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

Appendix No.	Poster Title
Appendix 1	Electronically monitoring release method as a proxy for Pacific halibut discard mortality rates in the directed Pacific halibut longline fishery
Appendix 2	Pacific halibut migration research at IPHC
Appendix 3	Can we reconstruct the growth history of the Pacific halibut *(Hippoglossus stenolepis)* population by otolith increment analysis?
Appendix 4	Re-ageing of archived otoliths from the 1920s to the 1990s
Appendix 5	Identification of molecular growth signatures in skeletal muscle of juvenile Pacific halibut *(Hippoglossus stenolepis)* for monitoring population growth patterns
Appendix 6	Genetic population structure of Pacific halibut *(Hippoglossus stenolepis)*: progress to date
Appendix 7	Genetic sex identification of Pacific halibut *(Hippoglossus stenolepis)* commercial landings
Appendix 8	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

Appendix 9	Identification and characterization of FSH β and LH β in female Pacific halibut *(Hippoglossus stenolepis)*
Appendix 10	Oocyte stages and development in female Pacific halibut *(Hippoglossus stenolepis)*

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).

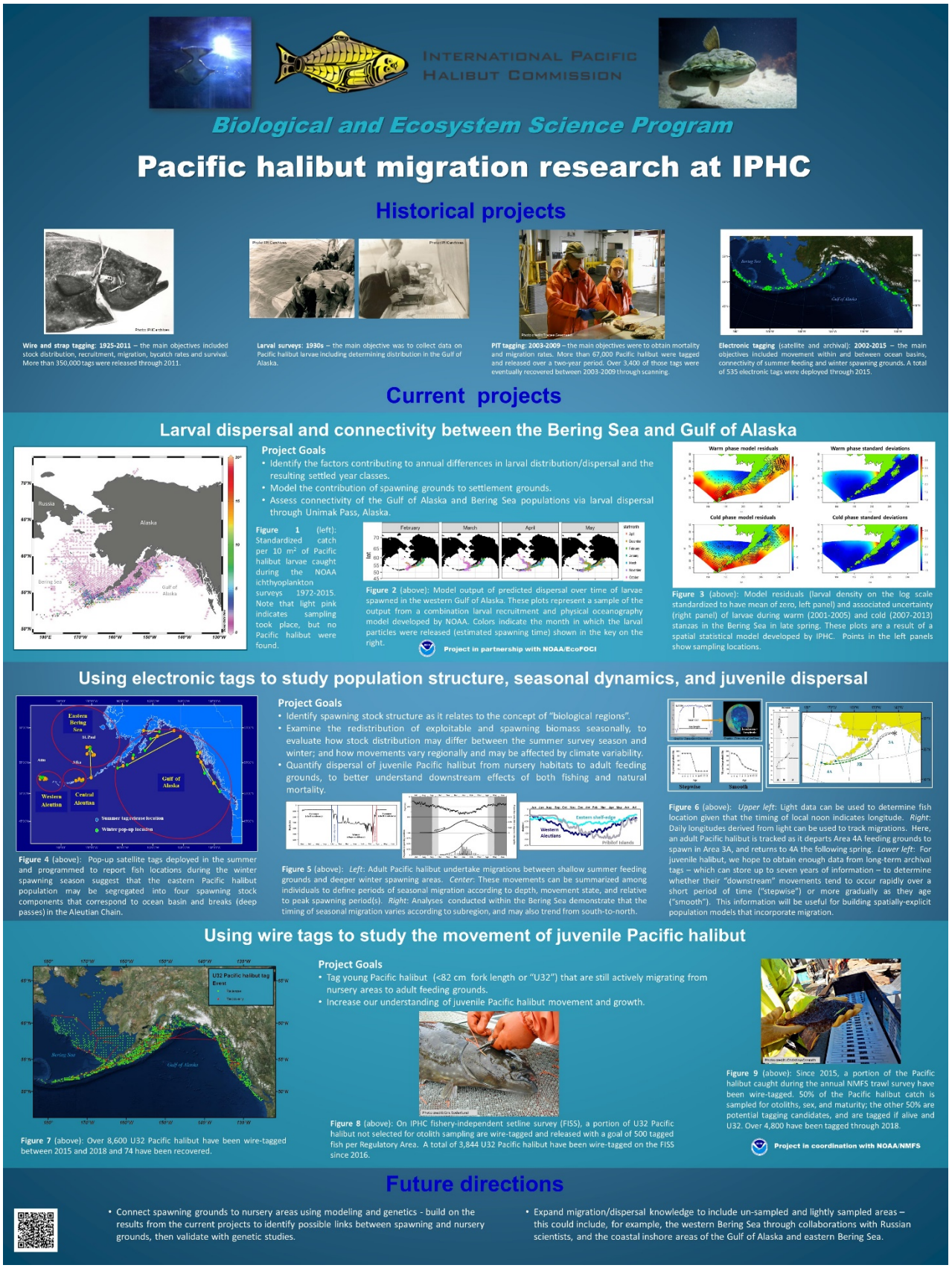
APPENDICES

As listed in Table 1

Appendix 1

Back to Table 1

Appendix 2



Back to Table 1

Appendix 3

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. Planas, Joan Forsberg
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 200 kg in body weight and 2.4 m in length and with the oldest individuals caught aged at 55 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 (1,2). Since the International Pacific Halibut Commission maintains a long-term, coastwide otolith collection, we aim to determine if otolith growth in Pacific halibut corresponds with their somatic growth.

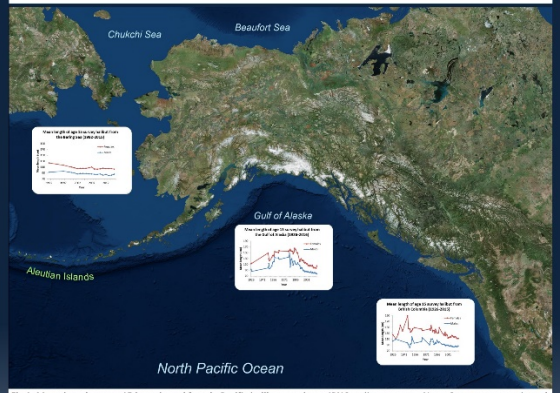


Fig 1. Mean length at age 15 in male and female Pacific halibut caught on IPHC setline surveys. Note: Surveys not conducted in all years

Objective

Determine whether otolith growth can be used as a proxy for somatic growth. Additionally, determine if otolith growth reflects the sexual dimorphism in adult Pacific halibut lengths, or reflects the length-at-age declines of the last 30 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 14 or 15 years old when captured; 11-year-olds were used for birth year 2002. Otoliths in this study had been aged by the break-and-bake technique, where the otolith is cut in half transversely and the posterior half is baked to enhance contrast between seasonal growth rings (Fig. 2A,B). The baked otolith halves were cut about 1.5 to 2 mm below the reading surface and mounted on glass slides. The mounted otolith sections were then photographed and measurements were made using Image-Pro Premier 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 sulcus groove (Fig. 2C).

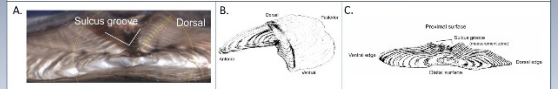


Fig.2 Images of Pacific halibut otoliths. A. Otolith cross-section showing transect where increment distances were measured B. 3D diagram of a halibut otolith C. Cross-section of otolith showing points of reference and measurement zone

Results

There is a 2.4% increase in mean otolith radius for age 15 females between the 1977 and 1992 year classes in the Gulf of Alaska, despite an 11.1% decline in body length

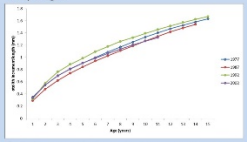


Fig 3. Mean cumulative otolith increment growth at age in survey-caught females from the Gulf of Alaska

Table 1. Mean fork length (cm) and mean otolith radius (mm) with standard deviations for Age 15 females from the Gulf of Alaska setline survey (area wide and subsample) for the 1977 and 1992 year classes

	1977	1992
Fork length (Gulf of Alaska)	119.4 ± 14.5	106.1 ± 15.4
Fork length (subsample)	120.9 ± 12.5	102.8 ± 11.3
Otolith radius (subsample)	1.63 ± 0.14	1.67 ± 0.15

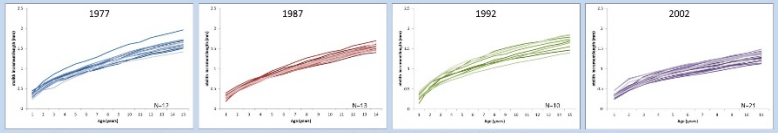


Fig.4 Individual cumulative otolith increment growth at age in survey-caught females from the Gulf of Alaska for 4 different year classes

There is 6.4% difference in the mean otolith radius for age 15 males and females in the 1992 year class in the Gulf of Alaska, despite a 26.4% difference in body length

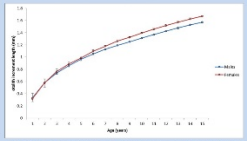


Fig.5 Mean cumulative otolith increment growth at age in survey-caught males and females from the Gulf of Alaska in 1992

Table 2. Mean fork length (cm) and mean otolith radius (mm) with standard deviations for Age 15 females and males from the Gulf of Alaska setline survey (area wide and subsample) for the 1992 year class

	Male	Female
Fork length (Gulf of Alaska)	83.9 ± 8.0	106.1 ± 15.4
Fork length (subsample)	80.8 ± 4.3	102.8 ± 11.3
Otolith radius (subsample)	1.57 ± 0.14	1.67 ± 0.15

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 area wide between those years. Otolith radius at age is therefore not a good proxy for length at age.
- Male 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 sexes (26.4% difference at age 15 in 1992 year class). Otolith radius does not reflect the sexual dimorphism 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

1. Vigliola, L. & Meekan M. (2009) The Back Calculation of Fish Growth from Otoliths. Tropical Fish Otoliths: Information for Assessment, Management, and Ecology, pp. 174-211

2. Sogard, S. (2011) Interpretation of Otolith Microstructure in Juvenile Winter Flounder (Pseudopleuronectes americanus): Ontogenetic Development, Daily Increment Validation, and Somatic Growth Relationships. Canadian Journal of Fisheries and Aquatic Sciences, 1991, 48(10): 1862-1871

Acknowledgements

Financial support for this project was provided by the North Pacific Research Board, Project 1309.

Back to Table 1

Appendix 4

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

Joan E. Forsberg, Dana Rudy, Chris Johnston, Robert Tobin and Ian J. Stewart
International Pacific Halibut Commission, Seattle, WA, USA

INTERNATIONAL PACIFIC HALIBUT COMMISSION

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 fishery-independent setline 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.

Microscope used by IPHC in the 1960s. New and historic surface ages were compared to see if there were differences that could be due to changes in equipment or ageing protocol.

Stored otoliths were transferred from vials to trays with individual cells.

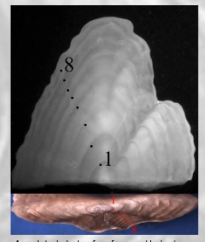
Study goals

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 1920s, IPHC age readers have cleared 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.

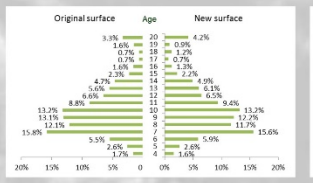
Methods

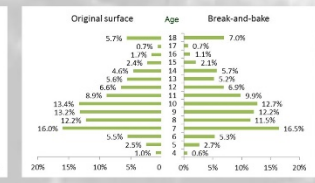
Years for which otoliths had been collected and aged were identified. One or two years per decade were selected based on number of geographical regions (IPHC regulatory areas) and otoliths available. For each selected year within a decade, otoliths were retrieved from storage. Otoliths collected prior to 2002 were stored in groups of ~25 per vial. Otoliths were separated within the vial by numbered paper labels. Almost 28,000 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.

Partially funded by North Pacific Research Board Project 1309.

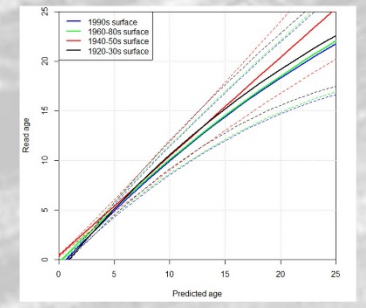


Annotated photo of surface and baked halves of an otolith collected in 1926.

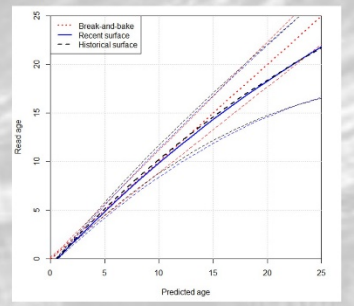




Age frequency distributions were compared for new and original surface readings and for original surface and break-and-bake readings for each decadal group. Example above is for the 1960s group.



Comparison of bias (solid lines) and imprecision (dashed lines) estimates for surface ages read during the 1990s, 1960s-1980s, 1940s-1950s, and 1920s-1930s.



Comparison of bias and imprecision for break-and-bake, recent (1998+) and pooled historical (1926-1993) surface ages.

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 reconciles 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-ageing 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-ageing of archived otoliths also provided an excellent opportunity to observe the condition of otoliths stored in glycerin solution for up to 88 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.

Back to Table 1

Appendix 5

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

Josep V. Planas*,[1] , Dana Rudy[1], Anna Simeon[1], Thomas P. Hurst[2]

[1]International Pacific Halibut Commission, Seattle, WA, USA
[2]Alaska Fisheries Science Center, NOAA, Newport, OR, USA

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.

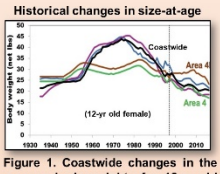
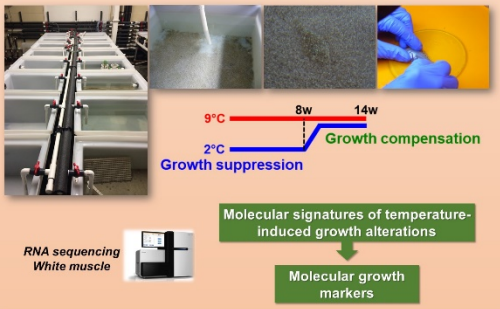


Figure 1. Coastwide changes in the average body weight of a 12-yr old female Pacific halibut (black line).

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 pit-tagged fish were acclimated for 8 weeks to 2°C and 9°C in duplicate tanks (N = 5) 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)**

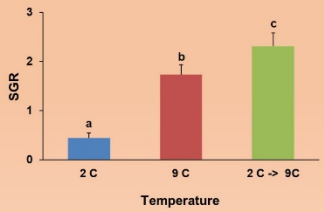


Figure 2. Effects of temperature on the specific growth rate in juvenile Pacific halibut. Different letters indicate statistically significant differences among the groups (N = 10).

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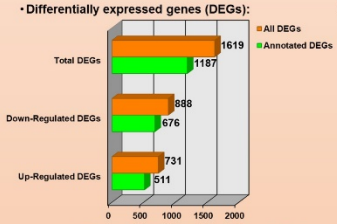
- **Transcriptomic responses to temperature-induced growth suppression**

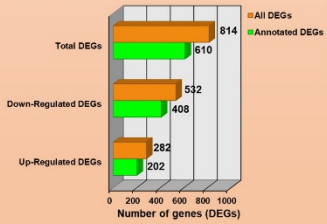


Functional categories (Down-regulated at 2°C):

Change	Functional category
Down	Muscle development and contraction
Down	Transcription and translation
Down	Protein and carbohydrate metabolism
Down	Energy metabolism and transfer
Down	Cell division
Down	Stress response
Down	Immune response

Figure 3. *Left*: Number of differentially expressed genes. *Right*: Functional categories of genes significantly down-regulated under growth suppression.

- **Transcriptomic responses to temperature-induced growth stimulation**



Functional categories (Up-regulated from 2°C to 9°C):

Change	Functional category
Up	Muscle development and contraction
Up	Protein metabolism and modification
Up	Carbohydrate metabolism (for ATP)
Up	Iron transport and binding
Up	Hemoglobin synthesis
Up	Cell adhesion and proliferation
Up	Transcription and translation

Figure 4. *Left*: Number of differentially expressed genes. *Right*: Functional categories of genes significantly up-regulated under growth stimulation.

CONCLUSIONS

- Acclimation at 2°C resulted in a significant reduction in the specific growth rate (SGR) whereas a significant increase in SGR was observed as 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 structure and function and metabolic activation.
- The resulting molecular growth signatures will be useful to investigate potential changes in growth patterns in Pacific halibut.

ACKNOWLEDGEMENTS. This study was conducted with funding from IPHC and the North Pacific Research Board (Project NPRB 1704).

Back to Table 1

Appendix 6

INTERNATIONAL PACIFIC HALIBUT COMMISSION
Biological and Ecosystem Science Program

Lorenz Hauser, Dan Drinan, Heather Galindo[1]
Timothy Loher[2]
[1] University of Washington School of Fisheries and Aquatic Sciences, Seattle, WA, USA (lhauser@uw.edu)
[2] *In attendence:* International Pacific Halibut Commission, Seattle USA (tim.loher@iphc.int)

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

Species distribution and management structure

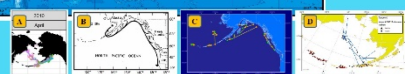
Pacific halibut *(Hippoglossus stenolepis)* are distributed throughout the North Pacific Ocean (**Fig. 1**), from northern California in the east, northward throughout the Bering Sea, and westward throughout the Sea of Okhotsk and northern Bering Sea. Stocks in the eastern Pacific are managed by the US and Canada via the International Pacific Halibut Commission, with legal retention restricted largely to hook-and-line fisheries that are composed of commercial, recreational, and subsistence sectors. Mortality rates in all harvest sectors and catch limits in target fisheries (**Fig. 2, upper**) are calculated and applied, respectively, within a series of regional regulatory areas **(Fig. 2, lower)**, and a variety of policy analyses and harvest considerations are structured similarly. However, the numerical stock assessment is conducted at the coastwide scale on an assumption of population-level panmixis.

The species can be highly dispersive at nearly all life history stages – from larval through spawning adult (**Fig. 3**) – at temporal scales ranging from seasonal to generational. But, behaviors such as philopatry, homing (Loher, *Fish. Res.* **92**:63-69), and limited home ranges (Nielsen *et al.*, *Mar. Ecol. Prog. Ser.* **517**:229-250), in association with bathymetric and environmental heterogeneity, may contribute to isolation and local adaptation that has the potential to result in significant population structure. Here, we endeavor to identify genetic population structure that might warrant attention at scales not addressed within current management structures, as well to identify genetic signatures that may serve as a mechanism for further understanding dispersal and mixing.

Figure 1: Pacific halibut are distributed throughout the North Pacific Ocean to at least 800 m depth, from northern California in the east to Hokkaido in the west, and northward throughout the Bering Sea and Sea of Okhotsk. larval settlement and early juvenile nursery areas are likely distributed throughout most of this range in relatively shallow (<50 m) water.

Figure 2: The Pacific halibut resource in the eastern Pacific Ocean is managed within a series of Regulatory Areas, some of which were established to reflect perceived biological stock structure (e.g., Area 4B in the Aleutian Islands region) and others of which were intended to represent socioeconomic or political units within broader biological regions (e.g., Area 2B, defined as all Canadian waters).

	2A	2B	2C	3A	3B	4A	4B	4CDE	Total
O26 Non-FCEY									
Commercial discard mort.	0.01	0.07	NA	NA	0.13	0.06	0.03	0.02	0.32
Bycatch	0.11	0.33	0.02	1.01	0.45	0.30	0.30	1.88	4.30
Recreational	NA	NA	1.43	1.86	0.01	0.02	0.00	0.00	3.31
Subsistence	NA	0.41	0.44	0.22	0.04	0.04	0.00	0.05	1.14
Total Non-FCEY	0.12	0.71	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
O26 FCEY									
Commercial discard mort.	NA	NA	0.06	0.30	NA	NA	NA	NA	0.36
Recreational (+discard mort.)	0.21	0.46	0.69	1.70	NA	NA	NA	NA	3.06
Subsistence	0.03	NA	NA	NA	NA	NA	NA	NA	0.03
Commercial landings	0.23	2.65	3.01	5.99	1.95	1.32	0.99	1.36	16.49
Total FCEY	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TCEY	0.59	3.04	5.05	12.07	2.56	1.69	1.21	3.33	31.00



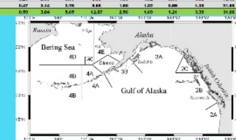


Figure 3: Pacific halibut may be highly dispersive across life history stages. Advection modelling suggests that dispersal during the 6 month pelagic larval phase (panel A) may be on the order of 100s of kilometers (E. Goldstein, NMFS-AFSC Seattle, unpublished), balanced by contranatant migrations of on average the same magnitude conducted as benthic juveniles (panel B) (Hilborn et al., IPHC Sci. Rep. 51). As adults, basin scale seasonal (panel C) and interannual (panel D) migrations have been documented (Seitz et al., Fish. Mgmt. Ecol. 24:339-346; Loher and Clark, IPHC Rep. Assmt. Res. Act. 2010:527-552).

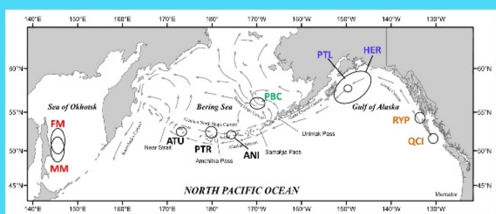


Figure 4: Locations at which adult Pacific halibut were sampled for genetic analyses; note that the 3 letter site designations used here are the same as those used in the table of results and DACP plot, presented subsequently. Locations spanning British Columbia (QCI and RYP) through the eastern Bering Sea (PBC) and central Aleutian Islands (ANI) were visited during the winter and targeted halibut spawning aggregations. Samples from the western Aleutians (PTR and ATU) were collected during the IPHC's summer stock assessment survey; Russian samples (FM and MM) were also collected during summer.

Prior research

Results from previous analyses of genetic population structure have suggested subtle genetic differentiation between the eastern and western Pacific, despite the fact that the signal of each individual study has been limited. Early allozyme analyses (Grant *et al.*, *Can. J. Fish. Aquat. Sci.* **41**:1083-1088) observed differentiation between samples from Japan compared to those from the Bering Sea and Gulf of Alaska; subsequent microsatellite analyses detected differences between Russia and both the Gulf of Alaska and Washington (Bentzen *et al.*, *IPHC Rep. Assmt. Res. Act.* ***1998***:229-241). Most recently, Nielsen *et al*. (*Genetics* **11**:999-1012) observed significant differentiation between samples collected in the Aleutian Islands relative to individuals from the eastern Bering Sea and Gulf of Alaska.

Recent methodology

Here, samples (N = 198 female, 168 male) from adult Pacific halibut that were collected in the eastern Pacific Ocean from British Columbia to the southeastern Bering Sea and westward into the Aleutian Islands, plus samples from two locations in the Sea of Okhotsk (N = 56 female, 38 male) (**Fig. 4**) were genotyped using 23 anonymous microsatellite loci and 38 EST-linked microsatellites to investigate potential selective differences. Expected and observed heterozygosity were calculated using Genepop v4.2 and the allelic richness (A_R) and private alleles (A_P) were estimated for each sampling location using rarefaction with five individuals per location using HP-Rare. After removal of sex-linked loci, loci deviating from Hardy-Weinberg equilibrium, and linked loci, overall and pairwise F_{ST} was estimated and tested using the techniques of Weir and Cockerham (*Evolution* **38**:1358-1370) implemented by Genepop v4.2.

{For full methodology and results see: Drinan *et al.*, *J. Fish. Biol.* **89**:2571-2594.}

Findings

Three out of 16 neutral microsatellites were found to be linked to sex (present in females and generally absent in males;) and suggest that – unlike their Atlantic congener (*Hippoglossus hippoglossus*) – females are the heterogametic sex in Pacific halibut (see Galindo *et al.*, *Mar. Biotechnol.* **13**:1027-1037). These loci were used to develop sex-identification markers (Drinan *et al.*, *J. Heredity* **109**:326-332) that are now routinely used by the IPHC for stock assessment purposes (**see poster by Simeon *et al.*, this session**).

Genetic diversity (A_R, A_P, H_O, and H_E) was similar among sampling locations and few private alleles were present. Overall F_{ST} was 0.0032 and highly significant ($p < 0.001$) and median F_{ST} value for all site-based pairwise comparisons (**Table 1**) was 0.0014 (25 and 75% quantiles = -0.0001 and 0.0074). For individual pairwise comparisons, significant differentiation was found in 17 of 28 tests after correcting for multiple comparisons (**Table 1**). Pairwise comparisons that included samples from ATU or PTR (western Aleutian Islands) had the greatest median F_{ST} values while other sampling locations had median F_{ST} values an order of magnitude smaller (**Table 1**). Discriminant analysis of principal components (DAPC) plotting showed evidence of separation into distinct geographic groups with the first dimension separating ATU and PTR (both western Aleutian Islands) from all other samples; a second dimension separated ANI (central Aleutians) from all non-Aleutian Islands samples; and all other samples clustered together (**Fig. 5**).

	Sea of Okhotsk		Aleutian Islands			Bering Sea	Gulf of Alaska		Haida Gwaii	
	FM	MM	ATU	PTR	ANI	PBC	HER	PTL	RYP	QCI
FM		0.0006 (0.759)	0.0012 (0.730)	0.0015 (0.903)	0.0007 (0.909)	0.0008 (0.560)	0.0007 (0.337)	0.0008 (0.707)	0.0029 (0.284)	0.0015 (0.747)
MM	-0.0004 (0.605)		0.0028 (0.041)	-0.0016 (0.277)	0.0014 (0.050)	0.0035 (0.001)	0.0011 (0.047)	0.0001 (0.437)	0.0023 (0.01)	0.0013 (0.041)
ATU	0.0060 (<0.001)	0.0071 (<0.001)		0.0008 (0.267)	0.0001 (0.580)	0.0014 (0.032)	0.0000 (0.410)	0.0001 (0.571)	0.0025 (0.454)	0.0006 (0.598)
PTR	0.0073 (<0.001)	0.0080 (<0.001)	0.001 (0.006)		-0.0003 (0.093)	0.0030 (0.105)	0.0018 (0.021)	0.0008 (0.027)	0.0017 (0.101)	-0.0007 (0.177)
ANI	0.0007 (0.145)	0.0028 (<0.001)	0.0056 (<0.001)	0.0103 (<0.001)		0.0005 (0.339)	0.0000 (0.434)	0.0004 (0.461)	0.0001 (0.701)	-0.0005 (0.721)
PBC	-0.0001 (0.531)	0.0012 (0.046)	0.0088 (<0.001)	0.0080 (<0.001)	0.0016 (0.005)		0.0001 (0.065)	0.0000 (0.103)	0.0021 (0.167)	0.0000 (0.004)
HER								0.0004 (0.404)	0.0025 (0.530)	-0.0007 (0.457)
PTL	-0.0001 (0.574)	0.0007 (0.127)	0.0077 (<0.001)	0.0103 (<0.001)	0.0013 (0.075)	-0.0012 (0.529)			-0.0005 (0.957)	-0.0011 (0.936)
RYP										0.0005 (0.378)
QCI	0.0002 (0.235)	0.0004 (0.046)	0.0063 (<0.001)	0.0070 (<0.001)	0.0008 (0.885)	0.0009 (0.506)		0.001 (0.607)		

Table 1: Genetic differentiation among Pacific halibut from the sites depicted in Fig. 4. Below the diagonal are pairwise F_{ST} and P values (in parentheses) ultimately based on 14 anonymous and 32 expressed sequence-tag (EST) linked microsatellite markers. Above the diagonal are measures that were based solely upon anonymous microsatellites, representing those which had been included in a prior analysis of Pacific halibut population structure (Nielsen et al., Genetics 11:999-1012).

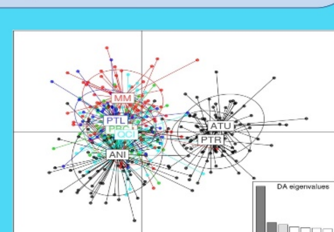


Figure 5: Discriminant analysis of principal components (DAPC) and DA eigenvalues from a study of Pacific halibut population structure using 14 anonymous and 32 EST-linked microsatellite markers. Samples from the western Aleutian Islands (ATU and PTR) show distinct separation from the remaining regions along the first dimension; the central Aleutians (ANI) show weaker separation along the second dimension.

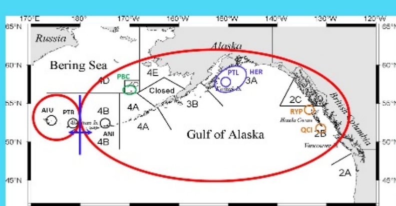


Figure 6: Population structure of Pacific halibut in the eastern Pacific ocean, as implied by genetic analysis. While the population appears to be well-mixed along the coast of North America – and perhaps westward into the central Aleutian Islands – an isolated substock may exist in the Western Aleutians that is separated from the remainder of the stock at Amchitka Pass. Of note is that this separation would not correspond to the boundaries of an existing IPHC regulatory area; rather, Area 4B may be composed of two substocks characterized by different dynamics and productivity characteristics, for which combined stock metrics are calculated and to which equivalent management actions are applied.

Implications

The results of the population genetic study suggest that IPHC Regulatory Area 4B may be composed of two distinct substocks of Pacific halibut units: its eastern half representing a westerly extension of a genetically well-mixed population inhabiting the continental shelf of North America; and its western half representing a somewhat-isolated substock. If true, regional productivity and stock dynamics may in western Area 4B relative to eastern 4B, warranting the development of spatially-explicit assessment or management procedures that can accommodate any significant differences between these substocks.

We hypothesize that isolation of the Western Aleutian pacific halibut may arise is due to limited migration of benthic-phase individuals across Amchitka Pass (minimum depth ~1150 m) in conjunction with localized spawning (Seitz *et al.*, *Fish. Mgmt. Ecol.* **24**:339-346), retention of those larvae within cyclonic currents that encircle the major Aleutian Island Groups (i.e., the Fox, Andreanof, and Near-Rat Islands), and localized settlement (IPHC unpublished data) that results in regional self-recruitment.

Ongoing and future research

At this juncture, the study's main result is considered provisional due to the nature of the sample collections: i.e., the western Aleutians were sampled during summer, which represents the species' dispersive feeding period and therefore represents the highest probability of encountering reproductive stock mixtures. Efforts are under way to obtain winter samples from both the central and western Aleutian Islands for further analysis. Additionally, modern sequencing technologies including whole-genome resequencing may provide further insights into population structure, dispersal, and mixing; and may allow for the identification of genes under selection. Such genes may reveal temporal adaptation to rapidly changing environments and to spatial and temporal variance in fishing pressure.

Back to Table 1

Appendix 7

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

INTERNATIONAL PACIFIC HALIBUT COMMISSION

Anna Simeon[1], Dan Drinan[2], Lorenz Hauser[2], Timothy Loher[1], Lara Erikson[1], Ian J. Stewart[1] and Josep V. Planas[1]

[1]International Pacific Halibut Commission, Seattle, WA, USA. E-mail: anna.simeon@iphc.int

[2]University of Washington, School of Aquatic and Fishery Sciences, Seattle, WA, USA

Background

- Throughout the fishery's history, the sex ratio of commercially-caught Pacific halibut has remained unknown as landed individuals are eviscerated at sea and the sexes are otherwise indistinguishable. The sex ratio from the IPHC's fishery independent setline 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 described 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 catch sex ratio through SNP genotyping

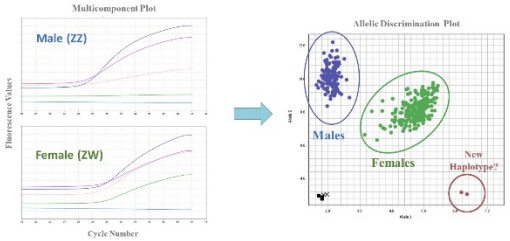
Methods

10,137 fin clips collected by IPHC port samplers in 2017

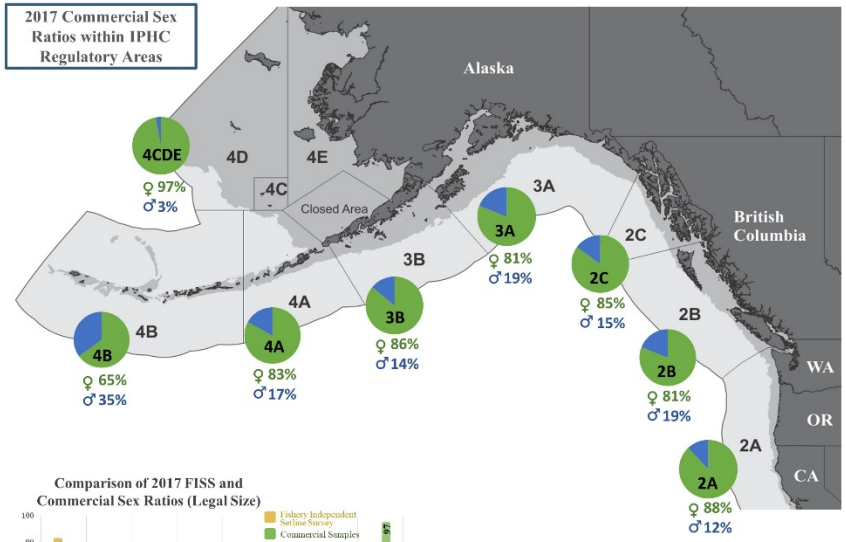
DNA isolated through simple, cost effective NaOH extraction

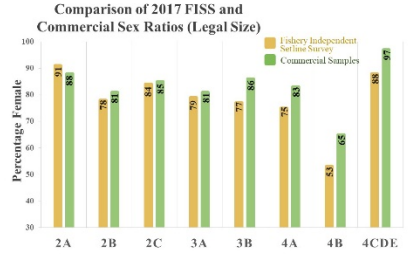
Sample genotyping via multiplexed TaqMan qPCR assay

- A multiplexed TaqMan assay was designed to genotype both SNPs (hs10183, hs23885) simultaneously using reporter dye pairs FAM/VIC and ABY/JUN and reference dye Mustang Purple. Target sequences were based on those described in Drinan et al. 2017.



Results





- 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.

- 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.

With this technique, the sex ratio of the commercial catch will be monitored annually and used in future stock assessments.

References

- Drinan D.P., Loher T., & Hauser L. (2017) Identification of Genomic Regions Associated with Sex in Pacific Halibut. Journal of Heredity. 109(3): 326-332.
- View more information and data at www.iphc.int

Back to Table 1

Appendix 8

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

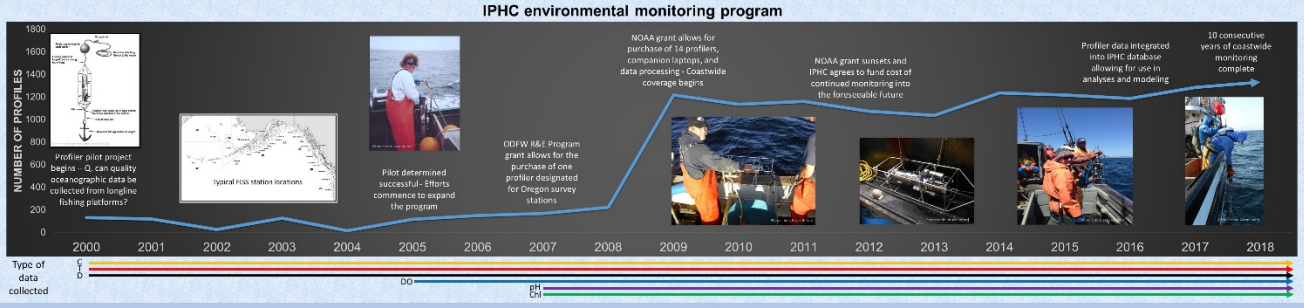
Lauri L. Sadorus and Raymond Webster
International Pacific Halibut Commission, Seattle, WA, USA, E-mail: lauri.sadorus@iphc.int

INTERNATIONAL PACIFIC HALIBUT COMMISSION

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 (FISS), water column profilers are deployed to collect conductivity (C), temperature (T), pressure (depth; D), dissolved oxygen (DO), pH, and fluorescence (Chl). 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.

IPHC environmental monitoring program



Oceanographic conditions on the Pacific halibut grounds

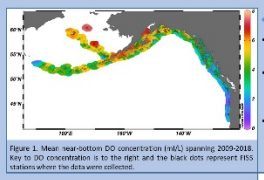


Figure 1. Mean near-bottom DO concentration (ml/L) spanning 2009-2018. Key to DO concentration is to the right and the black dots represent FISS stations where the data were collected.

Dissolved oxygen

- Mean near-bottom DO has varied from hypoxic (< 1.4 ml/L) to relatively high (8 ml/L).
- Hypoxia is sometimes detected at the deepest FISS stations where deep basin water has flowed up onto the slope and outer shelf.
- Shallow water hypoxia is occasionally detected at FISS stations off the U.S.A. west coast.

pH

- During IPHC monitoring, ocean acidity has generally correlated with DO concentration, i.e. near-bottom pH levels are higher where DO is higher and lower where DO is lower.
- pH tends to be lower in the east than elsewhere.
- pH tends to be lower at deeper stations compared to shallow stations.

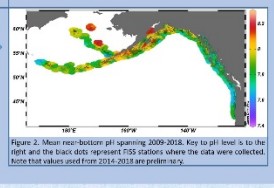


Figure 2. Mean near-bottom pH spanning 2009-2018. Key to pH level is to the right and the black dots represent FISS stations where the data were collected. Note that values used from 2014-2018 are preliminary.

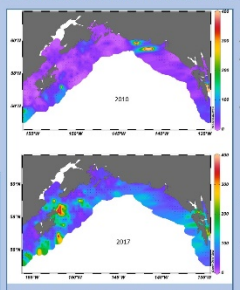


Figure 3. Depth integrated chlorophyll *a* concentration (mg/m²) for two example survey years, 2010 and 2017, in the Gulf of Alaska. Key is to the right and the black dots represent FISS stations where the data were collected.

Chlorophyll

- Highly variable spatially and temporally.
- In example year 2010, mean chlorophyll was 32 mg/m³ and ranged from 0-1,334 mg/m³.
- In example year 2017, the mean was greater at 86 mg/m³, but the range was more narrow at 0-428 mg/m³.

Temperature

- Near-bottom mean temperature increased in all regions profiled over the 2009-2018 time period.
- All areas indicate fluctuations between warmer and colder years at the higher end of the range.
- Increase in minimum temperature in the most recent years in all regions except Region 2.

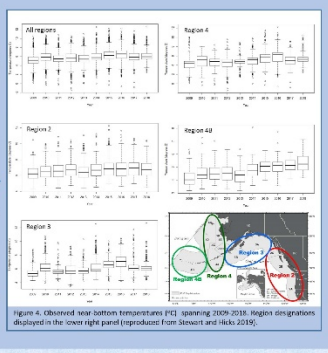


Figure 4. Observed near-bottom temperatures (°C) spanning 2009-2018. Region designations displayed in the lower right panel (reproduced from Stewart and Hicks 2019).

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

Purpose

- The IPHC uses a spatio-temporal model (Webster et al. In review) to examine distribution and density changes of Pacific halibut throughout the monitored area.
- Modelling output is used in the IPHC stock assessment, and ensures standardized treatment of data inputs across areas and time, while accounting for process and sampling uncertainty.
- Recently, environmental covariates have been added to the analysis to examine distribution changes driven by oceanographic conditions.
- To date, spatio-temporal modeling has provided strong evidence of relationships between Pacific halibut catch rates and environmental covariates, such as dissolved oxygen and temperature.

Example

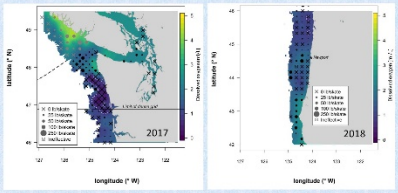


Figure 5. Estimated DO in northern Regulatory Area 2A in 2017 (left panel) and central Area 2A in 2018 (right panel) with DAS Pacific halibut WPUE values from the FISS overlaid with black symbols.

- In 2017, the FISS encountered a large number of tightly clustered stations off the northwest USA coast that caught zero Pacific halibut, where fish are normally encountered.
- A previous study (Sadorus et al. 2014) found that Pacific halibut have a minimum DO concentration threshold of about 0.9 ml/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 interspersed with stations above the minimum DO threshold. The result was that hypoxia did not have nearly the distributional impact in 2018 as it did in 2017.

References

Sadorus, L. L., Mantua, N. J., Essington, T., Hickey, B., and Hare, S. 2014. Distribution patterns of Pacific halibut (Hippoglossus stenolepis) in relation to environmental variables along the continental shelf waters of the US West Coast and southern British Columbia. Fish. Oceanogr. 23(3): 225-241.
Sadorus, L., Webster, J., and Sullivan, M. 2016. IPHC Oceanographic data collection program 2000-2014. Int. Pac. Halibut Comm. Tech. Rep. 60. 32 p.
Stewart, I., and Hicks, A. 2019. Pacific halibut (Hippoglossus stenolepis) stock assessment. Development. Int. Pac. Halibut Comm. Scientific Review Board Meeting, June 26-28, 2018, Seattle, WA. U.S.A. Report IPHC-2019-SRB01-07
Webster R. A., Soderlund, E., Dykstra, C. L., and Stewart, I. J. In review. Monitoring change in a dynamic environment: spatio-temporal modeling of calibrated data from different types of fisheries surveys of Pacific halibut.

Acknowledgements

We would like to thank all of the people who have been instrumental in making the environmental data collection program a big success, especially:
- The FISS team along with all of the vessels, crews, and sea samplers who have worked tirelessly to deploy the profilers and collect high quality data;
- Jay Walker of IPHC who has been the program's technical partner and sees that the data are collected properly and make it from the profilers to the database;
- Peggy Sullivan from NOAA/JISAO who has processed thousands of our profiles, making the data available to scientists worldwide to utilize for their research.

Back to Table 1

Appendix 9

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

Kennedy Bolstad[1], Anna Simeon[1] and Josep V. Planas[1]

[1]International Pacific Halibut Commission, Seattle, WA, USA

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 Setline Survey (FISS) that is conducted annually by the IPHC. However, this assessment method has not been verified histologically, so the codes assigned to females may not represent their actual maturity status. Gonadotropic hormones such as follicle stimulating hormone beta (FSHβ) and luteinizing hormone beta (LHβ) are key orchestrators of reproduction in teleosts and tetrapods. 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 full-length cDNA sequences obtained by RNA sequencing of male and female Pacific halibut pituitaries. Housekeeping genes, EEF1A1 and GAPDH were used as the controls.

Extract RNA from pituitaries

RT-PCR into cDNA

qPCR: FSHβ and LHβ primers

Compare non-spawning and spawning samples

RESULTS

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

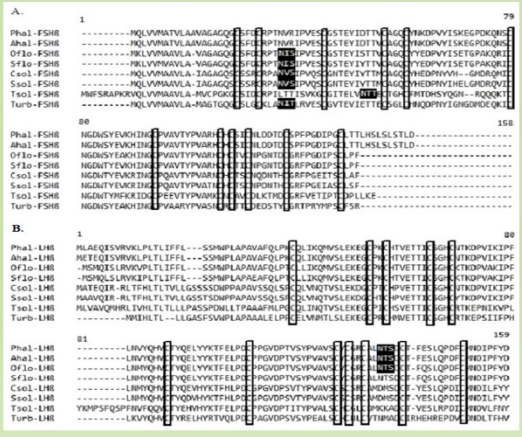


Figure 1. Protein sequence alignments of Pacific halibut FSHβ (A) and LHβ (B) with other flatfish species. The inserted dashes serve to align the cysteine residues which are outlined by rectangles. Potential N-glycosylation sites are marked by solid boxes. Species abbreviations are: Pacific halibut (Phal),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

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

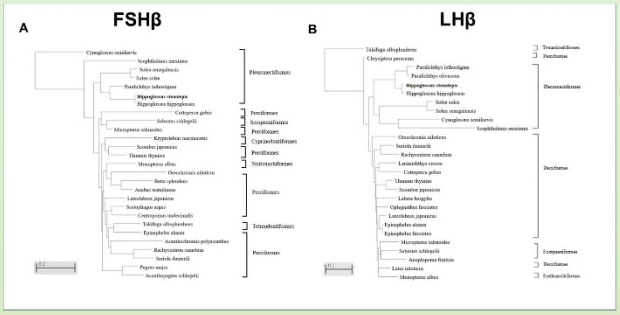


Figure 2. A phylogenetic tree comparing the FSHβ (A) and LHβ (B) protein sequence of Pacific halibut (*Hippoglossus stenolepis*) to other teleosts. This tree was constructed using the neighbor-joining method.

- **FSHβ and LHβ mRNA sequences share the highest percent identify with Atlantic halibut *(Hippoglossus hippoglossus)***

FSHβ

Description	Percent identity
Hippoglossus hippoglossus, mRNA for FSH β protein	99.60
Paralichthys lethostigma, mRNA for FSHβ precursor	88.15
Paralichthys olivaceus, mRNA for GTH IIβ subunit	85.11

LHβ

Description	Percent identity
Hippoglossus hippoglossus mRNA for LHβ protein	96.92
Paralichthys lethostigma, mRNA for LH precursor	91.81
Paralichthys olivaceus, mRNA for GTH IIβ subunit	92.89
Solea senegalensis, mRNA for LHβ precursor	83.42
Solea solea, LHβ subunit	85.33

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

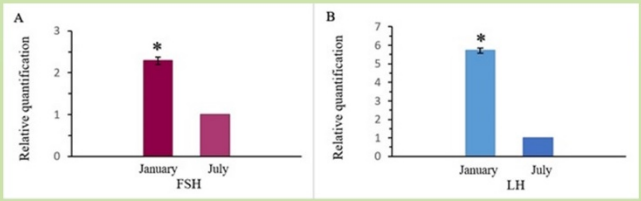


Figure 3. Relative expression levels for FSHβ (A) and LHβ (B) from spawning (January) and non-spawning (July) Pacific halibut. July samples are set as the reference (=1).

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 gonadotropic 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

Back to Table 1

Appendix 10

INTERNATIONAL PACIFIC HALIBUT COMMISSION

Biological and Ecosystem Science Program

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, incurring 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.

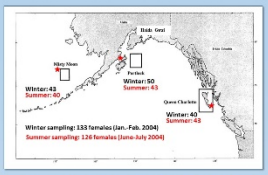


Figure 1. Geographic location of sample collection sites. Summer collection sites (non-spawning season) are indicated by a red star and winter collection sites (spawning season) are indicated by a black box. The number of females collected at each site is indicated.

RESULTS

- **Oocyte classification**

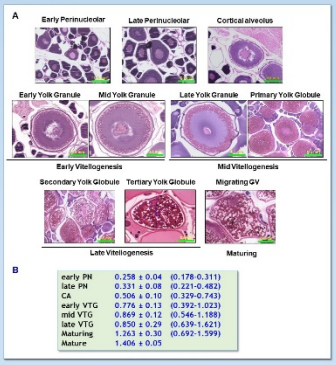


Figure 2. Pacific halibut oocyte stages and diameters. A) Pictures of representative oocytes at the various stages during oocyte development. B) Oocyte diameters (in millimeters) at different stages in oocyte development. Oocyte stage classification included oocytes at the early and late perinucleolar (PN), cortical alveoli (CA), mid and late vitellogenesis (VTG), maturing (migrating germinal vesicle [GV]) and mature stages. The range of oocyte diameters is indicated within parenthesis.

- **Oocyte size distribution**

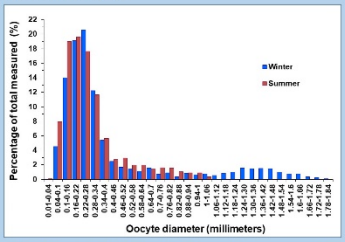


Figure 3. Pacific halibut oocyte distribution in females caught in summer and winter periods. Oocyte size categories are in millimeters and are shown as percentage of the total number of oocytes measured.

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

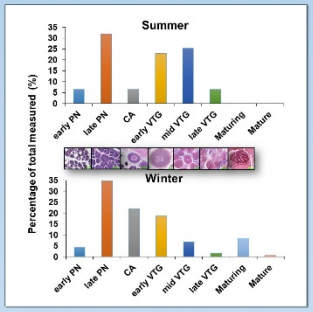


Figure 4. Pacific halibut oocyte stages in females caught in the Summer (A) and Winter (B). Oocyte stage classification included oocytes at the early and late perinucleolar (PN), cortical alveoli (CA), mid and late vitellogenesis (VTG), maturing and mature stages.

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.

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Back to Table 1