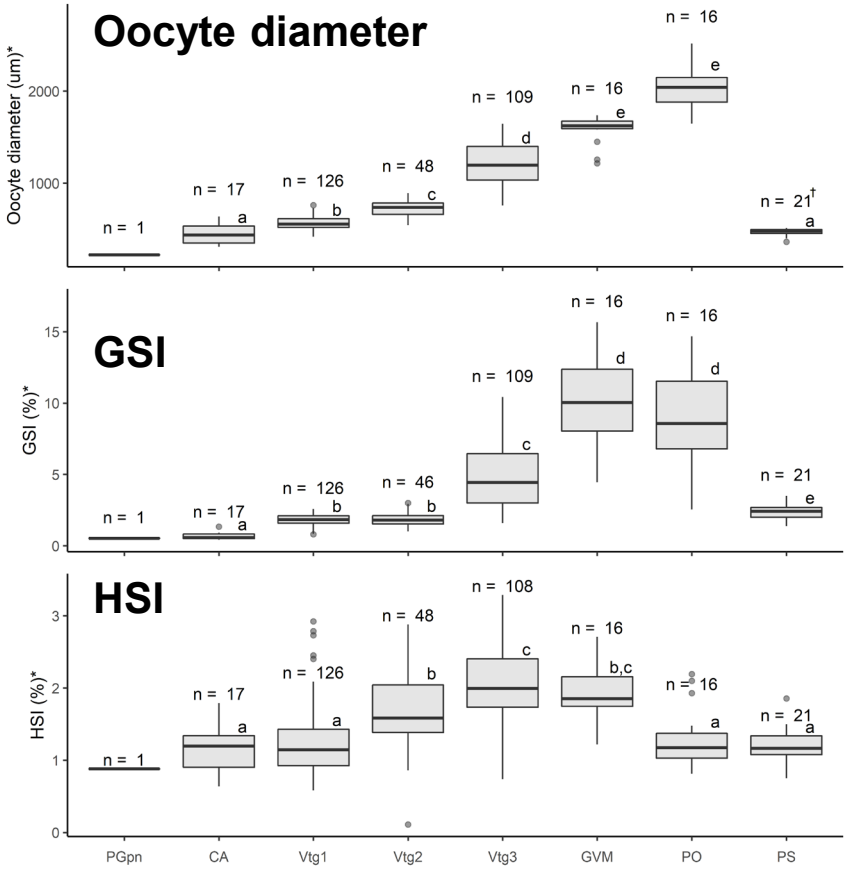
Developing
↓
Spawning capable
↓
Regressing

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

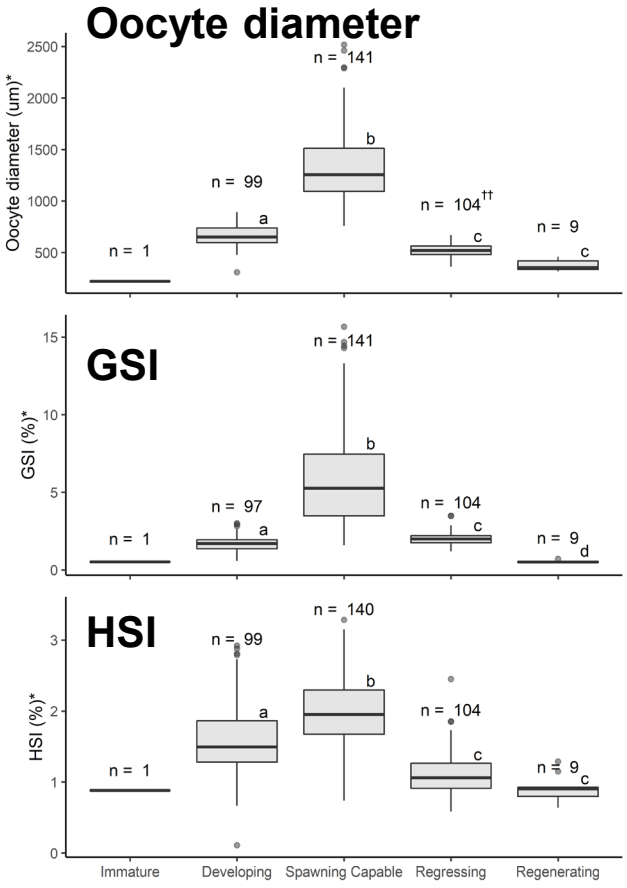
Results: Reproductive parameters by month



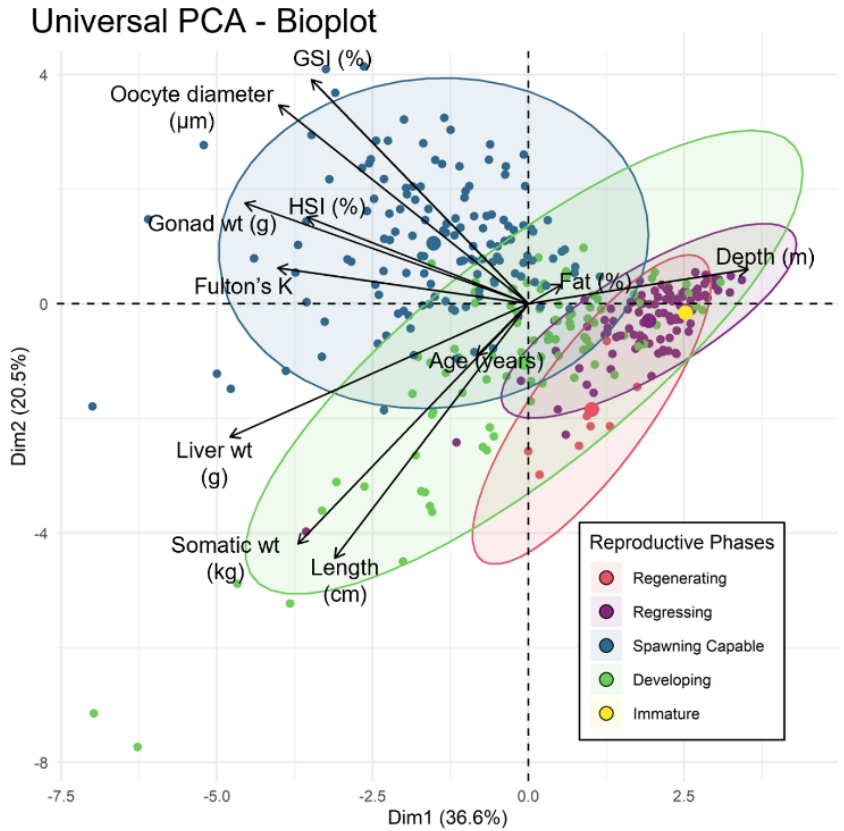
Results: Reproductive parameters by stage



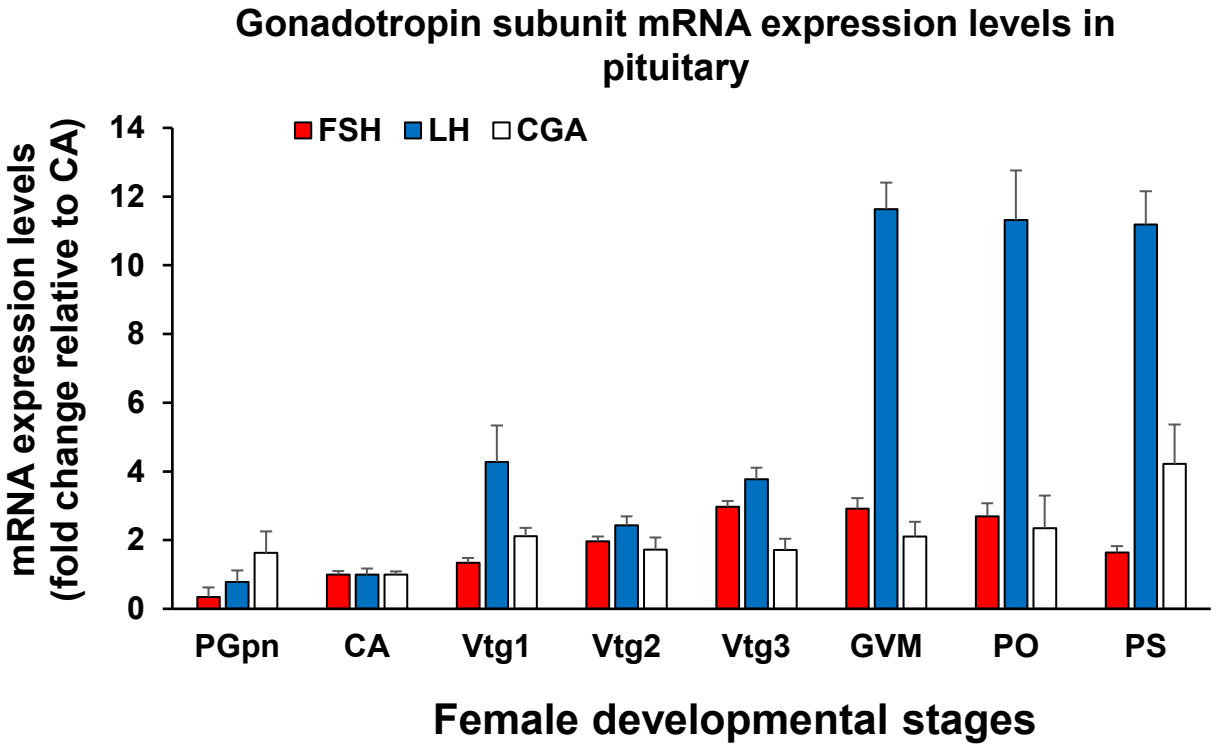
Results: reprod. parameters by reproductive phase



Results: predictive value of variables for staging



Results: endocrine reproductive markers



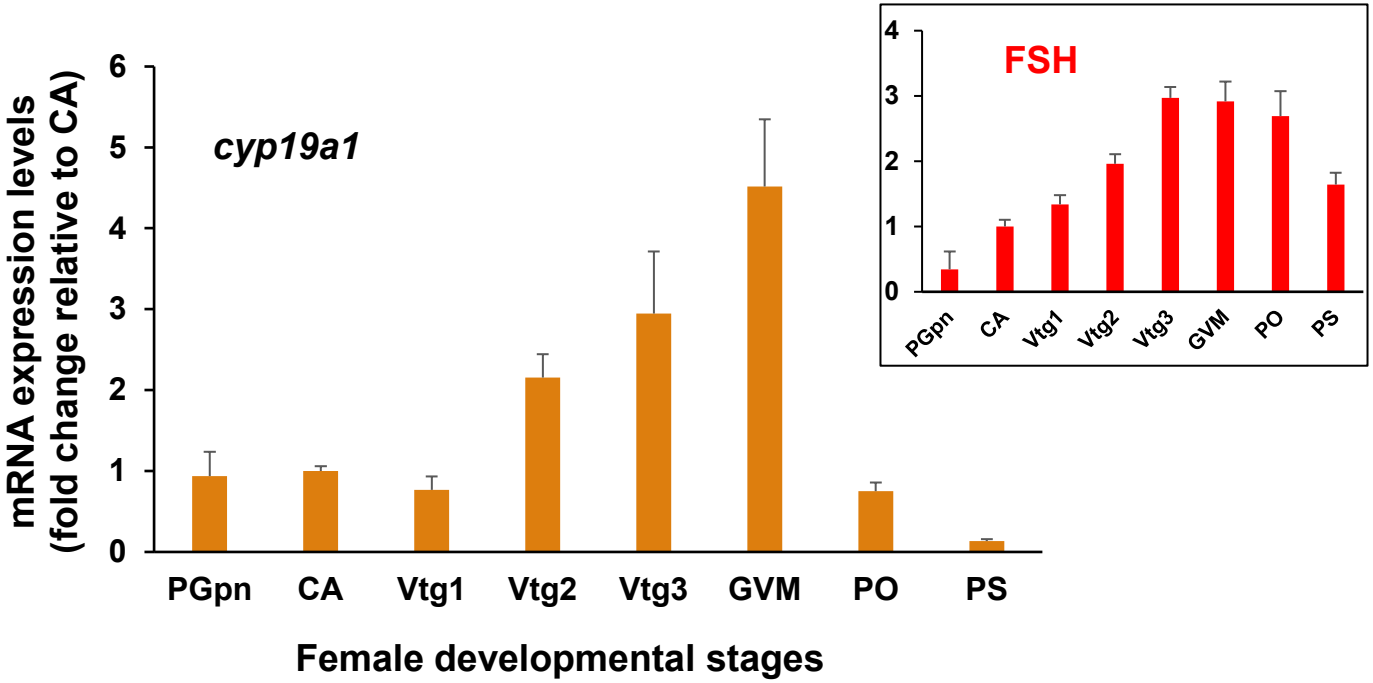
Results: endocrine reproductive markers

Testosterone

FSH (+) → *cyp19a1*

17β-estradiol

Ovarian expression of aromatase (*cyp19a1*)



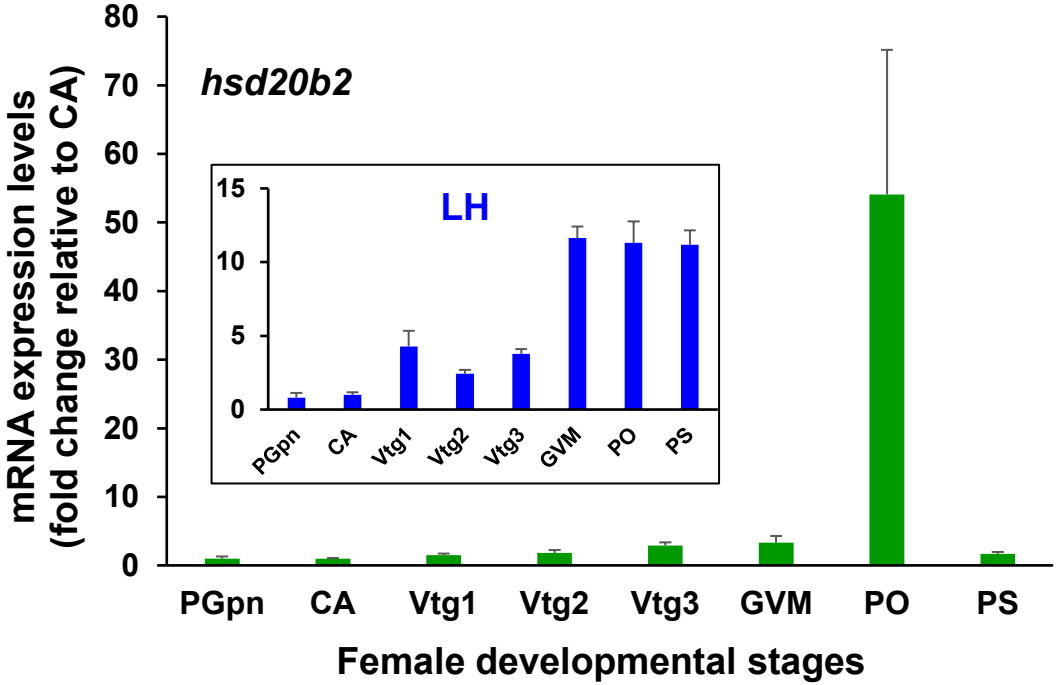
Results: endocrine reproductive markers

17α-hydroxy-progesterone

LH ⊕ → | *hsd20b2*

17α,20β-P (maturation-inducing steroid)

Ovarian expression of 20β-hydroxysteroid dehydrogenase (*hsd20b2*)



Conclusions

- First characterization of oocyte development in female Pacific halibut.
- Pacific halibut females are group synchronous, batch spawners and have determinate fecundity.
- Pacific halibut females follow a clear annual reproductive cycle, with developmental stages advancing in the spring from early, to mid and late vitellogenesis, and progressing through oocyte maturation and ovulation, with peak spawning occurring in January and February.
- This information is key for ongoing revision of maturity ogives through histological analyses to inform stock assessment.
- Changes in the expression of key endocrine markers drive the progression of female reproductive development during the reproductive cycle.