Report on Current and Future Biological and Ecosystem Science Research Activities

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, 20 AUGUST 2021)

PURPOSE

To provide the Scientific Review Board with a description of progress on IPHC's five-year Biological and Ecosystem Science Research Plan (2017-21).

BACKGROUND

The primary biological and ecological research activities at IPHC that follow Commission objectives are identified and described in the IPHC Five-Year Biological and Ecosystem Science Research Plan (2017-21). These activities are integrated with stock assessment and the management strategy evaluation processes (Appendix I) and are summarized in five main areas, as follows:

1) Migration and Distribution. Studies are aimed at further understanding reproductive migration and identification of spawning times and locations as well as larval and juvenile dispersal.
2) Reproduction. Studies are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity.
3) Growth and Physiological Condition. Studies are aimed at describing the role of some of the factors responsible for the observed changes in size-at-age and to provide tools for measuring growth and physiological condition in Pacific halibut.
4) Discard Mortality Rates (DMRs) and Survival. Studies are aimed at providing updated estimates of DMRs in both the longline and the trawl fisheries.
5) Genetics and Genomics. Studies are aimed at describing the genetic structure of the Pacific halibut population and at providing the means to investigate rapid adaptive changes in response to fishery-dependent and fishery-independent influences.

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

SRB RECOMMENDATIONS AND REQUESTS

The SRB issued the following recommendations and requests in their report of SRB018 (IPHC-2021-SRB018-R):

*Request 1 (*SRB018–Req.08 (para. 39))

> ***"****The SRB* ***REQUESTED*** *that the IPHC Secretariat focus future reproductive biology studies on the development of updated regulatory area-specific maturity ogives (schedules of percent maturity by age).."*

The IPHC Secretariat is focusing studies on the development of updated maturity ogives (please see Section 2.2.2., page 04)

*Request 2 (*SRB018–Req.09 (para. 40))

> ***"**The SRB **REQUESTED** that the IPHC Secretariat provide information on the age distribution of all females collected to characterize reproductive development throughout the annual cycle in order to refine efforts to identify potential skip-spawning females."*
>
> The IPHC Secretariat has provided the age distribution of Pacific halibut females collected throughout an annual cycle to characterize reproductive development in this document (please see Section 2.2.4, page 05).

*Request 3 (*SRB018–Req.10 (para. 41))

> ***"**The SRB **REQUESTED** that planned studies on fecundity assessment are prioritized and that the sampling design be developed in coordination with the SA to ensure that the results are as informative as possible for assessment purposes. Effective sample stratification along age, weight and length gradients that maximise the contrast in the effect of these variables will be key to precise estimates of fecundity. Oocyte diameter in contrast may be a important covariate to provide but cannot be used in stratification. The primary goal of the fecundity research should be to estimate the exponent of the fecundity vs. weight relationship for incorporation in the SA"*
>
> The Secretariat has prioritized studies on fecundity assessment. Sampling design considerations are currently being evaluated and will be discussed at SRB019.

*Request 4 (*SRB018–Req.11 (para. 42))

> ***"**The SRB **REQUESTED** that the Secretariat explicitly describe how the gene regions identified as 'over' or 'under' expressed would be used. For example, research has yet to determine mechanisms for transcriptional differences other than there is over- or under-representation of mRNA transcripts associated with different treatment groups (e.g. warm vs. cool water) from a heterogeneous set of individuals collected from a single location. The Secretariat has not yet established that results can be generalized to other regions in the species range. Neither has the transcriptional patterns been generalized to individuals of different size/age. These questions should be investigated."*

The IPHC Secretariat is currently working towards fulfilling this request.

*Request 5 (*SRB018–Req.12 (para. 43))

> ***"**The SRB **REQUESTED** that the Secretariat use these gene regions and align sequences to the whole genome sequence data. Specifically, the Secretariat should investigate whether there is sequence variability within gene coding regions or in regions around gene coding regions that may be transcriptional modifiers (e.g. promoters). If genetic variation exists in or near these genes, these variable base pair position(s) (i.e. single nucleotide polymorphisms*

or SNPs) should be incorporated in other aspects of the Secretariat research; for example for research activities under the Migration and Population Dynamics Research area."

The IPHC Secretariat is currently working towards fulfilling this request and initial efforts are described in this document (please see Section 3.2) and results will be presented at SRB019.

*Request 6 (*SRB018–Req.13 (para. 44))

***"**The SRB **REQUESTED** that the analysis of seasonal patterns in gonad development be explicitly tied to the development/improvement of the maturity ogive (the vector of proportion mature at age that SA requires)."*

The IPHC Secretariat has explicitly tied the analysis of seasonal patterns in gonad development to the development/improvement of the maturity ogive (Appendix IV).

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Migration and Distribution.

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

   1.1. Larval distribution and connectivity between the Gulf of Alaska and Bering Sea.
   Principal Investigator: Lauri Sadorus (M.Sc.)

   No updates to report.

   1.2. Wire tagging of U32 Pacific halibut.
   Principal Investigator: Joan Forsberg (B.Sc.)

   No updates to report.

2. Reproduction.

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

research outcomes for the management and strategy evaluation (MSE) process is in the improvement of the simulation of spawning biomass in the Operating Model (Appendix III).

2.1. Sex ratio of the commercial landings.
Principal Investigator: Anna Simeon (M.Sc.)

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

2.2. Maturity assessment.
Principal Investigator: Josep Planas (Ph.D.)

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) histological assessment of the temporal progression of female developmental stages and reproductive phases throughout an entire reproductive cycle; 2) update of maturity schedules based on histological-based data; 3) fecundity determinations, and 4) investigations on skip-spawning.

2.2.1. Histological assessment of the temporal progression of female developmental stages and reproductive phases throughout an entire reproductive cycle. Details on sample collection, histological protocols and analyses, and results on reproductive developmental characteristics by month, by ovarian developmental stage and by reproductive phase in Pacific halibut females were provided in document IPHC-2021-SRB018-08. A manuscript describing the temporal progression of reproductive development in female Pacific halibut and the relationship of reproductive development with physiological condition indicators (e.g. hepatosomatic index, Fulton's condition factor, fat content) is currently being finalized for submission to a peer-reviewed journal (Fish et al., in preparation).

2.2.2. Update of maturity schedules based on histological-based data. An important outcome of the work conducted on the seasonal characterization of female reproductive development (Section 2.2.1; Appendix IV) has been to determine that the months of July and August represent an appropriate time during the FISS for the collection of ovaries for updating maturity schedules and fecundity estimations. The IPHC Secretariat is currently investigating various sampling designs for ovarian sample collection during the 2022 FISS effort.

2.2.3. Fecundity estimations. Methods for fecundity determinations are currently being researched and will be selected based on accuracy and feasibility for Pacific halibut field collections. Ovaries from three females that are classified as maturing (stage 2) have been collected during the 2021 FISS for testing selected fecundity assessment methods in the Fall of 2021.

2.2.4. Investigation on skip spawning. As reported in document IPHC-2021-SRB018-08, only eight out of 180 Pacific halibut females (4.4%) collected during the spawning capable phase (August to February) showed histological signs of reproductive delay and were only identified in the months of November (1) and December (7). Ages of these females were 10 yrs (1), 11 yrs (2), 12 yrs (2), 14 yrs (1) and 15 yrs (2). The age distribution of the entire collection of aged Pacific halibut females collected between September 2017 and August 2018 and used for characterizing seasonal reproductive development (n=342; Section 2.2.1) is shown in Figure 1. Therefore, the proportion of sampled females that showed reproductive delay was 11.1% at 10 yrs (n=9), 12.5% at 11 yrs (n=16), 4.7% at 12 yrs (n=42), 1.7% at 14 yrs (n=57), and 3.6% at 15 yrs (n=56). Given that 11.6 years is the estimated average age at which 50% of female Pacific halibut are sexually mature (Stewart and Webster, 2021), with nearly all fish estimated to mature by approximately age 17, it cannot be fully determined if the observed reproductive delays in eight females of ages 10 to 15 represent a delay of immature females entering puberty (initiation of the first reproductive cycle) or a delay in the initiation of a given reproductive cycle after having successfully spawned previously (i.e. mature females skipping a reproductive cycle). A larger sample size during the spawning capable phase (ideally during the late FISS season) would be needed to further characterize the observed reproductive delays, likely in combination with the work proposed in Section 2.2.2.

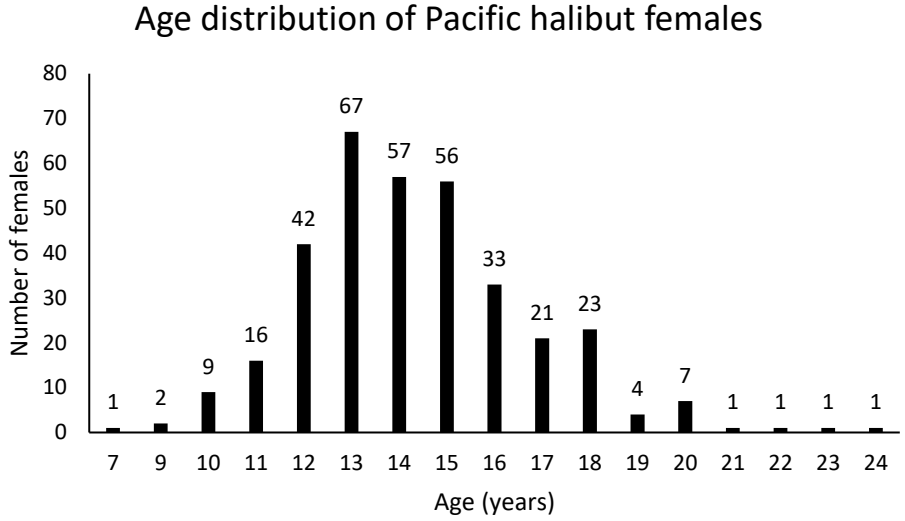

Figure 1. Distribution of ages of Pacific halibut females collected from September 2017 until August 2018 for analyses of reproductive progression over an annual reproductive cycle.

3. Growth.
Principal Investigator: Josep Planas (Ph.D.)

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.

3.1. Identification and validation of physiological markers for somatic growth. A manuscript describing the procedures and results of this study is in preparation (Planas et al., in preparation; provided previously).

3.2. Application of molecular growth markers for evaluating growth patterns in the Pacific halibut population. The IPHC Secretariat is conducting a test of a set of real time qPCR-validated gene markers (alpha actin, asparagine synthetase, fast muscle myosin heavy chain, myosin regulatory light chain 2, ornithine carbamoyltransferase, fructose-2,6-bisphosphatase) on skeletal muscle samples from juvenile Pacific halibut. These muscle samples correspond to a total of 30 age-matched individuals (4 years-old) of different sizes and are being used to test the hypothesis that size differences in age-match individuals are reflected by differences in the mRNA expression levels of growth marker genes, as assessed by real time qPCR. The muscle samples that are currently being processed correspond to three size categories of juvenile Pacific halibut: 30-36 cm (N=10), 44 cm (N=10) and 53-61 cm (N=10) in fork length.

In response to SRB018–Req.12 (para. 43), The IPHC Secretariat has selected ten putative growth marker genes that showed significant down-regulation during temperature-induced growth suppression and significant up-regulation during temperature-induced compensatory growth stimulation at the mRNA level in skeletal muscle from juvenile Pacific halibut, as described in the supplementary data provided

for SRB018-08 (Table 6). These transcripts were mapped to the Pacific halibut genome to identify the presence of sequence variability (SNPs) within coding and non-coding regions of these genes. In brief, Minimap2 (v2.17) (Li 2018) was used to align the assembled transcripts associated with the putative growth marker genes to the Pacific halibut genome (GCF_013339905.1). Transcripts were aligned using the "--splice" preset option enabled. For transcripts that aligned to the genome, the NCBI RefSeq annotation of the Pacific halibut genome was searched for any genes that overlapped the alignment and were oriented in the same direction as the alignment. The coding regions of the largest transcript for each of these genes were initially compared to the positions of 10,474,925 SNPs identified by low-coverage whole-genome resequencing of 285 individual Pacific halibut (please see Section 5.1.1 for details on sequencing and SNP identification). An additional region defined as 5kb upstream from the start of these genes was also interrogated to identify SNPs that may be involved in the transcriptional regulation of these genes. The preliminary results indicate that the transcripts associated with growth in Pacific halibut overlapped with genes in the Pacific halibut NCBI RefSeq annotation. A total of 1,299 SNPs were located in the regions examined and may potentially have some influence on growth (Table 1). Current efforts are now devoted to characterizing SNPs in the coding sequence and upstream regulatory regions of these putative growth marker genes. As a result of this effort, a bioinformatic pipeline is now in place to interrogate SNPs in and around gene regions that can be incorporated into future Secretariat research.

Transcript ID	Gene	Annotation	Non-coding	Coding	Five prime flanking
TRINITY_DN102963_c0_g1_i1	LOC118098571	glycine--tRNA ligase-like	86	11	94
TRINITY_DN98755_c4_g1_i1	LOC118105518	myosin heavy chain, fast skeletal muscle-like	60	39	30
TRINITY_DN88997_c0_g1_i1	LOC118110038	troponin I, slow skeletal muscle-like	52	6	94
TRINITY_DN105325_c2_g1_i1	LOC118118854	zinc finger protein 638-like	529	52	101
TRINITY_DN104023_c1_g2_i2	LOC118124806	asparagine synthetase [glutamine-hydrolyzing]-like	242	23	77
TRINITY_DN105033_c2_g1_i1	acta1a	actin alpha 1, skeletal muscle a	18	7	104
TRINITY_DN97221_c0_g3_i1	mylpfb	myosin light chain, phosphorylatable, fast skeletal muscle b	29	2	71
TRINITY_DN97789_c1_g1_i1	rhcga	Ammonium transporter, Rh family, C glycoprotein a	30	7	28
TRINITY_DN87895_c0_g1_i2	ttn.1	titin, tandem duplicate 1	420	205	124
TRINITY_DN106670_c2_g1_i1	ubp1	upstream binding protein 1	121	7	84

Table 1. Summary of SNPs present in genes associated with growth in Pacific halibut.

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

Information on all Pacific halibut removals is integrated by the IPHC Secretariat, providing annual estimates of total mortality from all sources for its stock assessment. Bycatch and wastage of Pacific halibut, as defined by the incidental catch of fish in non-target fisheries and by the mortality that occurs in the directed fishery (i.e. fish discarded for sublegal size or for 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 parametization (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.
Principal Investigator: Claude Dykstra (M.Sc. candidate)

The objective of this study was to evaluate the effects of capture conditions and different hook release techniques on injury levels and associated physiological condition and survival of longline-discarded Pacific halibut.

A detailed description of fish capture conditions and related environmental parameters, hook release techniques, hook injury assessment, physiological condition and blood stress indicators was provided in document IPHC-2021-SRB018-08.

Initial data exploration focused on investigating the relationships of the hook release treatments to the biological (size, sex, somatic fat levels, Fulton's condition factor, physiological blood stress indicators) and environmental (soak time, depth, sea state, time on deck, and temperature influences) conditions and the resultant injuries and release viability classifications of the test fish. The data showed less severe injuries and

nearly identical injury profiles for the two regulatorily approved removal methods (careful shake and gangion cut), and more severe injuries from the mechanical hook stripper (Section 4.1 Figure 1; provided separately). Similarly, the approved release methods resulted in significantly greater outcomes of fish in the Excellent viability category, while the hook stripper resulted in significantly higher number of fish in the Moderate and Poor viability categories.

The interplay of these variables was further investigated by conducting correlation analyses among the numeric variables collected (Section 4.1 Figure 2; provided separately). Of the three physiological blood stress indicators only lactate proved to have significantly different blood levels across release viabilities, with fish classified as "dead" having significantly higher blood lactate levels than fish in other viability categories (one-way ANOVA, $F(3,502)=16.82$, $p<0.001$) (Section 4.1 Figure 3; provided separately). This is likely related to the fact that 89% of dead sub-legal fish had sand flea presence and these fish had presumably been struggling to get away from them while hooked. Pacific halibut exhibited a wide range in the blood levels of stress indicators (glucose, lactate and cortisol) that were largely not correlated to other biological or environmental variables. Similarly, no significant differences were found between the blood parameters and individual injury or the severity of injuries incurred.

Categorical variables (release condition, injury type) where then analyzed by logistic regression through the use of generalized linear models (GLM) of the binomial family (Section 4.1 Figure 4, provided separately). Release method was examined in the model as both an additive and an interactive variable. Interactive effects were not found to improve the models. Again, the wide range of values for each numeric variable led to minimal significance in the results. When using the full set of data (including legal fish) the weight of the fish was found to have some significance for injury outcome in fish subjected to the hook stripper release method; however, when restricting to sublegal fish, this relationship disappeared. Additional analyses were attempted by making different groupings based on the injury incurred such as injury location (jaw, cheek, etc.), type (tear, puncture, other) and injury severity (minimal, fair, severe), but this did little to affect the outcome of the models.

Treating the categorical injuries as ordinal (different degrees of severity) allowed for exploration of the relationship of fish weight to injury. This was achieved through Paired Ordinal Linear Regression (POLR) analysis (Section 4.1 Figure 5; provided separately). POLR predictions for Hook Stripper are dynamically affected by the weight of the fish. In this particular hook release method, the hook is mechanically forced out through a "path of least resistance". As a result, Pacific halibut of low weight (<10kg) predominantly suffer "Torn Face" injuries, slightly larger fish (10kg ~ 20 kg) suffer from "Cheek and Jaw" injuries, larger fish (20kg - 30kg) suffer from "Torn Jaw" injuries, even larger fish (30kg - 45kg) suffer the more typical "Torn Cheek" injuries, and finally the largest fish tend to show no severe injuries, likely due to the hook never penetrating fully through the cheek, either due to its thickness or due to stronger bones in the very large fish. Mechanistically this is an interesting observation in the dynamics of injuries in Pacific halibut and the influence of fish weight in those injuries. Results from the POLR

analysis do not produce confidence bounds, so an effort is ongoing to generate an equivalent through the additive GLM models and bootstrapping of the data. The ordinality of the injuries are not fully straight forward (i.e. injuries are not uniformly distributed from one another, and many are confounded, i.e. a torn cheek hooking injury inevitably tears through the cheek, or the jaw, the more force or the less resistance provided) and this is likely constraining this form of analysis.

Principal component analysis (PCA) and Random Forest (RF) methods were also explored as part of the analysis. The underlying variability of the data made these methods largely uninformative, other than pointing to some influence of size (length correlated with weight) and the influence of sand flea presence and fish categorized as dead (Section 4.1 Figure 6, provided separately).

Survival of discarded fish was directly assessed by biotelemetric monitoring of released fish with the use of satellite-transmitting electronic archival tags equipped with accelerometers (sPAT tags), as described in document IPHC-2021-SRB018-08. Post-release behavioral data were evaluated for 75 sPAT-tagged Pacific halibut that were at liberty for 2-96 days. Three fish were confidently inferred to have died after periods at liberty of 41-80 days and another three fish may have died 96 days after release; resulting in minimum and maximum estimated 96-day post-release discard mortality rates (DMRs) of 4.2% (range = 0.0-8.7%) and 8.4% (range = 1.7-14.6%), respectively. These ranges are consistent with the currently-applied DMR value of 3.5%.

A manuscript describing discard mortality rate estimations in the directed longline fishery is currently in review in the Journal of North American Fishery Management (Loher et al., in review; provided separately).

4.2. Estimation of discard mortality rates in the charter recreational sector.
Principal Investigator: Claude Dykstra (M.Sc. candidate)

The IPHC Secretariat is conducting a research project to better characterize the nature of charter recreational fisheries with the ultimate goal of better understanding discard practices relative to that which is employed in the directed longline fishery. This project has received funding from the National Fish and Wildlife Foundation and the North Pacific Research Board (Appendix V) and the project narratives of both projects have been provided in previous meeting documentations. The experimental field components of this research project took place in Sitka, Alaska (IPHC Regulatory Area 2C) from 21-27 May 2021, and in Seward, Alaska (IPHC Regulatory Area 3A) from 11-16 June 2021, with methods and analyses detailed in the project narratives provided.

The fishing vessels were required to fish 6 rods at a time, three (3) rigged with 12/0 circle hooks and three (3) rigged with 16/0 circle hooks (Figure 1C) in order to establish a comparison of the two most common gear types used in the Pacific halibut recreational fishery, as informed by the survey conducted in 2019 and subsequent discussions. The overall goal was to capture at least 240 Pacific halibut in 2C and in 3A (480 total) over five days of fishing per Regulatory Area. In IPHC Regulatory Area

2C, we aimed to sample 60 fish in each of the following size classes: ≤ 68 cm, 69 cm – 77 cm, 78 cm – 93 cm, ≥ 94 cm (or ≤ 26.67”, 27” – 30.5”, 31” – 36.5”, ≥ 37”). In IPHC Regulatory Area 3A, we aimed to sample 60 fish from each of the following size classes: ≤ 61 cm, 62 cm – 69 cm, 70 cm – 83 cm, ≥84 cm (or ≤ 24”, 24.25” – 27”, 27.25” – 32.75”, ≥ 33”).

In IPHC Regulatory Area 2C (Sitka, AK), we captured, sampled and released 243 Pacific halibut that were on average 80.1 ± 19.0 cm in fork length (range from 52 to 149 cm) and 7.4 ± 7.5 Kg in weight (range from 1.5 to 49.75 Kg). In IPHC Regulatory Area 3A (Seward, AK), we captured, sampled and released 118 Pacific halibut that were on average 72.5 ± 14.1 cm in fork length (range from 42 to 110 cm) and 5.0 ± 3.3 Kg in weight (range from 0.55 to 17 Kg). Therefore, a total of 361 Pacific halibut were captured, sampled and released in the two research charters conducted. The distribution of lengths of all encountered fish is shown in Figure 2A, showing a similar length distribution between fish captured in the two sites. In addition, the distribution of fish lengths by hook size (12/0 and 16/0) was similar (Figure 2B).

For all Pacific halibut captured, we recorded the time from hooking to release, length and weight, the injury code and release viability category using the standard IPHC criteria, and air and fish temperature. In addition, from each fish we collected a blood sample by caudal puncture, we measured somatic fat content with the use of a Distell Fat Meter, we took a picture of the hooking injury, collected a fin clip for genetic sexing and tagged the fish with an opercular wire tag prior to release (Figure 3A). Pacific halibut captured in IPHC Regulatory Area 3A were subjected to the same sampling protocol with the exception of the 80 fish that were tagged with acceleration-logging survivorship pop-up archival transmitting (sPAT) tags. sPAT-tagged fish were selected only among those fish that were classified in the “excellent” viability category and did not have a blood sample taken to minimize handling-related stress (Figure 3B). The distribution of fish lengths by tag type (wire tag or sPAT) in fish captured and released in IPHC Regulatory Area 3A is shown in Figure 2C.

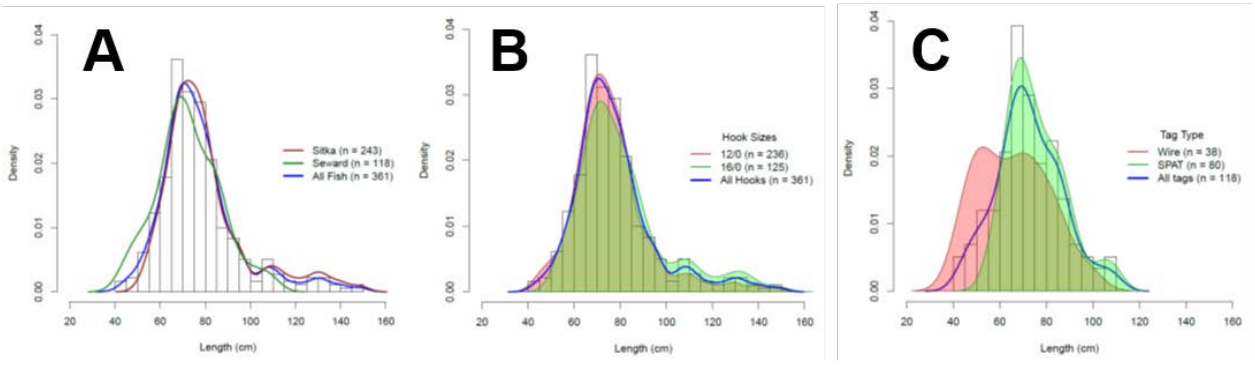

Figure 2. Length distributions of Pacific halibut captured, sampled and released by IPHC Regulatory Area (A: Sitka, AK for 2C and Seward, AK for 3A), by hook size (B: 12/0 and 16/0) and by type of tag (C: wire tag and sPAT).

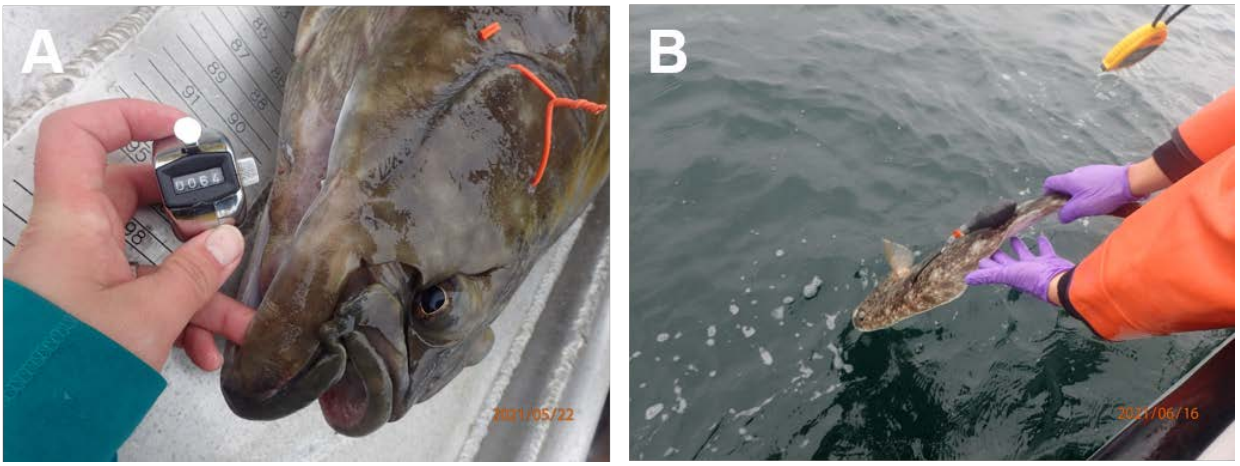

Figure 3. Tags used in this study: A) orange wire tag through the operculum; B) sPAT attached to the dorsal musculature, as fish is being carefully released.

The deployed sPAT tags were programmed to be released after 96 days and we expect to recover the accelerometer data by 25 September 2021 or earlier (i.e. due to mortality or capture).

Processing of blood samples for the determination of stress indicators (cortisol, glucose and lactate) is in progress and analysis of injuries and viability by hook size and fish size is currently being conducted.

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

5.1. Population genomics.
Principal Investigator: Andy Jasonowicz (M.Sc.)

The primary objective of the studies that the IPHC Secretariat is currently conducting is to investigate the genetic structure of the Pacific halibut population and to conduct

genetic analyses to inform on Pacific halibut movement and distribution within the Convention Area.

5.1.1. Studies to resolve the genetic structure of the Pacific halibut population in the Convention Area. Details on sample collection, bioinformatic processing and proposed analyses utilizing low-coverage whole genome sequencing (lcWGR) to investigate Pacific halibut population structure were provided in document IPHC-2021-SRB018-08. The bioinformatic processing pipeline has been successfully migrated to Microsoft Azure cloud computing services and the raw sequence data from a second sequencing run of 250 samples has been processed. This includes alignment to the Pacific halibut reference genome and quality filters to ensure integrity of the data prior to analysis. Quality metrics are comparable to those obtained from a preliminary sequencing run of 36 samples (Table 2). A single sample failed to produce any sequence reads and was omitted from any summaries, single nucleotide polymorphism (SNP) identification and downstream analyses.

These sequence alignments were combined with the alignments from a previous sequencing run (n=36) and used to identify SNPs and estimate genotype likelihoods using the samtools model implemented in ANGSD (v0.934) (Korneliussen et al. 2014). SNPs were retained if they had a global minor allele frequency (MAF) ≥ 0.01 or greater, p-value of 1e-6 or less for a site being variable, and present in at least 214 out of 285 (~75%) of the individuals. A total of 10,474,925 SNPs were identified using these parameters.

Library	**iphc_001**	**iphc_002**
Number of samples	36	250
Sequencing Platform	Illumina HiSeq 4000	Illumina NovaSeq S4
Raw Reads Per Sample (Millions)*	26.5 (21.8 - 42.9)	24.7 (10.7 – 47.2)
Reads Retained (%)*	60 (54 - 69)	63 (22 - 70)
Coverage Per Sample (x)*	3.2 (2.6 – 5)	3.5 (1.0 - 5.6)

Table 2. Summary of raw sequence data and genome alignments for two Pacific halibut lcWGR sequencing runs. *expressed as mean (min – max)

With this dataset, principal component analysis was used to gain a preliminary look at population structure and signals of natural selection in the genome. Prior to these analyses, the dataset was filtered to remove SNPs in any unplaced scaffolds, the mitochondrial genome, and chromosome 9 (RefSeq: NC_048935.1), which contains a large sex-associated region. PCAngsd (v1.02) (Meisner and Albrechtsen 2018; Meisner et al. 2021) was run using default

parameters (MAF ≥ 0.5 by default) to estimate a covariance matrix among individuals using genotype likelihoods for 285 Pacific halibut. Numpy (v1.21.2) (Harris et al. 2020) was then used to compute the eigenvalues and eigenvectors for the covariance matrix obtained using PCAngsd. A genome-wide selection scan was also carried out using the "-selection" flag in PCAngsd.

A total of 4,850,093 sites were retained by PCAngsd. These preliminary results suggest that there may be some degree of spatial and temporal separation among these sampling collections (Fig. 4), and regions of the genome that are potentially under natural selection (Fig. 5). However, additional samples are to be processed to reach our target sample size of 50 per collection with collections from British Columbia (2007) and Central Gulf of Alaska (1999 & 2018) to be included in the next sequencing run. The inclusion these additional samples of will help resolve these patterns further.

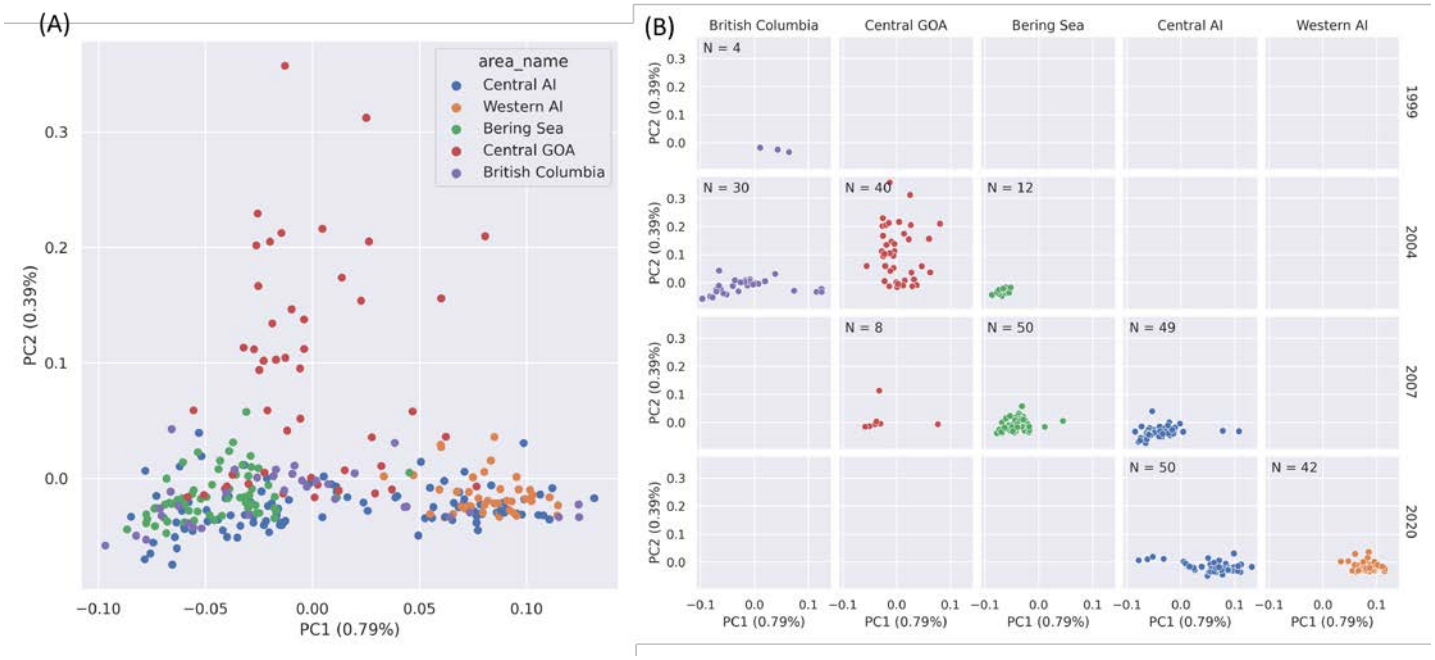

Figure 4. Principal component analysis scores of genotype likelihoods from 4,850,093 SNPs in 285 Pacific halibut sequenced to date. A) Plot of PC1 vs PC2 for all populations together. B) PC1 vs PC2 plotted separately for each geographic area and collection year. Number of samples analyzed for each collection are listed in each facet.

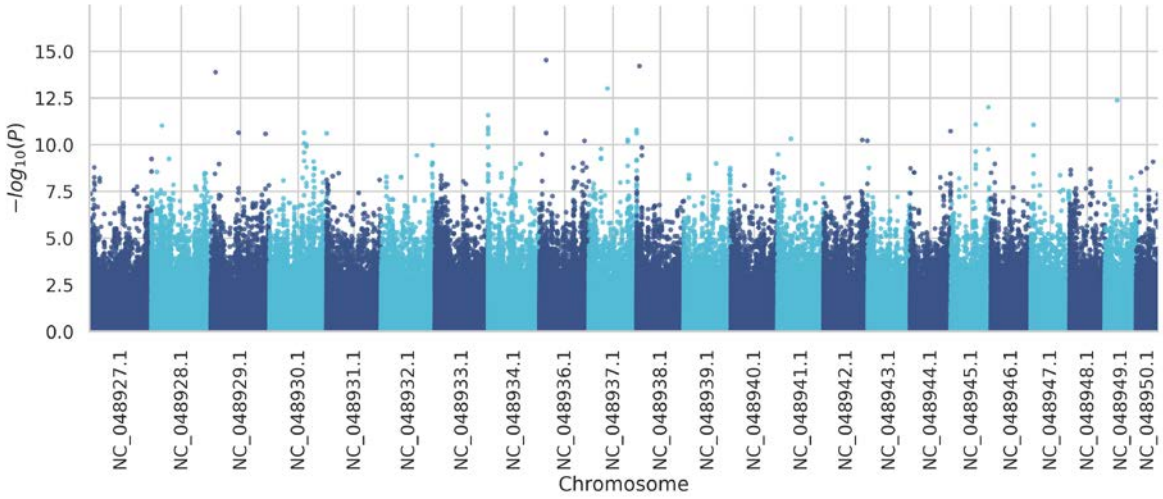

Figure 5. Manhattan plot based on the genome-wide selection scan implemented in PCAngsd.

RECOMMENDATION/S

That the SRB:

a) **NOTE** paper IPHC-2021-SRB019-08 which provides a response to requests from SRB018, and a report on current research activities contemplated within the IPHC Five-Year Biological and Ecosystem Science Research Plan (2017-2021).

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APPENDIX I

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

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

APPENDIX II

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

SA Rank	Research outcomes	Relevance for stock assessment	Specific analysis input	Research Area	Research activities
1. Biological input	Updated maturity schedule	Scale biomass and reference point estimates	Will be included in the stock assessment, replacing the current schedule last updated in 2006	Reproduction	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

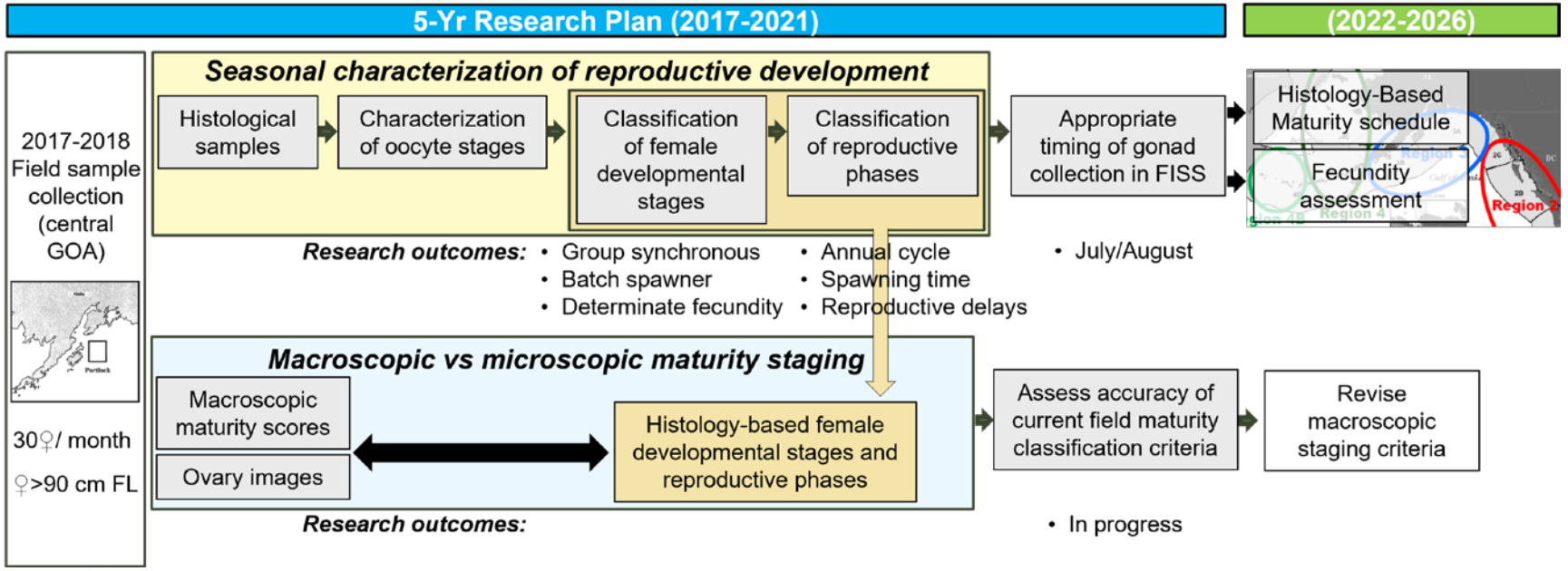
APPENDIX III

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

MSE Rank	Research outcomes	Relevance for MSE	Research Area	Research activities
1. Biological parameterization and validation of movement estimates	Improved understanding of larval and juvenile distribution	Improve 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

Flow of research activities and outcomes on Reproduction during the 5-Year Research Plan (2017-2021) and their link with planned research activities for the 5-Year Research Plan (2022-2026)



APPENDIX V

Summary of current awarded research grants

Project #	Grant agency	Project name	PI	Partners	IPHC Budget ($US)	Management implications	Grant period
1	**National Fish & Wildlife Foundation**	Improving the characterization of discard mortality of Pacific halibut in the recreational fisheries (NFWF No. 61484)	IPHC Dr J. Planas and Mr Claude Dykstra	Alaska Pacific University, U of A Fairbanks, charter industry	$98,902	Bycatch estimates	1 April 2019 – 1 November 2021
2	**North Pacific Research Board**	Pacific halibut discard mortality rates (NPRB No. 2009)	IPHC Dr. J. Planas	Alaska Pacific University,	$210,502	Bycatch estimates	1 January 2021 – 31 March 2022
Total awarded ($)					**$309,404**		