Report on Current and Future Biological Research Activities

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PURPOSE

To provide the Scientific Review Board with an update of current progress on research projects conducted and planned by the Biological and Ecosystem Science Research Program.

BACKGROUND

The primary biological research activities at IPHC that follow Commission objectives are identified and described in the proposed Five-Year Research Plan for the period 2017-21. These activities are summarized in five broad categories, as follows:

1) Migration. 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.

   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. Work is nearing completion on this cooperative project between NOAA EcoFoci and the IPHC. Larval advection modeling is producing information about possible connectivity pathways during cold and warm years as well as quantifying the degree of connectivity between known spawning grounds and settlement both between and within the Gulf of Alaska and Bering Sea. Application of the IPHC-developed space-time model is being used to assess distribution of young fish from 2-year-old to adult ages as they move away from the settlement grounds. Results will provide a new understanding of linkages between spawning grounds, eventual settlement, and subsequent migration of young fish, as well as variability in these pathways under different environmental scenarios. This work will fill a gap in knowledge of early life history dispersal utilized by Pacific halibut. Final results and manuscript draft are expected later this year.

1.2. Wire tagging of U32 Pacific halibut. Wire tagging of Pacific halibut caught in the NOAA/NMFS trawl surveys which began in 2015, is continuing in 2019. Through 2018, 4,749 tags had been released and 39 recovered to date. The wire tagging effort that has taken place during the FISS in recent years is not taking place in 2019 due to work load commitments on the surveys. Through 2018, a total of 3,112 U32 Pacific halibut had been wire tagged and 39 of those have been recovered to date.

1.3. Electronic archival tagging. Electronic archival tags that allow for daily light-based geo-positioning as well as depth and temperature recording will be deployed on U32 Pacific halibut caught in the eastern Bering Sea during 2019. The project began in 2018 aboard the IPHC's Fishery Independent Setline Survey (FISS), during which 255 fishery-recovery long-term (7 year recording capacity) archival tags were deployed coastwide and 13 Pop-up Archival Transmitting (PAT) tags were deployed in the Aleutian Islands. This year's effort will deploy fishery-recovery archival tags (n = 62) from the NOAA/NMFS Eastern Bering Sea (EBS) trawl survey; a combination of fishery-recovery (n = 35) and PAT (n = 9) tags around the Pribilof Islands in collaboration with the Central Bering Sea Fishermen's Association (CBSFA); and a combination of fishery-recovery (n = 50) and PAT (n = 16) tags in the Norton Sound and St. Lawrence Island region in collaboration with the Norton Sound Economic Development Corporation (NSEDC). Of the tags that are planned for deployment in the EBS trawl survey, roughly half will be deployed along the Alaska Peninsula and half at stations on or northward of 58º50' N latitude and west of 162º W longitude. In addition, a small number of PAT tags (n = 6) will be deployed north of St. Lawrence Island via the NMFS Northern Bering Sea trawl survey. These efforts will be accompanied by tagging of large (>100 cm) Pacific halibut by NSEDC in the Norton Sound region, so as to produce data that are comparable to the IPHC's prior PAT-tagging research conducted to examine adult connectivity and spawning stock structure throughout the managed range,

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 estimations.

2.1. Sex ratio of the commercial landings. For the first time, the IPHC has generated sex information of the entire set of age commercial landings in 2017. Genetic assays developed in collaboration with the University of Washington (Drinan *et al*. Identification of genomic regions associated with sex in Pacific halibut. *J. Heredity*, 2018, 326-332) consisting in a multiplex Taqman assay for two single nucleotide polymorphisms (SNPs) that are exclusive for females have been conducted at the IPHC biological laboratory on a QuantStudio6 instrument. Fin clips from over 10,000 aged Pacific halibut collected coastwide by IPHC port samplers in 2017 were used for genomic DNA extraction in 96 well plates and Taqman assays were conducted in 384 well plates.

2.2. Maturity estimations. In order to characterize the gonadal maturation schedule, the IPHC is conducting a full characterization of the annual reproductive cycle in female and

male Pacific halibut. Biological samples (gonads, blood, pituitary, otolith, fat content) were collected at monthly intervals from female (N=30) and male (N=30) Pacific halibut captured from the Portlock region in the central Gulf of Alaska throughout an entire calendar year, from September 2017 until August 2018. Formalin-fixed gonadal samples were processed for histology in early 2019 and duplicate histological slides for each sampled Pacific halibut gonad (N = 360 per sex) were stained with Hematoxylin and Eosin and are now available for staging. An MSc student from Alaska Pacific University, with funding from IPHC, was trained for this purpose in March 2019 and will begin staging the entire collection of ovarian histological samples in June 2019. The revision of maturity schedules and the comparison of macroscopic and microscopic ovarian staging will constitute the basis of her MSc dissertation. Preliminary results include the temporal progression of the four maturity classification stages used for staging females in the IPHC FISS (Fig. 1) and of the gonadosomatic index (gonad weight/round weight x 100; GSI) for both females and males and a classification of the different oocyte developmental stages that is critical for accurate staging.

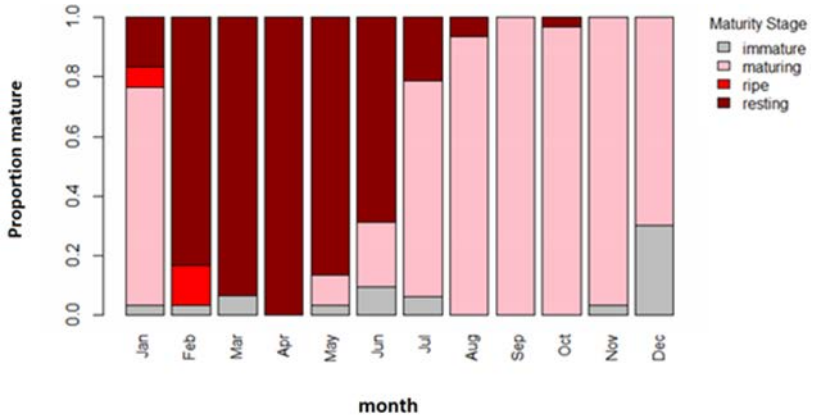

Figure 1. Temporal changes in the proportion of female Pacific halibut staged macroscopically according to the maturity classification criteria used in the FISS throughout an entire calendar year in the Portlock region (Central Gulf of Alaska).

Future plans include: 1) analysis of the entire collection of testicular histological samples and 2) the temporal characterization of reproductive hormones in the blood (17β-estradiol, testosterone and 17α, 20 β-dihydroxy-4-pregnen-3-one for females and 11-ketotestosterone, testosterone and 17 α, 20 β-dihydroxy-4-pregnen-3-one for males) and the gene expression profiles of gonadotropic hormones (follicle-stimulating hormone and luteinizing hormone) in the pituitary of female and male Pacific halibut. In addition to characterizing the progression of reproductive development throughout an entire annual reproductive cycle (intraseasonal) reproductive samples, the IPHC will collect samples in June 2019 to compare with those collected in June 2018 and June 2017 in the Portlock region in order to evaluate possible differences in interseasonal variation in maturity schedules.

3. Growth.

In order to improve our understanding of the possible role of growth alterations in the observed historical changes in size-at-age in Pacific halibut, the IPHC Secretariat is conducting studies aimed at: 1) the identification and validation of physiological markers for growth; and 2) the use of growth markers for evaluating growth patterns in the Pacific halibut population and the effects of environmental influences. The IPHC Secretariat is conducting 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 are partially funded by a grant from the North Pacific Research Board to the IPHC (Appendix I).

3.1. Effects of temperature. Temperature acclimation laboratory studies were conducted in collaboration with the Alaska Fisheries Science Center in Newport, OR and resulted in the successful manipulation of growth patterns: growth suppression by acclimation to low water temperature and growth stimulation by temperature-induced growth compensation in juvenile Pacific halibut. White skeletal muscle samples from the control and treatment groups resulting from the two types of growth manipulations were collected and processed for transcriptomic (i. e. RNAseq) and proteomic analyses. Temperature induced growth suppression resulted in a significantly decrease in the mRNA expression levels of 676 annotated genes and in a significantly decrease in the abundance of 150 annotated proteins. In contrast, temperature-induced growth stimulation resulted in a significant increase in the mRNA expression levels of 202 annotated genes and a significant increase in the abundance of 149 annotated proteins. Efforts are currently underway to analyze these data and prepare a manuscript for submission to a peer-reviewed journal. Based on the transcriptomic results, a set of potential growth marker genes has been selected for validation by qPCR as well as a set of potential housekeeping genes for normalization of expression levels.

3.2. Effects of density. In order to investigate the effects of density on somatic growth, laboratory experiments have been conducted. Fish were held in groups of 8 fish per tank (with 4 replicate tanks), 4 fish per tank (with 4 replicate tanks) and also individually (with 10 replicate tanks) under restricted feeding (at 50% of maximal feeding rate) for a period of 6 weeks. White skeletal muscle samples and liver samples were collected from fish at different densities for target gene expression analyses by qPCR.

3.3. Effects of hierarchical dominance and handling stress. Laboratory experiments designed to investigate the effects of hierarchical dominance and handling stress are currently being conducted. Muscle and liver samples will be collected for target gene expression analyses by qPCR.

4. Discard Mortality Rates (DMRs) and Survival Assessment. 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 tagging. These studies are partially funded by a grant from the Saltonstall-Kennedy Grant Program NOAA to IPHC (Appendix I).

4.1. Evaluation of the effects of **hook release techniques** on injury levels and association with the physiological condition of captured Pacific halibut. The IPHC has evaluated the effects of different release techniques on injury levels (Fig. 2) and the results indicate that a majority (more than 70%) of Pacific halibut released by careful shake and by gangion cutting are classified in the excellent injury category. In contrast, Pacific halibut that encounter the hook stripper are primarily classified in the medium and poor injury categories.

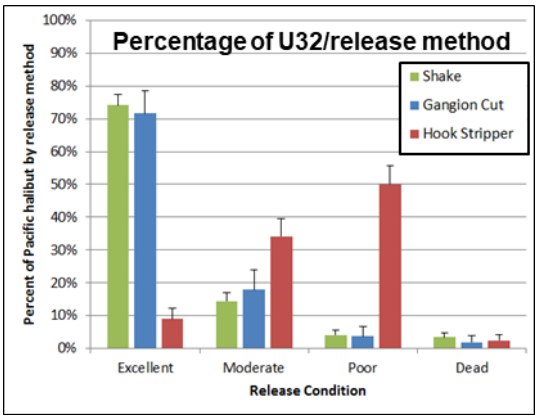

Figure 2. Prevalence of types of injuries (as indicated by injury classification or release condition) in U32 fish released by different hook release techniques (careful shake, gangion cut and hook stripper).

The physiological condition of Pacific halibut subjected to the different hook release techniques is currently being assessed by relating the injury category assigned to each fish with the condition factor, fat levels and levels of blood stress indicators. Blood glucose levels from all fish released have been determined using a colorimetric method. A colorimetric method for measuring blood lactate levels and an enzyme-linked immunoabsorbance (ELISA) method for measuring blood cortisol levels have been validated for Pacific halibut plasma samples and will be used next to measure blood lactate and cortisol levels.

4.2. Post-release survival estimations. In order to evaluate the survival of discarded fish, two types of tagging approaches were used. 1) Classical mark-and-recapture of released fish with wire tags: 1,027 fish (under 33 inches in length) were tagged. 2) Biotelemetric monitoring of released fish with the use of satellite-transmitting electronic archival tags equipped with accelerometers: results from a total of 79 Pacific halibut ranging from 53-81 cm FL allowed us to estimate that the DMR of U32 Pacific halibut that were categorized as being in excellent-condition at the time of their release was approximately 4%.

4.3. Application of electronic monitoring (EM) for capturing the hook release methods. Evaluation of EM data whereby reviewers recorded the release method and condition of

released fish evidenced a high degree (95%-100%) of agreement between the actual release method used and that captured by EM. Therefore, once the survival estimates of fish released by the different hook release techniques are determined, these results strongly suggest that mortality rates could be deduced from EM-captured hook release techniques.

4.4. Discard mortality rates of Pacific halibut in the charter recreational fishery. The IPHC will begin shortly a research project aimed at experimentally deriving DMRs from the charter recreational fishery for the first time. This project has received funding from the National Fish and Wildlife foundation (Appendix I). As an initial step in this project, information from the charter fleet on types of gear and fish handling practices used will be collected through stakeholder meetings and on dock interviews with charter captains and operators. This information will inform the design of the experimental test fishing that will take place in 2020 and in which fish mortality will be estimated as described in 4.2.

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. In an effort to revisit past studies on the genetic structure of the Pacific halibut population conducted with the use of a panel of microsatellites (Drinan *et al*. Subtle genetic population structure in Pacific halibut *Hippoglossus stenolepis*. *J. Fish Biol*., 2016, 89: 2571-2594) on a set of samples covering the entire distribution range of the species, the Secretariat is planning on collecting additional winter samples in those geographic areas that only provided summer samples for that particular study (i.e. Western Aleutian Islands). The additional winter samples from spawning groups in the Western Aleutian islands are critical since the subtle population structure differences observed in the past study were precisely from fish sampled in those areas. Revised genetic analyses will be conducted using state-of-the-art genetics techniques that incorporate a much larger number of markers (e.g. SNPs) and that will provide improved genetic resolution, such as RADseq or whole genome sequencing.

5.2. Genomics. The IPHC Secretariat is currently conducting a project aimed at generating a first draft sequence of the Pacific halibut genome. This study is being conducted in collaboration with the National Institute of Agrogenomic Research (INRA, Rennes, France) and the University of Washington. An initial sequencing effort using genomic DNA from one Pacific halibut female in half an Illumina lane in 2 x 250 pair end mode resulted in a total size of assembled scaffolds of 700 Mb, likely corresponding to the size of the Pacific halibut genome. This non-contiguous genomic sequence is currently being complemented by long read sequencing using the Nanopore technology (i.e. PromethION) combined with Hi-C sequencing for chromosome-scale scaffolding of the genome assembly. The sequencing effort is expected to be completed by the end of summer 2019. Plans to establish a collaboration with Canadian scientists to establish a genomic comparison between Pacific and Atlantic halibut genomes are being discussed, including the possibility of a joint publication highlighting the comparative genomics

approach. In addition to genome sequencing, the IPHC Secretariat has completed transcriptome 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. Current plans regarding this extensive transcriptomic dataset include generating a reference transcriptome for the species and to create a user-friendly, searchable database to be made public in the IPHC website.

APPENDIX I

Summary of current 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 2019 (no cost extension requested)
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
3	**Bycatch Reduction Engineering Program - NOAA**	Adapting Towed Array Hydrophones to Support Information Sharing Networks to Reduce Interactions Between Sperm Whales and Longline Gear in Alaska	Alaska Longline Fishing Association	IPHC, University of Alaska Southeast, AFSC-NOAA	TBD	Whale Depredation	September 2018 – August 2019
4	**Bycatch Reduction Engineering Program - NOAA**	Use of LEDs to reduce Pacific halibut catches before trawl entrainment	Pacific States Marine Fisheries Commission	IPHC, NMFS	TBD	Bycatch reduction	September 2018 – August 2019
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	$98,902	Bycatch estimates	January 2019 – December 2019
				Total awarded ($)	**$516,914**		