

Report on Current and Future Biological and Ecosystem Science Research Activities

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PURPOSE

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

BACKGROUND

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

- 1) <u>Migration and Population Dynamics</u>. 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) <u>Reproduction</u>. Studies are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity and fecundity.
- 3) <u>Growth</u>. 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) <u>Mortality and Survival Assessment</u>. 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) <u>Fishing Technology</u>. 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 stock assessment (<u>Appendix II</u>) and the management strategy evaluation process (<u>Appendix III</u>) 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 SRB021 (<u>IPHC-2022-SRB021-</u><u>R</u>):

SRB021–Rec.09 (para. 41) **NOTING** the information on recent wire tagging of Pacific halibut as part of the recreational DMR study and intent to characterize movements of Pacific halibut

among IPHC Regulatory Areas, the SRB **RECOMMENDED** that the data available be summarized to map and analyze existing trends in the data.

A summary of Pacific halibut movement from available data generated during the recreational DMR study will be provided for SRB022.

SRB021–Rec.10 (para. 44) **NOTING** the Secretariat's interest in applications of molecular markers for somatic growth and evaluation of growth patterns, the SRB **RECOMMENDED** that the Secretariat devote attention to annotation of sequence data that may be relevant to understanding spatial, temporal, and demographic (size/age) variation growth and maturation.

The Secretariat is discussing avenues to address the SRB recommendation.

SRB021–Rec.11 (para. 47) **NOTING** the flow chart presented in Figure 1 of paper <u>IPHC-2022-SRB021-09</u>, the SRB **RECOMMENDED** that (i) additional analyses be conducted in areas of unsupervised clustering for individuals, and (ii) estimate measures of genetic variation among individuals within and among sampling groups to characterize interindividual relationships, which could provide further indication of admixture. The coefficients of relationship among individuals within sampling location and levels of pair-wise variance in SNP allele frequency between sampling locations can be used to identify 'source' and 'sink' regions.

The IPHC plans to conduct K-means clustering using individuals principal component scores and perform analyses, a range of vales for K will be tested and model selection criteria (eg. Bayesian information criterion) will be used to select the best fit model. Admixture proportions will also be estimated, and used to infer the true number of genetic groups present in the data. The use of inter-individual measures of relatedness will be explored. Figure 1 in document IPHC-2023-SRB022-09 has been updated to reflect this.

SRB021–Rec.12 (para. 48) The SRB **NOTED** that in the sub-area of Population Genetics and Structure, the Secretariat intends to use Site Frequency Spectral (SFS) analyses. Both selection and population growth can produce similar SFS patterns in data. As such, the SRB **RECOMMENDED** testing using a 'Tajima D' analysis and estimate levels of excess of low frequency SNP alleles within sampling areas (or reporting units).

The IPHC Secretariat has begun incorporating the estimation of Tajima's D for each collection in their analysis of low-coverage whole genome resequencing data. Figure 1 IPHC-2023-SRB022-09 has been updated to reflect this.

SRB021–Rec.13 (<u>para. 49</u>) **NOTING** that Secretariat's intention to use Multiple Dimensional Scaling to visualise inter-individual and inter-location genetic similarity, the SRB **RECOMMENDED** that the Secretariat develop a data baseline of background information at the individual level to better develop hypotheses to explain visual patterns in data.

The biological data and sample attributes for the individuals used for low-coverage whole genome resequencing will be used for this. Relationships between these attributes and the results obtained from the ordination methods (eg. PCA & MDS) planned for this analysis will

be examined, aiding in the interpretation of the resulting visual patterns produced by these methods.

SRB021–Rec.14 (para. 50) **NOTING** the Secretariat's interest in describing linkage relationships, and that descriptions of linkage disequilibrium can be fraught with difficulty in situations of admixture and due to vagaries in breeding structure, the SRB **RECOMMENDED** that the Secretariat explore other literature not cited in <u>IPHC-2022-SRB021-09</u> in this area.

The IPHC Secretariat acknowledges this and will explore additional literature that pertains to this issue to ensure that these analyses are consistent with current literature.

SRB021–Rec.15 (para. 51) The SRB **RECOMMENDED** that the Secretariat (i) develop a rapid screening panel of SNP markers (e.g. GTseq, RADcapture) for future use in Close-Kin Mark recapture (CKMR), population assignment, or other applications (CKMR applications may necessitate the development of microhaplotypes to achieve adequate accuracy in multi-generational pedigree analyses), and (ii) begin developing potential SNP panels and evaluate accuracy of population-based or pedigree-based assignment under scenarios likely to be encountered in future IPHC applications.

The low-coverage whole genome resequencing dataset that the IPHC Secretariat has recently generated could be leveraged to develop application specific marker panels in the future.

SRB REQUESTS

The SRB issued the following requests in their report of SRB021 (IPHC-2022-SRB021-R):

SRB021–Req.05 (<u>para. 37</u>) The SRB **REQUESTED** that the Secretariat amend the priorities under bullet "2. Reproduction" (<u>IPHC-2022-SRB021-09</u>) to include other avenues of investigations such as size/age specific fecundity and spatial variation in same.

Fecundity estimations by size/age and spatial variation are now incorporated as priorities for the research area of Reproduction.

SRB021–Req.06 (<u>para. 39</u>) The SRB **NOTED** and **APPRECIATED** details provided concerning ongoing or anticipated statistical analyses of data that enhanced the SRB's ability to understand and critique methods to expected research outcomes and **REQUESTED** continued consistency in the presentation in these areas.

The Secretariat will continue efforts to provide details of data analysis approaches used and planned.

SRB021–Req.07 (<u>para. 40</u>) **NOTING** the progress update on Migration and Distribution and the specific research goal of creating a map of suitable juvenile Pacific halibut settlement habitat, the SRB **REQUESTED** (i) a clearer statement of the relevance of this research to management, MSE, and/or the stock assessment and (ii) clarification regarding the types of data to be collected and used to determine occupancy (and preference), and by what data sources.

The Secretariat will clarify the relevance and data sources and types used for mapping suitable juvenile habitat in SRB022.

SRB021–Req.08 (<u>para. 43</u>) **NOTING** the Secretariat's interest in growth and size-at-age relationships, the SRB **REQUESTED** clarification of narrative regarding collection of environmental covariate data for projecting future short-term size-at-age trends.

The Secretariat is working towards better defining future work on the influence of environmental covariate data on size-at-age trends.

SRB021–Req.09 (para. 45) **NOTING** the Secretariat's interest in identification of evidence for spatial population structure, and given the IPHC manages stocks on the basis of biological reporting regions, the SRB **REQUESTED** clarification on how the Secretariat may alter assessments if 'functionally isolated components of the population are found'.

A summary of this topic is included in IPHC-2022-SRB022-08.

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 I). 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 II).

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 biophysical transport larval model (Sadorus al. (2021): on а et https://doi.org/10.1111/fog.12512). Although it is known that Pacific halibut initiate their demersal stage as roughly 6-month-old juveniles following the pelagic larval phase and settle in shallow nursery (settlement) areas, near or outside the mouths of bays (Carpi et al. (2021): https://doi.org/10.1007/s11160-021-09672-w), very little information is available on the geographic location and physical characteristics of these areas. Currently, the IPHC Secretariat has 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 (Table 1), has resulted in catch locations for a total of 52,356 Pacific halibut aged 0-2 encountered from 1946 to 2022.

		Number of records					
Data source	Age-0*	Age-1	Age-2	Total	Sites where absent [#]		
IPHC historical projects (prior to 1961)	288	1,494	1,234	3,016			
IPHC/NOAA joint trawl projects (1961- 1996)	40	368	1,032	1,440			
Other IPHC projects	1	6	91	98			
NMFS trawl surveys	76	2,897	16,427	19,400			
DFO commercial	1	113	836	950			
DFO research	1	145	398	544			
NMFS observer program	42	456	23,948	24,446			
ADFG beam trawl surveys	34	677	1,463	2,174			
ACOR research projects (2018-2022)	69			69	393		
NOAA Nearshore Fish Atlas	128	76	2	206	1,037		
Literature	13			13			
Total	693	6,232	45,431	52,356	1,430		

Table 1. The number of age-0, age-1, and age-2 Pacific halibut recorded by data source. * Ages were determined through either direct otolith reading or estimated using fork length (i.e. 0-9 cm = age-0; 10-19 cm = age-1; 20-29 = age-2). #Absence indicates those geographical sites located in what was determined as plausible nursery habitat areas for flatfish in Alaska, based on bottom depth (< 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.

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 will commence in cooperation with Alaska Coastal Observations and Research (ACOR) and University of Alaska Fairbanks to mine data from unpublished sources that was recorded in the 1990s on juvenile Pacific halibut encounters in beach seines. Also in cooperation with ACOR, juvenile Pacific halibut data and genetic samples will be collected from juveniles encountered during non-targeted research taking place around Kodiak Island and the Alaska Peninsula..

- 1.2. Wire tagging of U32 Pacific halibut. The patterns of movement of Pacific halibut among IPHC Regulatory Areas have important implications for management of the Pacific halibut fishery. The IPHC Secretariat has undertaken a long-term study of the migratory behavior of Pacific halibut through the use of externally visible tags (wire tags) on captured and released fish that must be retrieved and returned by workers in the fishing industry. In 2015, with the goal of gaining additional insight into movement and growth of young Pacific halibut (less than 32 inches [82 cm]; U32), the IPHC began wire-tagging small Pacific halibut encountered on the National Marine Fisheries Service (NMFS) groundfish trawl survey and, beginning in 2016, on the IPHC fishery-independent setline survey (FISS). 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 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. <u>Population genomics</u>. 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. <u>Studies to resolve the genetic structure of the Pacific halibut population in the</u> <u>Convention Area</u>. 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 <u>IPHC-2021-SRB018-08</u> and <u>IPHC-2022-SRB021-09</u>.

1.3.1.1. <u>Bioinformatic Processing of Sequence Data and Read Alignment</u>. To ensure consistent quality across the three completed sequencing runs and to identify samples that may not be suitable for further analysis, an initial quality check of the raw sequence reads were conducted using FastQC (Andrews et al. 2015) (Figure 1A). The raw sequence reads for each sample were processed as follows. First, reads were trimmed using multiple filters implemented in fastp (v0.23.2) (Chen et al. 2018). To remove low quality bases at the end of the sequence reads, a sliding window approach was used. If average base quality was less than 20 in a 4 bp window, the remainder of the read was trimmed. Poly-G trimming was also performed to remove poly-G tails that can occur when platforms such as the Illumina NovaSeq that utilize a two-channel sequencing chemistry. Sequencing adapters were trimmed from the raw reads using two approaches implemented in fastp, automatic adapter detection, and by supplying the Illumina Nextera transpose adapter sequences directly.

Genomic Sequencing	Sequencing Run # 1	Sequencing Run # 2	Sequencing Run # 3
Number of samples*	250 (247)	250 (249)	110 (108)
Sequencing Platform	Illumina NovaSeq S4	Illumina NovaSeq S4	Illumina NovaSeq S4
Raw Reads Per Sample (Millions)**	24.8 (11.5-47.2)	24.9 (13.0-51.6)	27.7 (14.1-85.8)
Reads Retained (%)**	71 (62-77)	71 (57-77)	70 (59-75)
Coverage Per Sample (x)**	3.7 (1.8-5.9)	3.7 (1.8-7)	4.2 (1.8-11.6)

Table 2. Summary of raw sequence data and genome alignments for three Pacific halibut IcWGR sequencing runs. Summary statistics are only calculated for samples retained for further analyses (>1.5x coverage) *numbers in parenthesis indicate number of samples with > 1.5x coverage. **expressed as mean (min – max).

Trimmed sequence reads were aligned to the Pacific halibut reference genome (Ref Seq: GCF 022539355.2; Jasonowicz et al., 2022) using bwa-mem2 (v2.2.1) (Vasimuddin et al. 2019) and the resulting alignments were coordinate sorted and converted to the binary alignment map format (BAM) using samtools (v1.16) (Li et al. 2009). Mate-pair information was verified and fixed if needed using Picard tools (v2.27.4) (broadinstitute.github.io/picard/). Next, PCR and optical duplicates were removed using Picard tools, supplying a maximum pixel distance of 2500 to detect optical duplicates. Overlapping read pairs were then clipped using bamUtil (v1.0.15) (Jun et al. 2015). Next, realignment around insertion/deletion elements was performed using GATK (v3.8) (Van der Auwera and O'Connor 2020). Metrics for the resulting alignments were obtained using samtools to summarize the bit values set in the FLAG field of each BAM file and mosdepth (v0.3.3) (Pedersen and Quinlan 2018) was used to calculate the sequencing depth for each individual (Figure 1B). Individuals were removed from the data set if sequencing depth was less than 1.5x in fully assembled autosomal regions of the genome, retaining 604 individuals for further analysis. Sequencing yield per sample was 25.4 million reads on average (range = 11.5 - 85.8 million reads), and an average coverage per sample of 3.43x (Table 2).

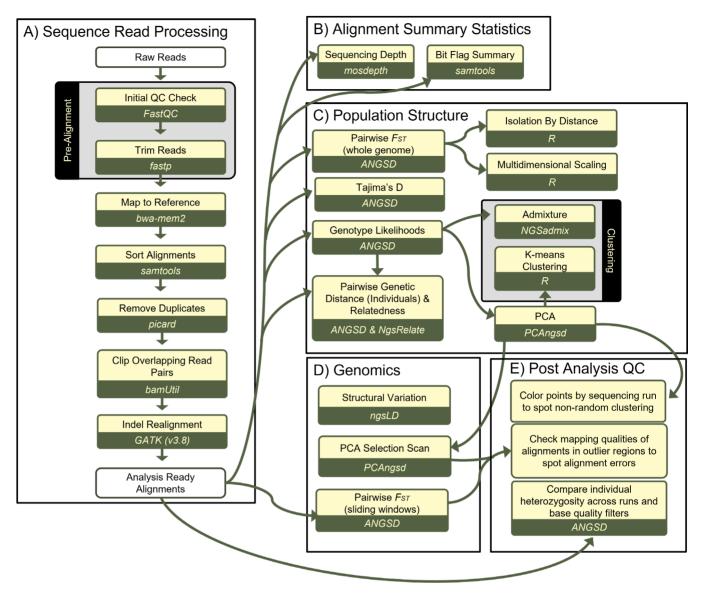


Figure 1. Proposed bioinformatic workflow for the interrogation of low-coverage whole genome sequence data. This diagram tracks the flow of data through the main stages of this project, (A) raw sequence read processing, (B) alignment summaries, (C) analysis of population structure, (D) genomic analyses,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

1.3.1.2. <u>Genotype Likelihood Estimation and SNP Detection</u>. Genotype likelihoods were estimated using the GATK model in ANGSD (Figure 1C). This model assumes that sequencing errors are independent at a given site and the base quality scores accurately reflect the probability of sequencing error. This is in contrast to the other models implemented in ANGSD which may fail to correctly identify low frequency mutations and classify them as sequencing errors instead (Lou and Therkildsen 2021). Low quality base calls and reads with low mapping qualities were removed using ANGSD's input filters. A minimum base quality of 20 and mapping quality of 20. Quality scores are encoded as -10log10(e) where *e* is the probability of an error, therefore a quality score of 20 corresponds to a base call or read alignment accuracy of 99%. Reads mapping to multiple genomic locations were also removed. A p-value threshold of 1e-6 for a site being variable was used and SNPs were only retained if they had a minor allele frequency (MAF) ≥ 0.01 and covered in at least 80% of the individuals. Minimum and maximum sequencing depth filters were imposed and sites were excluded if the total sequencing depth for all samples combined was not between 604 and 3624. This was to exclude regions of the genome that may be poorly covered or might represent repetitive regions. This resulted in 10,230,908 SNPs being identified in fully assembled autosomal regions of the genome, with 4,725,899 SNPs having a MAF ≥ 0.05 .

1.3.1.3. <u>Population Genomics Analyses.</u> Initial results in this area are provided in the Supplementary Documentation. Furthermore, additional analyses are being conducted based on recommendations from the SRB. To accommodate *SRB021– Rec.12*, Tajima's D will be estimated by calculating 1 dimensional site frequency spectra (SFS) for each and sample collection and the realSFS tool included with ANGSD will be used to obtain estimates for Tajima's D in a sliding window fashion (15 Kb windows, 7.5 Kb step) across the genome (Figure 1C). Additionally, interindividual genetic distances will be estimated (*SRB021–Rec.11*) using the single read sampling approach in ANGSD, and NgsRelate (Korneliussen and Moltke 2015) will also be used to obtain relatedness estimates using genotype likelihoods directly (Figure 1C).

2. <u>Reproduction</u>.

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 (<u>Appendix II</u>), and represent the most important biological inputs for stock assessment (please see document <u>IPHC-2021-SRB018-06</u>). 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 (<u>Appendix III</u>).

2.1. Sex ratio of the commercial landings.

The IPHC Secretariat is currently 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 scoring samples for maturity using histological 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 (MARVLS repository for reproductive available R code 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 will continue 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, we will be sampling 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. <u>Fecundity estimations.</u> The IPHC Secretariat have 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 are currently receiving training on this method by experts in the field. The IPHC Secretariat will be collecting ovarian samples for fecundity estimations as part of the 2023 FISS. Sampling will take 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. <u>Growth</u>.

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 our estimates of discard mortality rate in the directed longline fishery (Loher et al., 2022; https://doi.org/10.1002/nafm.10711), 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.

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.

By the end of 2022, of the 281 fish that were tagged with opercular wire tags (243 fish in IPHC Regulatory Area 2C and 38 in IPHC Regulatory Area 3A) 32 tags have been recovered: 30 from IPHC Regulatory Area 2C and 2 from IPHC Regulatory Area 3A. Tags recovered by fisheries are summarized in Table 3 and shown in Figure 2. Ten tags were recovered within 5.5 km (3 nm) of their initial release (5 in year 1, and 5 one year later).

Тад Туре	Release Area	Recovery Year	Ν	Distance Traveled (km)			Days at Large		
				Average	Min	Max	Average	Min	Max
Wire	2C	2021	14	99.2	0.9	571.3	56*	10	112
Wire	2C	2022	16	20.8	0.1	105	396	325	459
Wire	3A	2021	1	0.1	-	-	51	-	-
Wire	3A	2022	1	39.8	-	-	267	-	-
Satellite	3A	2021	7	0.5	0.1	2.4	59	38	70
Satellite Tether	3A	2022	1	23.9	-	-	438	-	-

* 3 with no recovery location information

Table 3. Summary of distances traveled (km) and days at large for fishery recoveries of recreationally captured and released Pacific halibut fitted with a wire opercular tag or a sPAT tag or tether.

For the 80 fish in excellent viability, 76 provided sufficient data for survival analysis. Of the 4 sPAT tags that did not provide data, 2 sPAT tags never reported and 2 tags did not have sufficient data for successful interpretation. Track plots of the spatial release to recovery for these tags can be found in Figure 3, and general recovery metrics can be found in Table 4. The one sPAT listed in Table 3 as being recovered in 2022 represents the recovery of a fish with the anchor tether still attached and whose satellite tag reported successfully after 96 days.

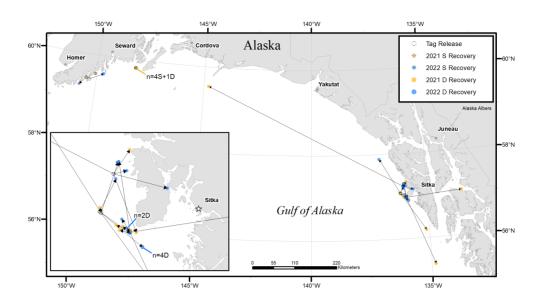


Figure 2. Release and fishery recovery locations of recreationally captured and released Pacific halibut fitted with a wire opercular tag (D) or a sPAT (S) by year of recovery.

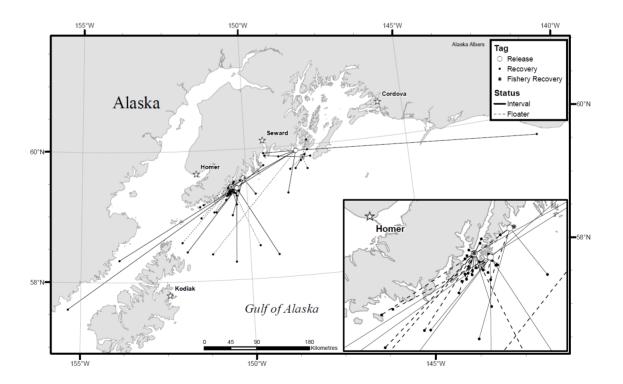


Figure 3. Release and recovery (satellite report or fishery removal) locations of recreationally captured and released Pacific halibut fitted with a sPAT. Status tracks indicate whether a tag reported prematurely (floater) or after the maximum 96-day retention period (interval). The majority of floaters were fully active at the time of tag loss.

Of the seven sPAT fishery recoveries in the first year (Table 4), one was recovered 2.4 km from its release location, and the remaining 6 tags were recovered less than 0.5 km from their release location, so effectively all were recaptured on or about their release location.

Tag Type	Release Ar	Recovery Year	Recovery Type	Ν	Distance Traveled (km)			Days at Large		
					Average Min Max		Average	Min	Max	
Satellite	3A	2021	Broadcast	73*	43.0	0.45	415.6	78.07	3.6	96
Satellite	3A	2021	Fishery	7	0.5	0.1	2.4	59	38	70

* 2 tags failed to report, 2 tags didn't record sufficient information for survival analysis.

Table 4. Summary of distance traveled (km) and days at large for recreationally captured and released Pacific halibut fitted with a sPAT tag.

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. 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; <u>Appendix IV</u>). 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 to the previous SRB meeting: <u>IPHC-2022-SRB020-08</u>.

During the second phase of the project, the IPHC Secretariat has worked with catch protection device manufacturers for the design of two different types of devices for field testing: one based on a modification of Sago's catch protection device (i.e., shuttle) and one based on a modification of a slinky pot (i.e., shroud) deployed on branchline gear. The two different devices will be tested on a chartered fishing vessel off Newport, OR in late May of 2023. The focus of the testing will be 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. These two different devices are the following:

• <u>Shuttle system</u>. Manufactured in Norway by Sago, two shuttle devices were modeled after the Sago Extreme but smaller at 80% size (Figure 4). 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 will be deployed from the surface, during the haulback event.

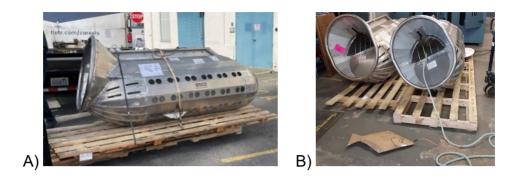


Figure 4. Images of the prototype shuttle devices to be used in this study in profile (A) and frontal view (B).

• <u>Shroud system</u>. Several shroud systems are currently under construction and will consist of a modified slinky pot designed to slide down the branch covering the catch during hauling (Figure 5).

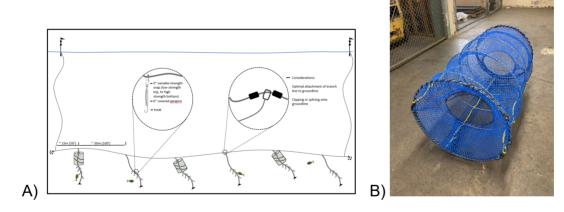


Figure 5. Schematic of shrouded branchline actively fishing on seabed (A) and an unmodified slinky pot to be modified into a shroud (B).

RECOMMENDATION/S

That the SRB:

a) **NOTE** paper IPHC-2023-SRB022-09 which provides a response to requests from SRB021, and a report on current research activities contemplated within the IPHC's five-year Program of Integrated Research and Monitoring (2022-26).

REFERENCES

- Andrews, S., Krueger, F., Seconds-Pichon, A., Biggins, F., and Wingett, S. 2015. FastQC. A quality control tool for high throughput sequence data. Babraham Bioinformatics. Babraham Institute 1(1): 1. Available from <u>http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ https://www.bioinformatics.babraham.ac.uk/projects/fastqc/</u> \% 0Ahttp://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/ [accessed.
- Carpi, P., Loher, T., Sadorus, L., Forsberg, J., Webster, R., Planas, J.V., Jasonowicz, A., Stewart, I. J., Hicks, A. C. Ontogenetic and spawning migration of Pacific halibut: a review. Rev Fish Biol Fisheries. 2021. 31: 879-908. <u>https://doi.org/10.1007/s11160-021-09672-w</u>
- Chen, S., Zhou, Y., Chen, Y., and Gu, J. 2018. Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34(17): i884--i890. doi:10.1093/bioinformatics/bty560.
- 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.
- 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. <u>doi: https://doi.org/10.1111/jfb.14551</u>
- 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
- 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* (In press). DOI: <u>https://doi.org/10.1111/1755-0998.13641</u>.
- Jun, G., Wing, M.K., Abecasis, G.R., and Kang, H.M. 2015. An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. Genome Research 25(6): 918--925. doi:10.1101/gr.176552.114.
- Korneliussen, T.S., and Moltke, I. 2015. NgsRelate: a software tool for estimating pairwise relatedness from next-generation sequencing data. Bioinformatics 31(24): btv509. doi:10.1093/bioinformatics/btv509.
- Li, H. 2018. Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics 34(18): 3094--3100. doi:10.1093/bioinformatics/bty191.
- Lou, R.N., and Therkildsen, N.O. 2021. Batch effects in population genomic studies with lowcoverage whole genome sequencing data: Causes, detection and mitigation. Molecular Ecology Resources(July): 1--15. doi:10.1111/1755-0998.13559.
- Pedersen, B.S., and Quinlan, A.R. 2018. Mosdepth: quick coverage calculation for genomes and exomes. Bioinformatics 34(5): 867--868. doi:10.1093/bioinformatics/btx699.
- 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: <u>https://doi.org/10.1111/fog.12512</u>

- 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: <u>IPHC-2018-AM094-10</u>.
- 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.
- Van der Auwera, G.A., and O'Connor, B.D. 2020. Genomics in the Cloud: Using Docker, GATK, and WDL in Terra (1st Edition). 1 ed. O'Reilly Media.
- Vasimuddin, M., Misra, S., Li, H., and Aluru, S. 2019. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. 314--324. doi:10.1109/IPDPS.2019.00041.
- 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



<u>APPENDIX I</u>

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

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



<u>APPENDIX II</u>

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

SA Rank	Research outcomes	Relevance for stock assessment	Specific analysis input	Research Area	Research activities
	Updated maturity schedule		Will be included in the stock assessment, replacing the current schedule last updated in 2006		Histological maturity assessment
1. Biological	Incidence of skip spawning	Scale biomass and	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
input	Fecundity-at-age and -size information	reference point estimates	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	Reproduction	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	Population structure
3. Biological	Assignment of individuals to source populations and assessment of distribution changes	Improve estimates	Will be used to define management targets for minimum spawning biomass by Biological Region	Genomics	Distribution
input	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	Sex ratio-at-age	Scale biomass and	Annual sex-ratio at age for the commercial fishery fit by the stock assessment		Sex ratio of current commercial landings
and processing	Historical sex ratio-at-age	fishing intensity	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		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		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	Improve estimates of unobserved mortality	May reduce discard mortality, thereby increasing available yield for directed fisheries	SURVIVA	Best handling practices: recreational fishery

<u>APPENDIX III</u>

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

MSE Rank	Research outcomes	Relevance for MSE	Research Area	Research activities
1. Biological parameterization and	Improved understanding of larval and juvenile distribution	Improve parametization of the	Migration	Larval and juvenile connectivity studies
validation of movement estimates	Stock structure of IPHC Regulatory Area 4B relative to the rest of the Convention Area	Operating Model		Population structure
2. Biological parameterization and	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	Genetics and Genomics	Distribution
validation of recruitment variability and 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	Identification and application of markers for growth pattern evaluation			
parameterization and validation for growth	Environmental influences on growth patterns	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
projections	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



INTERNATIONAL PACIFIC HALIBUT COMMISSION

IPHC-2023-SRB022-09

APPENDIX IV

Summary of active 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	\$193,685	Stock structure	December 2021- January 2024
		Total awarded (\$)	\$293,385				