Pacific halibut (*Hippoglossus stenolepis*) maturity status:
Preliminary assessment using histology and macroscopic staging methods

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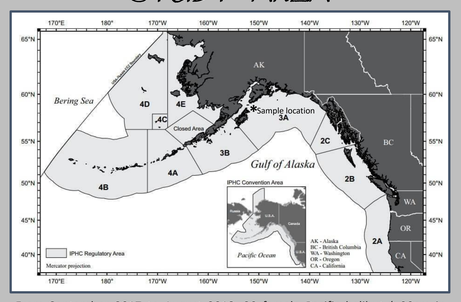
STUDY AREA



From September 2017 to August 2018, 30 female Pacific halibut (>90cm in length) were collected each month by longline survey from the Gulf of Alaska (∗ on map). Ovaries were staged using the standard IPHC macroscopic maturity stage (*see table right*) assessment approach and ovarian samples were collected and processed for histological assessment.

INTRODUCTION AND OBJECTIVES

Accurate reproductive information is foundational to successful fisheries management. Presently, female Pacific halibut (*Hippoglossus stenolepis*) maturity status is derived from macroscopic assessment of ovaries sampled over a limited 2-3 month period during the annual fisheries-independent setline survey (FISS) conducted by the International Pacific Halibut Commission (IPHC). This relatively narrow sampling window may not be adequate to describe the actual potential reproductive contribution of stages occurring over an annual cycle. Further, relative accuracy of macroscopic maturity assessments has yet to be compared to histologically-derived assignments, the highest standard of stage classification as histology was previously undefined.

Objectives of this study are to:

1) define female Pacific halibut maturity stages with histology;

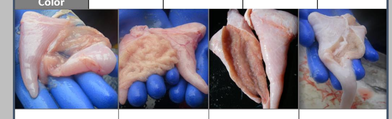
1) examine histology stages over an annual reproductive cycle;

3) compare staging results from histological and macroscopic assessment methods; and

4) update the female Pacific halibut maturity schedule using 2017-2018 macroscopic maturity status.

IPHC MACROSCOPIC MATURITY STAGES

Female Maturity	Stage 1 *Immature*	Stage 2 *Maturing*	Stage 3 *Spawning*	Stage 4 *Resting*
Capillaries Develop-ment	Slight develop-ment	Well formed, branched, purple	Thin and small	Large and deflated
Membrane Thickness	Thinnest	Very thin	Thin	Thick
Egg Develop-ment	Not visible white grainy	Visible, mostly opaque	Visible, fully developed	May be re-absorbing eggs
Membrane Color	Pink to red	Clear	Clear	Opaque

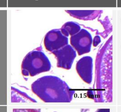
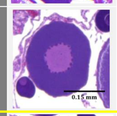
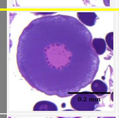
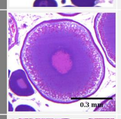
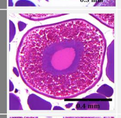
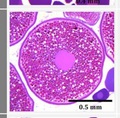
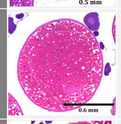
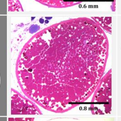


Stage 1 | ***Stage 2*** | ***Stage 3*** | ***Stage 4***

Objective #1

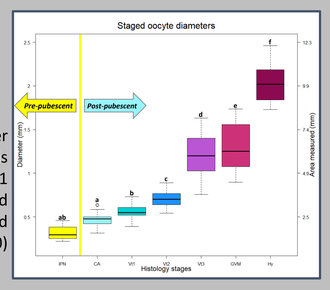
Methods: Visual characteristics of histology stages were documented, oocytes staged, and oocyte areas measured per staged individual. Differences in stage diameters were compared using a one-way Analysis of Variance followed by Tukey's Honestly Significant Difference test.

HISTOLOGY OF PACIFIC HALIBUT OOGENESIS

Female Pacific halibut histology stages		Stage photo	Microscopic visual characteristics	Sample size, diameter range, and mean (mm)
Primary Growth (PG)	Early perinuclear (ePN)		Oocytes are small often angular and compact with a single large nucleolus. Cytoplasm stains dark purple.	N = 0
	Late perinuclear (lPN)		Oocytes are larger and rounder than ePN, cytoplasm stains lighter purple, and nucleoli develop.	N = 4 Diameter range 0.22-0.46 Mean diameter = 0.32
Vitellogenic	Cortical alveolus (CA)		First cortical alveoli appear as white stain in the periphery of the oocyte.	N = 41 Diameter range 0.32-0.64 Mean diameter = 0.46
	Early-vitellogenic (Vtg1)		Yolk globules first appear and stain pink at the periphery of the oocyte and fill inwards occupying up to 1/3 of the cytoplasm.	N = 110 Diameter range 0.39-0.73 Mean diameter = 0.56
	Mid-vitellogenic (Vtg2)		Yolk globules transition from only the periphery (outer 1/3 as in Vt1) of the ooplasm and fill inwards to the nucleus.	N = 59 Diameter range 0.54-0.89 Mean diameter = 0.71
	Late-vitellogenic (Vtg3)		Yolk globules become larger as they begin to fuse and completely fill the ooplasm, making contact with the central nucleus.	N = 69 Diameter range 0.76-1.63 Mean diameter = 1.21
Post-vitellogenic	Germinal vesicle migration (GVM)		The nucleus begins to migrate to the edge of the cell wall through a cytoplasm fully filled with yolk globules.	N = 59 Diameter range 0.90-1.74 Mean diameter = 1.30
	Germinal vesicle breakdown (GVBD)		Nucleus is no longer visible and yolk globules coalesce into dark pink stained yolk masses.	N = 0
Hydration	Hydration (Hy)		Yolk coalesces into a central mass in the cell occupying over 50% of the area. Oocyte is still within the follicle wall.	N = 14 Diameter range 1.73-2.46 Mean diameter = 2.03

Pre-pubescent / *Post-pubescent*

Histological characteristics used to describe stages of oogenesis of Pacific halibut were defined (above). All oocyte stages were observed, but none classified as perinuclear (ePN) or germinal vesicle breakdown (GVBD). Individuals staged beyond the primary growth phase (as ePN or lPN) should be considered post-pubescent and are expected to contribute to the subsequent spawn.

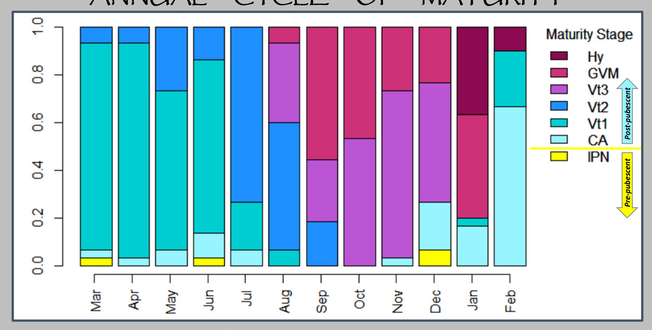


The one-way ANOVA showed significant difference ($p < 2e\text{-}16$) in diameter size by stage. Oocyte diameters at the transition into and out of vitellogenesis (CA-Vt1 and Vt3-GVM) showed weaker significance (adj p = 0.03 and 0.01 respectively) than the remaining relationships of post-pubescent staged oocytes ($p < 0.001$), however the diameters of pre-pubescent oocytes (staged as lPN in yellow), were not found to be different than either CA (adj p = 0.60) or Vt1 (adj p =0.06) stages (right).

Objective #2

Methods: Proportions of staged individuals were tracked through the annual cycle to understand the progression of maturity and determine peak reproductive season.

ANNUAL CYCLE OF MATURITY



Although a small portion of ovaries staged as pre-pubescent (late perinuclear in yellow) can be found throughout the season, maturity generally builds from predominantly early vitellogenic stages (shades of blue) starting in March through to the highest proportion of later stages in October, with spawning (hydrated oocytes in dark red) occurring only in January and February.

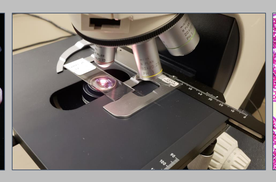
Objective #3

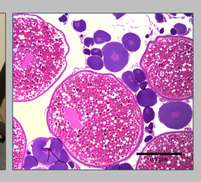
Methods: Spearman's ranked correlation test was utilized to offer insight between paired histological and macroscopic staging methods.

HISTOLOGICAL AND MACROSCOPIC PAIRED STAGING COMPARISON

Histology categories	IPHC macroscopic assessment categories				Histology-based biological categories	IPHC macroscopic assessment categories			
	Immature	Resting	Maturing	Spawning		Immature	Resting	Maturing	Spawning
	1	4	2	3		1	4	2	3
IPN	4	0	0	0	Immature	4	0	0	0
CA	13	27	0	1	Resting	15	141	53	1
Vt1	2	102	6	0					
Vt2	0	12	47	0	Developing	1	1	126	0
Vt3	0	0	69	0					
GVM	1	1	57	0					
Hy	0	0	9	5	Spawning	0	0	9	5

Spearman's ranked correlation test revealed a moderate correlation (rho = -0.52, $p < 2.2e\text{-}16$) between the histology and macroscopic assessment of stages. Macroscopic assessment of post-pubescent individuals in this study was observed to be very accurate with no disagreement between histology and macroscopic assignments. Conversely, pre-pubescent macroscopic assessments contained both pre- and post-pubescent histology stages (area outlined in red).

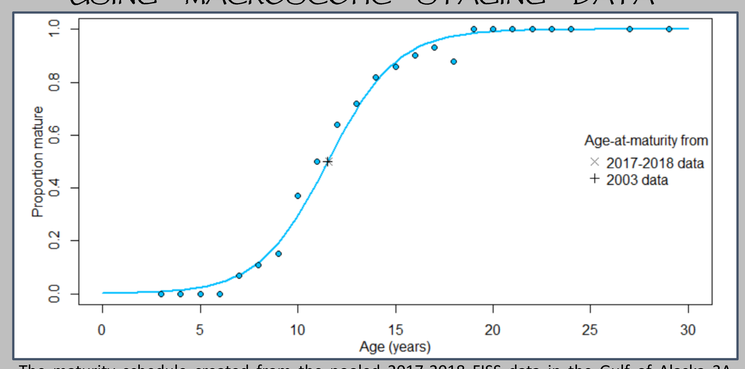




Objective #4

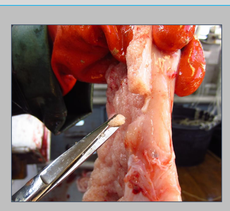
An updated maturity schedule was produced using historic (e.g. macroscopic) maturity data pooled from the 2017 and 2018 FISS.

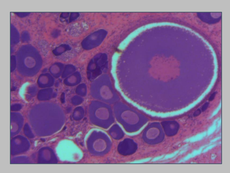
UPDATED MATURITY SCHEDULE USING MACROSCOPIC STAGING DATA



The maturity schedule created from the pooled 2017-2018 FISS data in the Gulf of Alaska 3A region (n = 2056) fits the data well and has both a slope and intercept significantly different than zero (p = 0.02 and 0.03). The updated age at maturity (-m/b) of 11.5 years from these data is biologically equivalent to the age at maturity of 11.6 years previously modeled by IPHC using data from 2003.

FUTURE RESEARCH





1) Investigate the causes of the post-pubescent histology stages within the macroscopically staged immature and apply results to refine the macroscopic maturity staging protocols.
2) Determine the seasonality of gravidity with oocyte size frequency distributions.
3) Use the know season of gravidity to investigate the proportion of post-pubescent individuals contributing to subsequent spawning events (*i.e.* search for skip-spawning).
4) Use the know season of gravidity to sample mature and immature individuals to construct a histology-based maturity schedule
5) Examine the biological changes associated with gonadal development including: gonadosomatic index, hepatosomatic index, and growth rates.
6) Use relationships examined above to investigate maturity models for use in the fisheries stock assessment.

Research intended to be included in my MSES study. Stay tuned!

SUPPORT BY

International Pacific Halibut Commission
Groundfish Forum
At-sea Processors Association
Partners for Healthy Fisheries