IPHC science posters for AM096

PREPARED BY: IPHC SECRETARIAT (31 JANUARY 2020)

PURPOSE
To provide the Commission and the public with copies of the IPHC Secretariat science posters displayed at the 96th Session of the IPHC Annual Meeting (AM096).

BACKGROUND
The IPHC Secretariat is engaged in multiple lines of research under the IPHC 5-year Biological and Ecosystem Science Research Plan (IPHC-2020-AM096-11), and results from several projects will be displayed in posters at AM096 for the benefit of the Commission and the public.

DISCUSSION
Table 1 lists the science posters on display at AM096.

Table 1. Science posters on display at AM096

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RECOMMENDATION

That the Commission:

1) **NOTE** paper IPHC-2020-AM096-INF05, which provides copies of the IPHC Secretariat science posters displayed at the 96th Session of the IPHC Annual Meeting (AM096).

APPENDICES

As listed in **Table 1**
Appendix 1

Electronically monitoring release method as a proxy for Pacific halibut discard mortality rates in the directed Pacific halibut longline fishery

Introduction:
- Regulations require release of sublegal (<81.3cm, <32") Pacific halibut (Hippoglossus stenolepis) in the directed longline fishery.
- Potential release mortality in the fishery is currently estimated through the application of discard mortality rates (DMRs) derived from injury or vitality data provided by observer programs. In 2017, wasteage in the fishery was estimated to be 453 (11.1 M lbs).
- Alaska is currently developing electronic monitoring (EM) as a tool to monitor this small vessel fleet (<17.4 m, <67'), but determining vitality data requires handling of the animal, something that cannot be achieved with cameras.
- Permitted hook release methods include careful shake, hook straightening, or cutting the gangion.
- Release methods can be easily assessed by EM, but the suite of injuries sustained by each hook release technique is unknown.

Objectives:
- Develop an injury profile for different hook release methods, which can then be used to calculate DMRs on vessels carrying EM rather than observers.
- Assessment of post-release survival (short- vs long-term) in relation to hook release method, associated injury levels, physiological condition, and size of Pacific halibut released in excellent condition.

Methods:
- Commercial longline vessel (24 m, 80') contracted to conduct test fishing with conventional fixed gear in western Gulf of Alaska in fall of 2017.
- EM system with 3 cameras, and hydraulic sensors installed.
- Standardized gear consisted of 250 m (1,400') skates with 100 #3 (100 Mustard) circle hooks, no snaps/swivels.
- Thirty-six (36) sets of eight skates of gear, with randomized hook release treatments were done:
  - Careful shake (5 skates/set).
  - Hook stripper (2 skates/set).
  - Gangion cut (1 skated/set).
- All Pacific halibut were assessed for length, weight, physical injury, release condition.
- Pacific halibut 53.3 cm (20 inch) were tagged and released after physiological sampling (blood, non-invasive fat content).
- EM footage reviewed by analysts at the Pacific States Marine Fish Commission.
- 2,467 fish caught, of which 1,100 were tagged and released.
- Short-term survival archival tags (70 SRAT releases scheduled for popup at 96 days after deployment).
- Long-term survival tags (1,027 wire tag releases, dependent on fishery recoveries).

Results:
- An almost perfect (95%-100%) agreement between the actual release method used and that captured by EM was observed (Figure 1).
- Assessment of injury profiles by release method evidenced that careful shake and gangion cutting are the release methods resulting in the highest proportion of fish in excellent condition (> 75%) for both small and large Pacific halibut (Figures 2 & 3).

Conclusions:
- EM was effective at capturing hook release method (Figure 4).
- Injury profiles for different sizes were developed and can be used as a proxy for DMR in the future.

Figure 1. Comparison of EM determined release method to actual.
Figure 2. Release condition of small (≤ 53.3 cm/ 20 inch) Pacific halibut by release method (careful shake, gangion cut, hook stripper).
Figure 3. Release condition of large (> 53.3 cm/ 20 inch) Pacific halibut by release method (careful shake, gangion cut, hook stripper).

Figure 4. EM capture of hook release methods: a) careful shake, b) gangion cut, and c) hook stripper.

Acknowledgment: this work is funded in part by the Saltonstall-Kennedy Grant Program, Project #NA17NMF4270240

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Biological and Ecosystem Science Program

Pacific halibut migration research at IPHC

Historical projects

Larval dispersal and connectivity between the Bering Sea and Gulf of Alaska

Project Goals
- Identify the factors contributing to annual differences in larval distribution/disparity and the resulting settled year classes.
- Model the contribution of spawning grounds to settlement grounds.
- Assess connectivity of the Gulf of Alaska and Bering Sea populations via larval dispersal through Unimak Pass, Alaska.

Using electronic tags to study population structure, seasonal dynamics, and juvenile dispersal

Project Goals
- Examine the redistribution of exploitable and spawning biomass seasonally, to evaluate how stock distributions may differ between the summer, spring, summer, and winter; and how movements vary regionally, and may be affected by climate variability.
- Quantify dispersal of juvenile Pacific halibut from nursery habitats to adult feeding grounds, to better understand downstream effects of both fishing and natural mortality.

Using wire tags to study the movement of juvenile Pacific halibut

Project Goals
- Tagging Pacific halibut: 625 cm fork length or “T2” that are still actively migrating from nursery areas to adult feeding grounds.
- Increase our understanding of juvenile Pacific halibut movement and growth.

Future directions

- Connect spawning grounds to nursery areas using modeling and genetics - build on the results from the current projects to identify possible links between spawning and nursery grounds, their validation with genetic studies.
- Expand migration/advisory knowledge to include un-sampled and lightly sampled areas - this could include, for example, the western Bering Sea through collaboration with Russian scientists, and the coastal intertidal areas of the Gulf of Alaska and eastern Bering Sea.

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Can we reconstruct the growth history of the Pacific halibut (*Hippoglossus stenolepis*) population by otolith increment analysis?

Dana M. Rudy, Chris Johnston, Robert Tobin, Tim Loher, Ian Stewart, Josep V. Planiol, Joan Fonsberg

International Pacific Halibut Commission, 2320 W. Commodore Way, Seattle, WA 98119

**Introduction**

The Pacific halibut (*Hippoglossus stenolepis*) is one of the largest and longest-lived flatfish in the world, reaching up to 300 kg in body weight and 2.4 m in length and with the oldest individual caught aged at 83 years. Although female Pacific halibut attain much larger sizes than males, the average length at age for both males and females has significantly decreased during the last 25 years, particularly in the Gulf of Alaska. This has led to a decrease in the exploitable biomass of Pacific halibut stocks. Several factors, including environmental, fisheries-related, and even anthropogenic, could be responsible for the observed decrease in the growth potential of this species. Otolith measurements have been used as a proxy for fish length in other species (LJ). Since the International Pacific Halibut Commission maintains a long-term, crooked otolith collection, we aim to determine if otolith growth in Pacific halibut corresponds with their somatic growth.

**Objective**

Determine whether somatic growth can be used as a proxy for somatic growth. Additionally, determine if otolith growth reflects the actual description in early Pacific halibut lengths, or reflects the length-at-stage descriptions of the last 10 years.

**Materials and Methods**

A subsample of otoliths from survey-caught Pacific halibut were selected for birth years 1977, 1987, 1992, and 2002. Most halibut were 1+ or 2+ years old when captured; 10-year-olds were used for birth year 2002. Otoliths in this study had been aged by the break-visible techniques, where the growth is cut in half transversely and the posterior half is biased to enhance contrast between seasonal growth (Fig. 3A+B). The dated otolith slices were cut about 0.5 to 2 mm below the reading surface and mounted on glass slides. The mounted otolith sections were then photographed and measurements were made using ImageJ (Image Software). Measurements were taken in a standard zone on all otoliths, from the origin to the last annulus along a straight line in the area dorsal to the growth ring (Fig. 4).

![Image of Pacific Halibut Otolith](image)

**Results**

There is a 2.4% increase in mean otolith radius for age 5+ females between the 1977 and 1992-year classes in the Gulf of Alaska, despite a 1.1% decline in body length.

**Conclusions**

- In the Gulf of Alaska, the change in mean otolith radius of 15-year-old female halibut between the 1977 and 1992-year classes does not reflect the somatic length-at-age decreases seen in females between those years. Otolith radius at age is therefore not a good proxy for length at age.
- Males and female otolith increment at age measurements are similar (only a 6.4% difference at age 15 in the 1992 year class), despite very different somatic lengths between ages (26.4% difference at age 15 in 1992 year class). Otolith radius does not reflect the actual description in Pacific halibut length at age.
- Although the factors regulating otolith growth in Pacific halibut are not well understood, otolith growth appears to be decoupled from somatic growth. Therefore, otolith growth patterns cannot be used to infer changes in somatic growth in Pacific halibut.

**References**


**Acknowledgements**

Financial support for this project was provided by the North Pacific Research Board, Project 1300.
Appendix 4

Re-ageing of archived otoliths from the 1920s to the 1990s

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Background
The International Pacific Halibut Commission (IPHC) has collected otoliths for age determination since 1925. All otoliths that have been examined for age determination are kept and added to the IPHC’s otolith collection, which contains samples from over 1.6 million Pacific halibut. Age determination techniques used for Pacific halibut have changed over time; prior to 1992, all otoliths were surface aged. Between 1992 and 2001, otoliths that met certain criteria were also aged by break-and-burn or break-and-bake method in addition to surface ageing. Beginning in 2002, all otoliths collected from the IPHC fisheries-independent survey and the commercial catch have been aged by break-and-bake. Observed size-at-age (SAA) of Pacific halibut has changed over time and the reasons behind changes in Pacific halibut SAA are not well understood. Prior to this study, the potential contribution of changes in ageing methods to observed SAA was uncertain.

Study goals
To provide information on the bias and imprecision of historical surface ages relative to age data from the 1990s onward, subsets of otoliths from each decade from the 1920s to the 1980s were re-aged by both the surface and break-and-bake technique, and these new ages were compared to the original surface ages. Additionally, a subset of otoliths collected in the 1990s that were previously only surface-aged were re-aged by break-and-bake. Since the 1990s, IPHC age readers have cleaned Pacific halibut otoliths in glycerin solution (50% glycerin:50% water) to increase readability of the growth patterns. Otoliths are also kept in glycerin solution for long term storage. This study also provided an opportunity to observe the condition of otoliths stored for almost 90 years in glycerin solution.

Methods
Years for which otoliths had been collected and aged were identified. One or two years per decade were selected based on number of geographical regions (IPHC regulatory areas) and otolith availability. For each selected year within a decade, otoliths were retrieved from storage. Otoliths collected prior to 2002 were stored in groups of ~25 per vial. Otoliths were separated within the vial by numbered paper labels. Almost 26,000 otoliths were transferred from vials to containers that have individual cells. The transferred otoliths were further subsampled to 500 from each regulatory area for ageing. A total of 17,414 otoliths were re-aged by three experienced readers.

Results
Results indicated that historical samples contained very few fish aged older than 15 years by either method. Based on simultaneous estimation of bias and imprecision for up to four unique ages per-otolith, the properties of historical surface ageing methods were found to be very similar to current methods, becoming increasingly biased and imprecise beyond 15 years. This study reinforces two important questions for assessment and related analyses attempting to reconstruct the historical abundance and biological trends for Pacific halibut. These results support the conclusion that increasing trends in size-at-age observed from the 1930s through the late 1970s were not an artifact of changes in ageing methods, but represent a real biological phenomenon, for which probable mechanisms are currently being investigated. Second, there does not appear to be a need for extensive further re-aging of historical samples. The truncated age structure of most historical samples suggests that little information will be lost if ages are aggregated beyond age 20 (as has been done in most analyses) and both the bias and imprecision of the surface method are included in any analysis.

In addition to clarifying precision of ageing methods, the re-aging of archived otoliths also provided an excellent opportunity to observe the condition of otoliths stored in glycerin solution for up to 60 years. Most of the otoliths examined were in good condition; some samples from the 1920s and 1930s had a chalky coating that obscured surface growth patterns, but were readable when broken and baked.

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Identification of molecular growth signatures in skeletal muscle of juvenile Pacific halibut (Hippoglossus stenolepis) for monitoring population growth patterns

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INTRODUCTION
The International Pacific Halibut Commission has reported changes in the size-at-age (SAA) of Pacific halibut (Hippoglossus stenolepis) caught in the commercial fishery as well as in its own survey research for almost 100 years. Although an increase in SAA was observed between the 1930’s until the 1980’s, SAA has significantly declined since the 1990’s until today, as evidenced by a 50% reduction in body weight for a typical 12-year old female during this period (Figure 1). However, our understanding of the potential causes for the long-term variability in SAA is still rather scarce. Although a number of factors could be contributing to this variability, recent analyses have suggested that temperature variation may have been a contributing factor to the observed changes in SAA in the Pacific halibut. Therefore, there is an urgent need to better understand the physiological effects of temperature on growth in this species.

MATERIALS AND METHODS
Juvenile Pacific halibut of approximately 6 month of age were collected off the coast of Kodiak, Alaska, US and transferred to the aquatic facilities of the Hatfield Marine Science Center in Newport, Oregon, US. Individually tagged fish were acclimated for 8 weeks to 2°C and 9°C in duplicate tanks (N = 50) prior to sampling. Subsequently, half of the fish previously acclimated at 2°C were gradually brought up to 9°C and held at 9°C for 6 additional weeks prior to sampling. The transcriptomic responses of white skeletal muscle from fish experiencing temperature-induced growth suppression and growth compensation were analyzed by RNA sequencing ( Illumina).

RESULTS
• Temperature modulates the specific growth rate (SGR)

CONCLUSIONS
• Acclimation at 2°C resulted in a significant reduction in the specific growth rate (SGR) whereas a significant increase in SGR was observed in a result of temperature-induced growth compensation.
• Growth suppression by low temperature acclimation is associated with a decrease in the expression of genes involved primarily in muscle function, protein synthesis, transcription and stress and immune response.
• Growth stimulation by temperature-induced compensation is associated with an increase in the expression of genes involved primarily in muscle function and metabolic activation.
• The resulting molecular growth signatures will be useful to investigate potential changes in growth patterns in Pacific halibut.

ACKNOWLEDGMENTS. This study was conducted with funding from IPHC and the North Pacific Research Board (Project NPB 1104).

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Genetic population structure of Pacific halibut (Hippoglossus stenolepis): progress to date

Species distribution and management structure
Pacific halibut (Hippoglossus stenolepis) are found throughout the North Pacific Ocean (Fig. 1). Some northern California to the south known to be managed by the United States by the International Pacific Halibut Commission, with both countries. However, there are no commercially significant or management measures currently in place for the Atlantic. Halibut can be found throughout the region, from the Bering Sea to the Gulf of Alaska.

The species can be highly diverse in terms of life history stages, from larval to adult sizes. Juvenile halibut are often found in the coastal regions, while adults can be found in deeper waters. This diversity can be important for understanding the genetic structure of the population.

Recent methodology
Recent studies have focused on understanding the genetic diversity and structure of the population. One such study involved analyzing the genetic differences between individuals collected from the Bering Sea and the Gulf of Alaska. The study used a set of microsatellite markers to examine genetic differentiation among populations.

Findings
The study found that there was significant genetic differentiation between the Bering Sea and the Gulf of Alaska populations. The genetic diversity in the Bering Sea population was higher than in the Gulf of Alaska population. This suggests that the populations may have different evolutionary histories.

Implications
These findings have important implications for the management of the species. Understanding the genetic structure of the population can help inform conservation and management strategies. For example, the Bering Sea population may be more resilient to environmental changes, while the Gulf of Alaska population may be more vulnerable.

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Genetic Sex Identification of Pacific Halibut (Hippoglossus stenolepis) Commercial Landings

Anna Simeon1, Dan Drinan2, Lorenz Hauser3, Timothy Lohar1, Lara Erikson1, Ian J. Stewart1 and Josep V. Planas1

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Background

- Throughout the fishery’s history, the sex ratio of commercially-caught Pacific halibut has remained unknown as landed individuals are ovaclarified at sea and the sexes are otherwise indistinguishable. The sex ratio from the IPHC’s fishery-independent trawl survey (FISS) has thus far been the only direct source of sex-ratio information.
- Differences in size between individuals landed commercially and on the FISS suggested a greater proportion of females in the fishery.
- Drinan et al. (2017) identified two sex-linked single nucleotide polymorphisms (SNPs) able to distinguish between males and females and describe molecular assays to identify an individual’s sex by these genetic signatures.

Study Objectives

- Develop multiplex assay for both sex-determining SNPs (twice the data for half the price)
- Directly determine the 2017 commercial sex ratio through SNP genotyping

Methods

- 16,137 fin clips collected by IPHC port samplers in 2017
- DNA isolation through simple, cost-effective NaOH extraction
- Sample genotyping via multiplexed TaqMan qPCR assay

- A multiplexed TaqMan assay was designed to genotype both SNPs (13260 ApoE and 173007 SMC3) simultaneously using reporter dyes EASYVIO and ROX and reference dye Mowing Purple. Target sequences were based on those described in Drinan et al. (2017).

Results

- 2017 Commercial Sex Ratios within IPHC Regulatory Areas

- Female proportion of the commercial catch ranges from 81% in regions 2B and 3A to 97% in regions 4CDE.
- The higher proportion of females in commercial samples versus the FISS samples is likely due to their larger, targeted size.

Comparison of 2017 FISS and Commercial Sex Ratios (Legal Size)

- 1.5% of genotyped samples display a unique haplotype or combination of haplotypes that do not strictly correspond to either sex.
- May be caused by an additional SNP in the probe binding regions, chromosomal inversion, or something else. Additional sequencing of these regions (to be completed in 2020) will help clarify.

References

- View more information and data at www.iphc.com

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A decade of coastwide environmental monitoring on the annual IPHC fishery-independent setline survey and practical applications of the data in a spatio-temporal assessment model

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Abstract

In 2009, the International Pacific Halibut Commission (IPHC) commenced an annual coastwide environmental monitoring program. At each station surveyed during the IPHC's fishery-independent setline survey (FIS), water column profiles are deployed to collect conductivity (C), temperature (T), pressure (depth D), dissolved oxygen (DO), pH, and fluorescence (DFH). These data are used to monitor the conditions of Pacific halibut habitat in North American waters of the Pacific Ocean and Bering Sea. The data have led to a better understanding of the environmental conditions throughout Pacific halibut habitat, including spatial variability in environmental variables. The monitoring has also enabled the ability to detect annual anomalies such as seasonal hypoxic zones that can greatly affect local Pacific halibut density. Incorporation of environmental covariates into the IPHC spatio-temporal modelling of density indices allows for the exploration of relationships between Pacific halibut density and environmental variables. As an example, we present results from modelling of data from surveys of the west coast of the United States of America.

Oceanographic conditions on the Pacific halibut grounds

- Dissolved oxygen (DO): Mean near-bottom DO has varied from hypoxic (< 1.4 mg/L) to relatively high (8 mg/L). Hypoxia is sometimes observed at the steeped FIS stations where deep basin water has flowed up onto the slope and outer shelf.
- Shallow water hypoxia is occasionally detected at FIS stations off the U.S. west coast.
- Chlorophyll: Highly variable spatially and temporally.
  - In example year 2010, mean chlorophyll was 22 mg/m³ and ranged from 0.134 mg/m³.
  - In example year 2017, the mean was greater at 68 mg/m³, but the range was more narrow at 0.428 mg/m³.
- Temperature: Near-bottom mean temperature increased in all regions, peaked over the 2009-2010 time period.
  - All areas indicate fluctuations between warmer and colder years at the higher end of the range.
  - Increase in minimum temperature in the recent years in all regions except Region 2.

Spatio-temporal modeling to examine environmental effects on Pacific halibut distribution

- In 2017, the FIS encountered a large number of lightly crowded stations, off the northeast U.S.A. coast that caught zero Pacific halibut, where fish are normally encountered.
- A previous study (Sadorsky et al. 2018) found that Pacific halibut have a minimum CO2 concentration threshold of about 9.2 mmol/L, i.e. they avoid DO below this level.
- Model results confirmed that there was strong evidence that Pacific halibut density indices were dependent on the DO covariate.
- In 2018, low levels of DO were also observed over a wide area. However, the affected zone typically has relatively low Pacific halibut catch rates, and the hypoxic stations were intermixed with stations above the minimum DO threshold. The result was that hypoxia did not have near the robustness index in 2018 as it did in 2017.

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Identification and characterization of FSHβ and LHβ in female Pacific halibut (Hippoglossus stenolepis)

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INTRODUCTION

Determining the maturity schedules of Pacific halibut (Hippoglossus stenolepis) is an important component of quantifying the spawning stock biomass used to establish management regulations by the International Pacific Halibut Commission (IPHC). Currently, this is assessed using macroscopic gonadal observations made during the Fishery Independent Stock Survey (FISS) that is conducted annually by the IPHC. However, this assessment method has not been verified histologically, so the ages assigned to females may not represent their actual maturity status. Gonadotropin hormones such as follicle stimulating hormone beta (FSHβ) and luteinizing hormone beta (LHβ) are key orchestrators of reproduction in teleosts and elasmobranches. Therefore, they may serve as reproductive markers for gametogenesis and vitellogenesis (FSHβ) and final maturation and spawning phases (LHβ). Using reproductive markers may contribute to resolving uncertainties about the stock’s spawning biomass through refining maturity estimates.

MATERIALS AND METHODS

Pituitary samples were collected from adult non-spawning (N = 7) and spawning (N = 5) Pacific halibut in the Portlock region of Alaska in 2018. From these samples, RNA was extracted and reverse transcribed into cDNA. Gene expression analysis was conducted using qPCR and FSHβ and LHβ primers designed against Pacific halibut 16S rDNA sequences obtained by RNA sequencing of male and female Pacific halibut pituitaries. Housekeeping genes, EEF1A1 and GAPDH were used as the controls.

Phylogenetic analysis of teleost FSHβ and LHβ deduced protein sequences nest Pacific halibut sequences in the flatfish clade

Results

- Pacific halibut FSHβ and LHβ deduced protein sequences show a high degree of homology with corresponding flatfish sequences.

Figure 1: Protein sequence alignments of Pacific halibut FSHβ (A) and LHβ (B) with other flatfish species. The inserted dashes serve to align the cysteine residues which are conserved. Potential N-glycosylation sites are marked by solid boxes. Species abbreviations are: Pacific halibut (Phii), Atlantic halibut (Ahha), olive flounder (Olaf), southern flounder (Sflh), common sole (Casd), Senegalese sole (Ssol), tongue sole (Tsol), and turbot (Turb).

- The expression levels of FSHβ and LHβ are higher in spawning than in non-spawning female Pacific halibut.

Figure 3: Relative expression levels for FSHβ (A) and LHβ (B) from spawning (January) and non-spawning (July) Pacific halibut. Joly samples are set as the reference (1).

CONCLUSIONS

- The nucleotide and deduced protein sequences of FSHβ and LHβ are now available for the first time in Pacific halibut.
- The high homologies of the FSHβ and LHβ nucleotide and protein sequences from Pacific halibut with respect to other flatfish species, indicate a high degree of evolutionary conservation of gonadotropin hormones.
- The higher overall relative FSHβ and LHβ mRNA expression levels in the pituitary from spawning over non-spawning female Pacific halibut are indicative of the functional conservation of these reproductive markers among teleost species.
- Overall, this study highlights the potential of the identified and characterized reproductive markers to help refine Pacific halibut maturity estimates.
Appendix 10

Oocyte stages and development in female Pacific Halibut (*Hippoglossus stenolepis*)

**INTRODUCTION**

Each year, the fishery-independent setline survey collects biological data on the maturity of female Pacific halibut that are used in the stock assessment. In particular, the female maturity schedule is used to estimate spawning stock biomass. Currently used estimates of maturity-at-age indicate that the age at which 50% of female Pacific halibut are sexually mature is 11.6 years on average. However, not only is maturity estimated with the use of macroscopic visual criteria, incating a relative level of uncertainty that is associated with semi-quantitative criteria, but the estimates of maturity-at-age have not been revised in recent years and may be outdated. For this reason, efforts need to be put in place to further understand reproductive maturity in female Pacific halibut. Unfortunately, relatively little is known regarding the changes that take place in the ovary during reproductive development leading to spawning in this species. This study aims to describe oocyte (immature egg) development in female Pacific halibut by comparing oocyte stages and characteristics between the non-spawning season (summer) and the spawning season (winter).

**MATERIALS AND METHODS**

Ovaries were collected from Pacific halibut females captured in three geographical regions (Fig. 1), two in the central and south Gulf of Alaska (Portlock and Haida Gwaii, respectively) and one in the southeast Bering Sea (Misty Moon), during the winter (Jan-Feb, 2004) and summer (June-July, 2004) periods. Ovaries were fixed in buffered formalin, embedded in paraffin and sections were mounted on glass slides. Two slides for each ovary were stained with Hematoxylin and Eosin. From each slide, the diameters of 10 randomly selected oocytes were measured, yielding a total of 20 measured oocytes per ovary analyzed. Measures were conducted using the Image-Pro Premier 9.1 software.

**RESULTS**

- **Oocyte classification**

- **Oocyte size distribution**

  ![Oocyte size distribution](image)

  Figure 3. Pacific halibut oocyte distribution in females caught in summer and winter periods. Oocyte size categories are in micrometers and are shown as percentages of the total number of oocytes measured.

- **Oocyte stage classification: Summer versus Winter**

  ![Oocyte stage classification](image)

  Figure 4. Pacific halibut oocyte stages in females caught in the summer (a) and Winter (b). Oocyte stage classification includes oocytes at the early and late perinucleolar (P), cortical alveoli (CA), and late vitellogenic (VT), maturing (M) and mature stages. The range of oocyte diameters is indicated within parentheses.

**CONCLUSIONS**

- This study represents the first attempt at describing ovarian development in Pacific halibut.
- Oocyte stages have been identified and can be used for accurate ovarian staging.
- The ovary of Pacific halibut contains a predominant population of early vitellogenic oocytes that is likely recruited during the Fall for Winter spawning.
- The observed differences in oocyte stages between Summer and Winter are indicative of the seasonal progression of ovarian development.
- Further studies are needed to complete the description of the annual reproductive cycle in this species.

**ACKNOWLEDGEMENTS**

Thanks to Collins Wiosnowski for her help with oocyte measurements and Joan Fordsberg, Chris Johnson and Robert Yost for their help with data analysis.

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