



Report on Current and Future Biological Research Activities

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, T. LOHER, L. SADORUS, C. DYKSTRA, J. FORSBERG, 16 AUGUST 2019)

PURPOSE

To provide the Scientific Review Board with an update of current progress on research projects conducted and planned by the Biological and Ecosystem Sciences Research Program.

BACKGROUND

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

- 1) Migration and Distribution. 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 and Distribution.

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. Manuscript preparation is underway on this cooperative project between NOAA EcoFoci and the IPHC and a draft is expected later this year. Two years, 2005 and 2009, were chosen as the primary focus of this project based on the fact that these years 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 ontogenic 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, is continuing in 2019. As of mid-July, three of four trips had been completed in the GOA trawl survey resulting in 759 tags released and two of four trips had been completed in the Bering Sea survey resulting in 412 tags released. Through 2018, 4,831 tags had been released bringing the total thus far to 6,002. To date, 42 have been recovered. The wire tagging effort that has taken place during the FISS in recent years was not implemented in 2019 due to work load commitments on the surveys. Through 2018, a total of 3,112 U32 Pacific halibut had been wire tagged and 47 of those have been recovered to date. The purpose of this work is to update our knowledge of U32 migration pathways and growth.
- 1.3. Electronic archival tagging. Electronic archival tags that allow for daily light-based geolocation as well as depth and temperature recording were deployed on U32 Pacific halibut caught in the eastern Bering Sea during 2019. The project began in 2018 aboard the IPHC's Fishery Independent Setline Survey (FISS), during which 255 fishery-recovery long-term (7 year recording capacity) archival tags were deployed coastwide and 13 Pop-up Archival Transmitting (PAT) tags were deployed in the Aleutian Islands. This year's effort deployed fishery-recovery archival tags from the NOAA/NMFS Eastern Bering Sea (EBS) trawl survey; PAT tags (n = ~10) tags from the Northern Bering (NBS) trawl survey; and PAT tags (n = ~40) in the Norton Sound and St. Lawrence Island region in collaboration with the Norton Sound Economic Development Corporation (NSEDC). 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 NSEDC. 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 samples (> 10,000 fin clips) collected in 2017. Genetic samples from aged commercial landings collected in 2018 are currently being processed for genetic sex identification and sex ratio information will be available before the end of 2019.

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. 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. An MSc student from Alaska Pacific University, with funding from IPHC, was trained for this purpose in March 2019 and has completed the initial staging of the entire collection of ovarian histological samples. The revision of maturity schedules and the comparison of macroscopic and microscopic ovarian staging will constitute the basis of her MSc dissertation.

Results. We have completed the analysis of the temporal progression of the four maturity classification stages (macroscopic) used for staging females in the IPHC FISS (Fig. 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 (Fig. 2). In addition, we have described the four maturity classification stages in relation to the GSH and the HISI (Fig. 3) and established criteria for the classification of the different oocyte developmental stages that is critical for accurate staging.

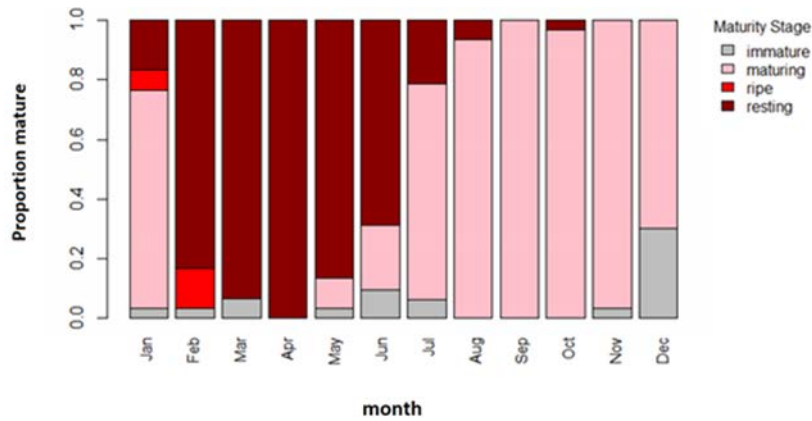


Figure 1. Temporal changes in the proportion of female Pacific halibut staged macroscopically according to the maturity classification criteria used in the FISS throughout an entire calendar year in the Portlock region (Central Gulf of Alaska).

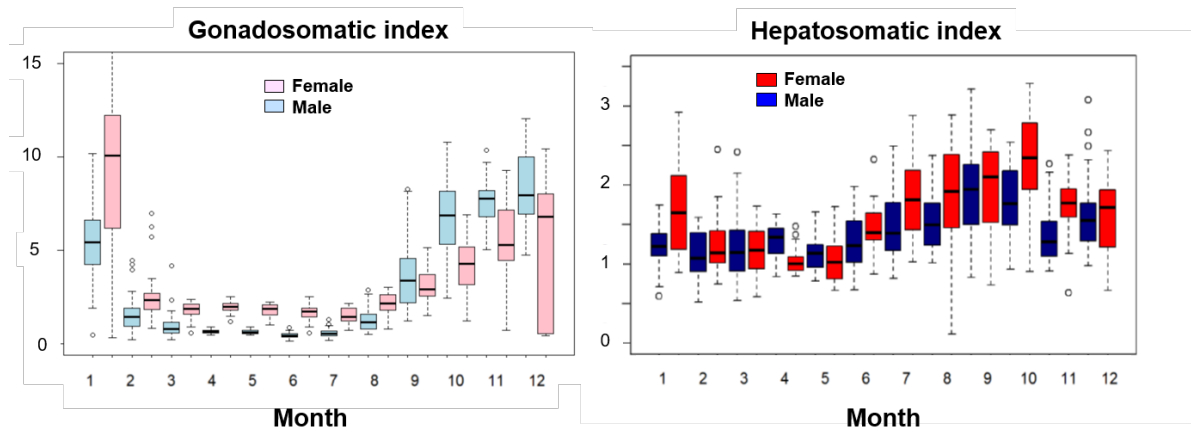


Figure 2. Temporal changes in the gonadosomatic (left) and hepatosomatic (right) indices in female and male Pacific halibut.

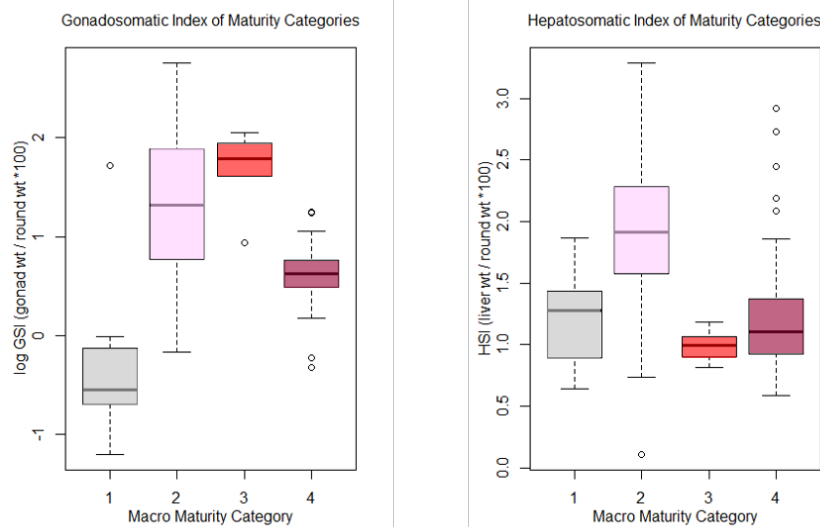


Figure 3. Macroscopic maturity categories in relation to the gonadosomatic (left) and hepatosomatic (right) indices in female Pacific halibut.

Future plans include: 1) analysis of the entire collection of testicular histological samples and 2) the temporal characterization of reproductive hormones in the blood (17β -estradiol, testosterone and 17α , 20β -dihydroxy-4-pregnen-3-one for females and 11 -ketotestosterone, testosterone and 17α , 20β -dihydroxy-4-pregnen-3-one for males) and the gene expression profiles of gonadotropic hormones (follicle-stimulating hormone and luteinizing hormone) in the pituitary of female and male Pacific halibut. In addition to characterizing the progression of reproductive development throughout an entire annual reproductive cycle (intra-seasonal) reproductive samples, the IPHC collected samples in June 2019 to compare with those collected in June 2018 and June 2017 in the Portlock region in order to evaluate possible differences in inter-seasonal variation in maturity schedules over a three-year period.

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. 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.

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 physiological condition of Pacific halibut subjected to the different hook release techniques (careful shake, gangion and hook stripping) 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. Blood glucose and lactate levels from all fish released have been determined using colorimetric methods. Once blood cortisol levels are measured, the relationship between blood stress parameters and capture and handling conditions will be investigated.

4.2. Discard mortality rates of Pacific halibut in the charter recreational fishery. The IPHC has begun 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,

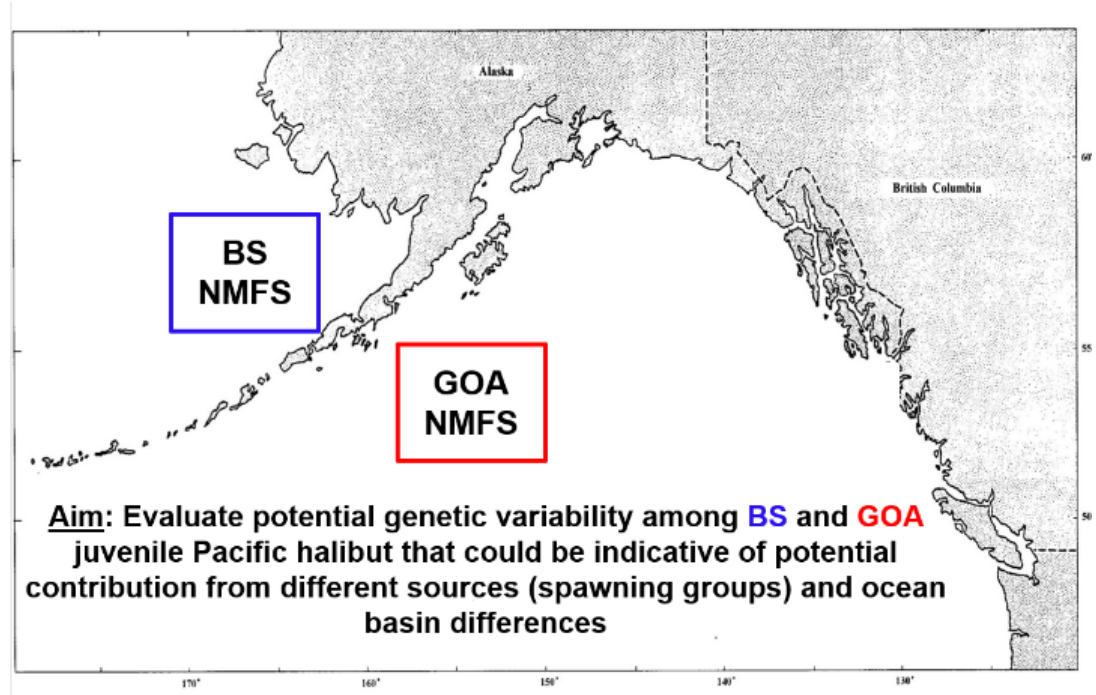
information from the charter fleet on types of gear and fish handling practices used is currently being collected through stakeholder meetings and on dock interviews with charter captains and operators. This information will inform the design of the experimental test fishing that will take place in 2020 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.

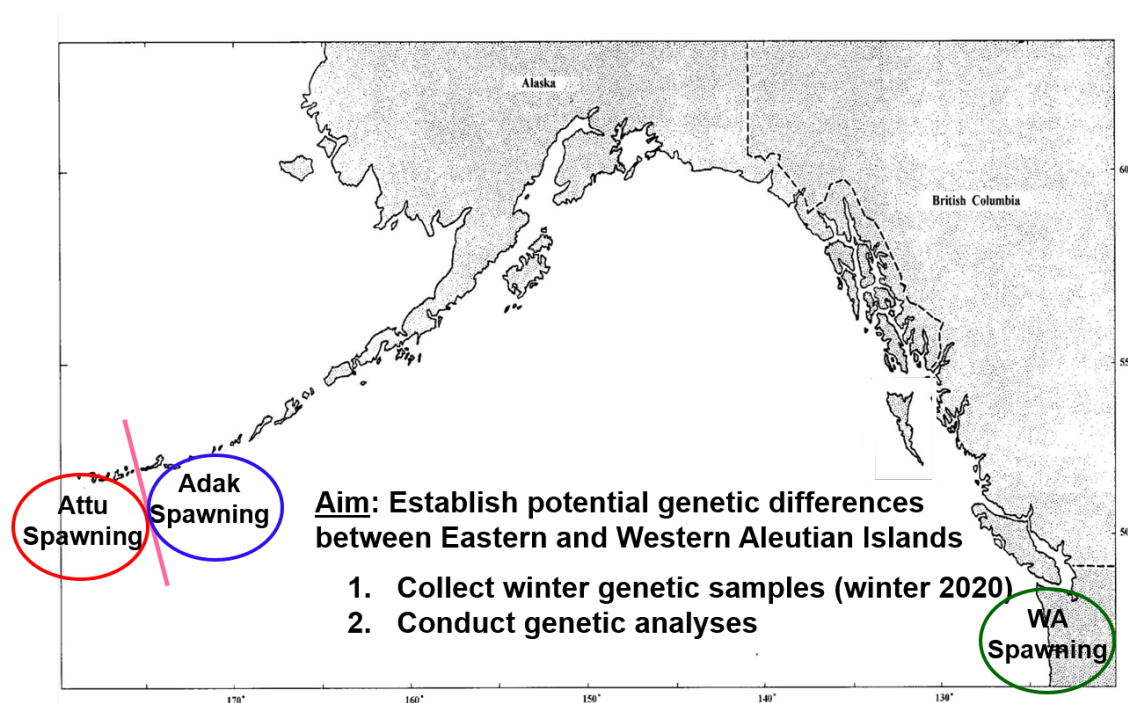


5.1.2. Analysis of genetic population structure in IPHC Regulatory Area 4B.

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 summer-collected (i.e. non-spawning season) samples 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 region. 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

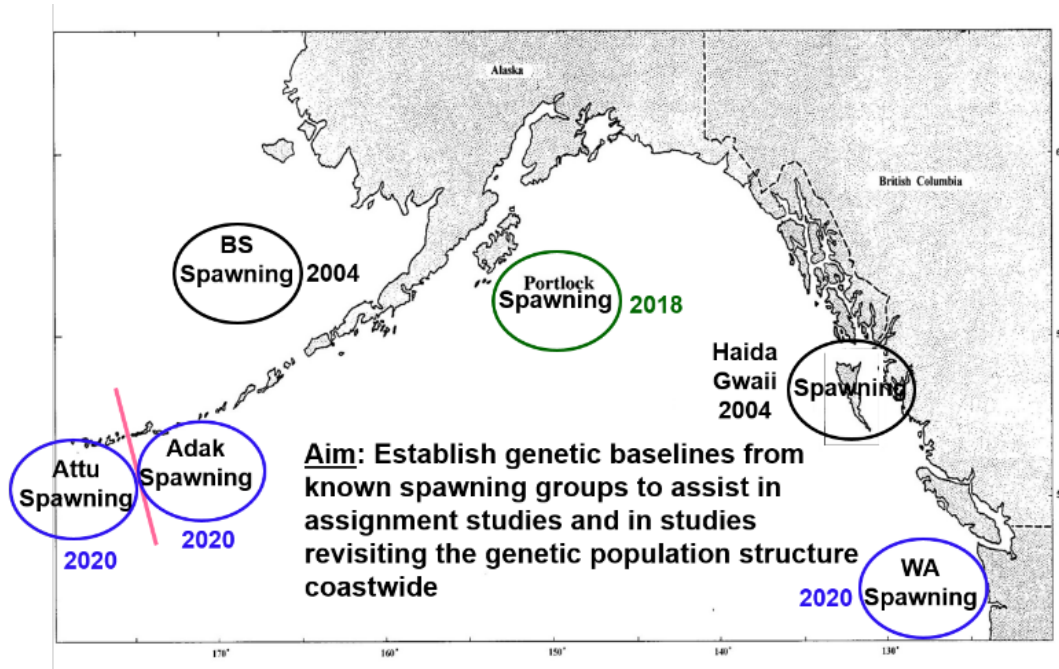
number of northern fish populations it has been shown that fish at the southern edge of the range display unique genetic composition.

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. In addition to the genetic samples that would be collected in 2) (i.e. eastern and western Aleutian Islands), we propose to collect additional winter samples from spawning fish off the Washington coast representing the southernmost spawning groups on the northeast Pacific Ocean. With these genetic samples, 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.



5.2. Genomics. The IPHC Secretariat is currently conducting a project aimed at generating a first draft sequence of the Pacific halibut genome. This study is being conducted in collaboration with the National Institute of Agrogenomic Research (INRA, Rennes, France) and the University of Washington. 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 end of summer 2019. Plans to establish a collaboration with Canadian scientists to establish a genomic comparison between Pacific and Atlantic halibut genomes are being discussed, including the possibility of a joint publication highlighting the comparative genomics approach. 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.



APPENDIX I

Integration of biological research, stock assessment and harvest strategy policy



Biological research

Stock assessment

Stock assessment MSE

Research areas	Research outcomes	Relevance for stock assessment	Inputs to stock assessment and MSE development
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
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
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. NA17NMF4270240)	IPHC	Alaska Pacific University	\$286,121	Bycatch estimates	September 2017 – August 2019 (no cost extension requested)
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	TBD	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	TBD	Bycatch reduction	September 2018 – August 2019
5	National Fish & Wildlife Foundation	Improving the characterization of discard mortality of Pacific halibut in the recreational fisheries	IPHC	Alaska Pacific University, U of A Fairbanks, charter industry	\$98,902	Bycatch estimates	January 2019 – December 2019
Total awarded (\$)					\$516,914		