Report on Current and Future Biological and Ecosystem Science Research Activities

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, 25 AUGUST 2023)

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 the IPHC that follow Commission objectives are identified and described in the IPHC Five-Year Program of Integrated Research and Monitoring (2022-2026). These activities are integrated with stock assessment (SA) and the management strategy evaluation (MSE) processes (Appendix I) and are summarized in five main areas, as follows:

1) Migration and Population Dynamics. Studies are aimed at improving current knowledge of Pacific halibut migration and population dynamics throughout all life stages in order to achieve a complete understanding of stock structure and distribution across the entire distribution range of Pacific halibut in the North Pacific Ocean and the biotic and abiotic factors that influence it.
2) Reproduction. Studies are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity and fecundity.
3) Growth. Studies are aimed at describing the role of factors responsible for the observed changes in size-at-age and at evaluating growth and physiological condition in Pacific halibut.
4) Mortality and Survival Assessment. Studies are aimed at providing updated estimates of discard mortality rates in the guided recreational fisheries and at evaluating methods for reducing mortality of Pacific halibut.
5) Fishing Technology. Studies are aimed at developing methods that involve modifications of fishing gear with the purpose of reducing Pacific halibut mortality due to depredation and bycatch.

A ranked list of biological uncertainties and parameters for SA (Appendix II) and the MSE process (Appendix III) and their links to research activities and outcomes derived from the five-year research plan are provided.

SRB RECOMMENDATIONS

The SRB issued the following recommendations in their report of SRB022 (IPHC-2023-SRB022-R):

*SRB022–Rec.08 (para. 32) The SRB **NOTED** that the current maturity sampling design does not determine whether the high rate of individuals at the cortical alveoli stage in the southeastern portion of the study area is a function of differences in seasonal reproductive timing or in size/age at maturity. The SRB **RECOMMENDED** additional investigations on the region-specific seasonal reproductive cycles and evaluating the extent to which differences among regions can be explained by size or age of the sampled individuals.*

The IPHC Secretariat is currently conducting a coastwide study on maturity with a significantly higher number of ovarian samples collected during the 2022 FISS and is expanding further the number of collected ovarian samples in the referenced study area during the current 2023 FISS (Section 2.2.1, this document).

*SRB022–Rec.09 (para. 35) The SRB **NOTED** the presentation on whale depredation avoidance devices and **RECOMMENDED** that the Secretariat pursue external funding opportunities for expanding this research and testing one or more devices in the presence of whales.*

The IPHC Secretariat submitted a grant proposal to test catch protection devices in the presence of killer whales that has been awarded for the period November 2023 – April 2025 (Appendix IV).

*SRB022–Rec.10 (para. 36) **NOTING** that in terms of bioinformatic quality filtering to exclude loci, filtering based on sequencing depth alone may not be sufficient to exclude mitochondrial sequences, the SRB **RECOMMENDED** that loci be mapped to the published Pacific halibut mitochondrial genome to ensure that non-autosomal loci are included in analyses. Filtering based on sequencing depth alone is likely not sufficient to exclude regions of the genome that represent repetitive elements. Suggest sites be checked for repetitive elements.*

The IPHC Secretariat used the following filtering criteria in addition to sequencing depth, as previously noted (p. 9, IPHC-2023-SRB022-09; slide 10, IPHC-2023-SRB022-09): a) Minor Allele Frequency (MAF): >= 0.01; b) missing data: a site must be covered in < 80% of individuals; and, c) SNP pval: remove sites with p-value < 1e-6 (i.e., probability of a site being variable). In addition, the RefSeq assembly used for read mapping (GCF_022539355.2) contains the published mitochondrial sequence. Since read mapping occurs prior to SNP detection, these reads can be filtered on the basis of genomic location. SNPs that were detected from reads mapping to non-autosomal regions, namely the mitochondrial genome and chromosome 9, which contains a large sex-associated region (Jasonowicz et al. 2022), were removed, and the number of SNPs (10,230,908) reported in SRB022 were referred to as autosomal, implying that mitochondrial SNPs and sex-linked SNPs are not included in the current summary of the dataset. We should note that the recommendation to ensure that non-autosomal loci are *included* in analyses is in contrast with the previous statement in this recommendation that filtering on sequencing depth alone may not be sufficient to exclude mitochondrial sequences. As stated above, non-autosomal loci would also include mitochondrial and sex-linked SNPs. We would argue that including non-autosomal SNPs in this dataset is not advisable since they have been shown to bias population genetic parameter estimates and analyses (Benestan et al. 2017) since mitochondrial and autosomal DNA are subject to different inheritance and evolutionary mechanisms. Finally, while filtering sites based on sequencing depth offers one means for excluding repetitive regions of the genome from downstream analyses, requiring sites to be covered in a minimum number of individuals can also be helpful for this purpose (Lou et al. 2021), as well as filtering sequencing reads based on mapping quality and their ability to map uniquely prior to SNP detection, as noted in IPHC-2023-SRB022-09. Part of the Pacific halibut genome annotation process conducted by NCBI includes the identification of repetitive regions so that we can also exclude these regions directly.

*SRB022–Rec.11 (para. 37) The SRB **RECOMMENDED** that the Secretariat include other genome-wide summary measures of diversity. Measures could include (a) measures of genome size, (b) percentages of genome as singleton and duplicated loci, (c) other summary measures of diversity including (i) number of loci with minor allele frequency (MAF)>0.01, (ii) number of loci with MAF>0.05, (iii) a measure of deviation of observed and expected heterozygosity (Fis), (iv) observed heterozygosity (Ho) and expected heterozygosity (He).*

The IPHC Secretariat has produced several of the suggested genome-wide summary measures of diversity. First, the complete Pacific halibut reference genome contains 52 scaffolds (602.1 Mbp), 24 of which represent fully assembled chromosomes (600.9 Mbp) (p.8, IPHC-2022-SRB020-08; Table 1, Jasonowicz et al. 2022). Second, genome assembly completeness, as estimated by BUSCO, indicates that 97.3% of the Pacific halibut reference sequence (GCF_022539355.2) is present in a single copy and 1.1% is duplicated (Table 1, Jasonowicz et al. 2022). Furthermore, as previously noted (p. 9, IPHC-2023-SRB022-09; slide 10, IPHC-2023-SRB022-09), the total number of SNPs identified in autosomal regions of the genome with a MAF ≥ 0.01 was 10,230,908, with 4,725,899 having a MAF ≥ 0.05. The estimation of additional diversity measures (Ho, He & Fis) is currently in progress and has been added to the updated proposed workflow (Figure 1).

*SRB022–Rec.12 (para. 38) The SRB **RECOMMENDED** that the Secretariat evaluate multiple 'windows' and inter-window 'spacing' to summarize diversity and differentiation. The SRB is unsure why a 15 Kb 'window was used with 7.5 Kb space for producing Manhattan plots. The size of the window will affect estimates of significance based on a measures of Fst significance. Specifically, the larger the 'window' likely the larger the standard deviation across a greater number of sites. Window size is also likely to affect levels of linkage disequilibrium and down-stream analyses based on it.*

The choice of window size is a starting point based on published literature using low-coverage whole- genome sequencing for studies of population structure of other commercially important groundfish species (Clucas et al. 2019). Given that the standard deviation will increase with the window size, we also estimated and plotted F_{ST} for single SNPs in order to visualize the dispersion of single SNP estimates of F_{ST} in relation to the estimate for the window, as previously provided in the supplemental documentation that was referenced in section 1.3.1.3. of document IPHC-2023-SRB022-09.

*SRB022–Rec.13 (para. 39) **NOTING** that different outlier tests are based on different assumptions and statistical approaches, the SRB **RECOMMENDED** that the Secretariat implement more than one method. Selection of specific markers would appropriately be based on concordant designation of highly population discriminatory loci identify across methods. The Secretariat is likely to have greater confidence in assignment of 'outliers' based on principles of concordance using multiple and semi-independent software packages and statistical approaches.*

The proposed workflow for the analysis of these data includes the identification of outlier loci using two approaches: 1) F_{ST} based outlier scans, and 2) PCA based selection scans, as previously noted (Fig. 1D; IPHC-2023-SRB022-09).

*SRB022–Rec.14 (para. 40) The SRB **RECOMMENDED** that after statistical significance of SNP loci has been established, the Secretariat use gene set enrichment analyses to establish functional annotations for genes associated with SNPs.*

Once statistical significance of SNPs has been established, we can proceed with this Recommendation. The IPHC Secretariat conducted enrichment analysis for genes identified in the large (12 Mb) sex-determining region in chromosome 9 of the Pacific halibut genome (Table S4, Jasonowicz et al. 2022). Therefore, the resources required for conducting enrichment analysis of genes associated with SNPs in the present study are readily available.

*SRB022–Rec.15 (para. 41) The SRB **APPRECIATED** that the Secretariat estimated Tajima's D as recommended (IPHC-2022-SRB021-R), and **RECOMMENDED** that:*

a) the Secretariat be cautious with filtering SNP loci based on minor allele frequency (MAF) at levels as low as 0.01 as employed in results described in IPHC-2023-SRB022-09, as this may affect values of Tajima's D; and

b) a range of values be explored.

The IPHC Secretariat used a minimal set of filters for any analyses requiring site frequency spectrum (SFS), as previously provided in the supplemental documentation referenced in section 1.3.1.3. of document IPHC-2023-SRB022-09. For such analyses, applying allele frequency-based filters will distort the SFS and the literature recommends that these types of filters should be avoided for analyses that rely on accurate estimation of the SFS (Matz 2018) and specifically when estimating Tajima's D from low coverage whole genome sequence data (See section 3.4.3 in Lou et al. 2021).

*SRB022–Rec.16 (para. 43) The SRB **RECOMMENDED** looking for genome regions (more than 2 or more co-located 'significant' SNPS) with high divergence as indication of regions containing structural variants. Measures of linkage disequilibrium can also be profitably used to identify structural variants.*

The IPHC Secretariat is currently working to follow up on this Recommendation. As previously shown (Fig. 1D, IPHC-2023-SRB022-09), the proposed bioinformatics workflow contemplates the use of the software ngsLD which estimates measures of linkage disequilibrium from genotype likelihoods (Fox et al. 2019).

*SRB022–Rec.17 (para. 44) The SRB **RECOMMENDED** plotting levels of heterozygosity as Manhattan plots across chromosomal regions.*

The IPHC Secretariat has begun estimating additional genetic diversity measures in response to SRB022–Rec.11 and has updated the proposed workflow to reflect this (Figure 1). This would include visualizing heterozygosity levels across chromosomal regions.

*SRB022–Rec.18 (para. 45) **NOTING** that use of high-throughput low-coverage DNA sequencing data can lead to biased estimates of the site frequency spectrum (SFS) due to high levels of uncertainty in genotyping, the SRB **RECOMMENDED** exploring other*

derivations from Secretariat proposed work described in IPHC-2023-SRB022-09 including visualisations of SFS in multi-dimensional space.

The IPHC Secretariat recognizes the uncertainty inherent in low coverage DNA sequencing data and is using methods that account for this uncertainty which is critical when dealing with low coverage data (Korneliussen et al. 2014; Mas-Sandoval et al. 2022). With regards to the estimation of the SFS, the method that we are currently using is detailed in Rasmussen et al. (2022), and was specifically developed to accommodate uncertainty inherent to low coverage whole genome resequencing (lcWGR) data. We would like to note that we are not planning to use any analyses that require hard called genotypes as this is not appropriate for lcWGR data. All current and planned analyses (and software) utilize the raw sequence alignments or genotype likelihoods directly, taking into account uncertainty due to low sequencing depth.

*SRB022–Rec.19 (para. 46) **NOTING** that one of the primary objectives of the Pacific halibut genome project is to provide spatial discrimination of 'populations' (IPHC reporting regions) and to assign individuals to these groups, and that the Secretariat described genetic relationships among individuals from different IPHC reporting region and years of collection based on multivariate ordination using principle component analyses (PCA), and that levels of variability explained associated with PCA axes projects is low, the SRB **RECOMMNEDED**:*

a) conducting additional analyses to evaluate statistical significance of measures of inter-population differentiation (Fst); and

b) re-analysis using only outlier loci.

The IPHC Secretariat is currently working to address these two Recommendations.

*SRB022–Rec.20 (para. 47) The SRB **RECOMMENDED**:*

a) that the Secretariat move forward to stock discrimination to satisfy the Secretariat objective of using genetic data to define spatial structuring including unsupervised clustering methods (e.g. K-means, Structure, etc.) as well as PCA-based clustering (e.g. Discriminant Analysis of Principle Component) clustering;

b) using assignment testing and mixture analyses such as leave-one-out cross-validation simulations to assess the potential accuracy of mixed stock analysis (MSA).

The IPHC Secretariat proposed the use of unsupervised clustering methods, specifically K-means (Fig.1, IPHC-2023-SRB022-09; slide 10, IPHC-2023-SRB022-09), and they are being implemented. However, NGSadmix (Skotte et al. 2013) will be used rather than structure since it was developed for lcWGR data and can handle genotype likelihoods as input. The use of Discriminant Analysis of Principle Components (Jombart et al. 2010) may be problematic since the current implementation requires hard called genotypes and would not be appropriate for genotype likelihood data generated from lcWGR. Assignment testing using genotype likelihoods can be done with WGSassign (Desaix et al. 2023) and this has been added to the updated proposed workflow (Figure 1).

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Migration and Population Dynamics.

   The IPHC Secretariat is currently conducting studies on Pacific halibut juvenile habitat and movement through conventional wire tagging, as well as studies that incorporate genomics approaches in order to produce useful information on population structure, 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 MSE process is in biological parameterization and validation of movement estimates, on one hand, and of recruitment distribution, on the other hand (Appendix III).

   1.1. Identification of Pacific halibut juvenile habitat. The IPHC Secretariat recently investigated the level of connectivity between spawning grounds and possible settlement areas based on a biophysical larval transport model (Sadorus et al. 2021). 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 (Carpi et al. 2021), very little information is available on the geographic location and physical characteristics of these areas. The IPHC Secretariat initiated studies to identify potential settlement areas for juvenile Pacific halibut throughout IPHC Convention waters and to identify suitable habitat characteristics for settlement grounds. Data mining of multiple sources ranging from IPHC's own historical databases to other public and private agencies who have collected data relevant to this project, has resulted in catch locations for a total of 52,356 Pacific halibut aged 0-2 encountered from 1946 to 2022 (data sources provided in Table 1 of IPHC-2023-SRB022-09).

   Estimated ages are based on either direct age determination through otolith reading or fork length if otolith-based ages are not available. An additional 1,430 locations that were study sites located in what was determined as plausible nursery habitat areas for flatfish in Alaska based on bottom depth information (< 50 m depth), and that were sampled with fishing gear that was appropriate for capturing small flatfish (e.g., beach seines and beam trawls) but that did not capture any Pacific halibut, have been noted as stations where Pacific halibut were absent. The IPHC Secretariat is also actively collecting substrate data, some of which has been recorded alongside species capture data (e.g. select records within NOAA's Nearshore Fish Atlas database: https://www.fisheries.noaa.gov/alaska/habitat-conservation/nearshore-fish-atlas-alaska), as well as overlays generated using the United States Geological Survey usSEABED sediment database (https://doi.org/10.5066/P9H3LGWM). The IPHC Secretariat is continuing to locate other sources of sediment and bottom-type data throughout the Convention Area.

In the summer of 2023, additional work was initiated in cooperation with Alaska Coastal Observations and Research (ACOR) and University of Alaska Fairbanks to mine data from unpublished sources that were recorded in the 1990s on juvenile Pacific halibut encounters in beach seines conducted off Kodiak Island, Afognak Island and Kachemak Bay, Alaska.

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 by using 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). A total of 1,499 Pacific halibut were 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 8,931 U32 Pacific halibut have been wire tagged and released on the IPHC FISS and 205 of those have been recovered to date (these totals include a subset of U32 releases that were part of a tail pattern project). 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.

1.3. 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

1.3.1. Studies to resolve the genetic structure of the Pacific halibut population in the Convention Area. Details on sample collection, sequencing, bioinformatic processing and proposed analyses utilizing low-coverage whole genome sequencing (lcWGR) to investigate Pacific halibut population structure were provided in documents IPHC-2021-SRB018-08, IPHC-2022-SRB021-09 and IPHC-2023-SRB022-09.

Additional components have been added to the proposed workflow for this project (Figure 1) to address SRB recommendations SRB022–Rec.11 and SRB022–Rec.20. The addition of estimating measures of genetic diversity from genotype likelihoods has been added to the workflow in Figure 1C and was carried out as follows. Genotype likelihoods estimated at 10,230,908 autosomal single nucleotide polymorphism (SNP) positions (methods on genotype likelihood estimation are detailed in IPHC-2023-SRB022-09) were used to estimate additional measures of genetic diversity (SRB022–Rec.11). ANGSD (v0.940) (Korneliussen et al. 2014) was used to test for departures from Hardy-Weinberg Equilibrium (-doHWE 1), estimate allele frequencies (-doMaf 1) and heterozygosity levels at each SNP for each sample collection and geographic area. A summary of these values is provided in Table 1. Population assignment testing has been added to the workflow

in Figure 1C and will be carried out using WGSassign (https://github.com/mgdesaix/WGSassign), a software package for conducting population assignment testing using genotype likelihoods from lcWGR data (Desaix et al. 2023).

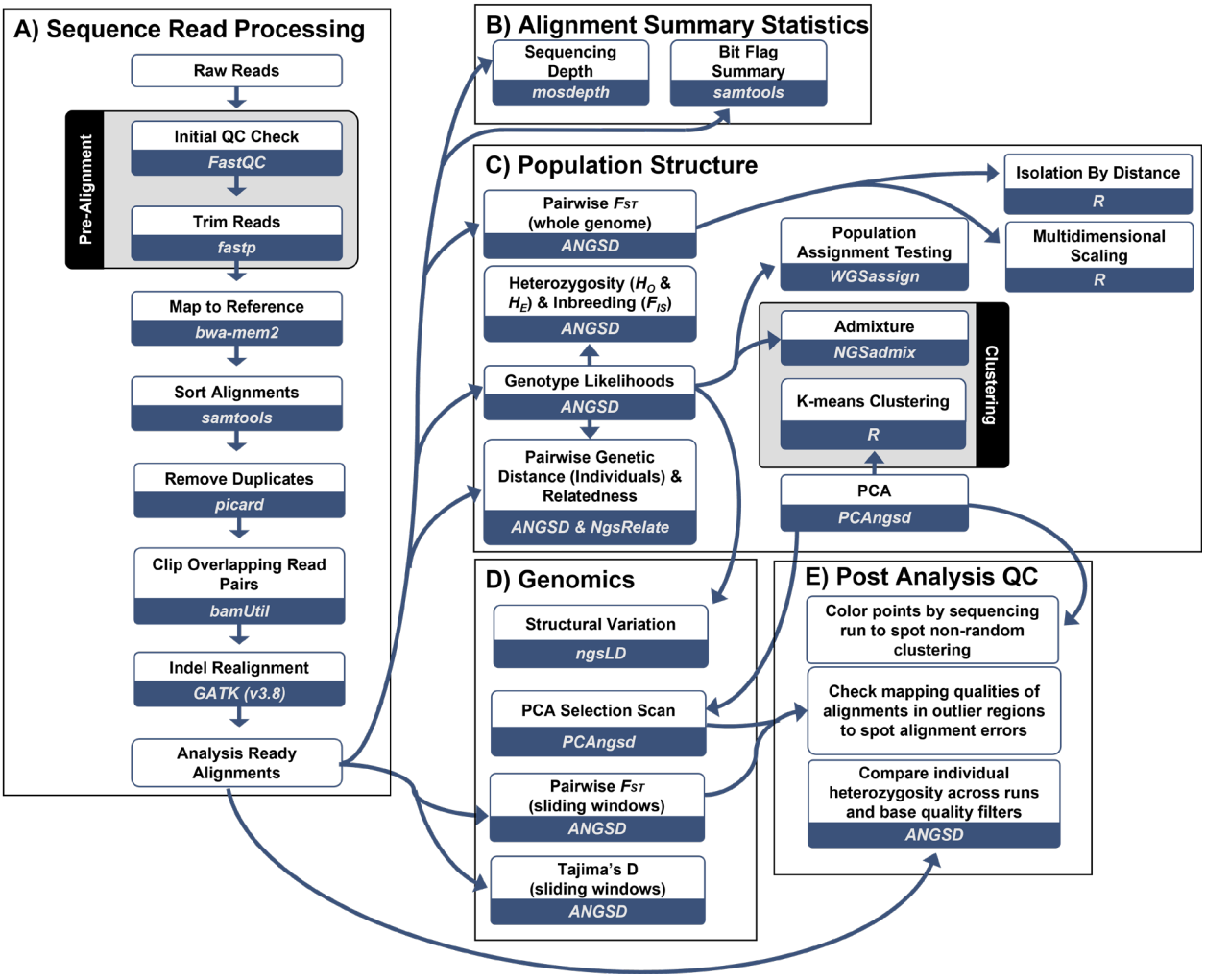


Figure 1. Proposed bioinformatic workflow for the interrogation of low-coverage whole genome sequence data. This diagram tracks the flow of data through the main stages of this project, (A) raw sequence read processing, (B) alignment summaries, (C) analysis of population structure, (D) genomic analyses,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

The IPHC Secretariat is currently conducting work to address components of SRB022–Rec.13, SRB022–Rec.14 and SRB022–Rec.20. Principal component analysis (PCA) was carried out using PCAngsd (v1.11) (Meisner and Albrechtsen 2018) to estimate a covariance matrix from the genotype likelihoods estimated from the lcWGR dataset (methods on genotype likelihood estimation are detailed in IPHC-2023-SRB022-09). A minor allele (MAF) threshold of 0.05 was applied,

retaining 4,725,899 autosomal SNPs for covariance matrix estimation. and Eigendecomposition was performed using the *eigen* function in R (v4.2.2). To determine an appropriate number of principal components (PCs) to retain for downstream analyses, a scree plot of the first 20 eigenvalues was visually inspected and following Cattell's rule (Cattell 1966), we retained the first three PCs (Figure 2) for further analyses.

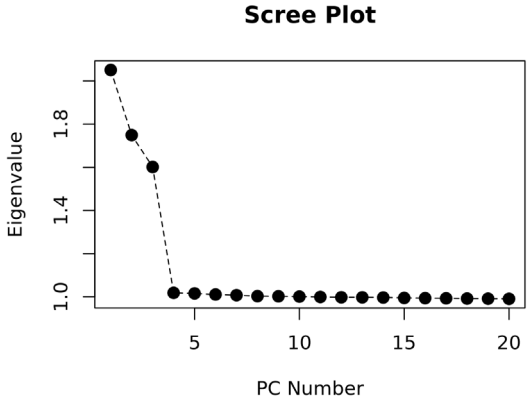


Figure 2. Scree plot of the eigenvalues for the first 20 principal components (PCs).

K-means clustering was performed on the retained PCs (SRB022–Rec.20) using the *kmeans* function in R. To determine the optimal number of clusters (*K*) present in the data we tested a range of *K* values (1 to 20) and used total within-cluster sum of squares (WSS) and Bayesian information criterion (BIC) to compare the *K* values tested (Figure 3). One method for choosing the optimal value of *K* is to look for an inflection point or "elbow" in the total WSS values and in the case of BIC, the lowest associated value (Jombart et al. 2010). In this data, a clear inflection point is not present in the WSS values (Figure 3A) and the lowest BIC value is associated with *K*=20, the maximal value of *K* tested. This could be taken as an indication that discrete clusters are not present in the dataset.

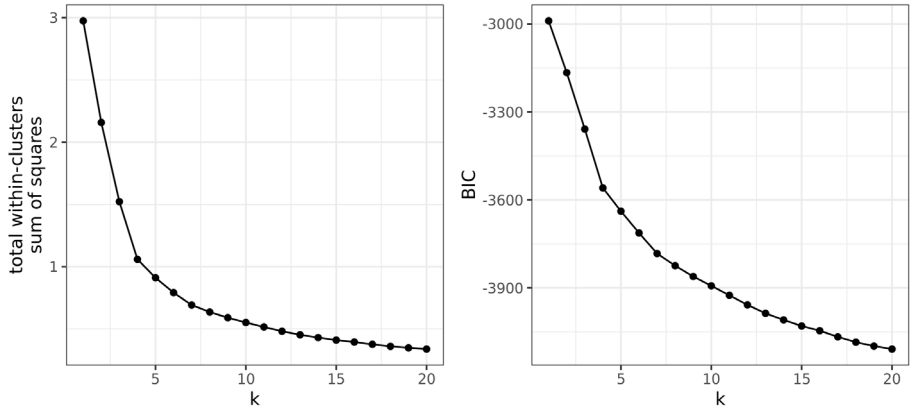


Figure 3. Plots of total within-clusters sum of squares (A) and Bayesian information criterion for each value of K tested (1-20).

Area	Collection Year	N	MAF > 0.01	MAF > 0.05	F_{IS}	H_O	H_E
British Columbia	1999	49	8,958,267	4,890,386	0.109	0.154	0.158
	2004	43	8,756,199	4,995,125	0.115	0.156	0.163
	2007	50	8,939,078	4,900,656	0.120	0.154	0.157
	all years	142	9,256,496	4,762,476	0.023	0.155	0.161
Central Gulf of Alaska	1999	50	9,131,547	4,993,279	0.049	0.158	0.171
	2004	50	9,065,567	5,163,204	0.029	0.162	0.189
	2007	50	9,052,210	5,052,609	0.058	0.159	0.176
	2018	49	8,627,118	4,893,881	0.172	0.153	0.153
	all years	199	9,561,613	4,862,986	-0.032	0.158	0.176
Bering Sea	2004	43	8,886,235	5,007,451	0.094	0.156	0.164
	2007	50	9,057,451	4,930,166	0.089	0.155	0.162
	all years	93	9,214,470	4,851,360	0.030	0.156	0.164
Central Aleutian Islands	2007	37	8,464,803	4,983,042	0.150	0.154	0.157
	2020	49	8,823,846	4,904,749	0.129	0.154	0.158
	all years	86	8,921,876	4,799,261	0.066	0.154	0.159
Western Aleutian Islands	2020	50	8,690,974	4,893,669	0.151	0.153	0.157
	all years	50	8,690,974	4,893,669	0.151	0.153	0.157

Table 1. Summary of diversity measures estimated from low coverage whole genome sequence data for sample collections of Pacific halibut. The table includes sample sizes (N), number of loci with minor allele frequency (MAF)>0.01, number of loci with MAF>0.05.

We also conducted PCA based selection scans along the top three PCs (Figure 2) to identify and establish statistical significance of outlier SNPs (SRB022–Rec.13, SRB022–Rec.14). An extended version of the pcadapt model (Luu et al. 2017) designed to accommodate genotype likelihoods implemented in PCangsd (Meisner et al. 2021) was used to identify SNPs that may be under selection. As with the covariance matrix estimation, a MAF threshold of 0.05 was applied, retaining 4,725,899 autosomal SNPs for the selection scan. The scores from PCAdapt were converted to p-values using the provided R script pcadapt.R (https://github.com/Rosemeis/pcangsd/blob/master/scripts/pcadapt.R). To correct for multiple testing and control the false discovery rate (FDR) p-values were adjusted using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995). Applying an FDR threshold of 0.001, we identified 16,272 candidate SNPs potentially under selection (Figure 4). We are currently exploring additional options of multiple testing corrections to determine optimal thresholds for outlier SNP detection (e.g. Storey and Tibshirani 2003).

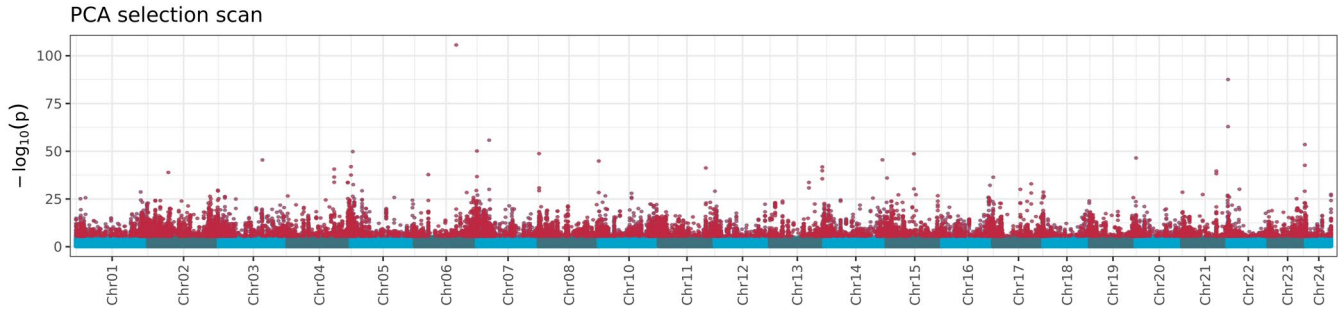


Figure 4. Manhattan plot of the -log10(p-values) obtained from the selection scan along the top three PCs carried out in PCangsd (using the pcadapt model). Points highlighted in read represent the 16,272 significant SNPs at an FDR threshold of 0.001.

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 has finalized processing genetic samples from the 2022 aged commercial landings.

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). To accomplish this objective, the IPHC Secretariat collected ovarian samples for histology during the 2022 FISS. The FISS sampling resulted in a total of 1,016 ovarian samples collected coastwide for histological analysis, with 437 ovarian samples from Biological Region 2, 348 samples from Biological Region 3, 180 from Biological Regions 4, and 51 samples from Biological Region 4B. Ovarian samples have been processed for histology and IPHC Secretariat staff is currently finalizing scoring samples for maturity using histological maturity classifications as previously described in Fish et al. (2020, 2022). 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 (MARVLS repository for reproductive analyses: https://github.com/MARVLS/Fish-Gonad-Staging/tree/main/analyses). 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. Variation in the proportion mature with length and age will be examined among all four IPHC biological regions based on data available.

IPHC Secretariat is continuing to collect ovarian samples in 2023 on the FISS. This will allow us to investigate both spatial and temporal differences in female Pacific halibut maturity. Due to the reduction in FISS design for 2023, sampling efforts are only taking place in IPHC Biological Regions 2 and 3. Targets are to collect 400 samples in Biological Region 2 and 1,000 in Biological Region 3.

2.2.2. Fecundity estimations. The IPHC Secretariat has initiated studies that are aimed at improving our understanding of Pacific halibut fecundity. This will allow us to estimate fecundity-at-size and -age and could be used to replace spawning biomass with egg output as the metric for reproductive capability in stock assessment and management reference points. Fecundity determinations will be conducted using the auto-diametric method (Thorsen and Kjesbu 2001; Witthames et al., 2009). IPHC Secretariat staff received training on this method by experts in the field (NOAA Fisheries, Northeast Fisheries Science Center, Wood Hole, MA) in May 2023. Ovarian samples for fecundity estimations are being collected during the 2023 FISS. Sampling is taking place in IPHC Biological Region 3, with a minimum target of 250-300 fecundity samples (from fish that will also have a maturity sample collected, as described in 2.2.1).

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. Mortality 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. After having reported on experimentally-derived estimates of discard mortality rate in the directed longline fishery (Loher et al., 2022), the second component of this study investigated the relationships among hook release techniques (e.g., gentle shake, gangion cutting, and hook stripping), injury levels, stress levels and physiological condition of released fish, as well as the environmental conditions that the fish experienced during capture. Gentle shake and gangion cutting resulted in the same injury and viability outcomes with 75% of sublegal fish in Excellent condition, while the hook stripper produced the poorest outcomes (only 9% in Excellent condition). Hook stripping also resulted in more severe injuries, particularly with respect to tearing injuries, whereas gentle shake and gangion cutting predominantly resulted in a torn cheek, effectively the injury incurred by the hooking event. Physiological stress indicators (plasma levels of glucose, lactate, and cortisol) did not significant change with viability outcomes, except for higher lactate plasma levels in fish categorized as Dead. Hematocrit was significantly lower in fish that were categorized as Dead. Furthermore, 89% of fish classified as Dead were infiltrated by sand fleas, present in several sets in deeper and colder waters. Our results indicated that avoiding the use of hook strippers and minimizing soak times in areas known to have high sand flea activity result in better survival outcomes. A manuscript describing this study has been submitted for publication in the peer-reviewed literature (Dykstra et al., submitted).

4.2. Estimation of discard mortality rates in the charter recreational sector. Results from the similar study conducted in fish captured using guided recreational fishery practices yielded an estimated discard mortality rate of 1.35% (95% CI 0.00-3.95%) for Pacific halibut released in Excellent viability category that were captured and released from circle hooks. This estimate is consistent with the supposition that fish discarded in the recreational fishery from circle hooks in excellent condition have a mortality rate that is arguably lower than 3.5%, as is currently used for Excellent viability fish released in the commercial fishery (Meyer, 2007). As this estimate does not factor in mortality rates on fish in less than Excellent condition, does not inform mortality rates on non-circle hooks (J-hooks, jigs, other), nor directly applies to fish captured and released from non-charter practices, changes to the overall recreational discard mortality estimation are not currently contemplated. These results represent the first report of experimentally derived estimates of mortality of Pacific halibut captured and discarded in the recreational fishery. Current efforts are devoted to analyze collected data on capture conditions (e.g., bottom, ambient and fish temperatures; time on hook and on deck), blood stress parameters and injury and viability classifications.

5. Fishing technology.

The IPHC Secretariat has determined that research to provide the Pacific halibut fishery with tools to reduce whale depredation is considered a high priority (Appendix I). 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 is

investigating gear-based approaches to catch protection as a means for minimizing whale depredation in the Pacific halibut and other longline fisheries with funding from NOAA's Bycatch Research and Engineering Program (BREP) (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 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 provided for the SRB020 meeting: IPHC-2022-SRB020-08.

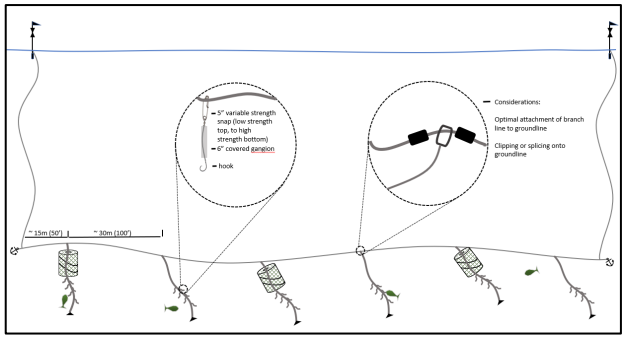
During the second phase of the project, the IPHC Secretariat worked with catch protection device manufacturers for the design of two different types of devices for field testing: one based on a modification of Sago Solutions SA's catch protection device (i.e., shuttle) and one based on a modification of a slinky pot (i.e., shroud) deployed on branchline gear. Pilot testing was designed to investigate (1) the logistics of setting, fishing, and hauling of the two pilot catch protection designs, and (2) the basic performance of the gear on catch rates and fish size compared to non-protected gear. Descriptions of the two different devices are as follows:

- Shuttle system. Manufactured in Norway by Sago Solutions AS, two shuttle devices were modeled after the Sago Extreme model but smaller at 80% size (Figure 5). Their dimensions are 2.60 m (8.5 ft) long by 0.80 m (2.6 ft) in diameter, each weighing approximately 100 kg (220 lb.) when empty. Typically, these devices are set with the gear; however, for this study the units were deployed from the surface, during the haulback event, by threading them onto a blank skate of gear between the control and the treatment skates.

A)  B) 

Figure 5. Images of the prototype shuttle devices used in this study in profile (A) and frontal view (B).

- Shroud system. Shrouds were constructed in house by modifying a slinky pot (opening one end and installing a rigid cap in the other end) and designed to slide down the branch line during haulback, clustering the snaps (and hooks) and covering any catch (Figure 6).

A) 

B) 

Figure 6. Schematic of shrouded branchline actively fishing on seabed (A) and a shroud made from a modified slinky pot (B).

Field work was conducted off Newport, OR, aboard the R/V Pacific Surveyor (56' length) in late May 2023. Ten sets were made with each gear type, with an even number of treatments (controls or protection devices) per set. Shuttles had a standard fixed gear skate of 100 hooks on 18 foot spacing, a blank half skate (on which to thread and allow the shuttle to reach the bottom before entraining catch) followed by a second section with 100 fixed hooks. Shroud treatments initially consisted of six branchlines (each with 10 hooks snapped on at four-foot spacing), three with shrouds to cover the catch, and three controls with no protective shroud. This was reduced to two protected and two control branchlines, all with two-foot hook spacing to provide more handling time and to reduce risk to crew. The pilot nature of the study provided the flexibility to adjust and react to observations in real time. A moderate learning curve was required for shuttles to be able to efficiently thread onto the gear, shuttles had good entrapment of catch (similar catch rates to the control) (Figure 7), and smaller hooks and weaker gangions incurred lower levels of damage to the entrained fish. The devices are rugged, and safely operational on a small vessel.

A) 

B) 

C) 

Figure 7. Shuttle being retrieved A), catch entrained in shuttle B), and catch being emptied onto the vessel deck.

Branchline fishing with shrouds had a steep learning curve and presented some safety concerns. Upon working through these concerns, this type of catch protection device had a very small effective footprint, with minimal catch with which to make comparisons between shrouded branches and controls (Figure 8), despite the high hagfish activity. Many logistical issues would

need to be worked out to scale this catch protection device up to real fishing conditions and would conceivably still provide opportunities for catch depredation to whales.

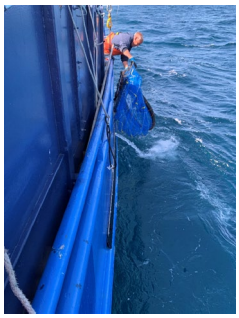
A)  B)  C) 

Figure 8. Shroud gear being retrieved A), skate covered by the shroud B), and a Pacific halibut and branchline hooks covered by the shroud C).

In a third phase of this project, the IPHC Secretariat has recently received another grant from the Bycatch Reduction Engineering Program-NOAA entitled “Full scale testing of devices to minimize whale depredation in longline fisheries” (NA23NMF4720414) to refine effective methods for protecting longline captured fish from depredation and to complete replicates in the presence of toothed whales in known depredation hotspots to demonstrate the efficacy and safety of the gear. Field work for this project is planned for mid-2024.

RECOMMENDATION/S

That the SRB:

a) **NOTE** paper IPHC-2023-SRB023-08 which provides a response to Recommendations from SRB022, and a report on current research activities contemplated within the IPHC’s five-year Program of Integrated Research and Monitoring (2022-26).

REFERENCES

Benestan, L., Moore, J.S., and Sutherland, B.J.G. 2017. Sex matters in massive parallel sequencing: Evidence for biases in genetic parameter estimation and investigation of sex determination systems. Molecular Ecology 26(24): 6767--6783. doi:10.1111/mec.14217.

Benjamini, Y., and Hochberg, Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 57(1): 289--300. doi:10.1111/j.2517-6161.1995.tb02031.x

Cattell, R.B. 1966. The Scree Test For The Number Of Factors. Multivariate Behavioral Research 1(2): 245--276. doi:10.1207/s15327906mbr0102_10.

Clucas, G.V., Lou, R.N., Therkildsen, N.O., and 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.

Desaix, M.G., Rodriguez, M.D., Ruegg, K.C., Anderson, E.C., Collins, F., Division, E., Fisheries, S., Marine, N., Service, F., Cruz, S., and Collins, F. 2023. Population assignment from genotype likelihoods for low-coverage whole-genome sequencing data. authorea(June). doi:10.22541/au.168569102.27840692/v1.

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.

Dykstra, C., Wolf, N., Harris, B.P., Stewart, I.J., Hicks, A., Restrepo. F., Planas, J.V. Letting Pacific halibut off the hook: relating capture and physiological conditions to viability and survival of fish discarded from commercial longline gear. 2023. Submitted.

Fish, T., Wolf, N., Harris, B.P., Planas, J.V. A comprehensive description of oocyte developmental stages in Pacific halibut, Hippoglossus stenolepis. Journal of Fish Biology. 2020. 97: 1880-1885. doi: https://doi.org/10.1111/jfb.14551

Fish, T., Wolf, N., Smeltz, T.S., Harris, B.P., Planas, J.V. Reproductive biology of female Pacific halibut (Hippoglossus stenolepis) in the Gulf of Alaska. Frontiers in Marine Science. 2022. 9: 801759. doi: https://doi.org/10.3389/fmars.2022.801759

Fox, E.A., Wright, A.E., Fumagalli, M., and 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., Donnadieu, 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 2022. 22: 2685-2700. DOI: https://doi.org/10.1111/1755-0998.13641.

Jombart, T., Devillard, S., and Balloux, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94. doi:10.1186/1471-2156-11-94. http://www.biomedcentral.com/1471-2156/11/94.

Korneliussen, T.S., Albrechtsen, A., and Nielsen, R. 2014. ANGSD: Analysis of Next Generation Sequencing Data. BMC Bioinformatics 15(1): 356. doi:10.1186/s12859-014-0356-4.

Loher, T., Dykstra, C.L., Hicks, A., Stewart, I.J., Wolf, N., Harris, B.P., Planas, J.V. Estimation of post-release longline mortality in Pacific halibut using acceleration-logging tags. North American Journal of Fisheries Management. 2022. 42: 37-49. doi: https://doi.org/10.1002/nafm.10711.

Lou, R.N., Jacobs, A., Wilder, A.P., and Therkildsen, N.O. 2021. A beginner's guide to low-coverage whole genome sequencing for population genomics. Molecular Ecology 30(23): 5966--5993. doi:10.1111/mec.16077.

Luu, K., Bazin, E., and Blum, M.G.B. 2017. pcadapt : an R package to perform genome scans for selection based on principal component analysis. Molecular Ecology Resources 17(1): 67--77. doi:10.1111/1755-0998.12592.

Mas-Sandoval, A., Pope, N.S., Nielsen, K.N., Altinkaya, I., Fumagalli, M., and Korneliussen, T.S. 2022. Fast and accurate estimation of multidimensional site frequency spectra from low-coverage high-throughput sequencing data. GigaScience 11: 1--9. doi:10.1093/gigascience/giac032.

Matz, M.V. 2018. Fantastic Beasts and How To Sequence Them: Ecological Genomics for Obscure Model Organisms. Trends in Genetics 34(2): 121--132. doi:10.1016/j.tig.2017.11.002.

Meisner, J., and 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., and Hanghj, K. 2021. Detecting selection in low-coverage high-throughput sequencing data using principal component analysis. BMC Bioinformatics 22(1): 470. doi:10.1186/s12859-021-04375-2.

Meyer, S. 2007. Halibut discard mortality in recreational fisheries in IPHC Areas 2C and 3A [online]. Discussion paper presented to the North Pacific Fishery Management Council, September 2007. Alaska Department of Fish and Game. Available from: https://www.npfmc.org/wp-content/PDFdocuments/halibut/HalibutDiscards907.pdf

Rasmussen, M.S., Garcia-Erill, G., Korneliussen, T.S., Wiuf, C., and Albrechtsen, A. 2022. Estimation of site frequency spectra from low-coverage sequencing data using stochastic EM reduces overfitting, runtime, and memory usage. Genetics 222(4). doi:10.1093/genetics/iyac148R. Core Team. 2022. R: A language and environment for statistical computing (v4.2.2).

Sadorus, L.; Goldstein, E.; Webster, R.; Stockhausen, W.; Planas, J.V.; Duffy-Anderson, J. Multiple life-stage connectivity of Pacific halibut (Hippoglossus stenolepis) across the Bering Sea and Gulf of Alaska. Fisheries Oceanography. 2021. 30:174-193. doi: https://doi.org/10.1111/fog.12512

Skotte, L., Korneliussen, T.S., and 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.

Storey, J.D., and Tibshirani, R. 2003. Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences 100(16): 9440--9445.

Thorsen, A., and Kjesbu, O.S. 2001. A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. J. Sea Res. 46: 295-308.

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 (SA) and management strategy evaluation (MSE): rationale for biological research prioritization

Research areas	Research activities	Research outcomes	Relevance for stock assessment	Relevance for MSE	Specific analysis input	SA Rank	MSE Rank	Research priorization
Migration and population dynamics	Population structure	Population structure in the Convention Area	Altered structure of future stock assessments	Improve parametization of the Operating Model	If 4B is found to be functionally isolated, a separate assessment may be constructed for that IPHC Regulatory Area	2. Biological input	1. Biological parameterization and validation of movement estimates and recruitment distribution	2
	Distribution	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	3. Biological input		2
	Larval and juvenile connectivity studies	Improved understanding of larval and juvenile distribution	Improve estimates of productivity		Will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region	3. Biological input	1. Biological parameterization and validation of movement estimates	2
Reproduction	Histological maturity assessment	Updated maturity schedule	Scale biomass and reference point estimates	Improve simulation of spawning biomass in the Operating Model	Will be included in the stock assessment, replacing the current schedule last updated in 2006	1. Biological input		1
	Examination of potential skip spawning	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			1
	Fecundity assessment	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			1
	Examination of accuracy of current field macroscopic maturity classification	Revised field maturity classification			Revised time-series of historical (and future) maturity for input to the stock assessment			1
Growth	Evaluation of somatic growth variation as a driver for changes in size-at-age	Identification and application of markers for growth pattern evaluation	Scale stock productivity and reference point estimates	Improve simulation of variability and allow for scenarios investigating climate change	May inform yield-per-recruit and other spatial evaluations of productivity that support mortality limit-setting		3. Biological parameterization and validation for growth projections	5
		Environmental influences on growth patterns			May provide covariates for projecting short-term size-at-age. May help to delineate between effects due to fishing and those due to environment, thereby informing appropriate management response			5
		Dietary influences on growth patterns and physiological condition			May provide covariates for projecting short-term size-at-age. May help to deleineate between effects due to fishing and those due to environment, thereby informing appropriate management response			5
Mortality and survival assessment	Discard mortality rate estimate: longline fishery	Experimentally-derived DMR	Improve trends in unobserved mortality	Improve estimates of stock productivity	Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits	1. Fishery yield	1. Fishery parameterization	4
	Discard mortality rate estimate: recreational fishery				Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits			4
	Best handling and release practices	Guidelines for reducing discard mortality			May reduce discard mortality, thereby increasing available yield for directed fisheries	2. Fishery yield		4
Fishing technology	Whale depredation accounting and tools for avoidance	New tools for fishery avoidance/deterence; improved estimation of depredation mortality	Improve mortality accounting	Improve estimates of stock productivity	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	1. Assessment data collection and processing		3

APPENDIX II

List of ranked biological uncertainties and parameters for stock assessment (SA) and their links to biological research areas and research activities

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	Histological 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		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	Migration	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/deterence; 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 links to biological research areas and research activities

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 parametization 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		Genetics and Genomics	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 parametization of recruitment distribution in the Operating Model		Distribution
	Establishment of temporal and spatial maturity and spawning patterns	Improve simulation of recruitment variability and parametization 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 awarded research grants

Project #	Grant agency	Project name	PI	Partners	IPHC Budget ($US)	Management implications	Grant period
1	**Bycatch Reduction Engineering Program - NOAA**	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 2023
2	**North Pacific Research Board**	Pacific halibut population genomics (NPRB No. 2110)	IPHC	Alaska Fisheries Science Center-NOAA (Juneau, Seattle)	$193,685	Stock structure	December 2021- January 2024
3	**Bycatch Reduction Engineering Program - NOAA**	Full scale testing of devices to minimize whale depredation in longline fisheries (NA23NMF4720414)	IPHC	NOAA Fisheries - Alaska Fisheries Science Center (Seattle)	$199,870	Mortality estimations due to whale depredation	November 2023 – April 2025
Total awarded ($)					**$493,255**		