



IPHC 5-year Biological and Ecosystem Science Research Plan: Update (J. Planas)

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, 16 DECEMBER 2019)

PURPOSE

To provide the Commission with a description of progress on Biological and Ecosystem Science Research by the IPHC Secretariat.

BACKGROUND

The main objectives of Biological and Ecosystem Science Research at IPHC are to:

- 1) identify and assess critical knowledge gaps in the biology of the Pacific halibut;
- 2) understand the influence of environmental conditions; and
- 3) apply the resulting knowledge to reduce uncertainty in current stock assessment models.

The primary biological research activities at IPHC that follow Commission objectives are identified and described in the [Five-Year Research Plan](#) for the period 2017-21. These activities are summarized in five broad research areas designed to provide inputs into stock assessment and the management strategy evaluation processes ([Appendix I](#)), as follows:

- 1) Migration. Studies are aimed at further understanding reproductive migration and identification of spawning times and locations as well as larval and juvenile dispersal.
- 2) Reproduction. Studies are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity.
- 3) Growth and Physiological Condition. Studies are aimed at describing the role of some of the factors responsible for the observed changes in size-at-age and to provide tools for measuring growth and physiological condition in Pacific halibut.
- 4) Discard Mortality Rates (DMRs) and Survival. Studies are aimed at providing updated estimates of DMRs in both the longline and the trawl fisheries.
- 5) Genetics and Genomics. Studies are aimed at describing the genetic structure of the Pacific halibut population and at providing the means to investigate rapid adaptive changes in response to fishery-dependent and fishery-independent influences.

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Migration.

Knowledge of Pacific halibut migration throughout all life stages is necessary in order to gain a complete understanding of stock distribution and the factors that influence it.

- 1.1. Larval distribution and connectivity between the Gulf of Alaska and Bering Sea. A manuscript resulting from work on the cooperative project between NOAA EcoFoci and the IPHC has been drafted and is being edited for submission to a peer-reviewed journal. Two year classes, 2005 and 2009, were chosen as the primary focus of this project based on the fact that these represented relatively large and weak year classes,

and “warm” and “cold” environmental regimes in the Bering Sea, respectively. Additional “warm” and “cold” years were added to the larval advection modeling component to study the environmental linkage. Larval advection modeling produced information about dispersal pathways and degree of connectivity between spawning and settlement grounds both within and between the Bering Sea and Gulf of Alaska. Results suggest that up to half of the larvae spawned in the western Gulf of Alaska have the potential to be advected into the Bering Sea through Unimak Pass, AK. While Bering Sea environmental regime did not appear to strongly correlate to region of larval delivery in the Bering Sea, there was annual variation. Application of the IPHC-developed space-time model was used to assess distribution of young fish from 2-6 years old as they move away from the settlement grounds. Dispersal is widespread with young Pacific halibut moving further offshore and to deeper depths as they age. A portion of the young fish, especially evident when modeling the 2009 cohort due to higher densities, appeared to move out of Bristol Bay southward along the Alaska Peninsula, arriving at Unimak Pass within 2-3 years. Results from this project provide a new understanding of linkages between spawning grounds, eventual settlement, and subsequent migration of young fish, as well as variability in these pathways under different environmental scenarios. This work fills a gap in knowledge of early life history dispersal and ontogenetic migration utilized by young Pacific halibut.

- 1.2. Wire tagging of U32 Pacific halibut. Wire tagging of Pacific halibut caught in the NOAA/NMFS trawl surveys, which began in 2015, was continued in 2019. In 2019, 963 and 811 Pacific halibut were tagged in the Bering Sea and Gulf of Alaska, respectively. The wire tagging effort of U32 Pacific halibut that has taken place during the IPHC’s Fishery Independent Setline Survey (FISS) in recent years was not implemented in 2019 due to work load commitments on the surveys. However, through 2019, a total of 10,770 U32 Pacific halibut had been wire tagged and 110 of those have been recovered to date.
- 1.3. Electronic archival tagging. In 2019, as part of a collaborative research project with the Norton Sound Economic Development Corporation (NSED) and the University of Alaska Fairbanks, Pacific halibut were tagged in the eastern Bering Sea shelf with pop-up archival satellite (PAT) tags. Pacific halibut (U32 and O32) were tagged in the Norton Sound and St. Lawrence Island regions ($n = 56$). The PAT tags were programmed to release from their host fish and report their location and archived data during three periods: January 2020 (representing the spawning season); summer of 2020 (investigating site fidelity versus emigration); and summer of 2021 (examining longer-term dispersal). Tags provided by the IPHC were used to tag relative small fish (i.e., 70-90 cm) and were accompanied by tagging of large (>100 cm) Pacific halibut using tags that were purchased by NSED. This is designed to produce data that are comparable to the IPHC’s prior PAT-tagging research that was conducted to examine adult connectivity and spawning stock structure throughout the managed range, while expanding the work to examine considerably broader stock demographics than any prior electronic archival tagging experiment. Of particular interest is anecdotal information that suggest that the northeastern Bering Sea Pacific halibut population may be composed of two functional components: one that moves seasonally between this

region and the continental shelf edge in US waters (e.g. Middle and Pervenets Canyons in Area 4D), and another that may spawn in Russian waters (e.g. Navarin Canyon) be largely derived of individuals that are reared in Russian nurseries.

2. Reproduction.

Efforts at IPHC are currently underway to address two critical issues in stock assessment for estimating the female spawning biomass: the sex ratio of the commercial landings and maturity estimations.

2.1. Sex ratio of the commercial landings. For the first time, the IPHC has generated sex information of the entire set of aged commercial landings in 2017 and 2018. Genetic assays developed in collaboration with the University of Washington have been conducted at the IPHC biological laboratory using a QuantStudio6 instrument. Fin clips from over 10,000 aged Pacific halibut collected coastwide by IPHC port samplers in 2017 were genotyped and the results indicated that commercial landings were 82% female coastwide. A similar number of tissues from commercial landings collected in 2018 have been genotyped and the results indicate that landings were 81% female coastwide, consistent with the results from the previous year. Plans are underway to genotype the entire set of aged commercial samples collected in 2019 and, therefore, the sex ratio data from commercial landings will be available for three consecutive years (2017, 2018 and 2019). The sex ratio data of the commercial landings are currently being used in stock assessment.

2.2. Maturity estimations. In order to characterize the gonadal maturation schedule, the IPHC is conducting a full characterization of the annual reproductive cycle in female and male Pacific halibut. Biological samples (gonads, blood, pituitary, otolith, fat content) were collected at monthly intervals from female (N=30) and male (N=30) Pacific halibut captured from the Portlock region in the central Gulf of Alaska throughout an entire calendar year, from September 2017 until August 2018 (Figure 1). Formalin-fixed gonadal samples were processed for histology in early 2019 and duplicate histological slides for each sampled Pacific halibut gonad (N = 360 per sex) were stained with Hematoxylin and Eosin and are now available for staging. An MSc student from Alaska Pacific University, with funding from IPHC, was trained for this purpose in March 2019 and began staging the entire collection of ovarian histological samples in June 2019. The revision of maturity schedules and the comparison of macroscopic and microscopic ovarian staging will constitute the basis of her MSc dissertation.

We have completed the analysis of the temporal progression of the four maturity classification stages (macroscopic) used for staging females in the IPHC FISS (Figure 1) and of the gonadosomatic index (gonad weight/round weight x 100; GSI) as well as the hepatosomatic index (liver weight/round weight x 100; HSI) for both females and males (Figure 2). In addition, we have described the four maturity classification stages in relation to the GSH and the HSI (Figure 3) and established criteria for the classification of the different oocyte developmental stages that is critical for accurate staging.

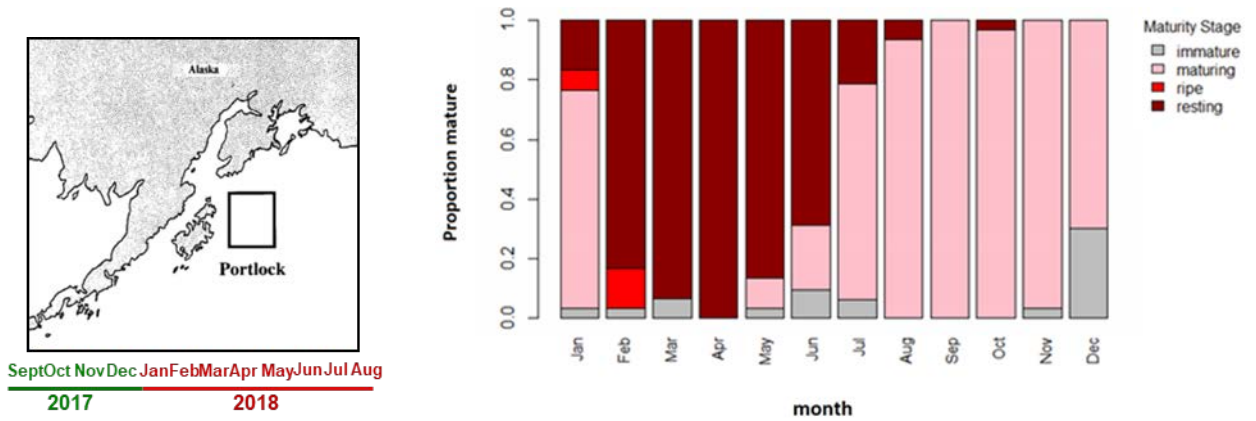


Figure 1. *Left.* Monthly sampling schedule in the Portlock area (Central Gulf of Alaska). *Right.* Temporal changes in the proportion of female Pacific halibut staged macroscopically according to the maturity classification criteria used in the FISS throughout an entire calendar year.

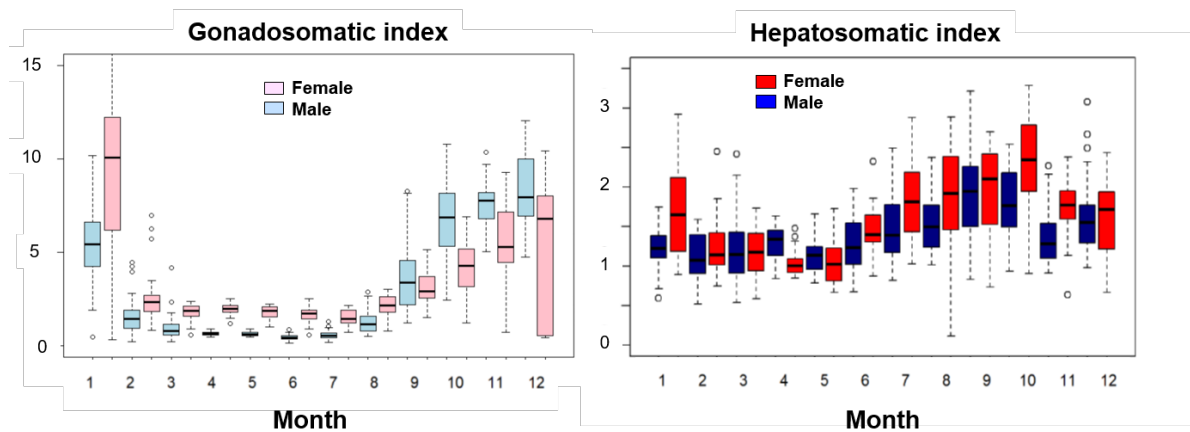


Figure 2. Temporal changes in the gonadosomatic (left) and hepatosomatic (right) indices in female and male Pacific halibut.

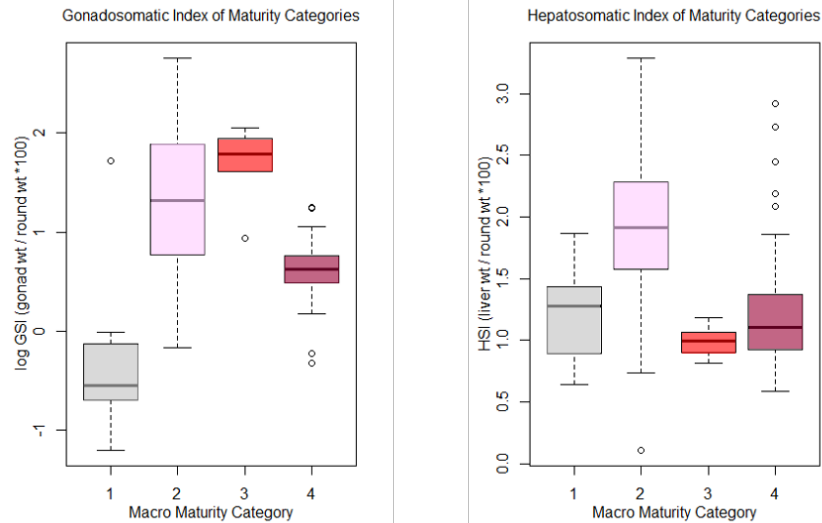


Figure 3. Macroscopic maturity categories in relation to the gonadosomatic (left) and hepatosomatic (right) indices in female Pacific halibut.

We have also identified and characterized two important physiological markers of reproductive status in the pituitary gland of female Pacific halibut: follicle stimulating hormone (FSH) and luteinizing hormone (LH). Assays to measure the gene expression levels of FSH and LH in the pituitary of female Pacific halibut have been developed. With the use of these assays, we have determined that the gene expression levels of LH are higher than FSH in pituitaries of spawning fish. In addition, the gene expression levels of FSH and LH are higher in spawning over non-spawning female Pacific halibut.

Future studies are aimed at characterizing the temporal profiles of reproductive hormones in the blood and of the gene expression profiles of FSH and LH, known key markers of the reproductive process, in the pituitary of female Pacific halibut. In addition to characterizing the progression of reproductive development throughout an entire annual reproductive cycle (intraseasonal) reproductive samples, the IPHC collected samples in June 2019 in the Portlock region to compare with those collected in the same location in June 2018 and June 2017 in order to evaluate possible differences in interseasonal variation in maturity schedules. Ovarian samples from these three years have been processed for histology and are in the process of being analyzed.

In order to determine whether there are spatial differences in maturity schedules, ovarian samples will be collected during the 2020 FISS season from a number of collection areas corresponding to the four biological regions.

3. Growth.

In order to improve our understanding of the possible role of growth alterations in the observed historical changes in size-at-age in Pacific halibut, the IPHC Secretariat is conducting studies aimed at: 1) the identification and validation of physiological markers for growth; and 2) the use of growth markers for evaluating growth patterns in the Pacific halibut population and the effects of environmental influences. The IPHC Secretariat is conducting

investigations on the effects of temperature variation on growth performance, as well as on the effects of density, hierarchical dominance and handling stress on growth in juvenile Pacific halibut in captivity (Figure 4). These studies are partially funded by a grant from the North Pacific Research Board to the IPHC ([Appendix II](#)) and the results on the effects of temperature on growth physiological indicators are being prepared for publication in a peer-reviewed journal.

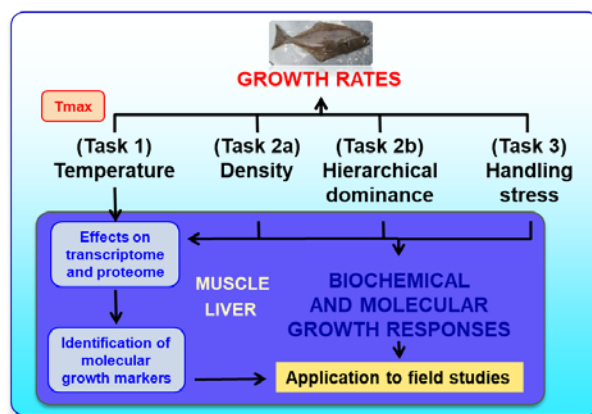


Figure 4. Diagram of the objectives of the NPRB-funded project with indication of the different tasks.

4. Discard Mortality Rates (DMRs) and Survival Assessment. In order to better estimate post-release survival of Pacific halibut caught incidentally in the directed longline fishery, the IPHC Secretariat is conducting investigations to understand the relationship between fish handling practices and fish physical and physiological condition and survival post-capture as assessed by tagging. These studies are partially funded by a grant from the Saltonstall-Kennedy Grant Program NOAA to IPHC ([Appendix II](#)).

- 4.1. Evaluation of the effects of **hook release techniques** on injury levels and association with the physiological condition of captured Pacific halibut. The IPHC has evaluated the effects of different release techniques on injury levels (Figure 5) and the results indicate that a majority (more than 70%) of Pacific halibut released by careful shake and by gangion cutting are classified in the excellent injury category. In contrast, Pacific halibut that encounter the hook stripper are primarily classified in the medium and poor injury categories.

The physiological condition of Pacific halibut subjected to the different hook release techniques is currently being assessed by relating the injury category assigned to each fish with the condition factor, fat levels and levels of blood stress indicators.

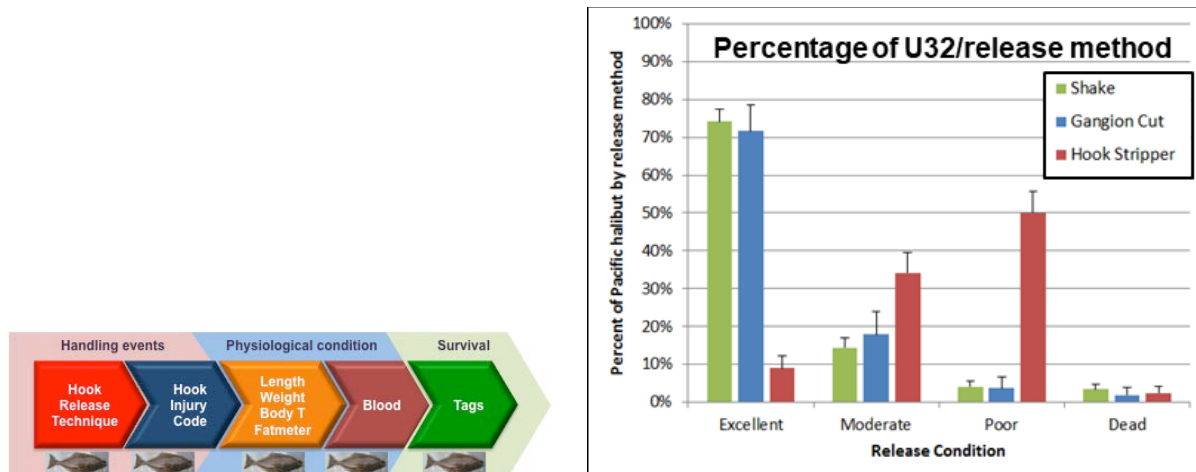


Figure 5. *Left.* Schematic diagram of workflow. *Right.* Prevalence of types of injuries (as indicated by injury classification or release condition) in U32 fish released by different hook release techniques (careful shake, ganglion cut and hook stripper).

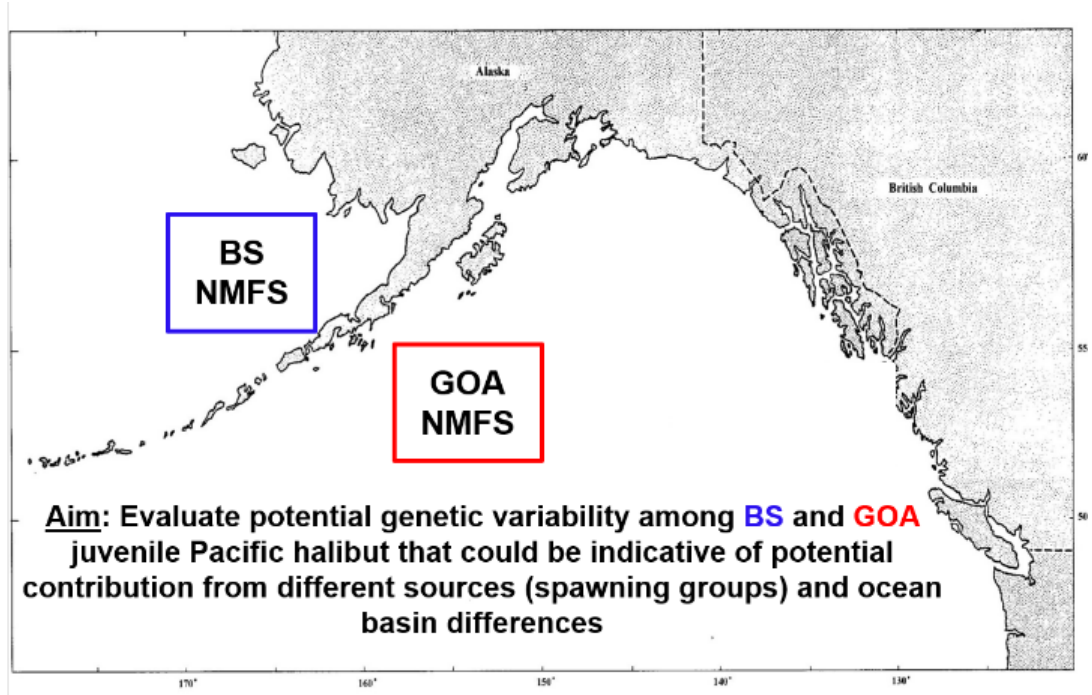
- 4.2. Post-release survival estimations. In order to evaluate the survival of discarded fish, two types of tagging approaches were used. 1) Classical mark-and-recapture of released fish with wire tags: 1,027 fish (under 33 inches in length) were tagged. 2) Biotelemetric monitoring of released fish with the use of satellite-transmitting electronic archival tags equipped with accelerometers: results from a total of 79 Pacific halibut ranging from 53-81 cm FL allowed us to estimate that the DMR of U32 Pacific halibut that were categorized as being in excellent-condition at the time of their release was approximately 4%.
- 4.3. Application of electronic monitoring (EM) for capturing the hook release methods. Evaluation of EM data whereby reviewers recorded the release method and condition of released fish evidenced a high degree (95%-100%) of agreement between the actual release method used and that captured by EM. Therefore, once the survival estimates of fish released by the different hook release techniques are determined, these results strongly suggest that mortality rates could be deduced from EM-captured hook release techniques.
- 4.4. Discard mortality rates of Pacific halibut in the charter recreational fishery. The IPHC has initiated in 2019 a research project aimed at experimentally deriving DMRs from the charter recreational fishery for the first time. This project has received funding from the National Fish and Wildlife Foundation ([Appendix II](#)). As an initial step in this project, information from the charter fleet on types of gear and fish handling practices used was collected through stakeholder meetings and on dock interviews with charter captains and operators. This information will inform the design of the experimental test fishing that will take place in 2020 and in which fish mortality will be estimated as described in 4.2.

5. Genetics and genomics. The IPHC Secretariat is exploring avenues for incorporating genetic approaches for a better understanding of population structure and distribution and is also building genomic resources to assist in genetics and molecular studies on Pacific halibut.

5.1. Genetics. The main purpose of the proposed studies is to incorporate genetic analyses into migration-related research in order to improve our understanding of Pacific halibut movement and dispersal and of the genetic structure of the Pacific halibut population. Three specific topics will be investigated:

5.1.1. *Analysis of genetic variability among juvenile Pacific halibut in the Bering Sea and the Gulf of Alaska*. The aim of this study is to evaluate the genetic variability among juvenile Pacific halibut in a given ocean basin in order to infer information on the potential contribution from fish spawned in different areas to that particular ocean basin. We hypothesize that genetic variability among juvenile Pacific halibut captured in one particular ocean basin (e.g. eastern Bering Sea) may be indicative of mixing of individuals originating in different spawning grounds and, therefore, of movement. By comparing the genetic variability of fish between two ocean basins (i.e. eastern Bering Sea and Gulf of Alaska), we will be able to evaluate the extent of the potential contribution from different sources (e.g. spawning groups) in each of the ocean basins and provide indications of relative movement of fish to these two different ocean basins. The use of genetic samples from juvenile Pacific halibut collected in the NMFS trawl survey in the eastern Bering Sea and in the Gulf of Alaska, aged directly or indirectly through the length-age key, will allow us to provide genetic information from fish that are at or near their settlement or nursery grounds.

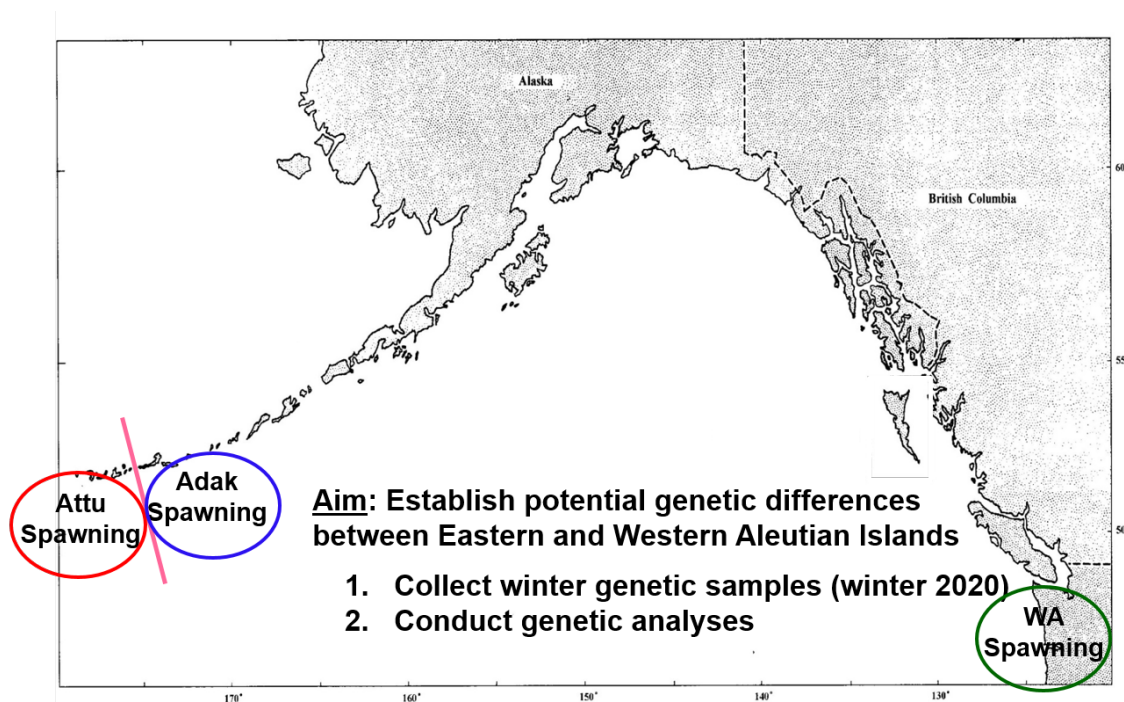
Fin clips from 150 fish from the eastern Bering Sea and from 150 fish from the Gulf of Alaska, all between 2 and 3 years of age, will be used for DNA extraction and purification. A pooled-sequencing approach will be used to obtain genome-wide data resulting from the sequencing of two libraries, each composed of all the individuals from each of the two areas. Pooled heterozygosity will be estimated for each of the two ocean basins as well as the mean difference in pooled heterozygosity between the two sample groups (i.e. ocean basins). For fish of unknown sex, genetic sex will be determined using SNPs to two sex-linked loci previously developed and used to determine the genetic sex of the commercial Pacific halibut landings.



5.1.2. *Analysis of genetic population structure in IPHC Regulatory Area 4B.* Understanding population structure is imperative for sound management and conservation of natural resources. Pacific halibut in US and Canadian waters are managed as a single, panmictic population on the basis of tagging studies and historical (i.e., pre-2010) analyses of genetic population structure that failed to demonstrate significant differentiation in the eastern Pacific. However, recent studies have reported significant genetic population on the basis of microsatellites that suggest that Pacific halibut residing in the Aleutian Islands may be genetically distinct from other regions. In particular, differentiation of the population on either side of Amchitka Pass is indicated, suggesting a possible basis for separating IPHC Regulatory Area 4B into two management subareas. However, in order to evaluate that possibility, it would be advisable to re-assess those conclusions using samples specifically collected to evaluate the implied stock delineation. In particular, the existing analyses employed tissue samples collected in the summer (i.e. non-spawning season) west of Amchitka Pass and may or may not be representative of the local spawning population. Although unlikely, one cannot exclude the possibility that the observed differentiation in the Aleutian Islands may be representative of differentiation caused by dilution of the west Aleutian sample by individuals from some other regions. The proposed work would sample the local population on either side of Amchitka Pass during the spawning season so as to best-characterize spawning structure and provide management advice regarding the relative justifiability for considering the western Aleutians as a genetically-distinct substock. Subsequently, genetic analyses will be conducted to evaluate the level of genetic differentiation between the two sampled areas. In addition, migration analyses have suggested that spawning occurs off the Washington coast, which would represent a component of the spawning population that has never before

been studied. In a number of northern fish populations it has been shown that fish at the southern edge of the range display unique genetic composition.

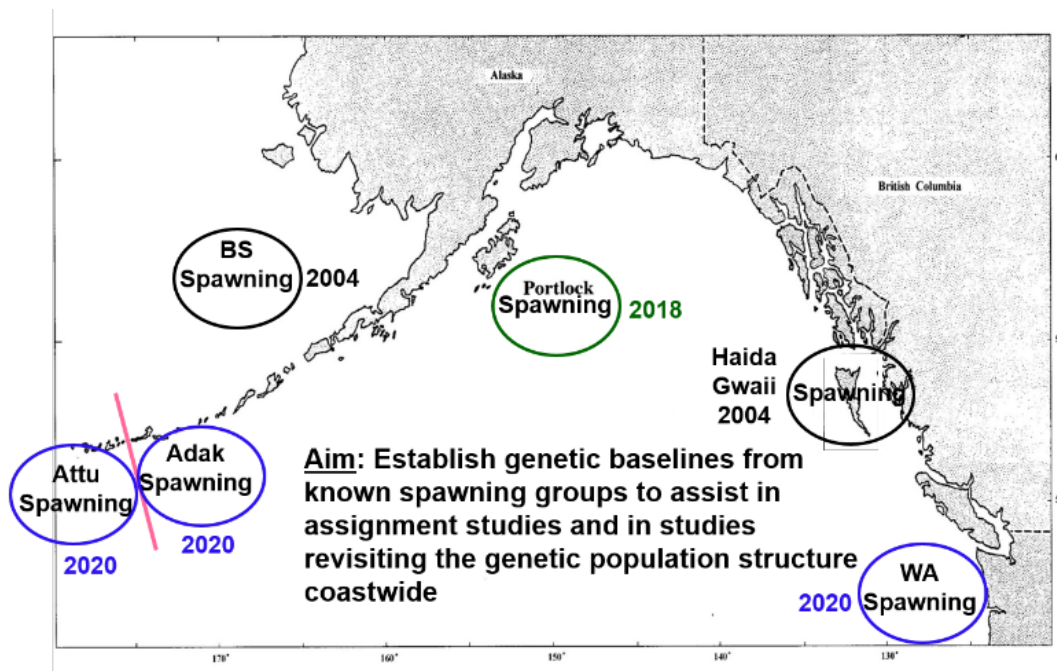
Sample collection will take place west and east of Amchitka Pass, and off the Washington coast, during the winter of 2020 in order to collect fish during the spawning season. Commercial fishing vessels will be chartered specifically for the purpose of collecting approximately 50 adult fish from each area and those samples will be subjected to DNA extraction and purification. A pooled-sequencing approach will be used to obtain genome-wide data resulting from the sequencing of three libraries, one from each area sampled.



5.1.3. *Identification of potential genetic signatures of origin or spawning groups to revise population structure.* In order to expand our proposed studies evaluating the Pacific halibut population genetic structure to the entire northeast Pacific Ocean covering the IPHC Convention Area, a broader genetic study is proposed that aims at establishing genetic baselines from known spawning groups throughout the geographic area in question. With the genetic samples that are planned to be collected in the winter of 2022, together with winter samples collected in the Portlock area (i.e. central Gulf of Alaska) in 2018 and in Haida Gwaii in 2004 and in the Bering Sea (i.e. Pribilof Canyon) in 2004, we plan on establishing genetic signatures of these spawning groups to revise the genetic population structure with up-to-date genetic techniques.

Fin clips from 50 fish from each of the six sampled geographic areas will be used for DNA extraction and purification. A pooled-sequencing approach will be used to obtain

genome-wide data resulting from the sequencing of 6 libraries, each composed of all the individuals from each of the six areas sampled.



In order to be able to conduct the proposed genetic studies, Mr. Andy Jasonowicz was initially hired as Research Biologist with a one-year contract starting in 26 August 2019.

5.2. Genomics. The IPHC Secretariat is currently conducting a project aimed at generating a first draft sequence of the Pacific halibut genome in collaboration with the French National Institute of Agricultural Research (INRA, Rennes, France) and the School of Aquatic and Fishery Science of the University of Washington (Seattle, WA). An initial sequencing effort using genomic DNA from one Pacific halibut female in half an Illumina lane in 2 x 250 pair end mode resulted in a total size of assembled scaffolds of 700 Mb, likely corresponding to the size of the Pacific halibut genome. This non-contiguous genomic sequence is currently being complemented by long read sequencing using the Nanopore technology (i.e. PromethION) combined with Hi-C sequencing for chromosome-scale scaffolding of the genome assembly. The sequencing effort is expected to be completed by the Spring of 2020. In addition to genome sequencing, the IPHC Secretariat has completed transcriptome sequencing of a wide variety of tissues (12) in Pacific halibut including white and red skeletal muscle, liver, heart, ovary, testis, head kidney, brain, gill, pituitary, spleen and retina. Current plans regarding this extensive transcriptomic dataset include generating a reference transcriptome for the species and to create a user-friendly, searchable database to be made public in the IPHC website.

RECOMMENDATIONS

That the Commission **NOTE** paper IPHC-2020-AM096-11 which outlines progress on Biological and Ecosystem Science Research by the IPhC Secretariat.

APPENDICES

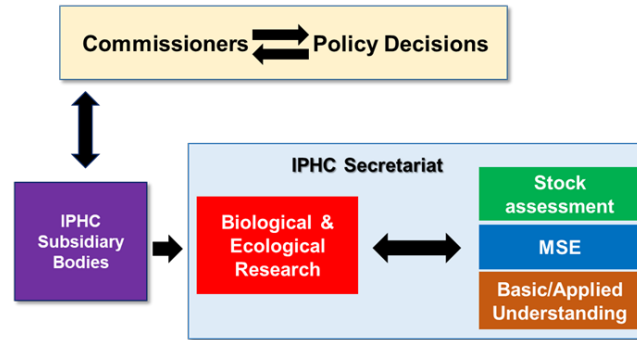
Appendix I: Integration of biological research, stock assessment and harvest strategy policy

Appendix II: Summary of external research projects awarded for funding



APPENDIX I

Integration of biological research, stock assessment and harvest strategy policy



Biological research

Stock assessment

Stock assessment MSE

<i>Research areas</i>	<i>Research projects</i>	<i>Relevance for stock assessment</i>	<i>Inputs to stock assessment and MSE development</i>
Migration	Larval distribution Juvenile and adult migratory behavior and distribution	Geographical selectivity Stock distribution	Information for structural choices Recruitment indices Migration pathways and rates Timing of migration
Reproduction	Sex ratio Spawning output Age at maturity	Spawning biomass scale and trend Stock productivity Recruitment variability	Sex ratio Maturity schedule Fecundity
Growth	Identification of growth patterns Environmental effects on growth Growth influence in size-at-age variation	Temporal and spatial variation in growth Yield calculations Effects of ecosystem conditions Effects of fishing	Predicted weight-at-age Mechanisms for changes in weight-at-age
Discard Survival	Bycatch survival estimates Discard mortality rate estimates	Scale and trend in mortality Scale and trend in productivity	Bycatch and discard mortality estimates Variability in bycatch and uncertainty in discard mortality estimates
Genetics and Genomics	Genetic structure of the population Sequencing of the Pacific halibut genome	Spatial dynamics Management units	Information for structural choices



APPENDIX II
Summary of current awarded research grants

Project #	Grant agency	Project name	PI	Partners	IPHC Budget (\$US)	Management implications	Grant period
1	Saltonstall-Kennedy NOAA	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival (Award No. NAI17NMF4270240)	IPHC	Alaska Pacific University	\$286,121	Bycatch estimates	September 2017 – August 2020
2	North Pacific Research Board	Somatic growth processes in the Pacific halibut (<i>Hippoglossus stenolepis</i>) and their response to temperature, density and stress manipulation effects (NPRB Award No. 1704)	IPHC	AFSC-NOAA-Newport, OR	\$131,891	Changes in biomass/size-at-age	September 2017 – February 2020
3	Bycatch Reduction Engineering Program - NOAA	Adapting Towed Array Hydrophones to Support Information Sharing Networks to Reduce Interactions Between Sperm Whales and Longline Gear in Alaska	Alaska Longline Fishing Association	IPHC, University of Alaska Southeast, AFSC-NOAA	-	Whale Depredation	September 2018 – August 2019
4	Bycatch Reduction Engineering Program - NOAA	Use of LEDs to reduce Pacific halibut catches before trawl entrainment	Pacific States Marine Fisheries Commission	IPHC, NMFS	-	Bycatch reduction	September 2018 – August 2019
5	National Fish & Wildlife Foundation	Improving the characterization of discard mortality of Pacific halibut in the recreational fisheries (NFWF Award No. 61484)	IPHC	Alaska Pacific University, U of A Fairbanks, charter industry	\$98,902	Bycatch estimates	February 2020 – January 2021
Total awarded (\$)					\$516,914		