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## Report on Current and Future Biological and Ecosystem Science Research Activities

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### PURPOSE

To provide the Scientific Review Board with a description of progress towards research activities described in the IPHC's five-year Program of Integrated Research and Monitoring (2022-2026).

### BACKGROUND

The primary biological and ecological research activities at IPHC that follow Commission objectives are identified and described in the Program of Integrated Research and Monitoring (2022-2026). These activities are integrated with stock assessment and the management strategy evaluation processes ([Appendix I](#)) and are summarized in five main areas, 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.

A ranked list of biological uncertainties and parameters for stock assessment ([Appendix II](#)) and the management strategy evaluation process ([Appendix III](#)) and their links to research activities and outcomes derived from the five-year research plan are provided.

### SRB REQUESTS

The SRB issued the following requests in their report of SRB020 ([IPHC-2022-SRB020-R](#)):

*SRB020–Req.07 ([para. 29](#)) The SRB **NOTED** continued progress toward integration of biological and ecosystem sciences activities with the needs of Stock Assessment (SA) and MSE programs, and **REQUESTED** that future presentations/documents identify (a) the planned statistical analysis of biological data and (b) parameters or structural decisions within SA and MSE to be informed by the results.*

*SRB020–Req.08 ([para. 30](#)) The SRB **NOTED** progress on further developing genomic resources through low-coverage whole genome sequencing and, therefore, **REQUESTED** that the Secretariat provide a detailed plan for bioinformatic interrogation and how data will be used to address IPHC questions related to stock assessment.*

## UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

### 1. Migration and Distribution.

Research activities in this Research Area aim at improving existing knowledge on Pacific halibut larval and juvenile distribution. The relevance of research outcomes from these activities for stock assessment (SA) is in the improvement of estimates of productivity. These research outcomes will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region and represent one of the top three biological inputs into SA (Appendix II). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in the improvement of the parametrization of the Operating Model and represent the top ranked biological input into the MSE (Appendix III).

- 1.1. Estimation of Pacific halibut juvenile habitat. The IPHC Secretariat recently conducted a study to investigate the connectivity between spawning grounds and possible settlement areas based on a biophysical larval transport model (please see paper in the journal *Fisheries Oceanography*: <https://doi.org/10.1111/fog.12512>). Although it is known that Pacific halibut initiate their demersal stage as roughly 6-month-old juveniles following the pelagic larval phase and settle in shallow nursery (settlement) areas, near or outside the mouths of bays (please see paper in *Reviews in Fish Biology and Fisheries*: <https://doi.org/10.1007/s11160-021-09672-w>), very little information is available on the geographic location and physical characteristics of these areas. In order to fill this knowledge gap, the IPHC Secretariat has initiated studies to identify potential settlement areas for juvenile Pacific halibut throughout IPHC Convention Waters. A first objective of this study is to create a map of suitable settlement habitat by combining available bathymetry information (e.g. benthic sediment composition and shoreline morphological data) and information on recorded presence of age-0, age-1 and age-2 Pacific halibut juveniles as well as absence of young Pacific halibut noted by various nursery habitat projects focused on other flatfish species. Data sources are currently being collected.
- 1.2. Wire tagging of U32 Pacific halibut. The patterns of movement of Pacific halibut among IPHC Regulatory Areas have important implications for management of the Pacific halibut fishery. The IPHC Secretariat has undertaken a long-term study of the migratory behavior of Pacific halibut through the use of externally visible tags (wire tags) on captured and released fish that must be retrieved and returned by workers in the fishing industry. In 2015, with the goal of gaining additional insight into movement and growth of young Pacific halibut (less than 32 inches [82 cm]; U32), the IPHC began wire-tagging small Pacific halibut encountered on the National Marine Fisheries Service (NMFS) groundfish trawl survey and, beginning in 2016, on the IPHC fishery-independent setline survey (FISS). As of 28 July 2022, 1,330 Pacific halibut have been tagged and released on the 2022 IPHC FISS but no tagging was conducted in the NMFS groundfish trawl surveys in 2022. Therefore, a total of 7,441 U32 Pacific halibut have been wire tagged and released on the IPHC FISS and 135 of those have been recovered to date. In the NMFS groundfish trawl surveys through 2019, a total of 6,421 tags have been released and, to date, 78 tags have been recovered.

## 2. Reproduction.

Research activities in this Research Area aim at providing information on key biological processes related to reproduction in Pacific halibut (maturity and fecundity) and to provide sex ratio information of Pacific halibut commercial landings. The relevance of research outcomes from these activities for stock assessment (SA) is in the scaling of Pacific halibut biomass and in the estimation of reference points and fishing intensity. These research outputs will result in a revision of current maturity schedules and will be included as inputs into the SA (Appendix II), and represent the most important biological inputs for stock assessment (please see document IPHC-2021-SRB018-06). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in the improvement of the simulation of spawning biomass in the Operating Model (Appendix III).

### 2.1. Sex ratio of the commercial landings.

The IPHC Secretariat finalized the processing of genetic samples from the 2021 aged commercial landings, completing five consecutive years of sex ratio information (2017-2020).

### 2.2. Maturity assessment.

Recent sensitivity analyses have shown the importance of changes in spawning output due to skip spawning and/or changes in maturity schedules for stock assessment (Stewart and Hicks, 2018). Information of these key reproductive parameters provides direct input to stock assessment. For example, information on fecundity-at-age and –at-size could be used to replace spawning biomass with egg output as the metric of reproductive capability in the stock assessment and management reference points. This information highlights the need for a better understanding of factors influencing reproductive biology and success of Pacific halibut. In order to fill existing knowledge gaps related to the reproductive biology of female Pacific halibut, research efforts are devoted to characterize female maturity in this species. Specific objectives of current studies include: 1) update of maturity schedules based on histological-based data; and, 2) fecundity determinations.

2.2.1. Update of maturity schedules based on histological-based data. The IPHC Secretariat is undertaking studies to revise maturity schedules in all four biological regions through histological (i.e. microscopic) characterization of maturity, as reported previously. The maturity schedule that is currently used in stock assessment was based on visual (i.e. macroscopic) maturity classification in the field (FISS). In order to be able to accomplish this objective, the IPHC Secretariat is currently collecting ovarian samples for histology in the 2022 FISS. The sample targets are to collect 400 ovarian samples from Biological Region 3, 300 from each Biological Regions 2 and 4, and 250 samples from Biological Region 4B. Ovarian samples will be processed for histology in the Fall of 2022 and, subsequently, histological maturity classifications will be conducted by IPHC

Secretariat staff. Females classified as developing, regressing and spawning capable, according to the classification of reproductive phases defined histologically in our recent publication in *Frontiers in Marine Science*: <https://doi.org/10.3389/fmars.2022.801759>, will be considered mature. Following this maturity classification criteria, all sampled Pacific halibut females will be assigned to either the mature or immature categories.

The proportion of Pacific halibut females that are mature at a given length or age will be evaluated through the generation of maturity ogives. Maturity ogives will be represented using a logistic curve to which the maturity data (each female will be assigned as mature or immature according to histological classification) will be fit applying a generalized linear model with a binomial data distribution and a logit link function, as described by Dominguez-Petit et al. (2017) and with publicly available R code. The length and age at 50% maturity will be calculated from fitted models using the `dose.p` function and the proportion of mature individuals ( $p$ ) set to 0.5.

- 2.2.2. Fecundity estimations. Methods for fecundity determinations were investigated and, based on the current literature and recommendations from experts in the field, the auto-diametric method was selected as the method of choice (Witthames et al., 2009). The IPHC Secretariat is currently designing plans for ovarian sample collection for fecundity estimations as part of the 2023 FISS. No further updates to report.

### 3. Growth.

Research activities conducted in this Research Area aim at providing information on somatic growth processes driving size-at-age in Pacific halibut. The relevance of research outcomes from these activities for stock assessment (SA) resides, first, in their ability to inform yield-per-recruit and other spatial evaluations for productivity that support mortality limit-setting, and, second, in that they may provide covariates for projecting short-term size-at-age and may help delineate between fishery and environmental effects, thereby informing appropriate management responses (Appendix II). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in the improvement of the simulation of variability and to allow for scenarios investigating climate change (Appendix III).

The IPHC Secretariat has conducted studies aimed at elucidating the drivers of somatic growth leading to the decline in SAA by investigating the physiological mechanisms that contribute to growth changes in the Pacific halibut. The two main objectives of these studies have been: 1) the identification and validation of physiological markers for somatic growth; and 2) the application of molecular growth markers for evaluating growth patterns in the Pacific halibut population.

No updates to report.

#### 4. Discard Mortality Rates (DMRs) and Survival Assessment.

Information on all Pacific halibut removals is integrated by the IPHC Secretariat, providing annual estimates of total mortality from all sources for its stock assessment. Bycatch and wastage of Pacific halibut, as defined by the incidental catch of fish in non-target fisheries and by the mortality that occurs in the directed fishery (i.e. fish discarded for sublegal size or regulatory reasons), respectively, represent important sources of mortality that can result in significant reductions in exploitable yield in the directed fishery. Given that the incidental mortality from the commercial Pacific halibut fisheries and bycatch fisheries is included as part of the total removals that are accounted for in stock assessment, changes in the estimates of incidental mortality will influence the output of the stock assessment and, consequently, the catch levels of the directed fishery. Research activities conducted in this Research Area aim at providing information on discard mortality rates and producing guidelines for reducing discard mortality in Pacific halibut in the longline and recreational fisheries. The relevance of research outcomes from these activities for stock assessment (SA) resides in their ability to improve trends in unobserved mortality in order to improve estimates of stock productivity and represent the most important inputs in fishery yield for stock assessment (Appendix II). The relevance of these research outcomes for the management and strategy evaluation (MSE) process is in fishery parametrization (Appendix III).

For this reason, the IPHC Secretariat is conducting two research projects to investigate the effects of capture and release on survival and to improve estimates of DMRs in the directed longline and guided recreational Pacific halibut fisheries:

##### 4.1. Evaluation of the effects of hook release techniques on injury levels and association with the physiological condition of captured Pacific halibut and estimation of discard mortality using remote-sensing techniques in the directed longline fishery.

The results of the study reporting discard mortality rate estimations in the directed longline fishery have been published in the journal *North American Journal of Fisheries Management*: <https://doi.org/10.1002/nafm.10711>. The results of the second component of this study, namely the description of relationships among hook release techniques, injury levels, stress levels and physiological condition of released fish, are presently being analyzed using random forest analyses (Breiman 2001) in which viability category is used as the response variable, and hook release method, physiological characteristics, and physical and environmental conditions are used as predictor variables (classification, 500 trees, 2 variables per split). Multinomial logistic regression (Tabachnick and Fidell 2001) modeling is performed on the five most common injuries seen by release method in relation to fish weight, a variable from the random forest analyses shown to have some predictive value on injury type. The multinomial regression is being conducted in the following manner: (InjuryType ~ RoundWeight) wherein the levels of InjuryType were c(torn cheek, torn jaw, cheek and jaw, eye, torn face). Owing to non-normal distributions, relationships among injury types, physiological characteristics, and environmental conditions are examined using Kruskal-Wallis rank sum tests followed by Dunn's pairwise comparison tests. Specific

relationships between all variables are examined using Pearson's correlation coefficients. All statistical analyses and graphic outputs are performed in R version 3.6.2 (R Core Team 2019).

#### 4.2. Estimation of discard mortality rates in the charter recreational sector.

To date, of the 281 fish that were tagged with opercular wire tags (243 fish in IPHC Regulatory Area 2C and 38 in IPHC Regulatory Area 3A) 28 tags have been recovered (19 from IPHC Regulatory Area 2C and 9 from IPHC Regulatory Area 3A).

Seventy-six (76) of the 80 electronic accelerometer-based survivorship pop-up archival transmitting (sPAT tags) provided useable data reports. Survival analysis (R package = "survival") produces a mortality rate estimate of 2.04% with a 95% CI of 0.0-5.92%. These are the first field-corroborated estimates of recreational discard mortality and affirm the use of current methodologies embedded in recreational mortality estimates that feed into the SA and MSE process. Further analyses are being conducted on diurnal activity patterns overall, as well as in the periods shortly after capture and release, versus the periods shortly before tag detachment in order to determine if there are any typical patterns in activity rates as fish recover from the capture event.

Furthermore, the plasma levels of physiological stress indicators (i.e. cortisol, glucose and lactate) in captured and discarded Pacific halibut are currently being analyzed in order to relate stress levels with capture and handling conditions.

5. Genetics and genomics. The IPHC Secretariat is conducting studies that incorporate genomics approaches in order to produce useful information on population structure and distribution and connectivity of Pacific halibut. The relevance of research outcomes from these activities for stock assessment (SA) resides (1) in the introduction of possible changes in the structure of future stock assessments, as separate assessments may be constructed if functionally isolated components of the population are found (e.g. IPHC Regulatory Area 4B), and (2) in the improvement of productivity estimates, as this information may be used to define management targets for minimum spawning biomass by Biological Region. These research outcomes provide the second and third top ranked biological inputs into SA (Appendix II). Furthermore, the relevance of these research outcomes for the management and strategy evaluation (MSE) process is in biological parametrization and validation of movement estimates, on one hand, and of recruitment distribution, on the other hand (Appendix III).

##### 5.1. Population genomics.

The primary objective of the studies that the IPHC Secretariat is currently conducting is to investigate the genetic structure of the Pacific halibut population and to conduct genetic analyses to inform on Pacific halibut movement and distribution within the Convention Area.

- 5.1.1. Pacific halibut genome and characterization of the sex determining region in Pacific halibut. The IPHC Secretariat has updated the Pacific halibut genome assembly. The updated Pacific halibut genome has an estimated size of 602 Mb, 24 chromosome-length scaffolds that contain 99.8% of the assembly and a N<sub>50</sub> scaffold length of 27.3 Mb. The Pacific halibut whole genome sequencing data are openly available in NCBI at <https://www.ncbi.nlm.nih.gov/bioproject/622249>, under BioProject PRJNA622249, and the updated assembly is openly available in NCBI at [https://www.ncbi.nlm.nih.gov/assembly/GCA\\_022539355.2/](https://www.ncbi.nlm.nih.gov/assembly/GCA_022539355.2/) with GenBank assembly accession number GCA\_022539355.2. The master record for the whole genome shotgun sequencing project has been deposited at DDBJ/ENA/GenBank under the accession JAKRZP000000000 and is openly available in NCBI at <https://www.ncbi.nlm.nih.gov/nucleotide/JAKRZP000000000>. Sample metadata is openly available in NCBI at [https://www.ncbi.nlm.nih.gov/biosample?Db=biosample&DbFrom=bioproject&Cmd=Link&LinkName=bioproject\\_biosample&LinkReadableName=BioSample&ordinalpos=1&IdsFromResult=622249](https://www.ncbi.nlm.nih.gov/biosample?Db=biosample&DbFrom=bioproject&Cmd=Link&LinkName=bioproject_biosample&LinkReadableName=BioSample&ordinalpos=1&IdsFromResult=622249), under BioSamples SAMN14503176, SAMN25516224, SAMN25600010 and SAMN25600011. A detailed description of the genome of Pacific halibut and its sex-determining region has been published in the journal *Molecular Ecology Resources*: <https://doi.org/10.1111/1755-0998.13641>. No further updates to report.

Genomic sequencing	Sequencing Run # 1	Sequencing Run # 2	Sequencing Run # 3
Number of samples	249	249	102
Sequencing Platform	Illumina NovaSeq S4	Illumina NovaSeq S4	Illumina NovaSeq S4
Raw Reads Per Sample (Millions)	24.7 (10.7-47.2)	24.9 (13.0-51.6)	25.8 (10.9-85.8)
Reads Retained (%)	62 (22-69)	61 (46-70)	In progress
Coverage Per Sample (x)	3.0 (0.9-5.0)	3.0 (1.3-5.9)	In progress

**Table 1.** Summary of raw sequence data and genome alignments for three Pacific halibut lcWGR sequencing runs. \*numbers in parenthesis indicate number of samples with > 1,000,000 raw sequence reads. \*\*expressed as mean (min – max).

- 5.1.2. Studies to resolve the genetic structure of the Pacific halibut population in the Convention Area. This project has recently received funding from the North Pacific Research Board (NPRB Project No. 2110; Appendix IV; project narrative provided in the supplementary documentation). Details on sample collection, bioinformatic processing and proposed analyses utilizing low-coverage whole

genome sequencing (IcWGR) to investigate Pacific halibut population structure were provided in document [IPHC-2021-SRB018-08](#). Further details on bioinformatic processing are provided below, including a summary flow chart in Appendix V. All libraries have now been constructed, quantified, pooled, and sequenced on an Illumina NovaSeq 6000 platform using an S4 flow cell (2x150 bp paired end reads) on three separate lanes. Preliminary results show that the sequencing yield per sample was 25.1 million reads in average (range = 10.7 – 85.8 million reads), with 61% retained reads (in average) and an average coverage per sample of 3x (Table 1).

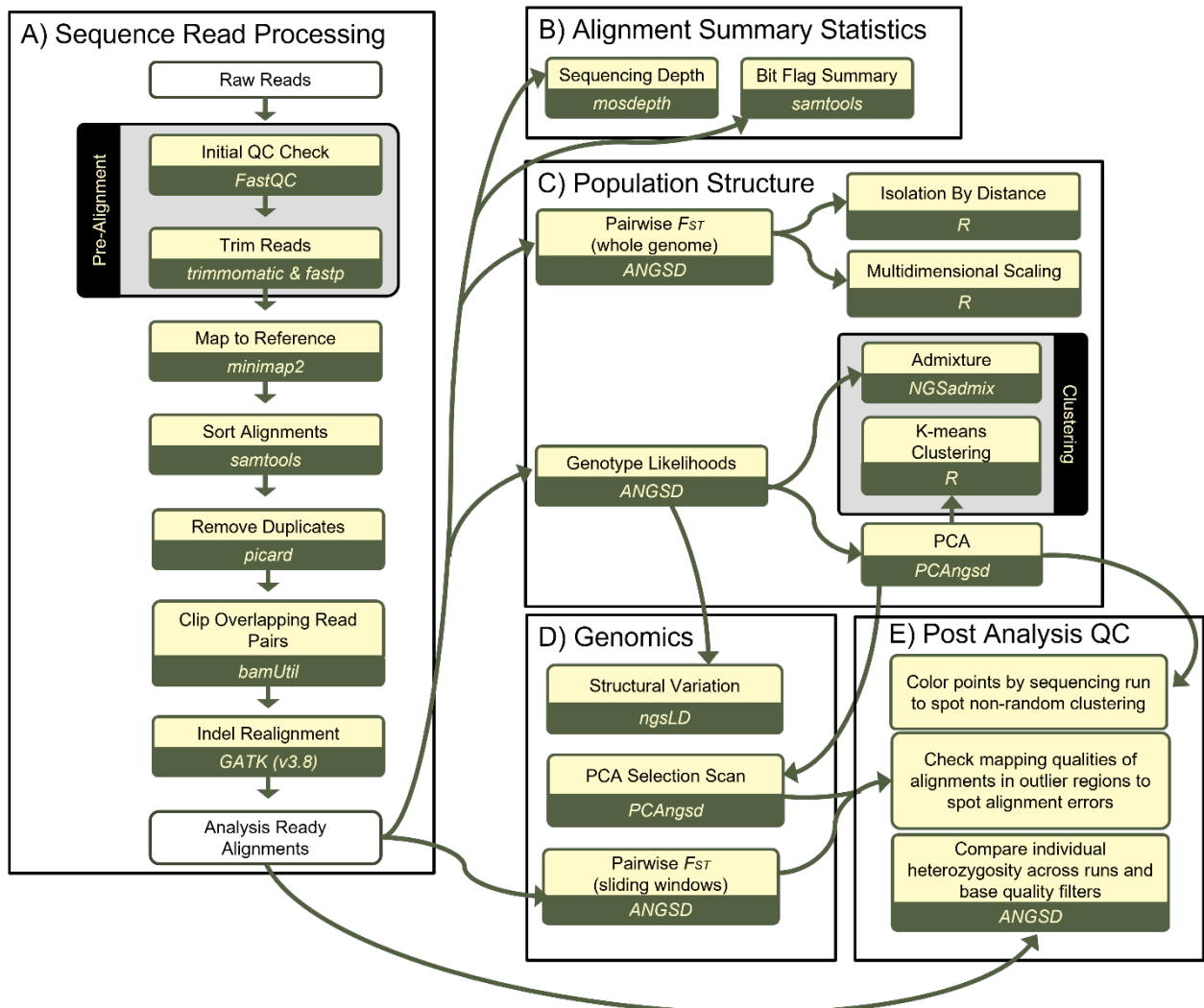
5.1.2.1. Initial QC. FastQC (Andrews, Krueger, Secks-Pichon, Biggins, & Wingett, 2015) will be used to perform an initial quality check of raw sequence reads (Figure 1A). This is to ensure consistent quality across sequencing runs and identify samples that may not be suitable for further analysis. Specifically, the raw base quality scores for each sample will be used to identify samples that were poorly sequenced and should be omitted from downstream analyses. Additionally, the presence of other sequencing artifacts may be detected at this step as well. Per base sequence content will be used to identify the presence of poly-G tails that are common when using the NovaSeq platform (Lou & Therikildsen, 2021).

5.1.2.2. Bioinformatic Processing and Read Alignment. The raw sequence reads will then be processed to remove Illumina adapter sequences and poly-G tails using Trimmomatic (Bolger, Lohse, & Usadel, 2014) and fastp (Chen, Zhou, Chen, & Gu, 2018) (Figure 1A). Adapter sequences will be removed using the following parameters; maximum of 2 mismatches allowed, palindrome clip threshold of 30, simple clip threshold of 10, minimum adapter length of 1, retaining both reads after palindromic trimming is done. In addition to poly-G trimming implemented in fastp, sliding window trimming will also be used to trim the ends of sequence reads read if the average base quality score drops below 15 in a window of 4 bases. Lou and Therikildsen (2021) have demonstrated this to be an effective means of poly-G tail removal.

Trimmed sequence reads will be aligned to the Pacific halibut reference genome (RefSeq assembly accession: [GCF\\_022539355.2](#)) using the short read preset option in minimap2 (Li, 2018) (Figure 1A). The resulting sequence alignment map (SAM) files will be coordinate sorted and converted to the binary alignment map format (BAM) using samtools (Li et al., 2009). The MarkDuplicates module in Picard (<http://broadinstitute.github.io/picard/>) will be used to remove PCR and optical duplicate reads (Figure 1A). Overlapping read pairs will be clipped to reduce redundancy using the clipOverlap tool in BamUtil (Jun, Wing, Abecasis, & Kang, 2015) (Figure 1A). Finally, local realignment around insertion/deletions (indels) will then be performed using GTAK (v3.8) (Van der Auwera & O'Connor, 2020) to produce analysis ready alignments (Figure 1A). Metrics (Figure 1B) for the final sequence alignments will be obtained using samtools to summarize the bit values set in the FLAG field of each BAM file for each sample and mosdepth



(Pedersen & Quinlan, 2018) to calculate the average sequencing depth per sample.



**Figure 1.** Proposed bioinformatic workflow for the interrogation of low-coverage whole genome sequence data. This diagram tracks the flow of data through the main stages of this project, (A) raw sequence read processing, (B) alignment summaries, (C) analysis of population structure, (D) genomic analyses, and (E) quality control steps to be taken.

5.1.2.3. Analysis. Genotype likelihoods will be estimated from the low-coverage data (Figure 1C) using GATK model implemented in ANGSD (Korneliussen, Albrechtsen, & Nielsen, 2014). This model assumes that sequencing errors are independent at a given site and the base quality scores accurately reflect the probability of sequencing error. This is in contrast to the other models implemented in ANGSD which may fail to correctly identify low frequency mutations and classify them as sequencing errors instead (Lou &

Therkildsen, 2021). Sites will be retained that have a minimum minor allele frequency of 0.01, have a high confidence of being variable ( $p \geq 1e-6$ ), covered by at least one read in 75% ( $\geq 450$ ) of individuals. A maximum depth threshold of 3,600 will also be applied to reduce calling SNPs from reads that may have mapped to poorly assembled repetitive regions in the genome. Following Clucas, Lou, Therkildsen, and Kovach (2019), this threshold was chosen as twice the average sequencing depth of 3x multiplied by the number of samples.

5.1.2.4. Population Genetics & Structure. To quantify the level of differentiation among these sample collections, pairwise  $F_{ST}$  will be estimated using two-dimensional site frequency spectra (SFS) for population pairs (Figure 1C). The site frequency spectra will be calculated for all sites in ANGSD using the GATK model for genotype likelihood estimation and supplying the Pacific halibut reference genome as ancestral. The realSFS tool included with ANGSD will then be used to perform the calculation of  $F_{ST}$ . We propose to compare estimates among all sample areas (all collection years combined), areas within sampling years, and examine genetic change over time within specific areas by examining comparisons across collection years. Multidimensional scaling will be used to visualize these comparisons (Figure 1C). To examine patterns of isolation by distance (Figure 1C), a Mantel test will be used to test for a correlation between genetic and geographic distance.

Individual based methods that do not rely on *a priori* population groupings will also be used to investigate population structure. PCAngsd (Meisner & Albrechtsen, 2018) will be used to conduct principal component analysis (PCA), sites with a minor allele frequency  $\geq 0.05$  will be removed prior to conducting PCA (Figure 1C). The resulting principal component scores will be used as input for unsupervised clustering methods (e.g. k-means clustering) to identify groupings in the data (Figure 1C). Additionally, NGSadmix (Skotte, Korneliusen, & Albrechtsen, 2013) will be used to estimate individual ancestry coefficients and identify genetically homogeneous groups within the data (Figure 1C).

5.1.2.5. Genomics. Genome scans will also be conducted to identify regions of the genome that may be under selection. Pairwise  $F_{ST}$  will be calculated in a sliding window fashion across the genome from the two-dimensional SFS previously. We propose to use the realSFS utility to report  $F_{ST}$  values in overlapping 15 Kb windows with a 7.5 Kb step (Figure 1D). PCAngsd also implements a PCA based selection scan (Meisner, Albrechtsen, & Hanghj, 2021) and we propose to use the FastPCA model to complement the  $F_{ST}$  based selection scans (Figure 1D). Additionally, we will estimate intrachromosomal pairwise linkage disequilibrium (LD) for each sample collection using ngsLD (Fox, Wright, Fumagalli, & Vieira, 2019) (Figure 1D). This may point to stock specific structural variation (e.g. inversions) present in the genome that may be useful in stock delineation.

5.1.2.6. Post Analysis QC. We also intend to conduct additionally quality checks recommended by Lou and Therkildsen (2021) to ensure integrity of the data following the alignment of the raw reads to the genome and the proposed analyses (Figure 1E). To ensure that base quality scores are calibrated correctly among the sequencing runs, estimates of individual genome wide heterozygosity will be compared using relaxed (Q20) and stringent (Q33) base quality filtering thresholds. The outcome of this analysis will also help determine an appropriate base quality threshold to use for genotype likelihood estimation. This will be performed on a subset of samples within each run (e.g. 50) to save on computational resources. To determine whether differences in data quality among the sequencing runs represent a major source of variation in the data, individual points in the PCA plot will be colored by sequencing run and a visual inspection will be made. To ensure that any outlier regions identified are not an artifact of alignment errors, we will check the mapping qualities of reads in these regions. If a large number of low quality reads are mapping to these regions, alignment artifacts may be likely. Lou and Therkildsen (2021) offer a comprehensive set of suggestions for the mitigation of various sources of technical bias in low-coverage whole genome resequencing datasets and other suggestions will be implemented as needed.

5.1.2.7. Application to SA & MSE. Results from previous genetic studies have suggested that fish in the western Aleutian Islands may be genetically distinct from the rest of the stock (Drinan, et al, 2016). A distinct genetic stock in this region would have implications for the stock assessment and management of Pacific halibut in this area. An accurate understanding of stock structure is necessary for effective fisheries management and stock assessment, therefore, the analysis of population structure outlined here is intended to provide a tool that will advance our current understanding of Pacific halibut population structure using modern, high resolution genomic technology. Additionally, the IPHC Secretariat plans to leverage this genomic resource to explore the development of tools to address specific questions regarding stock specific harvest and movement rates among fisheries and regulatory areas, both of which are relevant to stock assessment and MSE efforts. In addition to the management implications of this work highlighted in the heading of section 5, spatial dynamics represent a major source of uncertainty in the Pacific halibut assessment and are, therefore, a research area of high priority.

6. Whale depredation avoidance strategies. The IPHC Secretariat has determined that research to provide the Pacific halibut fishery with tools to reduce whale depredation is considered a high priority. This research is now contemplated as one of the research areas of high priority within the 5-year Program of Integrated Research and Monitoring (2022-2026). Towards this goal, the IPHC secretariat has recently obtained funding from NOAA's Bycatch Research and Engineering Program (BREP) to investigate gear-based approaches to catch protection as a means for minimizing whale depredation in the Pacific halibut and other longline fisheries (NOAA Award NA21NMF4720534; Appendix IV). The objectives of this study are to: 1) work with fishermen and gear manufacturers, via direct communication and through an

international workshop, to identify effective methods for protecting hook-captured flatfish from depredation; and 2) develop and pilot test 2-3 simple, low-cost catch-protection designs that can be deployed effectively using current longline fishing techniques and on vessels currently operating in the Northeast Pacific Ocean.

The results and outcome of the first phase of this project were reported in the documentation to the previous SRB meeting: [IPHC-2022-SRB020-08](#).

During the second phase of the project, the IPHC Secretariat has worked with catch protection device manufacturers for the design of two different types of devices for field testing: one based on a modification of Sago's catch protection device (i.e. shuttle) and one based on a modification of a slinky pot. These two devices are currently being manufactured and will be tested on a chartered fishing vessel off a port in Alaska (to be determined) in the Spring of 2023.

## RECOMMENDATION/S

That the SRB:

- a) **NOTE** paper IPHC-2022-SRB021-09 which provides a response to requests from SRB020, and a report on current research activities contemplated within the IPHC's five-year Program of Integrated Research and Monitoring (2022-2026).

## REFERENCES

- Andrews, S., Krueger, F., Seconds-Pichon, A., Biggins, F., & Wingett, S. (2015). FastQC. A quality control tool for high throughput sequence data. *Babraham Bioinformatics*. *Babraham Institute*, 1(1), 1.
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), 2114--2120. doi:10.1093/bioinformatics/btu170
- Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34(17), i884--i890. doi:10.1093/bioinformatics/bty560
- Clucas, G. V., Lou, R. N., Therkildsen, N. O., & Kovach, A. I. (2019). Novel signals of adaptive genetic variation in northwestern Atlantic cod revealed by whole-genome sequencing. *Evolutionary Applications*, 12(10), 1971--1987. doi:10.1111/eva.12861
- Domínguez-Petit R., Anastasopoulou A., Cubillos L., Gerritsen H.D., Gonçalves P., Hidalgo M., Kennedy J., Korta M., Marteinsdottir G., Morgado C., Muñoz M., Quincoces I., Saínza M., Thorsen A., Vitale F. 2017. Chapter 3: Maturity. In Handbook of applied fisheries reproductive biology for stock assessment and management, ed. R. Domínguez-Petit, H. Murua, F. Saborido-Rey and E. Trippel. Vigo, Spain. Digital CSIC. <http://hdl.handle.net/10261/87787>.
- Drinan, D. P., Galindo, H. M., Loher, T., & Hauser, L. (2016). Subtle genetic population structure in Pacific halibut *Hippoglossus stenolepis*. *Journal of Fish Biology*, 89(6), 2571--2594. doi:10.1111/jfb.13148
- Fish, T., Wolf, N., Smeltz, T. S., Harris, B. P., and Planas, J. V. 2022. Reproductive Biology of Female Pacific Halibut (*Hippoglossus stenolepis*) in the Gulf of Alaska. *Frontiers in Marine Science*. 9:801759. DOI: <https://doi.org/10.3389/fmars.2022.801759>.

- Fox, E. A., Wright, A. E., Fumagalli, M., & Vieira, F. G. (2019). ngsLD: evaluating linkage disequilibrium using genotype likelihoods. *Bioinformatics*, 35(19), 3855--3856. doi:10.1093/bioinformatics/btz200
- Jasonowicz, A.C., Simeon, A., Zahm, M., Cabau, C., Klopp, C., Roques, C., Iampietro, C., Lluch, J., Donnadiu, C., Parrinello, H., Drinan, D.P., Hauser, L., Guiguen, Y., Planas, J.V. Generation of a chromosome-level genome assembly for Pacific halibut (*Hippoglossus stenolepis*) and characterization of its sex-determining genomic region. *Molecular Ecology Resources* (In press). DOI: <https://doi.org/10.1111/1755-0998.13641>.
- Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Research*, 25(6), 918--925. doi:10.1101/gr.176552.114
- Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics*, 15(1), 356. doi:10.1186/s12859-014-0356-4
- Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics*, 34(18), 3094--3100. doi:10.1093/bioinformatics/bty191
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., . . . Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16), 2078--2079. doi:10.1093/bioinformatics/btp352
- Loher, T., Dykstra, C.L., Hicks, A., Stewart, I.J., Wolf, N., Harris, B.P., Planas, J.V. 2022. Estimation of postrelease longline mortality in Pacific halibut using acceleration-logging tags. *North American Journal of Fisheries Management*. 42: 37-49. DOI: <https://doi.org/10.1002/nafm.10711>
- Lou, R. N., & Therkildsen, N. O. (2021). Batch effects in population genomic studies with low-coverage whole genome sequencing data: Causes, detection and mitigation. *Molecular Ecology Resources*(July), 1--15. doi:10.1111/1755-0998.13559
- Meisner, J., & Albrechtsen, A. (2018). Inferring Population Structure and Admixture Proportions in Low-Depth NGS Data. *Genetics*, 210(2), 719--731. doi:10.1534/genetics.118.301336
- Meisner, J., Albrechtsen, A., & Hanghj, K. (2021). Detecting Selection in Low-Coverage High-Throughput Sequencing Data using Principal Component Analysis. *bioRxiv*, 2021.2003.2001.432540. doi:10.1101/2021.03.01.432540
- Pedersen, B. S., & Quinlan, A. R. (2018). Mosdepth: quick coverage calculation for genomes and exomes. *Bioinformatics*, 34(5), 867--868. doi:10.1093/bioinformatics/btx699
- Skotte, L., Korneliussen, T. S., & Albrechtsen, A. (2013). Estimating Individual Admixture Proportions from Next Generation Sequencing Data. *Genetics*, 195(3), 693--702. doi:10.1534/genetics.113.154138
- Stewart, I., and Hicks, A. (2018). Assessment of the Pacific halibut (*Hippoglossus stenolepis*) stock at the end of 2017. Int. Pac. Halibut Comm. Annual Meeting Report: [IPHC-2018-AM094-10](#).
- Stewart, I., and Webster, R. (2021). Overview of data sources for the Pacific halibut stock assessment, harvest policy, and related analyses. Int. Pac. Halibut Comm. Stock Assessment Document: [IPHC-2021-SA-02](#).
- Van der Auwera, G. A., & O'Connor, B. D. (2020). *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra (1st Edition)*. (1 ed.): O'Reilly Media.
- Witthames, P.R., Greenwood, L.N., Thorsen, A., Dominguez, R., Murua, H., Korta, M., Saborido-Rey, F., Kjesbu, O.S., 2009. Advances in methods for determining fecundity: application of the new methods to some marine fishes. *Fishery Bulletin* 107, 148--164.



**APPENDIX I**

**Integration of biological research, stock assessment and harvest strategy policy (2017-21)**



**Biological research**

**Stock assessment**

**Stock assessment MSE**

Research areas	Research outcomes	Relevance for stock assessment	Inputs to stock assessment and MSE development
<b>Reproduction</b>	Sex ratio Spawning output Age at maturity	Spawning biomass scale and trend Stock productivity Recruitment variability	Sex ratio Maturity schedule Fecundity
<b>Growth</b>	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
<b>Discard Survival</b>	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
<b>Migration</b>	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
<b>Genetics and Genomics</b>	Genetic structure of the population Sequencing of the Pacific halibut genome	Spatial dynamics Management units	Information for structural choices



**APPENDIX II**

**List of ranked biological uncertainties and parameters for stock assessment (SA) and their links to potential research areas and research activities (2017-21)**

SA Rank	Research outcomes	Relevance for stock assessment	Specific analysis input	Research Area	Research activities
1. Biological input	Updated maturity schedule	Scale biomass and reference point estimates	Will be included in the stock assessment, replacing the current schedule last updated in 2006	Reproduction	Historical maturity assessment
	Incidence of skip spawning		Will be used to adjust the asymptote of the maturity schedule, if/when a time-series is available this will be used as a direct input to the stock assessment		Examination of potential skip spawning
	Fecundity-at-age and -size information		Will be used to move from spawning biomass to egg-output as the metric of reproductive capability in the stock assessment and management reference points		Fecundity assessment
	Revised field maturity classification		Revised time-series of historical (and future) maturity for input to the stock assessment		Examination of accuracy of current field macroscopic maturity classification
2. Biological input	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area	Altered structure of future stock assessments	If 4B is found to be functionally isolated, a separate assessment may be constructed for that IPHC Regulatory Area	Genetics and Genomics	Population structure
3. Biological input	Assignment of individuals to source populations and assessment of distribution changes	Improve estimates of productivity	Will be used to define management targets for minimum spawning biomass by Biological Region	Migration	Distribution
	Improved understanding of larval and juvenile distribution		Will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region		Larval and juvenile connectivity studies
1. Assessment data collection and processing	Sex ratio-at-age	Scale biomass and fishing intensity	Annual sex-ratio at age for the commercial fishery fit by the stock assessment	Reproduction	Sex ratio of current commercial landings
	Historical sex ratio-at-age		Annual sex-ratio at age for the commercial fishery fit by the stock assessment		Historical sex ratios based on archived otolith DNA analyses
2. Assessment data collection and processing	New tools for fishery avoidance/deterrence; improved estimation of depredation mortality	Improve mortality accounting	May reduce depredation mortality, thereby increasing available yield for directed fisheries. May also be included as another explicit source of mortality in the stock assessment and mortality limit setting process depending on the estimated magnitude	Mortality and survival assessment	Whale depredation accounting and tools for avoidance
1. Fishery yield	Physiological and behavioral responses to fishing gear	Reduce incidental mortality	May increase yield available to directed fisheries	Mortality and survival assessment	Biological interactions with fishing gear
2. Fishery yield	Guidelines for reducing discard mortality	Improve estimates of unobserved mortality	May reduce discard mortality, thereby increasing available yield for directed fisheries	Mortality and survival assessment	Best handling practices: recreational fishery

### APPENDIX III

## List of ranked biological uncertainties and parameters for management strategy evaluation (MSE) and their potential links to research areas and research activities (2017-21)

MSE Rank	Research outcomes	Relevance for MSE	Research Area	Research activities
1. Biological parameterization and validation of movement estimates	Improved understanding of larval and juvenile distribution	Improve parameterization of the Operating Model	Migration	Larval and juvenile connectivity studies
	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area			Population structure
2. Biological parameterization and validation of recruitment variability and distribution	Assignment of individuals to source populations and assessment of distribution changes	Improve simulation of recruitment variability and parameterization of recruitment distribution in the Operating Model	Genetics and Genomics	Distribution
	Establishment of temporal and spatial maturity and spawning patterns	Improve simulation of recruitment variability and parameterization of recruitment distribution in the Operating Model	Reproduction	Recruitment strength and variability
3. Biological parameterization and validation for growth projections	Identification and application of markers for growth pattern evaluation	Improve simulation of variability and allow for scenarios investigating climate change	Growth	Evaluation of somatic growth variation as a driver for changes in size-at-age
	Environmental influences on growth patterns			
	Dietary influences on growth patterns and physiological condition			
1. Fishery parameterization	Experimentally-derived DMRs	Improve estimates of stock productivity	Mortality and survival assessment	Discard mortality rate estimate: recreational fishery





**APPENDIX IV**  
**Summary of active research grants**

<b>Project #</b>	<b>Grant agency</b>	<b>Project name</b>	<b>PI</b>	<b>Partners</b>	<b>IPHC Budget (\$US)</b>	<b>Management implications</b>	<b>Grant period</b>
1	<b>Bycatch Reduction Engineering Program - NOAA</b>	Gear-based approaches to catch protection as a means for minimizing whale depredation in longline fisheries (NA21NMF4720534)	IPHC	Deep Sea Fishermen's Union, Alaska Fisheries Science Center-NOAA, industry representatives	\$99,700	Mortality estimations due to whale depredation	November 2021 – October 2022
2	<b>North Pacific Research Board</b>	Pacific halibut population genomics (NPRB No. 2110)	IPHC	Alaska Fisheries Science Center-NOAA	\$193,685	Stock structure	December 2021- January 2024
<b>Total awarded (\$)</b>					<b>\$293,385</b>		