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 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 AND REQUESTS

The SRB issued the following recommendation in their report of SRB027 (IPHC-2025-SRB027-R) in relation to presentation IPHC-2025-SRB027-06:

*SRB027–Rec.01 (para. 14). The SRB **RECOMMENDED** that that evaluation of epigenetic aging be expanded from random selection of cross-validation samples to include testing out-of-sample interannual predictive performance. That is, how well can an epigenetic aging method trained on data from one set of years predict age of individuals sampled in other years?*

The IPHC Secretariat has selected a set of genetic samples separate from those used for the epigenetic clock development to test out-of-sample interannual predictive performance, as detailed in section 1.2.1.2.

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Migration and Population Dynamics.

   The IPHC Secretariat is currently focusing on 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. Population genomics. The primary objective of these studies is to investigate the genetic structure of the Pacific halibut population and to conduct genetic analyses to inform on Pacific halibut population dynamics and distribution within the Convention Area

      1.1.1. Population genetic structure. 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, IPHC-2023-SRB022-09, IPHC-2024-SRB024-09 and IPHC-2025-SRB026-06. Results from these studies are currently being prepared for publication in a leading peer-reviewed journal.

      1.1.2. Development of a novel method for estimating genetic differentiation from genotype likelihoods. As part of IPHC's research on population genetic structure, the IPHC Secretariat has developed a bioinformatic method (named *fst-gl*) designed to estimate F_{ST} using low-coverage whole genome resequencing (lcWGR) data from multiple populations. F_{ST} (Weir and Cockerham, 1984) is a widely used measure of population differentiation that is applied in the identification of SNPs or localized regions of the genome that show high levels of differentiation for the purposes of SNP panel development, identification of genomic signals of natural selection or local adaptation, identification of sex-associated genomic regions (Pacific halibut: Jasonowicz et al. 2022), etc.

      Current available bioinformatic methods for estimating F_{ST} require hard-called genotypic data generated at high-coverages (> 10x) using methods such as restricted site associated DNA (RAD)-seq or whole genome sequencing (WGS) to obtain

accurate estimates of F_{ST}. When sequencing at lower coverages (< 5x), uncertainty arises at the level of individual genotypes; therefore, accounting for this uncertainty is necessary when analyzing lcWGR data. The estimation of F_{ST} from lcWGR data or genotype likelihoods is complicated by the very limited number of available bioinformatic methods (e.g. *angsd*) that only estimate pairwise F_{ST} between two populations from low-coverage sequencing data (Korneliussen et al. 2014; Rasmussen et al. 2022) at a high computational cost.

The IPHC Secretariat has developed a bioinformatic method (*fst-gl*) and applied it to estimate F_{ST} values that were used to select the top SNPs for assignment testing, detailed in IPHC-2024-SRB024-09. *fst-gl* offers substantial performance improvements over existing methods implemented in *angsd* and enabled the IPHC Secretariat to test different training-set and SNP panel designs for assignment testing. *fst-gl* enables the estimation of F_{ST} among any number of populations and implements resampling routines to provide bootstrapped significance values for estimates of $F_{ST.}$

1.1.2.1. Methods.

1.1.2.1.1. Implementation. *fst-gl* was written in the *nim* programming language and leverages the *hts-nim* (Pedersen and Quinlan 2018) library for efficient parsing of variant call format (VCF) (Danecek et al. 2011) and the binary compressed version of VCF (BCF) files by exposing the low level HTSlib to *nim*. *fst-gl* also leverages the OpenMP API to implement multicore processing to further improve performance. At the core of *fst-gl* is an expectation maximization (EM) algorithm for estimating allele and genotype frequencies from genotype likelihoods for each population and then those estimates are used to calculate Weir and Cockerham's (1984) $\hat{\theta}$, a widely used estimator of F_{ST}. We reimplemented the EM algorithm for estimating allele frequencies from the software *vt* (Tan et al. 2015) in the *nim* programming language for *fst-gl.*

1.1.2.1.2. Performance evaluation. We used both simulated and empirical data to evaluate the performance of *fst-gl* compared to existing methods for estimating F_{ST} from lcWGR data. We used *SLiM* (v4) (Haller and Messer 2023), an individual based, forward in time simulation framework to simulate individual level genetic data under different levels of migration expected to generate varying levels of population differentiation and F_{ST} levels at individual SNPs ranging from 0 to 1. Briefly, we simulated two, three, and five population scenarios, where each population contains 1,000 individuals with a sex ratio of 0.5, under four different migration rates; 0 (no migration), 0.01, 0.05, and 0.1 for 10,000 generations using a Wright-Fisher based model in *SLiM*. Each scenario (migration rate x number of populations) was simulated 10 times.

At the end of each *SLiM* simulation, we randomly sampled 50 individuals from each population and extracted their simulated (true) genotypes and used this individual level variation to simulate Illumina sequence data using *reseq* (v1.1) (Schmeing and Robinson 2021). A sequencing error profile was obtained from https://github.com/schmeing/ReSeq-profiles to generate raw sequence data

similar to what might be observed when using Illumina's TruSeq chemistry on the NovaSeq 6000 platform. We simulated reads to an approximate sequencing depth of 20x, and processed them similarly to the bioinformatic methods provided in IPHC-2023-SRB022-09. Following sequence read alignment, *samtools* (v1.22.1) (Li et al. 2009) was used to down sample the aligned reads to average sequencing depths of 0.1x, 0.5x, 2.5x, 5x, 10x, and 15x, to evaluate performance of *fst-gl* under varying levels of uncertainty due to low sequencing depth.

For each simulation, we first obtained estimates of F_{ST} at each SNP position using the simulated genotypes of the individuals subsampled at the end of each simulation using *vcftools* (v0.1.14) (Danecek et al. 2011) and compared these to estimates obtained from the down sampled alignments using genotype likelihood-based methods (*fst-gl* and *angsd*) and hard-called genotypes.

After estimates of F_{ST} were obtained using each method, we calculated bias at each SNP position as $\hat{F}_{st} - F_{ST}$. To evaluate the performance of *fst-gl* in a real-world use case, we also applied the newly developed method to the task of identifying a sex-associated region of the Pacific halibut (*Hippoglossus stenolepis*) chromosome 9 (Chr09) (NCBI: NC_061491.1). This analysis was previously carried out using pool-seq data (Jasonowicz et al. 2022) and provides a simple real-world application for *fst-gl* that we can compare to existing results and possibly gain new insights. We matched our use case to the study design of the pool-seq analysis presented in Jasonowicz et al. (2022) by analyzing lcWGR sequence reads from 30 adult female and 30 adult male Pacific halibut collected by a chartered commercial longline vessel near the Portlock Bank region of the Gulf of Alaska (56°59'N- 58°55'N, 148°41'W-152°44'W) and included in the previously reported study of Pacific halibut population structure (IPHC-2025-SRB026-06).

We used both *fst-gl* and *angsd* to obtain pairwise estimates of F_{ST} between males and females and calculate windowed estimates of weighted F_{ST} (50 kb window, 1000 bp step) to facilitate a direct comparison to the F_{ST} estimates obtained by Jasonowicz et al. (2022). Since *fst-gl* also produces estimates of heterozygosity, we were able to compare heterozygosity levels between the sexes which was not possible in the pool-seq analysis (Jasonowicz et al. 2022).

1.1.2.1.3. Performance Benchmarks. We also compared various performance metrics (run time and memory usage) between the *angsd* and *fst-gl* analysis workflows for our real-world use case scenario. *snakemake* (v8.10.7) (Mölder et al. 2025) was used to run our workflow and the benchmarking directive was used to gather performance statistics for each step of the analysis.

1.1.2.2. Results & Discussion. We observed that sequencing depth has a strong influence on the accuracy of the F_{ST} estimates obtained across all methods compared in our analysis, with increases in accuracy observed as sequencing depth increases (Figure 1). Our analysis of simulated data

reinforces that using genotypes called from lcWGR data to estimate population parameters introduces bias into results, resulting in less accurate estimates than methods that account for uncertainty in individual genotypes (Figure 1). Even at higher sequencing depths (≥ 10x) where levels of bias were very small for all methods, estimates from *fst-gl* produced slightly more accurate estimates compared to those obtained from called genotypes (Figure 1).

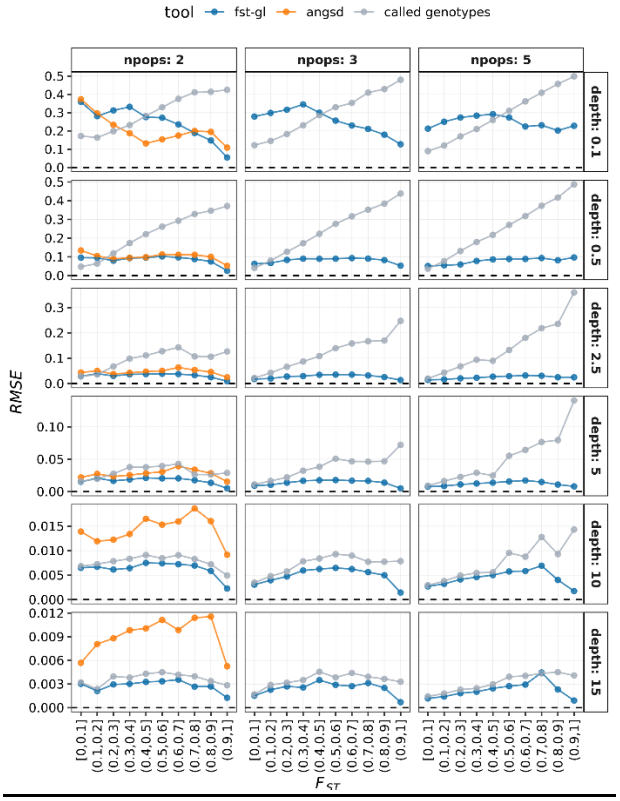

Figure 1. Comparison of RMSE observed at different simulated sequencing depths, number of populations, and methods for obtaining F_{ST} estimates from low-coverage sequence data. Note different y-axis scales for each level of sequencing depth and that angsd can only estimate pairwise F_{ST} between two populations.

When applied to our empirical data, *fst-gl* and *angsd* produced very similar estimates of F_{ST} across Chr09 (Figure 2A). Both methods captured a region of elevated differentiation from 14–26 Mbp on Chr09, with the largest peak of F_{ST} observed just downstream of the putative sex determining gene *bmpr1ba*, similar to Jasonowicz et al. (2022). Pacific halibut exhibit a ZZ/ZW system where females are the heterogametic sex (Drinan et al. 2018), and our analysis supports this notion as shown by the different levels of heterozygosity observed in this region of the genome, with many SNPs fully heterozygous in females (Figure 2B).

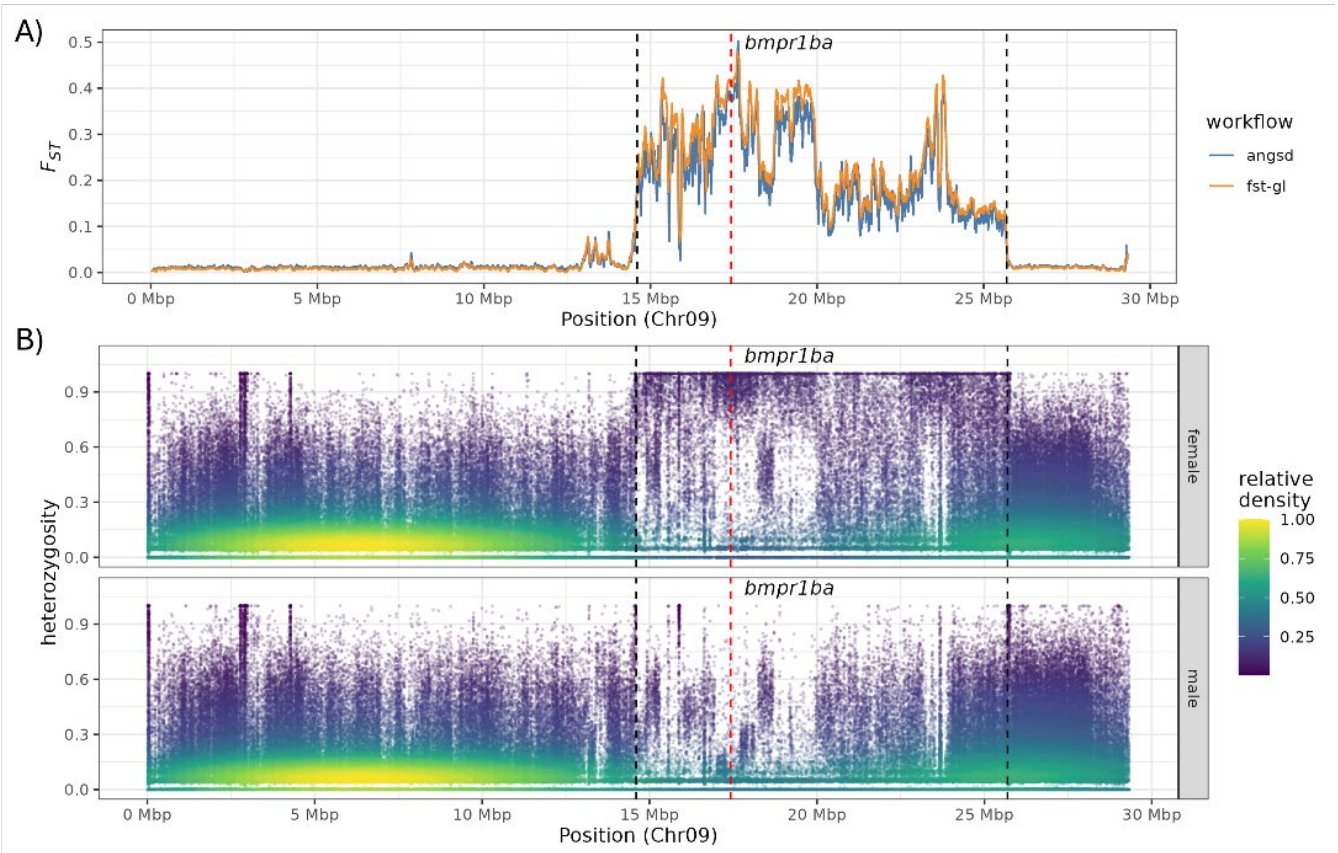

Figure 2. Windowed estimates of pairwise F_{ST} (50kb window, 100 bp step) between male and female Pacific halibut using lcWGR sequence data (2.9x) obtained using methods implemented in angsd and fst-gl (A). Estimates of heterozygosity at individual SNPs obtained from low-coverage sequence data for female (top) and male (bottom) Pacific halibut using fst-gl (B). Vertical dashed lines indicate the sex-linked region (black lines) and the location of bmpr1ba (red line), a candidate master-sex determining gene for Pacific halibut identified by Jasonowicz et al. (2022) using pool-seq.

Finally, we observed a substantial performance improvement of *fst-gl* over *angsd* in terms of both run time and memory usage. In our real-world example that analyzed a single 29.3 million base pair chromosome (Chr09), the total wall time for the *angsd* workflow was 32.53 mins and 19.74 mins for the *fst-gl* workflow, resulting in a 39.3% reduction in total wall time. After genotype likelihoods were obtained, *fst-gl* took only 11.23 seconds to run.

1.1.2.3. Conclusion. Through analysis of simulated and empirical data, we have shown that *fst-gl* reduces bias when estimating F_{ST} from low-coverage sequence data. Furthermore, *fst-gl* offers an improvement in performance over existing methods, especially if genotype likelihoods have already been estimated at SNPs of interest. In addition to developing a method for obtaining estimates of global F_{ST}, we have also added additional functionality that may be useful to future research in this area. First, this new method allows resampling routines to establish significance values under the null hypothesis of no differentiation Second, the ability to query genomic regions for indexed bcf and vcf.gz files enable targeted investigation of genomic

regions. Third, this second feature can also be used to achieve high levels of parallelism by partitioning the analysis into smaller units, further improving efficiency, particularly when working in high performance computing environments. This work is currently being prepared for publication in a leading peer-reviewed journal.

1.2. Genomics-based method for estimating age of Pacific halibut. The primary objective of this project is to develop a genetic method for aging Pacific halibut using fin tissue, a sample that can be easily collected from either live or dead individuals. This method is based on the identification of DNA methylation patterns in fin tissue that are associated with age through the development of an age estimation model (i.e., an epigenetic clock) for Pacific halibut. The first epigenetic clock was developed for humans in 2013 (Horvath, 2013), and it predicted age with great accuracy (r = 0.96) and with a mean aging error (MAE) of 3.6 years. Subsequently, epigenetic clocks have been developed for several fish species that demonstrated improved accuracy (r between 0.84 and 0.99) and lower average MAE (0.87 years, or 3.5% of the total lifespan of the species examined) (reviewed in Piferrer and Anastasiadi, 2023).

Patterns of DNA methylation (i.e. a natural process of regulation of gene expression that consists in the covalent modification of the nucleobase cytosine) in Pacific halibut will be investigated by performing genome-wide DNA methylation at single base-pair resolution using reduced representation bisulfite sequencing (RRBS) by leveraging the high-quality genome assembly available for Pacific halibut (Jasonowicz et al. 2022). This is an efficient and cost-efficient method to identify methylation patterns (i.e., CpG sites) in DNA because it targets bisulfite sequencing to a well-defined set of genomic regions with high CpG density that can be sequenced at high read depth. Age-associated DNA methylation patterns will be modelled to generate an epigenetic age predictor (i.e., epigenetic clock) for Pacific halibut constructed using elastic net penalized regression models that select a group of CpG sites that have a monotonically increasing relationship with age in the selected training data set. By implementing these linear models that select and weight age-correlated CpG sites, chronological age of Pacific halibut will be estimated based on the percentage methylation at these key CpG sites in fin tissue samples.

1.2.1. Methods.

1.2.1.1. Genetic samples used in the development of the epigenetic clock. For developing an epigenetic clock, we selected genetic samples (fin clips) from 249 individual Pacific halibut collected during IPHC's Fishery Independent Setline Survey (FISS) from 2021 to 2024 throughout IPHC Convention Waters (Figure 3) and covering most of IPHC's Regulatory Areas (Table 1). These genetic samples correspond to fish with known ages (read twice by the traditional break and bake aging method) between 6 to 30 years and include 6-10 individual samples (aiming at equal number of males and females) per year of age (Figure 4).

Table 1. Number of FISS samples by year and IPHC Regulatory Area with RRBS sequence data generated for the development of the Pacific halibut epigenetic clock.

Year	2A	2B	2C	3A	3B	4A	4B	4D	Total
2021	1	12	12	3	0	3	1	0	32
2022	2	22	17	20	2	2	3	1	69
2023	16	32	25	14	10	0	0	0	97
2024	0	15	26	7	1	0	0	2	51
Total	**19**	**81**	**80**	**44**	**13**	**5**	**4**	**3**	**249**

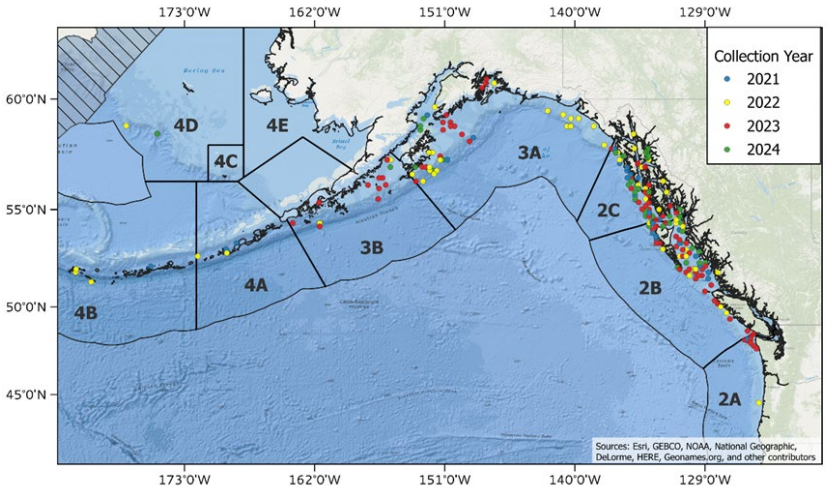

Figure 3. Map showing the collection locations of the samples with RRBS sequence data generated for the development of the Pacific halibut epigenetic clock.

Figure 4. Histograms showing the number of female (top) and male (bottom) genetic samples with RRBS sequence data generated for the development of the Pacific halibut epigenetic clock.

1.2.1.2. Genetic samples used in testing out-of-sample interannual predictive performance. For testing out-of-sample interannual predictive performance of the epigenetic clock,

we selected genetic samples (fin clips) from 46 individual Pacific halibut collected in commercial landings from 2017 to 2020 covering most of IPHC's Regulatory Areas (Table 2). These genetic samples correspond to fish with known ages (read twice by the traditional break and bake aging method) between 6 to 27 years (Figure 5).

Table 2. Number of market samples by year and IPHC Regulatory Area submitted for RRBS sequencing on the Aviti sequencing platform for out-of-sample validation of the Pacific halibut epigenetic clock.

Year	2C	3A	3B	4A	4B	4C	4D	Total
2017	0	0	0	4	4	0	4	12
2018	1	3	2	0	5	0	1	12
2019	0	3	2	3	2	1	0	11
2020	3	4	4	0	0	0	0	11
Total	**4**	**10**	**8**	**7**	**11**	**1**	**5**	**46**

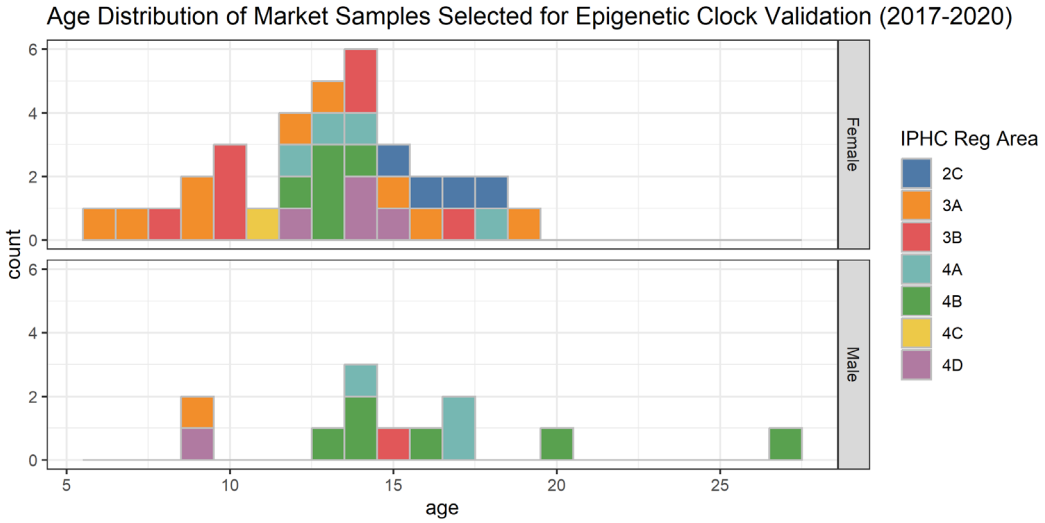

Figure 5. Age distribution of genetic samples from commercial landings ("market samples") selected for out-of-sample validation of the Pacific halibut epigenetic clock.

1.2.1.3. Development of a bioinformatic workflow. A bioinformatic workflow was developed to process reduced representation bisulfite sequencing (RRBS) data in house (Figure 6). Prior to start processing the raw sequence data, a reference genome was prepared for bisulfite alignments with the Pacific halibut reference genome (Jasonowicz et al., 2022; GCF_022539355.2) and control methylated and unmethylated sequences (see below) using the software Bismark.

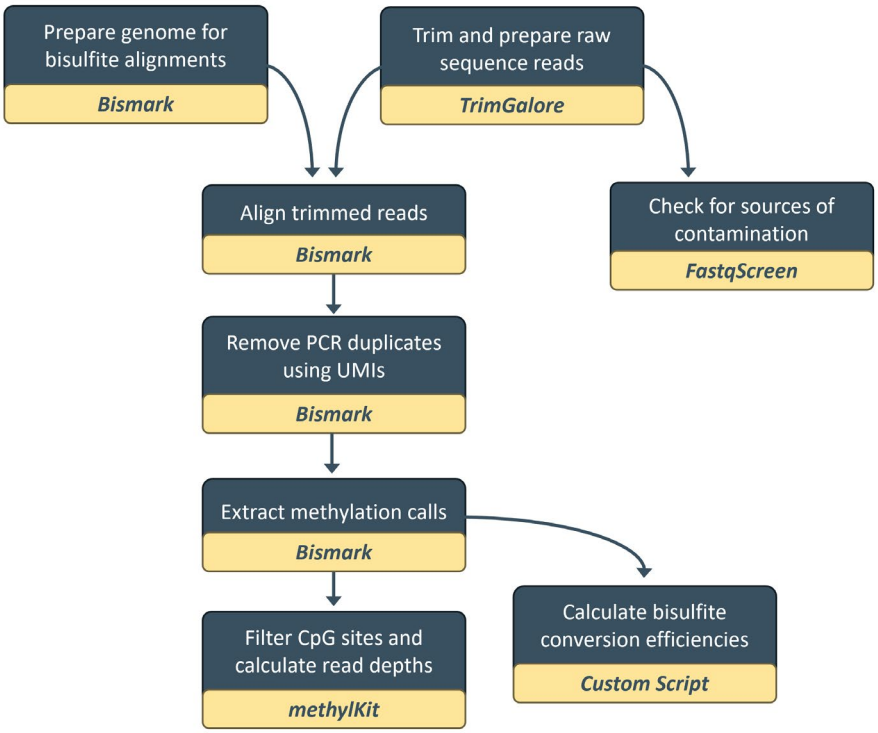

Figure 6. Basic bioinformatic workflow for processing RRBS data. The blue boxes describe each step and the software used is indicated in the yellow boxes.

1.2.1.4. Reduced representation bisulfite sequencing: library preparation and sequencing. High-quality genomic DNA was extracted from individual fin clips and purified using the DNeasy Blood and Tissue Kit (Qiagen). Genomic DNA was used to construct individual RRBS libraries using the Premium RRBS Kit V2 (Diagenode) following the manufacturer's specifications. In brief, RRBS libraries were prepared by digesting genomic DNA with the methylation sensitive restriction enzyme MspI, and the resulting DNA fragments were treated with bisulfite to convert non-methylated cytosines into uracils through chemical deamination, leaving methylated cytosines unaffected. A subsequent PCR amplification step converted uracils into thymines. Constructed RRBS libraries were first assessed for concentration and fragment size distribution by TapeStation (Agilent). Subsequently, individual libraries used for the development of the epigenetic clock were pooled and sequenced on an Illumina NovaSeq-X sequencing platform in 1.5B flow cells at 2 x 50PE at the Functional Genomics Facility at the University of Chicago (Chicago, IL). In addition, individual libraries used for the out-of-sample validation of the epigenetic clock were pooled and sequenced on an Element Aviti sequencing platform at 2 x 75PE at the Functional Genomics Facility at the University of Chicago (Chicago, IL).

1.2.1.5. Sequencing data analysis and methylation calling. Prior to analysis, raw sequence reads will be quality checked using *FastQC* (Andrews et al., 2015) to ensure consistent

quality across sequencing runs and to 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. The raw sequence reads will then be processed to remove Illumina adapter sequences and low quality reads using *Trim Galore!* (https://github.com/FelixKrueger/TrimGalore), a trimming tool designed specifically for RRBS data. Trimmed sequence reads will be aligned to a bisulfite converted index of the Pacific halibut reference genome (RefSeq assembly accession: GCF_022539355.2) excluding the sex chromosome (Chr09; Jasonowicz et al., 2022) to discard possible sex-associated methylation signals, using *bismark* (Krueger and Andrews, 2011) allowing for one mismatch. Having a high-quality reference genome available for Pacific halibut is a major benefit to this study as constructing one is costly and time consuming. Furthermore, the Pacific halibut genome has been annotated so that the locations and identity of genes are known, enabling the functional significance of methylated CpG sites present in protein coding gene regions to be inferred. 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 methylation module in *BS-Seeker2* (Guo et al., 2013) with default settings will be used for methylation calling. For all identified CpG sites, percentage methylation will be calculated as the percentage of the number of methylated reads over the number of total reads with a 95% confidence interval. Typically, RRBS produces in the order of hundreds of thousands of CpGs (Anastasiadi and Piferrer, 2023). CpG sites with at least 20x coverage and with methylation levels in > 90% of the samples will be used for downstream analyses.

1.2.1.6. Development of an age predicting model for Pacific halibut. The sequenced genetic samples will be randomly assigned to a training (200 samples) or a testing data set (50 samples) following an 80/20 data split. Sample assignments will be conducted using *caret* to maintain equal sex ratios in each data set. The training set will be used to fit the model and the testing set will be an independent set of data that will be used to evaluate the model fit.

The relationship between otolith-derived age and percent methylation across age-correlated CpG sites in the training data set will be characterized by performing elastic net penalized regression analysis using the R package *glmnet* (Friedman et al., 2010) set to a 10-fold cross validation with an α-parameter of 0.5 and automatically selecting the optimal penalty parameter (λ). We expect that the age-predicting model will retain in the order of a few hundred CpG sites with a low λ value. The performance of the model in the training and testing data set will be evaluated using Pearson correlations (i.e. measuring the degree of correlation between chronological and estimated age) as a measure of accuracy, MAE as a measure of precision (i.e., how well the model fits the actual data), and relative error rates (Piferrer and Anastasiadi, 2023). Comparison of MAE between the training and testing data sets will inform on the potential overfit of the model constructed using the training data set. The linear relationship between predicted and chronological (i.e., otolith-derived) age will be

visually represented and additional patterns in the data will be visualized using principal component analysis (PCA).

1.2.1.7. Identification of the genomic location of age markers. The Pacific halibut genome annotation (NCBI link) will also be used to determine if any functional genes are located within 400 bp of model selected CpG sites. This will inform whether clock CpG sites are proximal to specific annotated genes and whether methylation at those particular sites could have functional significance.

1.2.2. Results.

All aged fin clips used for the construction of the epigenetic clock have been processed for DNA extraction. The obtained genomic DNA was quantified, and all samples yielded enough high-quality genomic DNA to proceed with individual library construction. Library preparation was successfully completed for all 249 individual aged samples. RRBS libraries were combined into 6 pools of 41-42 libraries each and sequenced.

Based on initial processing of the six sequenced library pools, the average PCR duplication percentage was 43.08% across all pools, ranging from 33.77% to 64.82%. The average number of deduplicated reads per individual sample ranged from 5,288,760 reads to 7,264,685 reads. Average methylated controls were 1.58% across all libraries, ranging from 1.40% to 1.86%. Average methylated levels in the control samples below 2% indicate minimal false detection of unmethylated cytosines. On the other hand, average unmethylated controls were 99.25% across all libraries, ranging from 98.95% to 99.44%, providing a positive control to assess the efficiency of bisulfite conversion of DNA. The total number of obtained deduplicated reads that will be used for downstream analyses was 1,590,925,852 (i.e. over 1.5 billion reads) (Table 3).

Table 3. Initial metrics for all six library pools composed of individual libraries from project samples that will be used to develop an epigenetic clock.

Pool	Number of samples processed	Average PCR duplication rate (%)	Average number of remaining reads/sample	Average of methylated control (%)	Average of unmethylated control (%)	Sum of deduplicated reads (reads remaining)
1	42	64.82	5,288,760	1.40	98.95	222,127,927
2	41	49.56	6,506,924	1.65	99.20	266,783,900
3	42	34.99	7,264,685	1.64	99.37	305,116,779
4	42	38.35	6,509,939	1.86	99.44	273,417,435
5	41	33.77	6,443,062	1.41	99.29	270,608,611
6	41	36.99	6,167,590	1.51	99.25	252,871,200
Total	**249**	**43.08**	**-**	**1.58**	**99.25**	**1,590,925,852**

Current work is focused on processing the sequence data through the bioinformatic workflow depicted in Figure 6.

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 some of 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 is finalizing the processing of genetic samples from the 2025 aged commercial landings.

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

2.2.1. Update of maturity schedules based on histology and calibrated visual data. The IPHC Secretariat provided an update on spatial and temporal patterns in maturity ogives by Biological Region from 2022 to 2024, and a revised coastwide maturity ogive using histological based data in IPHC-2025-SRB026-06. At present, the IPHC Secretariat is preparing a manuscript for publication in a peer-reviewed journal describing the temporal and spatial changes in histology-derived maturity ogives and ovarian developmental stages. Furthermore, the IPHC Secretariat developed a calibration between histological and visual maturity ogives from the 2022-2024 data and produced calibrated maturity ogives based on FISS visual maturity data from 2002-2024. During this 23-year period, we observe significant shifts in the age at 50% (A_{50}) maturity (Figure 7). These results evidence two temporal shifts, one characterized by a gradual increase in the A_{50} (i.e. females maturing at a later age) from 2004 to 2016, and a second one characterized by a sharp decrease in A_{50} from 2017 to 2022 (i.e. females rapidly maturing at an earlier age). Studies are planned to identify possible drivers of these temporal shifts in maturity-at-age in female Pacific halibut.

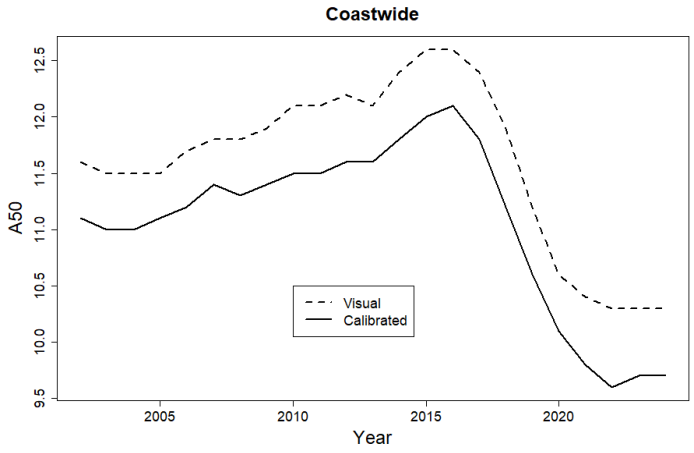

Figure 7. Coastwide A_{50} calculated from visual and calibrated maturity ogives from 2002 to 2024.

The IPHC Secretariat continued to collect ovarian samples for maturity in the 2025 FISS. 2025 FISS sampling resulted in the successful collection of 1,276 ovarian samples from all four Biological Regions: 275 samples in Biological Region 2, 380 samples in Biological Region 3, 355 samples in Biological Region 4, and 266 samples in Biological Region 4B. These samples will allow us to further investigate both spatial and temporal differences in histological-based female Pacific halibut maturity. In 2026, the IPHC Secretariat will continue to collect maturity samples across the entirety of the FISS by targeting the collection of 400 ovarian samples in Biological Region 2, 600 samples in Biological Region 3, 400 samples in Biological Region 4, and 300 samples in Biological Region 4B (Figure 8).

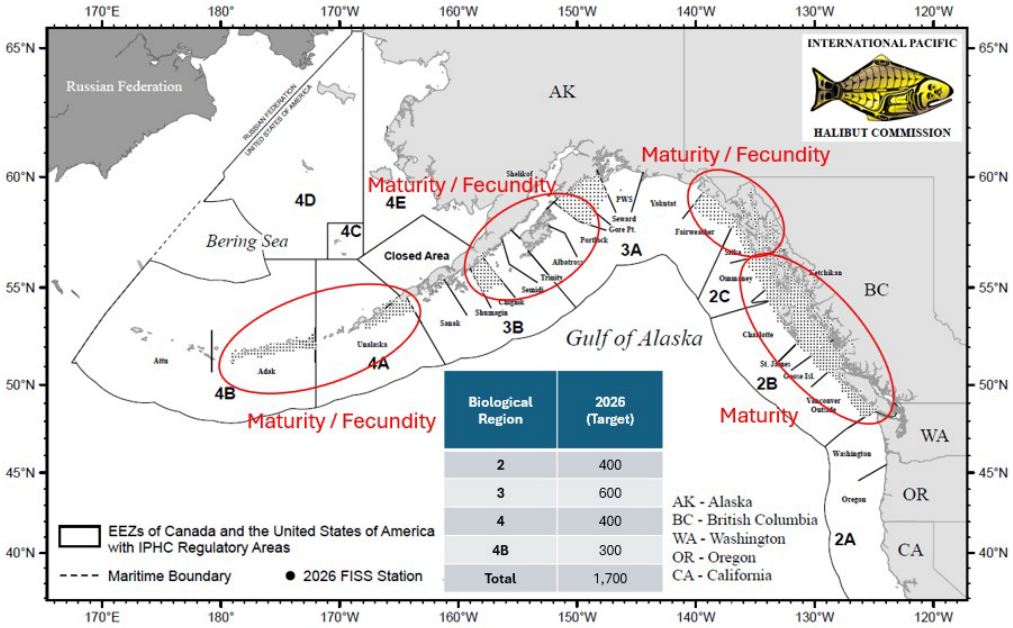

Figure 8. Coastwide map of sample areas and targets for maturity and fecundity collection in 2026 FISS.

2.2.2. Fecundity estimates. The IPHC Secretariat has initiated studies that are aimed at improving our understanding of Pacific halibut fecundity. These studies 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 SA and management reference points. Fecundity determinations will be conducted using the auto-diametric method (Thorsen and Kjesbu, 2001; Witthames et al., 2009) and 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 the development and application of the auto-diametric method to estimate fecundity in female Pacific halibut have been collected during the FISS in 2023, 2024 and 2025, as well as two special collections in IPHC Regulatory Area 2B in 2024 and 2025 (Figure 9). In 2023, sampling was conducted only in Biological Region 3, with a total of 452 fecundity samples collected. In 2024, sampling was conducted in Biological Regions 2 and 4, with 149 and 359 fecundity samples collected, respectively. In the Fall (Oct/Nov) of 2024, 271 additional fecundity samples targeting large females (85-200+ cm in fork length) were collected in Biological Region 2. This sampling was conducted to collect later developing females to help build the auto-diametric curve for fecundity estimations. For 2025, in addition to 878 samples collected in all four Biological Regions in the FISS, 242 fecundity samples were collected in Biological Region 2 in a special project targeting large females during the late summer months. This comprehensive collection of ovarian samples will be used initially for the development of the auto-diametric method, followed by actual fecundity estimations by age and by size (length and weight).

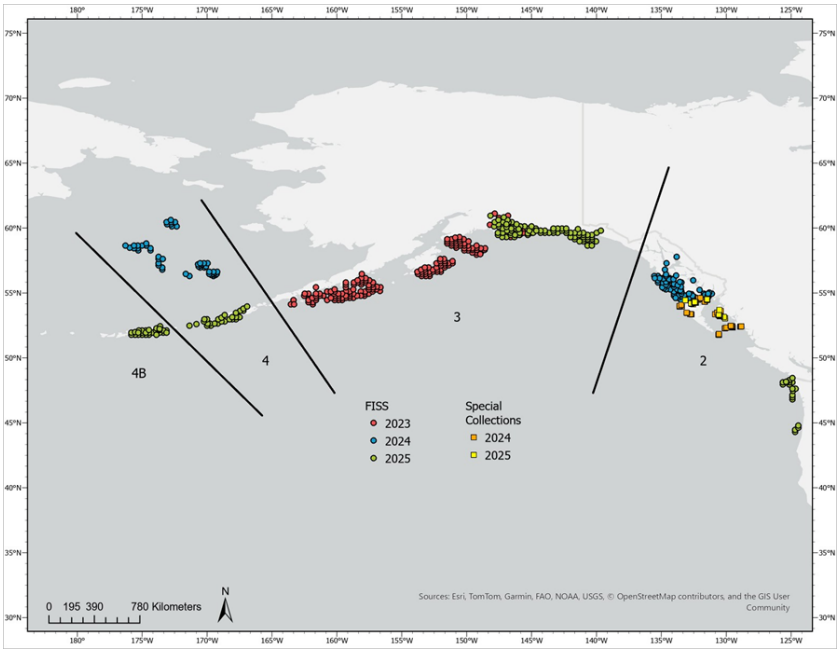

Figure 9. Coastwide map of 2023, 2024 and 2025 samples for fecundity collected in FISS (circle colors), and 2024 and 2025 special collection fecundity samples in IPHC Regulatory Area 2B (square colors).

To examine potential indicators of increased reproductive output of female Pacific halibut, IPHC Secretariat calculated gonadosomatic index (GSI) values for all fecundity samples that have been histologically staged. This includes all FISS samples in 2023 and 2024, as well as the special collection samples in Regulatory Area 2B in 2024 and 2025. GSI was calculated as $GSI = \frac{G}{W} x\ 100$. Where G = total gonad weight (kg), and W = dressed weight (kg). We compared GSI to age (Figure 10) and weight (Figure 11) across four different female ovarian developmental stages (Vtg1, Vtg2, Vtg3, and GVM).

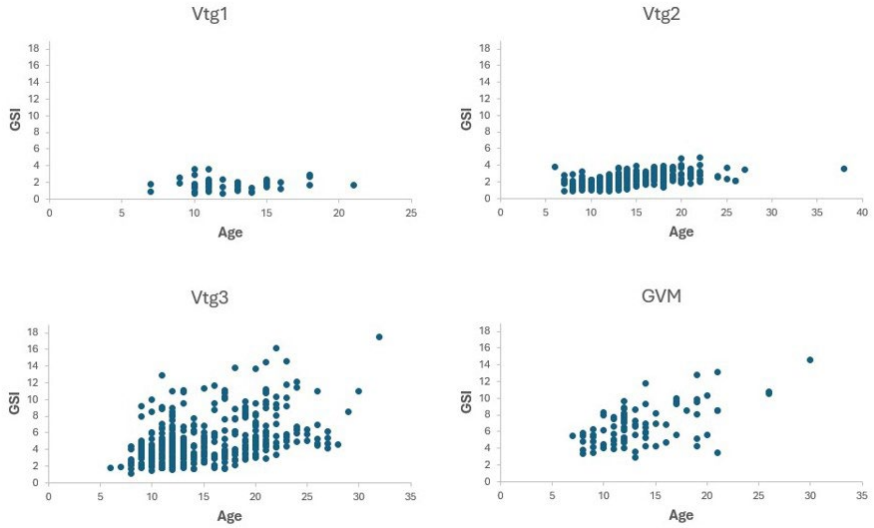

Figure 10. Gonadosomatic index (GSI) of female Pacific halibut according to age and female developmental stage (Vitellogenic 1, Vtg1; Vitellogenic 2, Vtg2; Vitellogenic 3, Vtg3; Germinal Vesicle Migration, GVM).

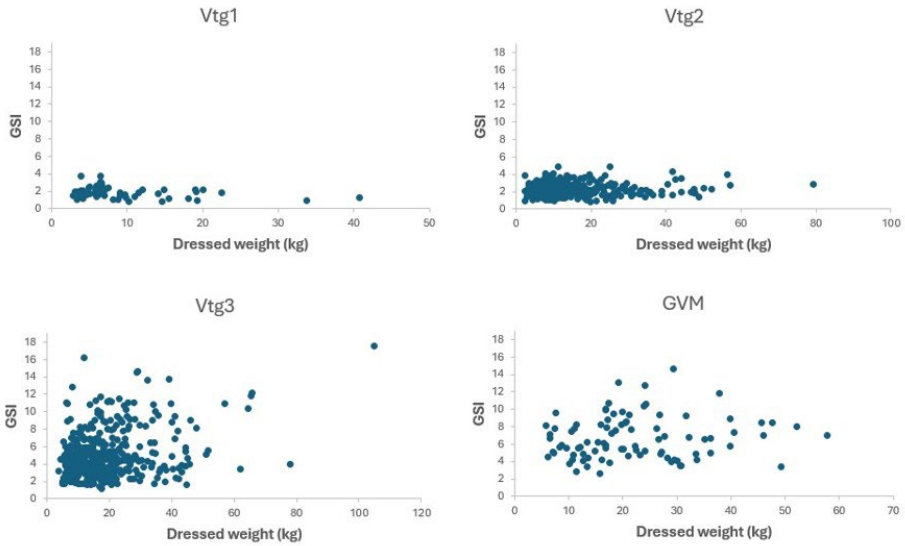

Figure 11. Gonadosomatic index (GSI) of female Pacific halibut according to weight and female developmental stage.

Our preliminary results show no increase in GSI with age for earlier developing females (e.g. Vtg1 and Vtg2), and a slight trend towards an increase in GSI with older females as they progress to the Vtg3 and GVM stages. When comparing GSI to weight, there is no indication of increasing gonad size with overall body weight, with higher variability in later stage developing females (Vtg3 and GVM). These preliminary results suggest that egg output might follow an isometric relationship with weight, which is the current assumption in the Pacific halibut SA.

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. A manuscript describing the results of these studies has been published (Planas et al., 2025).

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, constitute 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 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).

4.1. Estimation of discard mortality rates in the charter recreational sector. Results from a recently completed study investigating discard mortality rates and characteristics of fish captured and released using guided recreational fishery practices are currently being prepared for publication in a peer-reviewed journal.

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 has been 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 Awards NA21NMF4720534 and NA23NMF4720414; Appendix IV). The results and outcomes of the initial pilot phase of this project were reported in the documentation provided for the previous SRB meetings: IPHC-2022-SRB020-08 and IPHC-2024-SRB024-09.

The second phase of this project focused on further refinement and performance characterization of the shuttle device (Figure 12A) in the presence of toothed whales in IPHC Regulatory Area 4A. Field operations occurred from 21-28 May 2025 aboard the F/V Oracle (17.5 m, 58 ft) in the Bering Sea and Aleutian Islands off Alaska in known depredation hotspots and with higher expected Pacific halibut catch rates than seen in the pilot testing.

Eighteen sets were successfully completed, generating 15 sets of shuttle and control catch comparison data. Depredating orcas (identified by eaten or damaged Pacific halibut and by orca presence (Figure 12B, C)) were present at 6 of the paired sets. Camera systems developed by project participants were successfully deployed both on the control gear and on the shuttle itself (exterior and interior forward and reverse views), generating approximately 80 hours of underwater footage combined, enabling us to better quantify shuttle performance and retention rates (Figure 12D). Approximately 70 hours (10/15 paired sets) of the footage has been reviewed in detail, and 4,863 hook status observations have been recorded across all four cameras.

Preliminary results from data comparisons from 10 sets with completed video review suggest that the shuttle achieved good retention of Pacific halibut, and lower rates of retention for other frequently encountered fish (Table 4). Species morphology was likely the predominant reason for this observation and simple modifications to the entry tines and to optimal stopper fit should easily achieve much higher retention rates for these other species. Auto-removal of fish resulted in more severe hook release damage (compared to careful release by fishers at the vessel), which is expected to negatively affect survival outcomes of non-retained specimens.

Table 4. Numbers of fish encountered by the shuttle device that were either excluded, entered and escaped, entered and passed through still on the hook, and/or finally retained on 10 of 15 sets with video footage analyzed to date.

Common Name	Encountered	Excluded	Entered and Escaped	Entered and Passed Through	Retained
Pacific halibut	89	1 (1.1%)	0	8 (9.1%)	80 (90.9%)
Sablefish	160	2 (1.3%)	45 (28.5%)	30 (19.0%)	83 (52.5%)
Pacific cod	124	3 (2.4%)	13 (10.7%)	6 (5.0%)	102 (84.3%)
Rockfish	16	7 (43.8%)	2 (22.2%)	1 (11.1%)	6 (66.7%)
Skate	18	3 (16.7%)	0	2 (13.3%)	13 (86.7%)

Preliminary catch rate comparisons between protected and unprotected skates were mixed, and initial estimates of variability among catch rates within treatments were large. Overall, the shuttle demonstrated good retention capacity (up to 500 kg (1,000 lb) in the trials, with capacity for higher weights). Incidentally, rare footage of Orcas around the groundline at depth was captured.

Further testing of the shuttle device was conducted under typical commercial fishing conditions in the Bering Sea in October 2025 on the same fishing vessel with an IPHC field specialist aboard to assist with data collection. No cameras were used during this phase. The shuttle was only deployed for a total of 4 sets over this effort due to weather challenges and lack of whales present on many fishing days. Upon compilation, data from these sets will be included in the overall catch data analysis.

Data analysis and video review are ongoing. These preliminary results suggest potential for this approach to interrupt the reward cycle underpinning toothed whale depredation.

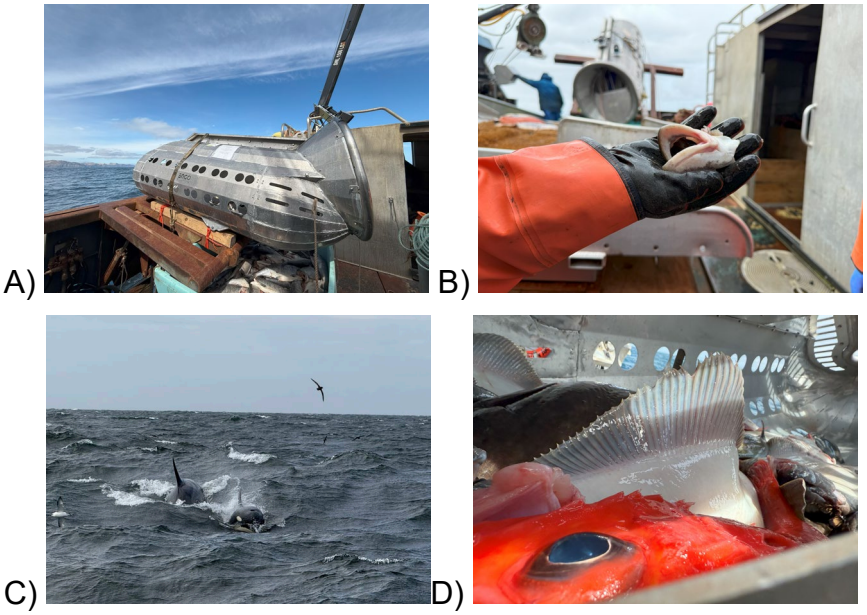

Figure 12. A) Shuttle device in transport. B) Typical evidence (lips only) of depredation. C) Killer whales rapidly approaching the hauling site. D). Catch retained within the shuttle.

RECOMMENDATION/S

That the SRB:

a) **NOTE** paper IPHC-2026-SRB028-06 which provides a response to Recommendations and Requests from SRB027, and a report on current biological research activities contemplated within the IPHC's five-year Program of Integrated Research and Monitoring (2022-26).

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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 current external research grants

Project #	Grant agency	Project name	PI	Partners	IPHC Budget ($US)	Management implications	Grant period
1	**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 2026
2	**Alaska Sea Grant**	Development of a non-lethal genetic-based method for aging Pacific halibut (R/2024-05)	IPHC, Alaska Pacific Univ. (APU)	Alaska Fisheries Science Center-NOAA (Juneau)	$60,374	Stock structure	December 2024-January 2027
Total awarded ($)					**$260,244**		