Report on Current and Future Biological and Ecosystem Science Research Activities

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, 12 MAY 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 SRB017 (IPHC-2020-SRB017-R):

Recommendation 1 (SRB017-Rec.02 (para. 31))

> *"the SRB **RECOMMENDED** that the research planning table shown in the meeting presentation for paper IPHC-2020-SRB017-08, be improved by adding clear prioritization of biological research needs for addressing uncertainties in the stock assessment and MSE programs. Ideally, this would be in the form of ranked biological uncertainties/parameters for the stock assessment and MSE operating model along with an explanation for deviations from this ranked list"*

The Secretariat has produced 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. Based on this information, the Secretariat has prioritized the biological research needs for addressing uncertainties in the stock assessment and MSE programs (Appendix IV).

Recommendation 2 (SRB017-Rec.03 (para. 49))

*"the SRB **RECOMMENDED** that the IPHC Secretariat work with collectors to develop a series of benchmark summary statistics that characterize the quality of the Pacific halibut genome developed."*

The Secretariat completed in 2020 the first chromosome-level assembly of the Pacific halibut genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_013339905.1) and was annotated by the NCBI Eukaryotic Genome Annotation Pipeline (NCBI Hippoglossus stenolepis Annotation Release 100; https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Hippoglossus_stenolepis/100/). The summary statistics of the genome assembly are provided in Table 2 of this report.

*Recommendation 3 (*SRB017–Rec.04 (para. 53))

*"The SRB **RECOMMENDED** that the IPHC Secretariat incorporate prioritization of research activities, as well as the timeline of available research outputs as inputs into the stock assessment and MSE processes."*

The IPHC Secretariat has prioritized the biological research needs for addressing uncertainties in the stock assessment and MSE programs (Appendix IV) and has produced a timeline of research outputs and their use as inputs into the stock assessment and MSE processes (please see document IPHC-2021-SRB018-10).

*Recommendation 4 (*SRB017–Rec.04 (para. 53))

*"The SRB **RECOMMENDED** that the IPHC Secretariat identify those research areas with uncertainty and indicate research questions that would require the SRB to provide input and/or decision in future documentation and presentations provided to the SRB."*

The Secretariat has identified the following research questions related to research areas with uncertainty that would require guidance and input from the SRB:

1. Genetics and Genomics Research Area. Research questions:
   1.1. Review proposed development of a genetic marker panel (GT-seq) for downstream applications (e.g. individual population assignments).
   1.2. Review proposed population assignment methods to inform on distribution with particular emphasis in IPHC Regulatory Area 4B.

1.3. Discuss potential interest and fishery sample collection designs for planning future coastwide assessment of stock composition with the use of a genetic marker panel.
1.4. Discuss potential interest and study design considerations for planning future close-kin mark recapture studies to provide estimates of population size, connectivity, fecundity, etc.

2. Reproduction Area. Research questions:
2.1. Review information presented on skip-spawning in Pacific halibut and discuss the scope and planning of research suggested in this area.
2.2. Discuss ovarian sample collection designs to assess maturity and fecundity at temporal and spatial scales.
2.3. Discuss strategies to scale maturity and fecundity information at the population level.
2.4. Discuss need for long-term monitoring of maturity and fecundity.

*Request 1 (*SRB017–Req.07 (para. 33))

*"The SRB **REQUESTED** that the IPHC Secretariat further develop planning for the remainder of the current 5-year planning period and to revise and submit a comparable synthesis planning document for review at SRB018. In terms of the current research activities and research outcomes, further detail is needed in several areas, including:*

a) further detail for (i) specific research outcomes, (ii) specific relevance for stock assessment relevance, (iii) specific relevance for MSE (see Section 8.1 for examples);

b) prioritize research activities and research outcomes.."

The IPHC Secretariat has provided a description of the planned research activities contemplated for the remainder of the current 5-year Biological and Ecosystem Science Research Plan (2017-2021) in this document (page 18).

*Request 2 (*SRB017–Req.08 (para. 34))

*"The SRB **REQUESTED** that further clarification on funding and staffing needs required to meet self-imposed deadlines"*

The Secretariat has provided information on staffing and funding availability in relation to the estimated timeline of research outputs presented in SRB017 (please see document IPHC-2021-SRB018-10).

*Request 3 (*SRB017–Req.10 (para. 43))

> ***"****The SRB* ***REQUESTED*** *that the IPHC should clarify how skip-spawning research contributes to stock assessment and MSE functions. In particular, future research should develop and present:*
>
> *i. models for forecasting or estimating skip-spawning for Pacific halibut taking into account the timing of the sample collection, size / age and potentially condition factor of females;*
>
> *ii. estimates of the potential impact of skip-spawning scenarios on management procedure performance;*
>
> *iii. clear plans for analyses of histological data, including incorporation of age variation and locational variation;*
>
> *iv. details of experimental and sampling designs, as well as expected analyses for "measures of fecundity"."*

The IPHC Secretariat has provided a description of the relevance of research on skip-spawning for stock assessment and MSE in this document as well as in document IPHC-2021-SRB018-08. The IPHC Secretariat is assessing the scope and planning of research suggested in this area and guidance and input from the SRB is needed in order to fulfill this request.

*Request 4 (*SRB017–Req.11 (para. 44))

> ***"****The SRB* ***REQUESTED*** *that the IPHC Secretariat provide a plan for integration of research outcomes in this research area with outcomes in the genetics and genomics research area"*
>
> The SRB request to integrate growth research conducted by the IPHC Secretariat with genomics research is under consideration due to research prioritization, staffing and funding reasons and will be addressed in SRB018.

*Request 5 (*SRB017–Req.12 (para. 47))

> ***"****The SRB* ***REQUESTED*** *that the IPHC Secretariat provide the grant proposal funding the DMR work, and provide a more detailed presentation at SRB018"*
>
> The IPHC Secretariat will kindly provide the project narratives of grant proposals awarded to IPHC by the National Fish and Wildlife Foundation and North Pacific Research Board that provide funding for this work. In addition, a detailed presentation on this project will be provided at SRB018

*Request 6 (*SRB017–Req.13 (para. 51))

> ***"****The SRB* ***REQUESTED*** *that the IPHC Secretariat prepare a research plan for describing and justifying how the knowledge (and all the resources expended in getting it) of the genome will be used to inform SA and MSE information needs (i.e. as per above request to further elaborate the research plan for this research area). This will likely require some form of interaction (e.g. collaborations, workshops) with outside researchers and/or agencies"*

The Pacific halibut genome represents a valuable and necessary resource to pursue population genomics studies that are aimed at defining population structure, identifying genetic baselines, assigning individuals to populations, identifying regions of the genome responsible for key biological traits, etc. The research activities that the IPHC Secretariat is planning to conduct regarding population genomics of Pacific halibut and that are relevant for stock assessment and MSE are concentrated on (1) establishing population structure, as the results may lead to revisit whether a single or separate stock assessment should be conducted in different IPHC regulatory areas, and (2) assigning individuals to source populations in order to derive stock composition and connectivity information, given that spatial dynamics are a major source of uncertainty in the stock assessment. Details of the initial planning and execution of these research activities are provided in this document and also in the form of a grant proposal that the IPHC Secretariat is preparing on these topics in collaboration with outside researchers and that would benefit from guidance and input from the SRB.

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.)

   Knowledge of the dispersal of Pacific halibut larvae and subsequent migration of young juveniles has remained elusive because traditional tagging methods are not effective on these life stages due to the small size of the animals. This larval connectivity project, in cooperation with NOAA EcoFOCI, used two recently developed modeling approaches to estimate dispersal and migration pathways of larval and young juvenile Pacific halibut in order to better understand the connectivity of populations both within and between

the Gulf of Alaska and Bering Sea. The results of this study have been published in the journal *Fisheries Oceanography* (Sadorus et al., 2021).

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

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). In 2020, 465 Pacific halibut were tagged and released on the IPHC FISS but no tagging was conducted in the NMFS groundfish trawl surveys because of its cancellation due to COVID-19. Therefore, a total of 3,577 U32 Pacific halibut have been wire tagged and released on the IPHC FISS and 96 of those have been recovered to date. In the NMFS groundfish trawl surveys through 2019, a total of 6,536 tags have been released and, to date, 69 tags have been recovered.

2. Reproduction.

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

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

The IPHC Secretariat has completed the processing of genetic samples from the 2017, 2018 and 2019 aged commercial landings. Given that additional years of commercial catch sex-ratio information are likely to further inform selectivity parameters and cumulatively reduce uncertainty in future estimates of stock size, the IPHC Secretariat is currently processing genetic samples from the 2020 age commercial landings.

The IPHC Secretariat is continuing work towards providing sex ratio information in years previous to 2017 through the use of genotyping techniques using historical otolith

samples. Initial tests conducted by the IPHC Secretariat have not been conclusive regarding the ability to extract sufficient amounts of quality DNA from clean archived otoliths. Further work in this area was postponed until work can be resumed in the IPHC laboratory.

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) investigation of skip-spawning in females; and 3) fecundity estimations.

2.2.1. Histological assessment of the temporal progression of female developmental stages and reproductive phases throughout an entire reproductive cycle.

Sample collection. Biological samples (gonads, blood, pituitary, otolith, fat content) from female Pacific halibut were collected at monthly intervals throughout an entire calendar year, from September 2017 until August 2018. At each month, 30 females > 90 cm in fork length were collected to select for mature females, as females of this size range have a greater than 0.5 probability of being mature (Clarke et al., 1999). Pacific halibut were captured by a contracted longline commercial fishing vessel in the Portlock region in the central Gulf of Alaska, historically known to contain major spawning grounds (St. Pierre, 1984), in order to attempt collecting fish from a single spawning population of Pacific halibut at various stages during their reproductive cycle. Length (fork length) and weight (round weight) measures were recorded. Blood samples were drawn from the caudal vein with the use of heparinized hypodermic needles and syringes, centrifuged at 1,500 x g for 30 min and plasma samples were frozen and kept at -20C. Somatic fat content was estimated using a Distell Fish Fatmeter (Model FM 692, Fauldhouse West Lothian, Scotland) by taking two readings from an area located midway between the lateral arch and the dorsal fin insertion on the non-pigmented side of the fish and applying the average to a fat calibration curve developed for Pacific halibut to derive percent fat content values. Ovaries and liver were excised and weighed to calculate the gonadosomatic index (GSI; ovary weight/round weight x 100) and hepatosomatic index (HSI; liver weight/round weight x 100). Two small ovarian fragments were dissected per ovary and one

fragment was fixed in buffered formalin for histological analysis and stored at room temperature and the other fragment was placed in pre-labeled 2 ml screw-cap microcentrifuge tubes containing 1 ml of RNAlater and kept frozen at -20C. Pituitary glands were also collected into RNAlater-prefilled microcentrifuge tubes and kept frozen at -20C.

All sampled Pacific halibut were assigned one of the four maturity stages for females (immature, maturing, ripe and resting) that are currently applied in IPHC's FISS for maturity assessment based on visual/macroscopic criteria of the gonads. Photographic images of the gonads were also taken in order to validate the visual assignment.

Histological analyses and developmental stage or reproductive phase classification criteria. Ovarian tissue samples (360 in total) were processed for histology by an independent laboratory (Histology Consultation Services, Everson, Washington, USA) where two series of four micrometer (µm) thick Paraffin sections, separated by approximately 500 µm, were mounted on two slides and stained with hematoxylin and eosin. Slides were examined visually with a compound microscope (1x – 100x magnification) and ovarian follicle developmental stages were assigned as described in Fish et al. (2020). In brief, the oocyte developmental stages described for Pacific halibut corresponded to Primary Growth (PG): one nucleolus (PGon), perinuclear (PGpn), cortical alveolar (PGca); Secondary Growth: primary-, secondary-, and tertiary-vitellogenesis (Vtg1, Vtg2, Vtg3): and Oocyte Maturation: germinal vesicle migration (GVM), and periovulatory (PO) (Fish et al., 2020). As shown in this previous study, female developmental stages were assigned on the basis of the most advanced oocyte stage present in the ovarian sections examined. Furthermore, female reproductive phases were determined by comparing female developmental stages with histological indications of past spawning events (e.g. presence of post-ovulatory follicles, atretic follicles, blood vessels, etc.) and assigned as immature, developing, spawning capable, regressing or regenerating according to Brown Peterson et al. (2011).

Results. The temporal analysis of female developmental stages showed a clear progression in reproductive development, with females in early vitellogenesis (Vtg1) predominantly from March until June, progressing to mid vitellogenesis during July and August and to late vitellogenesis from September to December (Fish et al., in preparation; figures provided separately). Females at the GVM stage appeared in low numbers in November and December and increased to almost 50% in January. Females at the PO stage were found only in January and February, coinciding with the period when females with the post-ovulatory follicles (i.e. evidence of spawning) were found. Therefore, these results clearly reflect the group-synchronous oocyte developmental reproductive strategy of Pacific halibut and confirm that the peak period of spawning takes place in January and February. Analysis of the temporal changes in female reproductive phase shows that spawning capable females are detected as early as August

and that they are prevalent until December, coinciding with the temporal progression of females in the late vitellogenic (Vtg3) developmental stage. These results indicate that the transition between mid and late vitellogenesis (Vtg2 to Vtg3) marks the beginning of the spawning capable reproductive phase, that for stock assessment purposes, contains females that are considered mature. Importantly, the detection of spawning capable females in July-August is conducive to conducting routine histological assessments of female maturity during the IPHC's FISS sample collection, as these are conducted between June and late August.

For all examined females, data on average oocyte diameter, GSI, HSI, Fulton's K, age and fat content was expressed by month of collection, by female developmental stage and by female reproductive phase. Significant positive correlations (Pearson, $p < 0.05$) were observed between oocyte diameter and GSI and also between Fulton's K and HIS, likely a reflection of ovarian development and the important role of the liver in lipid storage.

Current activities. Preparation of a manuscript for publication describing temporal progression of reproductive development in female Pacific halibut and relationship of reproductive development with physiological condition indicators is in progress (Fish et al., in preparation).

2.2.2. Investigation of skip-spawning in females.

Sample collection and methodology. Histological samples described in 2.2.1 were examined for the possible presence of skip spawning females (i.e. mature females that do not produce gametes in a given reproductive cycle), as described in Rideout et al (2005). Search for potential skip spawning females was focused on the period during the reproductive cycle when females initiate the progression of oocyte development towards oocyte maturation and spawning. This period, as indicated in section 2.2.1, begins with the transition between Vtg2 and Vtg3 developmental stages that marks the beginning of the spawning capable reproductive phase starting in August. Only females collected between the months of August and February (i.e. the end of the spawning period) and that were classified at a developmental stage less advanced than Vtg2 were examined. The presence of the following features characteristic of skip spawning females (Rideout et al., 2005) were recorded in the examined females: degenerating ovarian follicles, blood vessels, enlarged extracellular matrix, muscle bundles and thick ovarian wall (if present in the histological sections).

Results. During the spawning capable phase (August to February), eight females were classified at the CA developmental stage (one collected in November and seven in December) and one at the PGpn developmental stage (Table 2). During these two months, all remaining females were classified at the Vtg3 or GVM developmental stages. The female at the PGpn developmental stage that was collected in December was 9 years old, showed tightly compacted ovaries with

no signs of degeneration and was, therefore, classified as immature (average age at 50% maturity is estimated to be 11.6 years; Stewart and Webster, 2021). All other females at the CA stage ranged in age from 10 until 15 years and all showed presence of reabsorbing follicles, with various degrees of muscle bundle presence and blood vessels. With the available histological evidence it is difficult to distinguish between immature females that initiated their first reproductive cycle and failed to progress and mature females (i.e. previous spawners) that are true skip spawners.

Current activities. Examine biological measures of potential skip spawners and compare them with those of maturing females to try to establish if age, length, weight, condition or fat content could explain the ovarian developmental delay in these females. Blood and pituitary samples from these fish could be examined for potential differences in endocrine reproductive markers (e.g. plasma steroid hormone levels and pituitary mRNA expression levels of FSH and LH).

Table 1. Biological measures and developmental stage and reproductive phase classification of Pacific halibut females showing delayed ovarian development during the spawning capable phase.

Month	Female #	Weight (kg)	Length (cm)	Age (years)	Oocyte diameter (microns)	Gonadosomatic index (%)	Hepatosomatic index (%)	Fat content (%)	Developmental stage	Reproductive phase
Nov	27	14.73	108	15	394.33	0.71	0.64	2.22	CA	Regenerating
Dec	4	19.08	114	11	348.74	0.43	0.80	1.66	CA	Regenerating
Dec	5	24.13	124	15	328.57	0.51	0.90	1.90	CA	Regenerating
Dec	20	9.56	91	12	316.45	0.46	1.15	1.78	CA	Regenerating
Dec	23	20.72	120	14	336.67	0.47	0.92	2.74	CA	Regenerating
Dec	24	22.81	122	11	418.48	0.49	1.29	2.32	CA	Regenerating
Dec	26	19.65	119	10	438.52	0.55	0.89	1.66	CA	Regenerating
Dec	27	18.91	117	12	354.43	0.51	0.67	1.69	CA	Regenerating
Dec	25	8.85	90	9	221.90	0.52	0.88	1.21	PGpn	Immature

2.2.3. Fecundity estimations in Pacific halibut. The IPHC Secretariat is conducting a review of existing literature of described methods for fecundity measures in fish species with determinate fecundity, such as the Pacific halibut. In addition, contacts with experts in the field are also being pursued. Plans for collecting a small number of Pacific halibut ovaries in the field (FISS) for testing existing methodologies are currently in preparation (please see section on future research activities).

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. The IPHC Secretariat has recently completed a study funded by the North Pacific Research Board (Project No. 1704) that involved the combination of transcriptomic and proteomic approaches for the identification of physiological markers for somatic growth. This study resulted in the identification of 23 markers in skeletal muscle that were indicative of growth suppression and 10 markers in skeletal muscle that were indicative of growth stimulation. These markers represented genes and proteins that changed both their mRNA expression levels and abundance levels in skeletal muscle, respectively, in parallel with changes in the growth rate of Pacific halibut. From these, three markers showed patterns of expression and abundance that mirrored the change in growth rate: Asparagine synthetase, Ornithine carbamoyltransferase (both involved in amino acid and protein synthesis) and ubiquitin carboxyl-terminal hydrolase (involved in muscle contraction and development). A manuscript describing the procedures and results of this study is in preparation (Planas et al., in preparation).

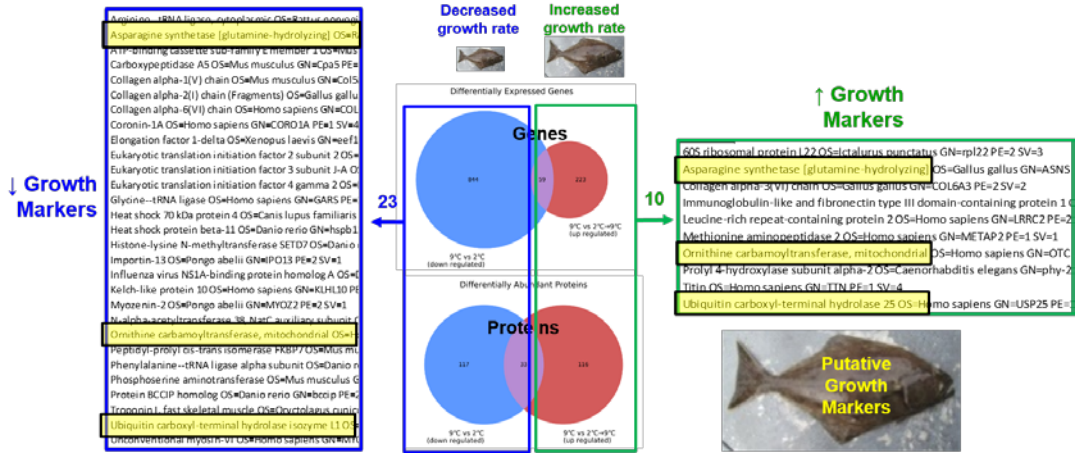

Figure 1. Identification of physiological growth markers. Markers on the left (blue box) and on the right (green box) correspond to markers that change both at the level of mRNA expression and protein abundance with decreased and increased growth rates, respectively. Markers highlighted in yellow correspond to markers that mirror changes in growth rate, irrespective of the direction of the change.

3.2. Application of molecular growth markers for evaluating growth patterns in the Pacific halibut population. The IPHC Secretariat has developed molecular assays to measure the mRNA expression levels by real time qPCR of growth markers identified in 3.1. These markers will be used to test the hypothesis that size differences among fish of the same age may be reflected by differences in the mRNA expression levels of growth markers and, therefore, validate the use of molecular growth markers to inform on growth patterns of Pacific halibut (please see section on future research activities).

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 (B.Sc.)

A manuscript describing discard mortality rate estimations in the directed longline fishery has been finalized and is being prepared for submission to the Journal of North American Fishery Management (Loher et al., in preparation). Additional updates on modeling analyses of potential relationships between individual physiological characteristics, environmental conditions and handling practices, as well as on the ability of electronic monitoring systems to capture release methods and individual lengths of captured fish, will be provided at SRB019.

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

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 are provided. The experimental field components of this research project will take 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. In brief, Pacific halibut will be captured with the use of 12/0 and 16/0 circle hooks that best capture the gear currently used in this fishery and fish sizes will be targeted to cover the Pacific halibut size distribution recorded by ADFG on an annual basis. All injuries will be documented, along with length, weight, somatic fat measurements (using the Distell Fatmeter), and a blood sample (for measuring the levels of physiological stress indicators in plasma) for each fish, before they are tagged and released. Environmental information on temperature (bottom/surface) and time (fight time, time on deck) will also be tracked. Eighty (80) Pacific halibut of Excellent release viability will be fitted with a satellite pop-up archival tag (sPAT – Wildlife Computers) for near term survival estimation in IPHC Regulatory Area 3A.

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. Determine the genetic structure of the Pacific halibut population in the Convention Area. Understanding population structure is imperative for sound management and conservation of natural resources (Hauser and Carvalho 2008). Pacific halibut in Canadian and USA waters are managed by the IPHC as a single coastwide unit stock since 2006 (Stewart and Martell 2014). The rationale behind this management approach is based on our current knowledge of the highly migratory nature of Pacific halibut as assessed by tagging studies (Webster et al. 2013) and of past analyses of genetic population structure that failed to demonstrate significant differentiation in the northeastern Pacific Ocean population of Pacific halibut by allozyme (Grant et al. 1984) and small-scale microsatellite analyses (Bentzen et al. 1998; Nielsen et al. 2010). However, more recent studies have reported slight genetic population structure on the basis of genetic analysis conducted with larger sets of microsatellites suggesting that Pacific halibut captured in IPHC Regulatory Area 4B may be genetically distinct from other areas (Drinan et al., 2016). These findings of subtle genetic structure in the Aleutian Island chain area are attributed to limited movement of adults and exchange of larvae between this area and the rest of the stock due to the presence of oceanographic barriers to larval and adult dispersal (i.e. Amchitka Pass) that could represent barriers to gene flow. Unfortunately, genetic studies suggesting subtle genetic structure (Drinan et al. 2016) were conducted using a relatively limited set of microsatellite markers and, importantly, using genetic samples collected in the summer (i.e. non-spawning season) that may not be representative of the local spawning population.

With the collection of winter (i.e. spawning season) genetic samples in the Aleutian Islands by the IPHC in early 2020, the IPHC has initiated efforts to re-examine population genetic structure using low-coverage whole-genome resequencing (lcWGR) (Therkildsen and Palumbi 2017; Clucas et al. 2019). Previous sample collections made during the spawning season will be used to investigate spatial and temporal patterns of population structure. The inclusion of temporal replicates will enable the investigation of variability of these patterns of time, ensuring confidence in the results (Waples, 1998). The available samples correspond to the following geographic areas and dates of collection:

- British Columbia (Haida Gwaii; 1999, 2004, 2007)
- Central Gulf of Alaska (Portlock region; 1999, 2004, 2007, 2018)
- Bering Sea (Pribilof Canyon; 2004, 2007)
- Central Aleutian Islands (Adak; 2007, 2020)
- Western Aleutian Islands (Attu; 2020)

DNA has been extracted and purified from these samples, sequencing libraries have been constructed, and we are now generating DNA sequence data. Qiagen DNeasy Blood & Tissue Kits (Qiagen, Valencia, California, USA) were used to extract and purify genomic DNA from a total of 50 samples per collection (600 total). Dual-indexed Illumina sequencing libraries were prepared for each sample using Illumina's Tagment DNA TDE1 Enzyme ran Buffer Kit (Illumina, San Diego,

California, USA) according to previously published protocols (Therkildsen and Palumbi 2017). In September of 2020, an initial sequencing run of 36 samples was conducted using the Illumina HiSeq 4000 (2x150 bp paired end reads) platform by the Novogene Corporation (Novogene, Sacramento, CA, USA). This sequencing run was carried out to ensure that the library preparation methods worked and to begin working on a bioinformatics pipeline for processing the raw sequence data, which was done as follows. FastQC (v11.9) (Andrews et al. 2015) was used to assess the quality of the raw sequence reads. Illumina adapter sequences were removed using trimmomatic (v0.39) (Bolger et al. 2014). The trimmed reads were then mapped to the Pacific halibut reference genome (NCBI RefSeq Accession: GCF_013339905.1) using the minimap2 aligner (v2.17) (Li 2018) with the genomic short-read mapping presets. Samtools (v1.12) (Li et al. 2009) was used to filter alignments based on mapping quality scores, retaining those alignments with a score >= 20. Polymerase chain reaction (PCR) and optical duplicates were filtered out using picard (v2.25.2) ("Picard toolkit" 2019) with a pixel distance of 2500 specified. Overlapping ends of each aligned read pair were clipped using the clipOverlap tool in bamUtil (v1.0.14) (Jun et al. 2015). Lastly local realignment around insertion/deletion elements was performed using GATK (v3.8) (Van der Auwera and O'Connor 2020). Single nucleotide polymorphisms (SNPs) were identified and genotype likelihoods were estimated using the GATK model implemented in ANGSD (v0.934) (Korneliussen et al. 2014). SNPs were retained if they had a global minor allele frequency of 0.05, p-value of 1e-6 or less for a site being variable, and present in at least 80% of the individuals.

An average of 26.5 million (range = 21.8 - 42.9 million) raw sequencing reads per sample were obtained from this sequencing run. The alignment of the reads to the Pacific halibut genome and quality filtering steps resulted in an average of 60% (range = 54% - 69%) of the raw reads being retained per sample and used for SNP calling. Individual genomic coverages for the quality filtered alignments were on average 3.2x (range = 2.6x – 5x). A total of 5,051,577 SNPs were identified using this preliminary dataset.

A second sequencing run of 250 samples was submitted to the Novogene Corporation in early 2021 for sequencing on the Illumina NovaSeq 6000 platform using an S4 flowcell (2x150 bp paired end reads). This data has been received and the run yielded an average of 24.7 million (range = 10.7 – 47.2 million) sequence reads per sample. Currently, the IPHC secretariat is working on setting up a cloud-computing environment in Micorsoft Azure for the bioinformatic processing of this data.

To date, 285 out of the 600 samples have been submitted for DNA sequencing. After sequencing is completed for all samples, measures of genetic differentiation (FST) will be estimated among the sample collections to examine levels of divergence between them and test for patterns of isolation by distance. The software ngsAdmix (Skotte et al. 2013), will be used to infer the number of genetic

clusters across the range of Pacific halibut without making a priori assumptions about sample origin. This program also attempts to estimate the ancestry of individual fish and therefore will be useful in the identification of potential migrants. Additionally, outlier tests will also be used to scan the genome for SNPs showing signals of divergent selection. These SNPs showing potential signatures of selection may offer more power to resolve population structure in highly migratory marine fish (Grewe et al. 2015; Anderson et al. 2019). We will compare the results of multiple methods of SNP outlier detection, in particular both FST based methods (eg. OutFLANK (Whitlock and Lotterhos 2015), tess3r (Caye et al. 2016)) and PCA based methods (PCAngsd (Meisner and Albrechtsen 2018)) will be used. Furthermore, SNPs showing signals of selection may be functionally relevant and linked to local adaptations. Transcriptomic resources developed by the IPHC Secretariat have been used by NCBI to annotate the Pacific halibut genome (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Hippoglossus_stenolepis/100/) which will be necessary for interpreting the functional significance of SNPs identified in this study.

5.2 Generation of genomic resources.

Principal Investigator: Josep Planas (Ph.D.)

The IPHC Secretariat has conducted studies aimed at generating genomic resources for Pacific halibut that are instrumental for a more in-depth understanding the genetic make-up of the species: a reference genome and a comprehensive collection of expressed sequence tags (ESTs). The generated genomic resources will greatly assist current studies on the genetic structure of the Pacific halibut population, on the application of genetic signatures for assigning individuals to spawning populations and for a thorough characterization of regions of the genome or genes responsible for important traits of the species.

5.2.1 Pacific halibut genome sequencing. The Pacific halibut genome represents a valuable and necessary resource to conduct population genomics studies that are aimed at defining population structure, identifying genetic baselines, assigning individuals to populations, identifying regions of the genome responsible for key biological traits, etc. The research activities that the IPHC Secretariat is planning to conduct regarding population genomics of Pacific halibut and that are relevant for stock assessment and MSE are concentrated on (1) establishing population structure, as the results may lead to revisit whether a single or separate stock assessment should be conducted in different IPHC regulatory areas, and (2) assigning individuals to source populations in order to derive stock composition and connectivity information, given that spatial dynamics are a major source of uncertainty in the stock assessment (Appendix II and Appendix III). Details of the initial planning and execution of these research activities are provided in this document and also in the form of a grant proposal that the IPHC Secretariat is preparing on these topics in collaboration with outside researchers and that would benefit from guidance and input from the

SRB. The IPHC Secretariat completed the first draft sequence of the Pacific halibut genome in collaboration with the French National Institute for Agricultural Research (INRA, Rennes, France). The Pacific halibut genome has a size of 594 Mb and contains 24 chromosome-size scaffolds covering 98.6% of the complete assembly with a N50 scaffold length of 25 Mb at a coverage of 91x. The Pacific halibut whole genome sequence has been deposited at DDBJ/ENA/GenBank under the accession JABBIT000000000 and the chromosome-level assembly is available in https://www.ncbi.nlm.nih.gov/assembly/GCF_013339905.1. In addition, the Pacific halibut genome was also annotated by NCBI and is available as NCBI Hippoglossus stenolepis Annotation Release 100 (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Hippoglossus_stenolepis/100/). The Pacific halibut genome assembly statistics and assembly completeness are shown in Table 2.

Table 2. Pacific halibut genome assembly statistics and assembly completeness.

		Complete assembly	**Chromosomes only**
Assembly metrics	Number of scaffolds	120	24
	Total size of scaffolds	594,269,479	585,884,243
	Longest scaffold	32,413,955	32,413,955
	Shortest scaffold	4,965	11,318,318
	Mean scaffold size	4,952,246	24,411,843
	Median scaffold size	13,681	24,662,186
	N50 scaffold length	24,986,857	24,986,857
	L50 scaffold count	11	11
	% of assembly in chromosomes	-	98.6 %
	% of assembly in unanchored scaffolds	-	1.4 %
Assembly completeness	Complete BUSCOs (C)	4,472 (97.6%)	
	C and single-copy BUSCOs	4,345 (94.8%)	
	C and duplicated BUSCOs	127 (2.8%)	
	Fragmented BUSCOs	33 (0.7%)	
	Missing BUSCOs	79 1.7%)	

5.2.2 Transcriptome sequencing. The IPHC Secretariat has completed transcriptome (i.e. RNA) sequencing of a wide variety of tissues (12) in Pacific halibut including white and red skeletal muscle, liver, heart, ovary, testis, head kidney, brain, gill, pituitary, spleen and retina. The raw sequence data have been deposited in NCBI's Sequence Read Archive (SRA) under the bioproject number PRJNA634339 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA634339) and with SRA accession numbers SAMN14989915 - SAMN14989926. As previously described, the transcript assemblies for each tissue were annotated using the Trinotate pipeline. TransDecoder (v5.5.0) was used to identify open reading frames longer than 100 codons and used to predict likely protein coding sequences. Transcripts and predicted proteins were queried against the Swiss-Prot database using BLASTx and BLASTp, respectively, and annotated. Importantly, raw sequence data were provided to NCBI for the annotation of the Pacific halibut genome (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Hippoglossus_stenolepis/100/).

EXTERNAL FUNDING AND PUBLICATION GENERATION THROUGHOUT THE FIVE-YEAR IPHC BIOLOGICAL AND ECOSYSTEM SCIENCE RESEARCH PLAN (2017-2021)

In relation to the research areas and research activities contemplated in the Five-Year IPHC Biological and Ecosystem Science Research Plan (2017-2021), the external research grants awarded to the IPHC and the peer-reviewed journal publications resulting from research activities (published, submitted to a peer-reviewed journal and in preparation) are indicated in Appendix VI.

REMAINING RESEARCH AREAS CONTEMPLATED IN THE FIVE-YEAR IPHC BIOLOGICAL AND ECOSYSTEM SCIENCE RESEARCH PLAN (2017-2021):

The following research activities are planned to be conducted prior to the finalization of the current 5-year Research Plan (2017-2021):

1. Migration and Distribution. Continuation of the wire tagging efforts of U32 Pacific halibut will take place in the FISS in 2021.

2. Reproduction.
    2.1. Sex-ratio information. Processing of fin clips from the 2020 commercial samples for DNA extraction and genotyping for sex will begin in the summer of 2021. In addition, efforts to attempt to purify DNA from archived otoliths will be resumed during the summer of 2021.
    2.2. Maturity assessment.
        2.2.1. Skip-spawning. Information on histological and biological characteristics of females with delayed ovarian development during the spawning capable reproductive phase will be contrasted with field observations (maturity classification and imaging).

2.2.2. Fecundity determinations. Current methods for fecundity determinations will be assessed and selected based on accuracy and feasibility for Pacific halibut field collections. Ovaries from three females that are classified as maturing (stage 2) will be collected in FISS for testing selected fecundity assessment methods in the Fall of 2021.

3. Growth. Following the identification of growth markers, as described in Planas et al. (in preparation), the IPHC Secretariat is planning on testing 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 collected in the field. These muscle samples correspond to a total of 30 age-matched individuals (4 years-old) of different sizes and will prove useful 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 will be 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.

4. Discard Mortality and Survival Assessment. Work contemplated in this area involves the field experimental component of the study on mortality rates and survival assessment of Pacific halibut discarded by the recreational fishery. This work is described in detail in the provided project narratives of the grants awarded from the National Fish and Wildlife Foundation and the North Pacific Research Board to conduct this work. This project will cease in 2021.

5. Genetics and Genomics. Planned research activities in this research area involve the completion of library construction and low coverage whole genome resequencing for the totality of 600 individual samples from Pacific halibut collected during the spawning season in order to establish the genetic structure and identify genetic baselines according to protocols presented at SRB017. The objectives, management implications, description of available sample collections and methodology are detailed in the Update on Progress on the Main Research Activities (Section 5) in the present document and in the provided project narrative of a grant proposal that the IPHC Secretariat is preparing for submission to a funding agency and that is requesting review from the SRB.

RECOMMENDATION/S

That the SRB:

a) **NOTE** paper IPHC-2020-SRB018-08 which provides a response to requests from SRB017, and a report on current and future research activities contemplated within the IPHC Five-Year Biological and Ecosystem Science Research Plan (2017-2021).
b) **REQUEST** any further analyses to be provided at SRB019, September 2021.

REFERENCES

Anderson, G., J. Hampton, N. Smith, and C. Rico. 2019. Indications of strong adaptive population genetic structure in albacore tuna (*Thunnus alalunga*) in the southwest and central Pacific Ocean. Ecology and Evolution 9(18):10354–10364.

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 Inst. 1(1): 1. Cambridge, UK. Available from http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Bentzen, P., Britt, J., and Kwon, J., 1998. Genetic variation in Pacific halibut (*Hippoglossus stenolepis*) detected with novel microsatellite markers. Report of Assessment and Research Activities. International Pacific Halibut Commission, Seattle, WA, pp. 229–241.

Bolger, A.M., Lohse, M., and Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30**(15): 2114–2120. doi:10.1093/bioinformatics/btu170.

Brown-Peterson, N. J., et al. (2011). "A Standardized Terminology for Describing Reproductive Development in Fishes." Marine and Coastal Fisheries 3(1): 52-70.

Caye, K., Deist, T.M., Martins, H., Michel, O., and Francois, O. 2016. TESS3: fast inference of spatial population structure and genome scans for selection. Mol Ecol Resour 16(2): 540-548. doi:10.1111/1755-0998.12471

Clucas, G.V., Lou, R.N., Therkildsen, N.O., and Kovach, A.I., 2019. Novel signals of adaptive genetic variation in northwestern Atlantic cod revealed by whole-genome sequencing. Evol Appl 12, 1971-1987.

Drinan, D.P., Galindo, H.M., Loher, T., and Hauser, L., 2016. Subtle genetic population structure in Pacific halibut *Hippoglossus stenolepis*. J Fish Biol 89, 2571-2594.

Drinan, D.P., Loher, T., and Hauser, L., 2018. Identification of Genomic Regions Associated With Sex in Pacific Halibut. J Hered 109, 326-332.

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

Fish, T., Wolf, N., Harris, B. P., and Planas, J. V. Characterization of the reproductive cycle in female Pacific halibut, *Hippoglossus stenolepis*. (In preparation).

Grant, W.S., Teel, D. J., and Kobayashi, T., 1984. Biochemical Population Genetics of Pacific Halibut (*Hippoglossus stenolepis*) and Comparison with Atlantic Halibut (*Hippoglossus hippoglossus*). Canadian Journal of Fisheries and Aquatic Sciences 41, 1083-1088.

Grewe, P. M., P. Feutry, P. L. Hill, R. M. Gunasekera, K. M. Schaefer, D. G. Itano, D. W. Fuller, S. D. Foster, and C. R. Davies. 2015. Evidence of discrete yellowfin tuna (*Thunnus albacares*) populations demands rethink of management for this globally important resource. Scientific Reports 5(October):1–9.

Hauser, L., and Carvalho, G. R., 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish and Fisheries 9, 333-362.

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., A. Albrechtsen, and R. Nielsen. 2014. ANGSD: Analysis of Next Generation Sequencing Data. BMC Bioinformatics 15(1):1–13.

Langmead, B., and Salzberg, S.L. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9(4): 357-359. doi:10.1038/nmeth.1923

Li, B., and Dewey, C.N. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12(1): 323. doi:10.1186/1471-2105-12-323.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., and Genome Project Data Processing, S. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25(16): 2078-2079. doi:10.1093/bioinformatics/btp352.

Loher, T., Dykstra, C., Simeon, A., Wolf, N., Harris, B.P., Hicks, A., Stewart, I.J., and Planas, J.V Estimation of post-release mortality using acceleration-logging archival pop-up tags for Pacific halibut (*Hippoglossus stenolepis*) discarded from longlines. (In Preparation).

Meisner, J., and Albrechtsen, A. 2018. Inferring Population Structure and Admixture Proportions in Low-Depth NGS Data. Genetics 210(2): 719-731. doi:10.1534/genetics.118.301336.

Nielsen, J.L., Graziano, S.L., Seitz, A.C., 2010. Fine-scale population genetic structure in Alaskan Pacific halibut (*Hippoglossus stenolepis*). Conservation Genetics 11, 999-1012.

Picard toolkit. 2019. Broad Institute, GitHub Repos. http//broadinstitute.github.io/picard/; Broad Inst.

Planas, J.V., Jasonowicz, A., Simeon, A., Rudy, D., Timmins-Schiffman, E., Nunn, B.L., Kroska, A., Wolf, N., and Hurst, T.P. Physiological signatures of temperature-induced growth manipulations in white skeletal muscle of juvenile Pacific halibut (*Hippoglossus stenolepis*). (In Preparation).

Rideout, R. M., Rose, G. A., Burton, M. P. M. (2005). "Skipped spawning in female iteroparous fishes." Fish and Fisheries 6: 50-72.

Robinson, M.D., and Oshlack, A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 11(3): R25. doi:10.1186/gb-2010-11-3-r25.

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

Skotte, L., T. S. Korneliussen, and A. Albrechtsen. 2013. Estimating individual admixture proportions from next generation sequencing data. Genetics 195(3):693–702.

Stewart, I.J., and Martell, S. J. D., 2014. A historical review of selectivity approaches and retrospective patterns in the Pacific halibut stock assessment. Fisheries Research 158, 40-49.

Stewart, I., and Hicks, A. (2018). Assessment of the Pacific halibut (*Hippoglossus stenolepis*) stock at the end of 2017. Int. Pac. Halibut Comm. Annual Meeting Report: IPHC-2018-AM094-10.

Stewart, I., and Webster, R. (2021). Overview of data sources for the Pacific halibut stock assessment, harvest policy, and related analyses. Int. Pac. Halibut Comm. Stock Assessment Document: IPHC-2021-SA-02.

Therkildsen, N.O., and Palumbi, S.R., 2017. Practical low-coverage genomewide sequencing of hundreds of individually barcoded samples for population and evolutionary genomics in nonmodel species. Mol Ecol Resour 17, 194-208.

Waples, R.S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J. Hered. **89**(5): 438–450. Available from http://jhered.oxfordjournals.org/content/89/5/438.short [accessed 8 April 2014].

Webster, R.A., Clark, W.G., Leaman, B.M., and Forsberg, J.E. 2013. Pacific halibut on the move: A renewed understanding of adult migration from a coastwide tagging study. Can. J. Fish. Aquat. Sci. **70**(4): 642–653. doi:10.1139/cjfas-2012-0371.

Whitlock, M.C., and Lotterhos, K.E. 2015. Reliable Detection of Loci Responsible for Local Adaptation: Inference of a Null Model through Trimming the Distribution of F(ST). Am Nat 186 Suppl 1(S1): S24-36. doi:10.1086/682949

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

Potential prioritization of proposed research activities (next period)

Research areas	Research activities	Research outcomes	Relevance for stock assessment	Relevance for MSE	Specific analysis input	SA Rank	MSE Rank	Research priorization
Reproduction	Sex ratio of current commercial landings	Sex ratio-at-age	Scale biomass and fishing intensity		Annual sex-ratio at age for the commercial fishery fit by the stock assessment	1. Assessment data collection and processing		1
	Historical sex ratios based on archived otolith DNA analyses	Historical sex ratio-at-age						1
Mortality and survival assessment	Whale depredation accounting and tools for avoidance	New tools for fishery avoidance/deterence; improved estimation of depredation mortality	Improve mortality accounting	Improve estimates of stock productivity	May reduce depredation mortality, thereby increasing available yield for directed fisheries. May also be included as another explicit source of mortality in the stock assessment and mortality limit setting process depending on the estimated magnitude	2. Assessment data collection and processing		2
Reproduction	Histological maturity assessment	Updated maturity schedule	Scale biomass and reference point estimates	Improve simulation of spawning biomass in the Operating Model	Will be included in the stock assessment, replacing the current schedule last updated in 2006	1. Biological input		3
	Examination of potential skip spawning	Incidence of skip spawning			Will be used to adjust the asymptote of the maturity schedule, if/when a time-series is available this will be used as a direct input to the stock assessment			3
	Fecundity assessment	Fecundity-at-age and -size information			Will be used to move from spawning biomass to egg-output as the metric of reproductive capability in the stock assessment and management reference points			3
	Examination of accuracy of current field macroscopic maturity classification	Revised field maturity classification			Revised time-series of historical (and future) maturity for input to the stock assessment			3
Genetics and genomics	Population structure	Population structure in the Convention Area	Altered structure of future stock assessments	Improve parametization of the Operating Model	If 4B is found to be functionally isolated, a separate assessment may be constructed for that IPHC Regulatory Area	2. Biological input	1. Biological parameterization and validation of movement estimates and recruitment distribution	4
	Distribution	Assignment of individuals to source populations and assessment of distribution changes	Improve estimates of productivity		Will be used to define management targets for minimum spawning biomass by Biological Region	3. Biological input		5
Migration	Larval and juvenile connectivity studies	Improved understanding of larval and juvenile distribution	Improve estimates of productivity	Improve parametization of the Operating Model	Will be used to generate potential recruitment covariates and to inform minimum spawning biomass targets by Biological Region	3. Biological input	1. Biological parameterization and validation of movement estimates	5
Mortality and survival assessment	Discard mortality rate estimate: longline fishery	Experimentally-derived DMR	Improve trends in unobserved mortality	Improve estimates of stock productivity	Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits	1. Fishery yield	1. Fishery parameterization	6
	Discard mortality rate estimate: recreational fishery				Will improve estimates of discard mortality, reducing potential bias in stock assessment results and management of mortality limits			6
	Best handling and release practices	Guidelines for reducing discard mortality			May reduce discard mortality, thereby increasing available yield for directed fisheries	2. Fishery yield		7
Growth	Evaluation of somatic growth variation as a driver for changes in size-at-age	Identification and application of markers for growth pattern evaluation	Scale stock productivity and reference point estimates	Improve simulation of variability and allow for scenarios investigating climate change	May inform yield-per-recruit and other spatial evaluations of productivity that support mortality limit-setting		3. Biological parameterization and validation for growth projections	8
		Environmental influences on growth patterns			May provide covariates for projecting short-term size-at-age. May help to delineate between effects due to fishing and those due to environment, thereby informing appropriate management response			8
		Dietary influences on growth patterns and physiological condition			May provide covariates for projecting short-term size-at-age. May help to deleineate between effects due to fishing and those due to environment, thereby informing appropriate management response			8

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 – 30 June 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**		

APPENDIX VI

Funding and publications resulting from research activities conducted during the 5-yr research plan (2017-2021)

Research areas	Research activities	Project participants	2017	2018	2019	2020	2021	Publications
Migration	Larval connectivity	AFSC-Seattle (lead), IPHC (Sadorus, Webster, Planas)				Ms prep; Ms submission	Pub	**Sadorus et al. 2021a**
	Adult and juvenile migration	IPHC (Loher, Sadorus, Dykstra, Forsberg, 2017 IPHC Intern, Planas)				Ms prep	Ms sub	Loher et al. 2021a (in review)
	Migration	IPHC				Ms prep	Ms sub	Carpi et al. 2021 (in review)
	Environmental variabiliy and distribution	IPHC (Sadorus, Webster), UW				Ms prep	Ms sub	Sadorus et al. 2021b (in review)
Reproduction	Sex ratio of current commercial landings	IPHC (Simeon, Planas, Stewart)					Ms prep	Stewart et al. 2022 (expected)
	Sex-marking program	IPHC (Loher, Simeon, Erikson, Planas)					Ms prep	Loher et al., 2021b (expected)
	Reproductive assessment	IPHC (lead, Planas), APU				Ms prep; Ms submission; Pub		**Fish et al., 2020**
							Ms prep	Fish et al., 2021 (expected)
		IPHC (lead, Simeon, 2019 IPHC Intern, Planas)					Ms prep	Simeon et al., 2022 (expected)
	Field maturity classification	IPHC (lead, Planas), APU					Ms prep	Fish et al., 2022 (expected)
Growth	Identification of growth markers	IPHC (lead, Simeon, Rudy, Planas), AFSC-Newport		NPRB 1704 Funding		Ms prep		Planas et al., 2021 (expected)
	Direct temperature effects on growth							
	Stress effects on growth						Ms prep	Hurst et al., 2022 (expected)
	Growth pattern evaluation	IPHC (Simeon, Planas)						2022 (expected)
Mortality and survival assessment	Trawl DMRs	AFSC (lead), IPHC (Loher)		Ms prep; Ms sub; Pub				**Rose et al., 2019**
	Longline DMRs	IPHC (lead, Dykstra, Loher, Stewart, Hicks, Planas), APU		Saltonstall-Kennedy NOAA Funding		Ms prep; Ms sub	Pub	**Kroska et al. 2021**
						Pub		**van Helmond et al. 2020**
							Ms prep	Loher et al., 2021c (expected)
							Ms prep	Dykstra et al., 2022 (expected)
	Recreational DMRs	IPHC (lead, Dykstra, Stewart, Hicks, Planas), APU		NFWF Funding				Dykstra et al., 2022 (expected)
							NPRB 2009 Funding	
	Pacific halibut trawl avoidance	PSMFC (lead), IPHC (Dykstra Simeon, Rudy, Planas)		BREP Funding		Ms prep; Ms sub	Pub	**Lomeli et al., 2021**
Genetics and Genomics	Genome sequencing	IPHC (lead, Jasonowicz, Simeon, Planas), INRA-France					Ms prep	Jasonowicz et al., 2021 (expected)
	Transcriptomic resources	IPHC (Jasonowicz, Simeon, Planas)					Ms prep	Jasonowicz et al., 2022 (expected)
	Population structure	IPHC (Jasonowicz, Planas)						Jasonowicz et al., 2022 (expected)

Pub (in bold): publication in peer-reviewed journal

Ms sub: manuscript submitted to peer-reviewed journal (in review)

Ms prep: manuscript in preparation for submission to peer -reviewed journal