Report on Current and Future Biological Research Activities

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, 20 AUGUST 2020)

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 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.

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Migration and Distribution.

   Knowledge of Pacific halibut migration throughout all life stages is necessary in order to gain a complete understanding of stock distribution and the factors that influence it.

   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. A manuscript describing this project is now under second revision in the journal *Fisheries Oceanography* (Sadorus et al., in review).

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 2019, a total of 821 Pacific halibut were tagged and released during the NMFS Gulf of Alaska trawl survey and 885 tags were released during the NMFS Bering Sea survey. Through 2019, a total of 6,536 tags have been released in the NMFS groundfish trawl survey and, to date, 52 tags have been recovered. On the IPHC FISS, a total of 3,112 U32 Pacific halibut had been wire tagged are released and 90 of those have been recovered to date. The wire tagging effort on the FISS was not implemented in 2019 due to work load commitments on the FISS operation. However, 54 U32 Pacific halibut were wire-tagged as part of other research projects in 2019. Recoveries by release and recovery Regulatory Area are reported in Table 1 and numbers recovered by release Regulatory Area and years at liberty are shown in Table 2. Wire-tagging efforts on U32 Pacific halibut are continuing in 2020 on IPHC's FISS but not on the NMFS groundfish trawl survey because of its cancellation due to COVID-19.

Table 1. Recoveries of tagged Pacific halibut from U32 wire tagging conducted between 2015 and 2019 by release and recovery Regulatory Area.

Release		**Recovery Regulatory Area**										
Reg Area	**Total Releases**	**2A**	**2B**	**2C**	**3A**	**3B**	**4A**	**4B**	**4D**	**4E**	**CLS**	**Total**
2A	34	1	3									**4**
2B	636	1	27									**28**
2C	747		8	22	1							**31**
3A	2,005				31	1						**32**
3B	2,309		1		3	25	1			1	1	**32**
4A	1,096				2		6	1		1		**10**
4B	369							5				**5**
4C	244						1					**1**
4D	469						1		2	1		**4**
4E	1,420								1	2	3	**5**
CLS	544				2	1	1			1		**5**
Total	**9,873**	**2**	**12**	**6**	**29**	**6**	**8**	**1**	**2**	**4**	**4**	**158**

Table 2. Number of Pacific halibut recovered by years at liberty and by release Regulatory Area from U32 wire tagging conducted between 2015 and 2019 (includes recoveries for which recovery area is not known).

Years at liberty	Number recovered by release Regulatory Area											
	2A	**2B**	**2C**	**3A**	**3B**	**4A**	**4B**	**4C**	**4D**	**4E**	**CLS**	**Total**
0		7	2	3	1	1	1	1	1	1		**18**
1	2	14	17	14	12	3	2		1	4	2	**71**
2	2	7	9	7	12	3	2		1	1		**44**
3		1	3	7	5	2	1		1		1	**21**
4			1	1	3	1					2	**8**
5				1								**1**
Total	**4**	**29**	**32**	**33**	**33**	**10**	**6**	**1**	**4**	**6**	**5**	**163**

2. Reproduction.

Efforts at IPHC are currently underway to address two critical issues in stock assessment for estimating the female spawning biomass: the sex ratio of the commercial landings and maturity assessment.

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

The IPHC Secretariat has recently completed the processing of genetic samples from the 2019 commercial landings and results indicate that the percentage of females coastwide in the commercial catch is approximately 78%, showing a decline in all regulatory regions since 2017. 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 also working towards providing information regarding the sex ratios in years previous to 2017 through the use of genotyping techniques using historical otolith samples. The IPHC Secretariat has recently tested whether DNA can be extracted from otoliths and whether the extracted DNA is of sufficient quantity and quality to be used in the genotyping assays currently used with DNA derived from fin clips. Preliminary results using recently collected otoliths with visible residue indicate that DNA can be extracted from otoliths, albeit at low concentration, and that the genotyping assays can successfully be used on otolith DNA for sex identification. Further studies will be completed by the SRB meeting regarding the viability of this protocol on clean archived otoliths.

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). These results highlight the need for a better understanding of factors influencing reproductive biology and success for 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) accurate description of oocyte developmental stages and their use to classify female maturity stages; 2) comparison of macroscopic (based on field observations) and microscopic (based on histological assessment) maturity stages and revision of maturity criteria; 3) revision of current estimates of female age-at-maturity; and 4) investigation of skip-spawning in females.

The IPHC Secretariat has described for the first time the different oocyte stages that are present in the ovary of female Pacific halibut and how these are used to classify females histologically to specific maturity stages. This information is contained in a manuscript that is currently in preparation for submission to a peer-reviewed journal (Fish et al., in preparation). Currently underway is a study assessing temporal changes in female maturity, as assessed by microscopic observations of ovarian samples collected throughout an entire annual reproductive cycle, and the comparison with macroscopic staging of maturity status as conducted in the field.

In addition, the IPHC Secretariat is conducting temporal and spatial analyses of female maturity schedules through the collection of ovarian samples in FISS. For the temporal analysis of maturity, ovarian samples have been collected in the Portlock region (central Gulf of Alaska) during the same period (June-July) for 30 females (>90 cm length) for four consecutive years: 2017, 2018, 2019 and 2020. These ovarian samples are being processed for histology and microscopic maturity staging will be conducted to compare the maturity status over time. Furthermore, for the spatial analysis of maturity, ovarian samples from 30 females (>90 cm length) are currently being collected in the FISS in 5 different regions in the Gulf of Alaska in order to determine potential spatial differences in maturity.

The IPHC Secretariat is also investigating the possible presence of skip spawning females by focusing on the histological characteristics of ovaries of females of reproductive age (older than 12 years of age) and that are classified as immature by macroscopic and microscopic staging at a time of the year when most females have oocytes at stages in late vitellogenesis or in later stages.

Plans are underway to measure fecundity in 2021 in order to be able to relate fecundity to age and size in female Pacific halibut.

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

   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 use of growth markers for evaluating growth patterns in the Pacific halibut population and the effects of environmental factors on somatic growth. In order to pursue these objectives, the IPHC Secretariat has conducted investigations on the effects of temperature variation on growth performance, as well as on the effects of density, hierarchical dominance and handling stress on growth in juvenile Pacific halibut in captivity. These studies have been partially funded by a grant from the North Pacific Research Board to the IPHC (Appendix II) and the preliminary results have been described in the final report of the project and a manuscript for publication is currently in preparation (Planas et al., in preparation).

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. 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.)

   In order to better estimate post-release survival of Pacific halibut caught incidentally in the directed longline fishery, the IPHC Secretariat is conducting investigations to understand the relationship between fish handling practices and fish physical and physiological condition and survival post-capture as assessed by electronic archival tagging with funding by a grant from the Saltonstall-Kennedy Grant Program NOAA (Appendix II). Currently, investigations are devoted to decipher potential relationships between individual physiological characteristics, environmental conditions, and handling practices, and final viability release classifications.

Electronic monitoring (EM) systems were proven to be effective at accurately capturing the release method applied to each animal. Ongoing work has focused on investigating the ability to estimate individual Pacific halibut lengths from EM systems in the longline fishery. The previously captured footage has been used to generate lengths for ~300 fish., Fish lengths are being compared to the actual measurement of fish from the same skates of gear. Additionally, efforts are currently underway to do a similar comparison from a current FISS operation, with a pre-calibrated camera, using imagery of the fish when they are located in the area of the screen where the fish would normally be shaken if not of legal size. .

4.2. Quantification of handling practices and physiological stress in Pacific halibut released in the charter recreational fishery.
Principal Investigator: Claude Dykstra (B.Sc.)

The IPHC has begun 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 (Appendix II). As previously reported, results show that the guided recreational fleet predominantly uses circle hooks (75-100%), followed by jigs. Predominant hook release methods included reversing the hook (54%), or twisting the hook out with a gaff (40%), fish are landed with the line and hook, followed by hand netting, and while aboard the fish were generally handled by supporting both the head and tail (65%), while other common techniques included handling by the operculum (10%) or by the tail alone (10%). We are now developing experimental designs for a field project that is being planned for the Spring of 2021 and in which fish condition and stress will be evaluated to identify best practices intended to minimize discard mortality in this fishery. The design effort is considering whether it is best to replicate field treatments in a way that reflects the questionnaire results in their entirety, generating an overall DMR estimate for that sector to be derived, or to focus on one set of conditions of a particular predominant interest and to develop a DMR that is less broad, but more transferable for best practices. Replicating questionnaire results involves many variables, several with uncontrollable features (10 variables: Reg Area, Port, Fish Size, Hook Type, Hook Size, Capture conditions, Landing method, Time on Deck, Fish Condition, and Release Method) allowing for an overall generic DMR estimate to be derived, with minimal parsing as to the influences of each variable. Selecting the more focused route would refine estimates for a specific hook type, and allow for fine tuning of one portion of the overall estimates of mortality (for instance circle hook effect of most predominant hook size, which is nested within several hook types contributing to an overall DMR estimate in a region). This work continues to be the subject of ongoing efforts to secure sufficient funding for a meaningful number of sPAT tags to estimate discard mortality.

5. Genetics and genomics. The IPHC Secretariat is exploring avenues for incorporating genetic approaches for a better understanding of population structure and distribution and is also building genomic resources to assist in genetics and molecular studies on Pacific halibut.

5.1. Genetics.
Principal Investigator: Andy Jasonowicz (M.Sc.)

The primary objective of the proposed studies is to investigate the genetic structure of the Pacific halibut population and to conduct genetic analyses to inform on Pacific halibut movement and distribution in the eastern North Pacific Ocean. Two specific objectives will be pursued:

5.1.1. Determine the genetic structure of the Pacific halibut population in the North-eastern Pacific Ocean. Understanding population structure is imperative for sound management and conservation of natural resources (Hauser, 2008). Pacific halibut in US and Canadian waters are managed by the International Pacific Halibut Commission (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 North-eastern Pacific Ocean population of Pacific halibut by allozyme (Grant, 1984) and small-scale microsatellite analyses (Bentzen, 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 the Aleutian Islands 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 recent collection of winter (i.e. spawning season) genetic samples in the Aleutian Islands by the IPHC in early 2020, winter collected samples from 5 different geographic areas across the North-eastern Pacific Ocean (i.e. British Columbia, Central Gulf of Alaska, Bering Sea, Central and Western Aleutian Islands) are now available to re-examine the genetic structure of the Pacific halibut population. Using low-coverage whole genome resequencing (Therkildsen and Palumbi, 2017; Clucas et al., 2019), and the recently sequenced Pacific halibut genome (deposited at DDBJ/ENA/GenBank under the accession JABBIT000000000), the IPHC Secretariat's main objective is to revise our current understanding of population genetic structure using novel, high-resolution genomic technology. The IPHC Secretariat will expand on previous work by including additional samples that have not yet been analyzed (winter collections from 2007, 2018, and 2020) and scanning the genome for signatures of natural selection. By including samples collected over multiple years, we can examine how spatial genetic variation and signatures of natural selection may change over time. The results from the proposed genomic studies would provide important information on spawning structure and provide management advice

regarding the relative justifiability for considering the western Aleutians as a genetically-distinct substock.

Methods

Collected fin clips preserved in ethanol from Pacific halibut during the spawning season (i.e. winter) will be processed for DNA extraction and purification using Qiagen kits. The available samples correspond to the following geographic areas and dates of winter collection: British Columbia (Haida Gwaii; 1998-1999, 2004, 2007), Central Gulf of Alaska (Portlock region; 1998-1999, 2004, 2007, 2018), Bering Sea (Pribilof Canyon; 2004, 2007), Central Aleutian Islands (Adak; 2007, 2020) and Western Aleutian Islands (Attu; 2020). Samples from 50 individuals from each of these collections, totaling 600 individuals, will be processed for genetic analyses. Libraries for low-coverage whole-genome resequencing will be prepared according to published protocols (Clucas et al. 2019) and sequencing will be conducted using the Illumina NovaSeq platform. With an output of 2.5 billion reads (750Gb) per NovaSeq S4 lane, we estimate that sequencing could be carried out in 3 lanes to achieve 5x sequencing coverage per individual. An initial sequencing run of 36 samples will be carried out using a single Illumina HiSeq 4000 lane to validate these numbers and library preparation methods.

An approach similar to the one used by Clucas et al. (2019) will be used to process the raw sequence reads prior to genotyping. Bowtie2 (Langmead and Salzberg 2012) will be used in end-to-end mode to align the raw sequence reads to the Pacific halibut genome. Samtools (Li et al. 2009) will be used to filter out alignments with a mapping quality score less than 20 (99% change of a correct alignment) and reads aligned to multiple locations in the genome. Polymerase chain reaction (PCR) duplicates will be removed using Piccard (https://broadinstitute.github.io/picard) and overlapping read pairs will be clipped using bamutil (Jun et al. 2015). Local realignment will be will be performed using GATK (Poplin et al. 2018) to improve alignments around insertion/deletion elements.

The software ANGSD (Korneliussen et al. 2014) and ATLAS (Link et al. 2017) will be used detect SNPs through the Pacific halibut genome. ANGSD will also be used to estimate measures of genetic diversity (allele frequencies and heterozygosity) for each sample collection. We expect to identify millions of SNPs taking this approach (Therkildsen and Palumbi 2017; Clucas et al. 2019). Measures of genetic differentiation (F_{ST}) will be estimated among the sample collections to examine levels of divergence between them and test for patterns of isolation by distance. To investigate the possibility of cryptic population structure, clustering methods will be used. 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 F_{ST} 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 currently under development by the IPHC Secretariat will be very useful in interpreting the functional significance of the many SNPs that we expect to identify in this study.

5.1.2. Analysis of genetic variability among juvenile Pacific halibut in the Bering Sea and the Gulf of Alaska. The aim of this objective is to evaluate the genetic variability or genetic diversity among juvenile Pacific halibut in a given ocean basin in order to infer information on the potential contribution from fish spawned in different areas to that particular ocean basin. We hypothesize that genetic variability among juvenile Pacific halibut captured in one particular ocean basin (e.g. eastern Bering Sea) may be indicative of mixing of individuals originating in different spawning grounds and, therefore, of movement. By comparing the genetic variability of fish between two ocean basins (i.e. eastern Bering Sea and Gulf of Alaska), we will be able to evaluate the extent of the potential contribution from different sources (e.g. spawning groups) in each of the ocean basins and provide indications of relative movement of fish to these two different ocean basins. The use of genetic samples from juvenile Pacific halibut collected in the National Marine Fisheries Service trawl survey in the eastern Bering Sea and in the Gulf of Alaska, aged directly by otolith reading or indirectly through a length-age key, will allow us to provide information on genetic variability among fish that are at or near their settlement or nursery grounds.

Methods

Fin clips from 150 fish from the eastern Bering Sea and from 150 fish from the Gulf of Alaska will be selected for genetic analysis. Fin clips have been collected in these areas between 2016-2019 (Table 3). Sample selection will be distributed among sampling years and age class. When possible, otolith reading will be used to directly age fish and an length-age key will be used to indirectly age fish that do not have otoliths samples available. For fish of unknown sex, genetic sex will be determined using SNPs to two sex-linked loci developed (Drinan et al., 2018) and used for determining the genetic sex of commercial Pacific halibut captures.

A similar technical approach with respect to sequencing and bioinformatics in section 5.1.1 will be used for this analysis. The software ANGSD and ATLAS will be used to estimate measures of genetic diversity (allele frequencies and heterozygosity) for sample collections made in the eastern Bering Sea and the Gulf of Alaska. Tests for Hardy-Weinberg equilibrium will also be performed using ANGSD. Clustering methods such as discriminant analysis of principal components (DAPC) (Jombart et al. 2010) and the estimation of admixture proportions (using ngsAdmix) will also be used to identify background population structure and identify individuals that may have originated in different ocean basins.

Table 3. Number of genetic samples available per year, aged or non-age, collected in the NMFS trawl survey.

All Samples by Year

Area		2016	2017	2018	2019
BS	**Total**	622	746	943	1,074
	Aged	188	195	167	138
GOA	**Total**		702		1,155
	Aged				340

Aged Samples (ages 1-5 only)

Area	Age	2016	2017	2018	2019
BS	**1**				25
	2	7		35	11
	3	39	40	34	10
	4	45	71	51	4
	5	25	36	30	4
GOA	**1**				57
	2				38
	3				28
	4				28
	5				19

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. Genome sequencing. The IPHC Secretariat has recently 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. In addition, the Pacific halibut genome has been annotated and is available in NCBI as NCBI Hippoglossus stenolepis Annotation Release 100.

5.2.2. Expressed Sequence Tags. 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 functional annotation of these transcriptomes to describe tissue-specific gene expression complements the genome sequencing efforts and represents a resource that will provide biological insights at a molecular level for ongoing and future IPHC research.

The IPHC Secretariat reported previously on the results of Illumina sequencing and assembly of the 12 individual tissues as well as the resulting combined assembly. 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.

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 (Figure 1) and BLASTp, respectively.

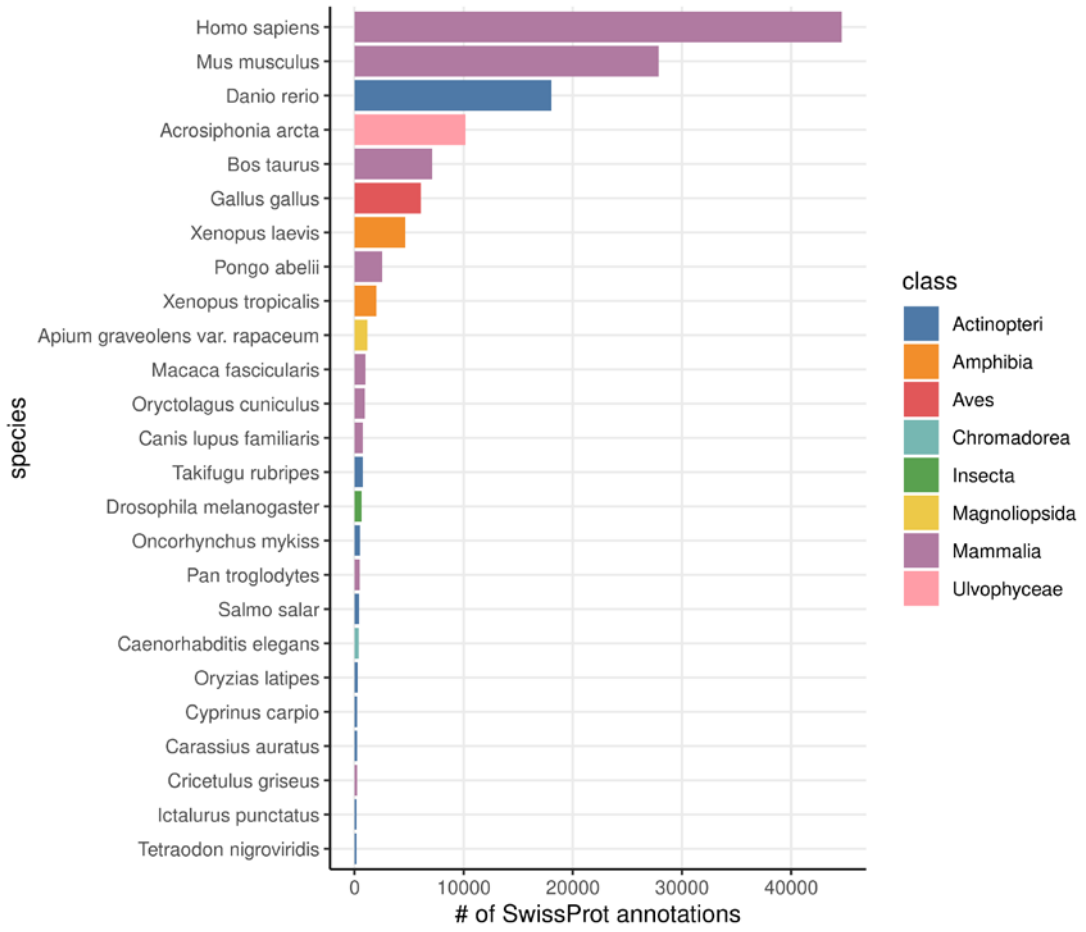

Figure 1. Most represented species assigned to top blastx match in the Swiss-Prot database.

Filtered reads were mapped back to the combined transcriptome assembly and differential gene expression analysis was performed by RSEM (v1.2.28) (Li and Dewey 2011). The raw RNA-seq read counts for each gene were normalized using the trimmed mean of M-values (TMM) method (Robinson and Oshlack 2010). The R package TissueEnrich (v1.6.0) (Jain and Tuteja 2019) was used to identify tissue-specific genes according to the expression categories defined by the Human Protein Atlas (HPA) (Uhlén et al. 2015), with the 'Tissue-enriched" category indicating genes with an expression level greater than or equal to 1 (TPM or FPKM) that also have at least five-fold higher expression levels in a particular tissue compared to all other tissues. Analysis of the three HPA expression categories across the 12 individual Pacific halibut tissues evidenced differences in the number of tissue-specific transcripts, with retina and pituitary containing the highest number of tissue-specific transcripts, followed by gill, testis and brain (Figure 2). Spleen and white muscle were the two tissues with the lowest number of tissue-specific transcripts.

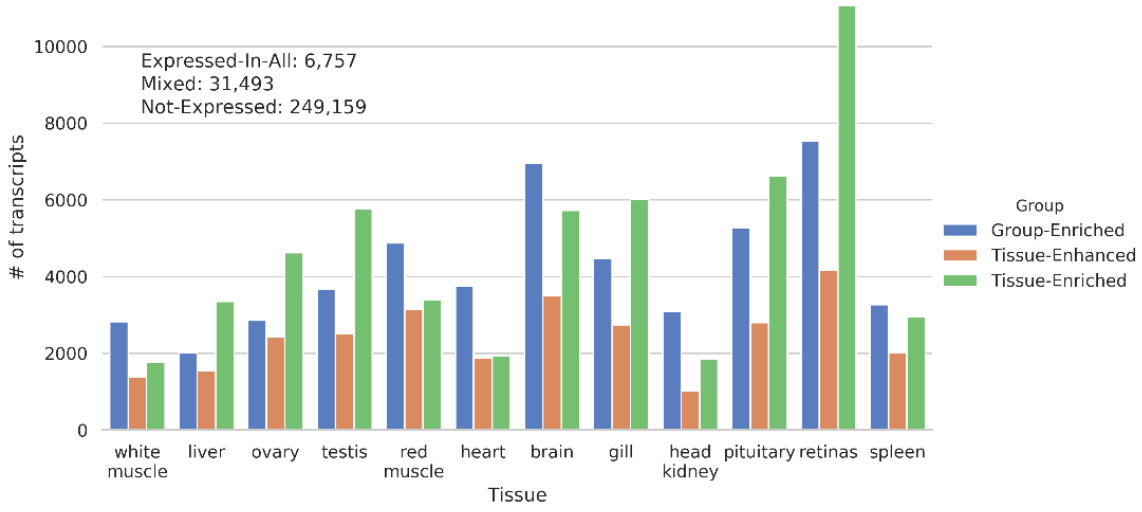

Figure 2. Number of transcripts in each expression category as defined by Uhlen et al. (2015).

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.

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.

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., and Planas, J. V. A comprehensive description of oocyte developmental stages in Pacific halibut, *Hippoglossus stenolepis*. Journal of Fish Biology (In review).

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.

Jain, A., and Tuteja, G. 2019. TissueEnrich: Tissue-specific gene enrichment analysis. Bioinformatics 35(11): 1966-1967. doi:10.1093/bioinformatics/bty890Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC genetics 11(1):94.

Jombart, T., Devillard, S. & Balloux, F. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet* 11, 94 (2010). https://doi.org/10.1186/1471-2156-11-94

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.

Poplin, R., Ruano-Rubio, V., DePristo, M.A., Fennell, T.J., Carneiro, M.O., Van der Auwera, G.A., Kling, D.E., Gauthier, L.D., Levy-Moonshine, A., Roazen, D., Shakir, K., Thibault, J., Chandran, S., Whelan, C., Lek, M., Gabriel, S., Daly, M.J., Neale, B., MacArthur, D.G., and Banks, E. 2018. Scaling accurate genetic variant discovery to tens of thousands of samples. doi:10.1101/201178.

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).

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.; Goldstein, E.; Webster, R.; Stockhausen, W.; Planas, J.V.; Duffy-Anderson, J. Multiple life-stage connectivity of Pacific halibut (*Hippoglossus stenolepis*) across the Bering Sea and Gulf of Alaska. Fisheries Oceanography (In Review).

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.

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.

Uhlén, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C.A., Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., and Ponten, F. 2015. Proteomics. Tissue-based map of the human proteome. Science 347(6220): 1260419. doi:10.1126/science.1260419

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

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

Summary of awarded research grants

Project #	Grant agency	Project name	PI	Partners	IPHC Budget ($US)	Management implications	Grant period
1	**Saltonstall-Kennedy NOAA**	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival (Award No. NA17NMF4270240)	IPHC	Alaska Pacific University	$286,121	Bycatch estimates	September 2017 – August 2020
2	**North Pacific Research Board**	Somatic growth processes in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress manipulation effects (NPRB Award No. 1704)	IPHC	AFSC-NOAA-Newport, OR	$131,891	Changes in biomass/size-at-age	September 2017 – February 2020
5	**National Fish & Wildlife Foundation**	Improving the characterization of discard mortality of Pacific halibut in the recreational fisheries	IPHC	Alaska Pacific University, U of A Fairbanks, charter industry	$98,902	Bycatch estimates	April 2019 – June 2021
Total awarded ($)					**$516,914**		