DRAFT: Progress Report on Biological Research Activities at IPHC

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PURPOSE

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

BACKGROUND

The main objectives of the Biological and Ecosystem Science Research Program at IPHC are to:

1) identify and assess critical knowledge gaps in the biology of the Pacific halibut;
2) understand the influence of environmental conditions; and
3) apply the resulting knowledge to reduce uncertainty in current stock assessment models.

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 2017-2021, as summarized in a previous document IPHC-2017-SRB10-INT02. These activities can be summarized in five broad categories: 1) Reproduction, 2) Growth and Physiological Condition, 3) Discard Mortality Rates (DMRs) and Survival, 4) Migration and 5) Genetics and Genomics, and have been selected for their important management implications, as follows.

1) The studies conducted on Reproduction are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity.
2) The studies conducted on Growth 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.
3) The proposed work on DMRs is aimed at providing updated estimates of DMRs in both the longline and the trawl fisheries.
4) The studies conducted on Migration are aimed at further understanding reproductive migration and identification of spawning times and locations as well as larval and juvenile dispersal.
5) The studies conducted on Genetics and Genomics 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 NEW AND CONTINUING BIOLOGICAL RESEARCH PROJECTS

For 2018, two new projects were approved that cover specific research needs related to larval migration and distribution (Project 650.22) and thermal growth history (Project 673.15) (Appendix I).

Project 650.22 ("*Larval connectivity*") proposes to study the movement and connectivity of Pacific halibut larvae both within and between the Gulf of Alaska and the Bering Sea. Larval

abundance and distribution in the Gulf of Alaska and the Bering Sea will be modeled over time and over oceanographic and environmental conditions.

Project 673.15 ("*Influence of thermal history on growth*") proposes to study the thermal profile experienced by fish at sea as assessed by electronic archival tagging and otolith microchemistry in order to investigate the relationship between growth patterns (or productivity) and both spatial and temporal variability in environmental conditions for growth. This study will allow us to relate temperature histories that are experienced by individual fish to the growth patterns that they display, examine spatial and temporal trends in rearing conditions and growth, and to extend thermal analyses to untagged Pacific halibut via otolith microchemical analyses. In addition, the data are expected to provide information regarding dispersal of U32 halibut, both seasonally and ontogenically.

Furthermore, twelve continuing projects were approved, including one project dealing with sex identification (621.16) and one dealing with reproductive maturity estimations (674.11), two projects monitoring the Pacific halibut population for mercury and *Ichthyophonus* contamination (642.00, 661.11), three projects continuing migration-related research with the use of wire and satellite tagging and tail imaging (650.21, 670.11, 675.11), one project dealing with the identification of markers for growth-related studies (673.14), one project investigating condition factor indices in wire-tagged fish (672.12), one project dealing with discard mortality rates in the longline fishery (672.13), one project continuing the sequencing of the Pacific halibut genome (673.13), and one project finalizing work conducted on the reevaluation of the weight-length relationship (669.11) (Appendix I). An update on progress on selected projects is provided below:

Project 621.16 ("*Development of genetic sexing techniques*") proposed to identify molecular markers for sex in order to provide a genetic validation of the physical marking of sex at sea (**Project 621.15**) and to provide a method for sex determination in settings in which direct observations of sex cannot be obtained. Three single nucleotide polymorphisms (SPNs) were identified to be associated with sex and molecular assays were developed for two of the identified SNPs. These assays were estimated to have an accuracy of 97.5% in a comparison between assayed sex and visually-determined sex in a sample of 199 fish, based on an assumption that no process or recording errors existed within the visually-determined data (Drinan et al., 2018). The assay was subsequently used to evaluate the accuracy of commercial sex-marking at sea, described below in Subsection 1.1 of "**PROGRESS ON THE MAIN RESEARCH ACTIVITIES**".

Project 642.00 ("*Assessment of mercury and other contaminants*") and **Project 661.11** ("*Ichthyophonus incidence monitoring*") were proposed to monitor levels of mercury contamination and *Ichthyophonus* prevalence, respectively, in Pacific halibut. Tissue samples for monitorization of these two parameters were collected in IPHC's fishery-independent setline survey in 2017.

Project 650.21 ("*Investigation of Pacific halibut dispersal on Bowers Ridge via Pop-up Archival Transmitting (PAT) tags*") proposed to study the migratory behavior of O32 Pacific halibut residing in summer on Bowers Ridge in IPHC Regulatory Area 4B, at both seasonal and interannual time scales. The primary goal of the project is to evaluate relative connectivity between Bowers Ridge, the western Aleutian Islands, and the broader eastern Pacific. Results will be placed in the context of data obtained from prior satellite-tagging experiments in which

more than 200 O32 Pacific halibut have been tagged in the eastern Bering Sea and Aleutian Islands region. In July of 2017, a total of 22 fish (13 female; 8 male; 1 of unknown sex) were successfully tagged on Bowers Ridge, with 16 of the PAT tags programmed to detach from their host fish and report via satellite on 15 January 2018 and the remaining six tags programmed to detach and report in July of 2018 (i.e., after 365 days at liberty). To date, broadcasts have been received from 15 tags, which reported between 24 December 2017 and 22 January 2018. Five fish remain at liberty with tags programmed to report from 5-10 July 2018.

Project 669.11 ("*At-sea collection of Pacific halibut weights to reevaluate conversion factors*") proposed to continue collecting round weights at sea to reevaluate the relationship between fork length and net weight. Data has been collected in IPHC's fishery-independent setline survey in 2017.

Project 670.11 ("*Wire tagging of Pacific halibut on NMFS trawl and setline surveys*") proposed to tag U32 Pacific halibut in order to further understand coastwide migratory and growth patterns of young Pacific halibut. In 2017, a total of 1,469 Pacific halibut were tagged on the NMFS trawl survey (713 in the Gulf of Alaska and 756 from the Bering Sea) and 1,927 Pacific halibut were tagged on the IPHC's fishery-independent setline survey.

Project 672.12 ("*Condition Factors for Tagged U32 Fish*") is continuing to study the relationship between the physiological condition of fish and migratory performance as assessed by tagging in U32 fish in order to better understand the potential use of quantitative physiological indicators in predicting migratory (as well as other types of) performance. Sample collection will continue on the 2018 IPHC fishery-independent setline survey.

Project 672.13 ("*Discard mortality rates and injury classification profile by release method*") proposed to study the relationship between hook release methods in the longline fishery and associated injuries with the physiological condition of fish in order to improve our understanding of factors influencing post-release survival in the directed fishery. Implementation of this project took place in early fall of 2017 during two trips of a chartered vessel, Various hook release methods were alternated randomly at each skate and electronic monitoring was conducted throughout the study (please see below for a full description).

Project 673.13 ("*Sequencing of the Pacific halibut genome*") proposed to characterize for the first time the genome of the Pacific halibut and provide genomic resolution to genetic markers for sex, reproduction, and growth that are currently being investigated in other projects. A first round of genomic sequencing has been performed resulting in a broad but discontinued coverage of the Pacific halibut genome. Further sequencing with more powerful sequencing technologies is currently being planned to achieve full coverage of the Pacific halibut genome.

Project 673.14 ("*Identification and validation of markers for growth in Pacific halibut*") proposed to identify and validate molecular and biochemical profiles that are characteristic of specific growth patterns and that will be instrumental to describe different growth trajectories in the Pacific halibut population and evaluate potential effects of environmental influences on growth. We have already initiated research to study somatic growth in juvenile Pacific halibut and its regulation by temperature and are in the process of identifying molecular signatures of slow versus fast growth patterns that will be used to describe environmental influences on growth trajectories (please see below for a full description).

Project 674.11 ("*Full characterization of the annual reproductive cycle in adult female Pacific halibut*") proposed to study the annual reproductive cycle of female and male Pacific halibut in order to further our understanding of sexual maturation in this species and to improve maturity assessments and maturity-at-age estimates. Sample collection in the Portlock area in central Gulf of Alaska began in September 2017 and is continuing on a monthly basis through August 2018 on chartered vessels (please see below for a full description).

Project 675.11 ("*Tail pattern recognition analysis in Pacific halibut*") is the continuation of a pilot study conducted in 2017 that investigated the identification of individual fish by way of photographic recognition of tail patterns to complement migratory studies. Various pattern-recognition software were used to examine uniqueness and longevity of patterns in both the blind and colored side of the tail, showing relative promise for identifying the same individuals over time. Cameras will be deployed on several vessels during the fisheries-independent setline survey in 2018 and tail images of wire tagged U32 fish will be collected and used to start building a database of tail images.

PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Reproduction. Efforts at IPHC are currently underway to address two critical issues in stock assessment based on estimates of female spawning biomass: the sex ratio of the commercial catch and maturity estimations.

    1.1. Sex ratio of the commercial catch. In the commercial fishery, Pacific halibut are eviscerated at sea and male and female fish cannot be distinguished at the shore-side processing plants, where biological information is collected by IPHC samplers. Therefore, the sex ratio of the commercial catch has not been determined to date. In order to obtain accurate sex information, IPHC initiated efforts to establish protocols for sex marking fish at sea aboard commercial longline vessels and to develop molecular assays to accurately determine the genetic sex in fin clip samples from offloaded fish. If protocols for sex marking at sea in commercial vessels prove to be successful, at-sea sex marking might be routinely employed to generate sex-ratio data for commercial offloads and genetic sex assays (see "**UPDATE ON PROGRESS ON NEW AND CONTINUING BIOLOGICAL RESEARCH PROJECTS",** Project 621.16, above) could then be used as a validation tool to determine and monitor the sex-marking accuracy. In 2015, a sex-marking protocol was developed that consisted of identifying females by making cuts in the dorsal fin and males by a cut in the operculum (McCarthy 2015). In 2016, at-sea marking was implemented aboard commercial longline vessels in a voluntary fashion in British Columbia (Loher et al., 2017). A total of 10 commercial vessels participated in the study by sex marking a total of 325 Pacific halibut that were sampled for fin clips at the ports by IPHC port samplers. The two molecular (SNP) assays were then applied to fin clip samples taken from the fish that had been marked at sea in order to identify their genetic sex. By comparing the sex-related markings to the genetic sex for each of these fish, and assuming 100% sexing accuracy via genetic assay, commercial sex-marking accuracy was determined to be 79% overall and varied from 48-100% among participating vessels. In 2017, the sex-marking project requested voluntary participation from the commercial longline fleet coastwide. During the course of the commercial season, a total of 929 samples were obtained from 84 sex-marked offloads coastwide.

Sex (SNP) assays on these samples are being conducted at the new biological laboratory at IPHC. At-sea marking has been halted pending analysis of 2017 results by the Quantitative Sciences Branch and their subsequent determination regarding the most appropriate direction in which to proceed in order to obtain the quality of sex-ratio data required for assessment and policy analysis.

1.2. Maturity estimations. Each year, the fishery-independent setline survey collects biological data on the maturity of female Pacific halibut that are used in the stock assessment. In particular, a female maturity schedule is used to estimate spawning stock biomass. Currently used estimates indicate that the age at which 50% of female Pacific halibut are sexually mature is 11.6 years on average. However, maturity is estimated with the use of macroscopic visual criteria of the ovaries collected in the field, implying a relative level of uncertainty associated with the employed semi-quantitative assessment. Furthermore, estimates of maturity-at-age have not been revised in recent years and may be outdated. For this reason, current research efforts are devoted to understand reproductive development and maturity in female Pacific halibut.

A recently completed project provided a first description of the changes that take place in the ovary during reproductive development leading to spawning in Pacific halibut by comparing oocyte stages and characteristics between fish caught during the non-spawning season (summer) and the spawning season (winter) in three different spawning areas (eastern Bering Sea, central Gulf of Alaska, and southern Gulf of Alaska) (Planas et al., 2017). In order to further characterize the gonadal maturation schedule, the IPHC is undertaking a full characterization of the annual reproductive cycle in female and male Pacific halibut. At monthly intervals, female (N=30) and male (N=30) Pacific halibut have been captured from the Portlock region in the central Gulf of Alaska and a variety of samples are being collected for physiological analyses of reproductive parameters throughout an entire annual reproductive cycle. Each individual gonad will be staged according to standard staging criteria, photographed, and weighed (in addition to the round weight of the fish) in order to calculate the gonadosomatic index. Individual gonad (ovary and testes) samples are being collected for histology by fixation in 10% buffered formalin and subsequently embedded in paraffin and stained with hematoxylin and eosin for staging. Gonad and pituitary samples are also being collected in RNAlater for transcriptomic analyses by RNAseq and individual gene expression by qPCR in order to identify changes in the expression of reproductive genes throughout the reproductive cycle. In addition, plasma samples (from 0.5 – 1ml of blood) are being collected from the caudal vein and will be used to measure the levels of reproductive hormones (i.e. sex steroids, prostaglandins, etc.) and nutrients (i.e. glucose, lipids) in order to characterize the activity of the endocrine system in relation to maturation and gonadal development. The combination of these various parameters will substantially improve the accuracy of current staging techniques of reproductive status, in addition to update current estimates of maturity-at-age and of the incidence of skipped spawning. Overall, the current effort to engage in a comprehensive reproductive monitoring of the adult Pacific halibut population will result in improved estimates of the actual spawning biomass.

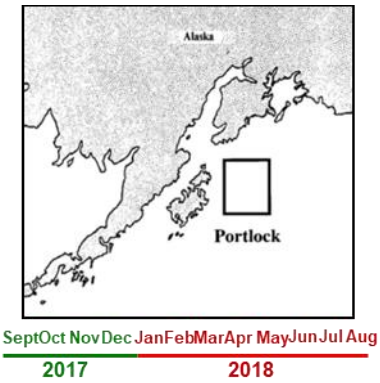

Figure 1. Pacific halibut monthly sampling schedule and location.

2. Growth. Important research efforts are aimed at understanding the possible role of somatic growth variation in the observed changes in size-at-age (SAA) and to develop tools for measuring growth and physiological condition in Pacific halibut. Changes in SAA in Pacific halibut have been hypothesized as being attributable to a variety of causes, including changes in population dynamics of the Pacific halibut stock due to a density effect, whereby high population densities would negatively affect growth, as well as changes in extrinsic factors (Loher, 2013). It is believed that extrinsic factors such as fishing can directly and indirectly impact SAA through size-selective harvest (as is the case in the Pacific halibut fishery), leading to the selective removal of faster growing individuals, and by its ability to alter ecological interactions, respectively. Importantly, environmental and ecological influences in the form of environmental changes (e.g. temperature) or in the competitive interaction with other species can have a direct impact on SAA by regulating somatic growth. Although other factors may be contributing, the results of a previous study funded by the North Pacific Research Board (NPRB) that had IPHC participation strongly suggested that temperature changes may have influenced halibut growth (Kruse et al., 2016). In view of our limited knowledge on the underlying physiological basis of somatic growth and, importantly, on the possible contribution of growth alterations in driving changes in SAA, we have initiated studies to develop and apply tools to evaluate spatial, temporal, and age-specific growth patterns and their response to environmental influences in Pacific halibut. The IPHC is leading efforts in this area within the framework of a 2-yr research project partially funded by NPRB that is led by the IPHC in collaboration with Dr. Thomas Hurst at the Hatfield Marine Science Center - Alaska Fisheries Science Center in Newport, OR. The awarded NPRB grant (NPRB 1704) period is from 1 September 2017 until 31 August 2019 (Appendix II) and its main aim is to investigate the effects of temperature, population density, social structure, and stress manipulations on biochemical and molecular indicators of somatic growth (IPHC-2018-SRB012-INF01). This study is expected to improve significantly our understanding of the physiological mechanisms regulating growth in the Pacific halibut in response to environmental and ecological influences but also, importantly, to identify molecular and biochemical growth signatures characteristic of growth patterns that could be used to monitor growth patterns in the Pacific halibut population. The specific objectives are (1) to investigate the physiological effects of **temperature** on growth in juvenile Pacific halibut by describing specific biochemical, transcriptomic (gene expression) and proteomic (protein) responses to temperature in skeletal muscle and liver, two key tissues that participate in growth regulation;

(2) to investigate the physiological effects of population **density** and **dominance hierarchies** on growth potential in order to understand how density and social interactions may influence growth potential in the nursery areas and (3) to investigate the physiological effects of **handling stress** on growth in juvenile Pacific halibut in order to understand the potential effects of handling-related stress on growth potential (Figure 2).

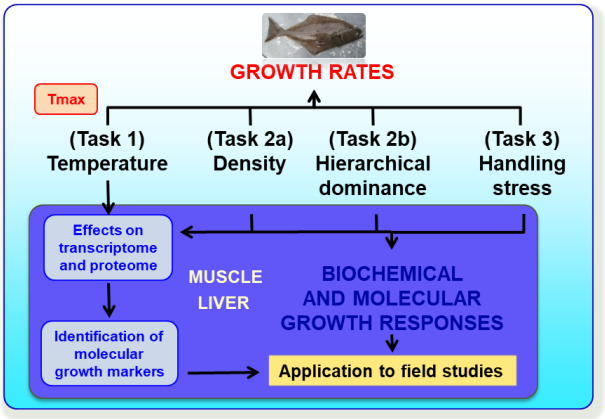

Figure 2. Diagram of the objectives of the NPRB project with indication of the different tasks.

Investigations on the effects of **temperature** variation on growth potential (Objective 1) are intended to show temperature-induced molecular and biochemical differences between juvenile Pacific halibut growing at different rates. The proposed experiments are aimed at describing molecular and biochemical features of skeletal muscle that are characteristic of growth patterns. The identified growth signatures will then be used as markers for growth in Pacific halibut in future studies aimed at understanding possible spatial and temporal changes in growth and, therefore, productivity.

Juvenile Pacific halibut (age 0, 5-7 cm length) were caught off Kodiak, AK and transferred to the aquatic facilities of the Hatfield Marine Science Center in Newport, OR. Fish were individually tagged (PIT tags) and acclimated at 9°C for 4 weeks. After the acclimation period, fish were divided into 2 groups (N=30 per group) and reared at 2°C and 9°C in triplicate tanks (N=10 per tank) for 8 weeks. After 2 weeks at each of these temperatures, fish were measured for weight and length (time 0) and growth monitored every 2 weeks, (at 4, 6, and 8 weeks from the beginning of the temperature experiment). During the experiment fish were fed ad-libitum daily rations. At the end of the experiment (week 8), 15 fish from each group were sacrificed by an overdose of anesthetic (MS-222), and muscle and liver samples were excised with one set of samples preserved for molecular analyses in RNAlater and stored at -20°C and a second set of samples frozen in liquid N_2 and stored at -80°C for biochemical and protein analyses. Subsequently, the temperature in the tanks containing the remaining fish at 2°C was gradually increased to 9°C and growth monitored every 2 weeks (at 2, 4, 6 and 8 weeks from the beginning of the temperature-switch experiment). As in the previous experiment, after the 8 week period at 9°C, fish were sacrificed by an overdose of anesthetic (MS-222), and muscle and liver samples were excised with one set of samples preserved for

molecular analyses in RNAlater and stored at -20°C and a second set of samples frozen in liquid N_2 and stored at -80°C for biochemical and protein analyses.

The results of this study indicate that after subjecting juvenile fish to two different temperatures (2ºC and 9ºC) for a period of 8 weeks, a clear suppressive effect of low temperature on the specific growth rate (SGR) is induced. In addition, when juvenile halibut that were previously acclimated to 2ºC for 8 weeks were subsequently acclimated gradually to 9ºC for an additional period of 6 weeks, a significant increase in SGR, representing compensatory growth, was observed (Figure 3). Therefore, these results validate the experimental design and confirm the ability of temperature to manipulate growth rates in the Pacific halibut.

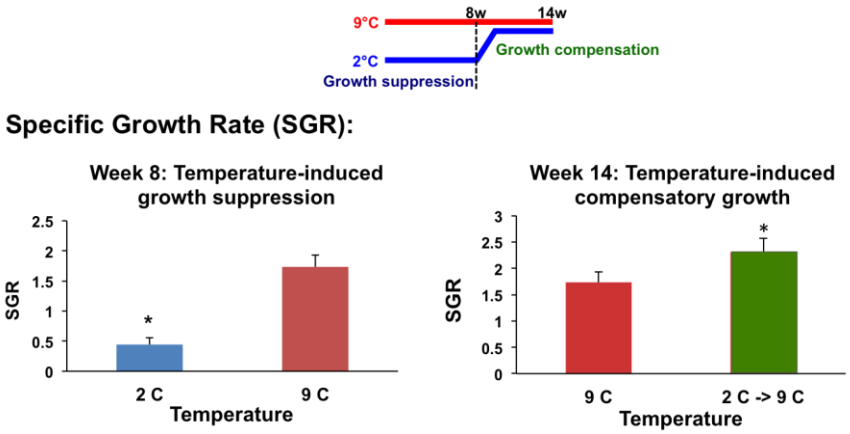

Figure 3. Effects of temperature manipulation on specific growth rate in juvenile Pacific halibut.

In order to identify molecular markers for growth, we initially set out to investigate changes in the expression of genes in skeletal muscle of juvenile Pacific halibut in response to the two growth manipulations: growth suppression by low temperature acclimation and growth stimulation by temperature-induced compensatory growth. A transcriptomic profiling approach was used by which RNA from skeletal muscle from individual fish from each group was extracted and sequenced. As a result of comparing the skeletal muscle transcriptome of fish acclimated at 2ºC with that of fish acclimated at 9ºC, we identified 1,187 genes that were differentially expressed in the temperature-induced growth suppression experiment. Among this gene set, 511 genes showed increased expression (up-regulated) and 676 genes showed decreased expression (down-regulated) under growth suppression. Functional classification of down-regulated genes revealed that categories of genes involved in muscle development and contraction, transcription and translation, protein and carbohydrate metabolism, energy metabolism and transfer, cell division and stress and immune response were all down-regulated under growth suppression. Analysis of the skeletal muscle transcriptome under growth stimulation conditions revealed that 610 genes were differentially expressed. Among this gene set, 202 genes were up-regulated and 408 genes were down-regulated under growth stimulation. Again, functional classification of up-regulated genes revealed that categories of genes involved in muscle development and contraction, protein

metabolism and modification, carbohydrate metabolism for ATP generation, iron transport and binding, hemoglobin synthesis, cell adhesion and proliferation and transcription and translation were all up-regulated under growth stimulation. Therefore, there is a clear correspondence of biological processes that are affected under growth suppression and growth stimulation. Consequently, we have initiated the identification of genes that show changes in expression under both growth manipulations and that are consistent with the type of growth modification (i.e. down-regulated under growth suppression and up-regulated under growth stimulation). A set of 13 genes has already been identified that show expression patterns consistent with the type of growth modification and that can be considered the first set of potential molecular markers for somatic growth.

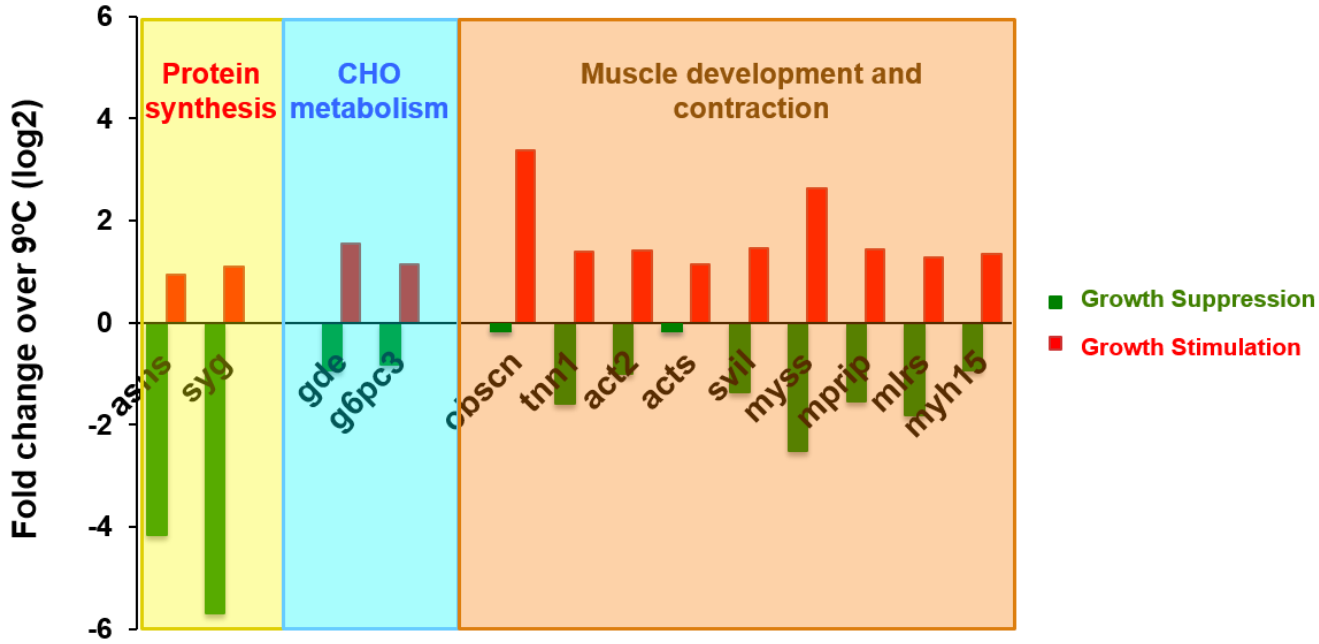

Figure 4. Expression pattern of individual genes under growth suppression and growth stimulation conditions. Genes are grouped according to their biological function.

At the present time, we are conducting investigations on the effects of **density** on growth. In a first set of experiments, 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. Growth data is currently being analyzed.

3. Discard Mortality Rates (DMRs) and Survival. DMRs are calculated from data that are collected by observers regarding the release viability or injury characteristics of Pacific halibut post-capture and are used to estimate the percentage of incidentally caught fish that die after release. Currently, post-capture DMR estimates are based on qualitative assessments of the physical condition of the fish (e.g., minor/moderate/severe/dead for longline gear) and have a certain degree of uncertainty associated with them, which represents a source of uncertainty in the estimation of total mortality within current stock assessment models. In

practice, assigned DMRs and their uncertainty translate into *a priori* adjustments to expected mortality in each upcoming year, and to the catch limits that are thereafter assigned to each harvest sector. Given current low halibut yields relative to long-term mean productivity, this potential to translate uncertainty into catch limit reductions can place undue hardship on some sector(s) relative to others. Therefore, there is an urgent need to improve our estimates of DMR as well as to provide strategies to improve survival of incidentally-caught Pacific halibut after release.

In order to address this important issue, we are conducting investigations to understand the relationship between fish handling practices and fish physical and physiological condition and survival post-capture as assessed by tagging in order to better estimate post-release survival in Pacific halibut caught incidentally in the directed and bycatch longline fisheries. The rationale of the proposed research is based on the notion that by understanding the relationship between handling practices, injury levels, and physiological condition, on one hand, and between these and post-release survival, on the other hand, estimates of DMR could be improved. An important underlying topic in this proposed research is to better understand how a detailed assessment of physiological condition prior to release can improve our estimates of survival after release. This research will attempt to develop and introduce quantitative measurable factors that are linked to fish handling practices, physiological condition and ultimately survival in order to improve current DMR estimates. These investigations are being conducted within the framework of a 2-yr project partially funded by the Saltonstall-Kennedy Grant Program that is led by IPHC in partnership with the Alaska Pacific University with a grant period of 1 September 2017 – 31 August 2019 (Appendix II) (IPHC-2018-SRB012-INF02). The specific objectives of this project are (1) to evaluate the effects of fish handling practices on injury levels and their association with the physiological condition of captured Pacific halibut, (2) to investigate the effects of fish handling methods and associated injury level and physiological condition on post-release survival, (3) to apply electronic monitoring in associating fish handling methods to survival in vessels without observer coverage and (4) to develop non-invasive methods for quantifying measurable physiological factors indicative of stress and physiological disturbance. The tasks delineated to pursue the abovementioned objectives are the following:

3.1. Evaluation of the effects of **hook release techniques** on injury levels and association with the physiological condition of captured Pacific halibut. The work involved evaluating the effects of different release techniques on injury levels and associated physiological condition levels from the large (16/0) circle hooks used in the Pacific halibut longline fishery.

Fish capture. One vessel chartered to operate in Alaskan waters (off Chignik, AK, within IPHC´s Regulatory Area 3B) was used for the study. The fishing location was selected based on the potential to catch adult fish of both legal (82 cm and above in length) and sub-legal (under 82 cm in length) sizes at rates that facilitate efficient completion of project goals. Functionally, however, the fleet has a tendency to discard fish under 84 cm to avoid landing fish that would appear to be sublegal (owing to shrinkage) post icing. Therefore, discarded fish were considered to be all fish under 84 cm in length. The vessel operated following the standard practices of the commercial Pacific halibut fleet; namely, in terms of the procedures and times of setting, soaking, and hauling baited longline

gear. Two fishing trips consisting of 6 fishing days per trip, were conducted. On each day, 3 hauls of 8 standard skates (i.e., 100 hooks) each were fished for a total of 288 skates of gear. The vessel had a secondary roller with automatic hook-removal setup inboard of the outboard roller, and a ramp through the gunwhale to prevent damage from landing the fish. A total of 2,487 Pacific halibut were caught, of which 79 were tagged and released with a motion sensing accelerometer tag (96-day deployments) and 1,048 were tagged and released with a traditional wire tag (fishery recovered).

Hook release techniques. Pacific halibut were released from the hook using two different careful release methods as well as by the use of automated hook-stripping devices (i.e. hook stripper), yielding a total of three (3) treatments. Careful release methods included: careful shaking and gangion cutting (approved under IPHC regulation and described in detail in Kaimmer and Trumble, 1998). Hook straightening is also a permitted release method, but is not used to release sub-legal fish in the directed fishery (sub-legal fish do not have enough mass to straighten the hook but instead the hook tears straight out when force is applied), so this treatment was not continued after an initial day of testing. Hook release with the use of automated hook-stripping devices was also evaluated given that, although this is not an accepted hook release method, it occurs nevertheless whenever fish fail to be manually released. The rate at which this occurs in both directed and non-directed longline fisheries is currently unknown, but patterns associated with the occurrence of prior-hooking injuries (Dykstra 2016) suggests that hook-stripping may be more prevalent than is currently assumed and may also vary spatially. Given that hook-stripping is likely to induce the highest DMRs in longline fisheries and that its occurrence might be easy to quantify via electronic monitoring, obtaining baseline data for this release method was important. For this experiment, five skates of careful shaking, two skates of hook stripping, and one skate of gangion cutting where randomly assigned by skate of gear.

Hook injury assessment. All captured fish corresponding to each of the hook release techniques or treatments were sampled for length and weight, and the extent of the current hooking injury was recorded. We followed the hook injury classification scheme initially outlined by Kaimmer (1994) and expanded by Kaimmer and Trumble (1998) into 14 different categories (i.e. injury codes) corresponding to four major severity levels (e.g., minor, moderate, severe, and dead).

Blood determinations. After assessing injury levels of Pacific halibut released using each of the three above-mentioned treatments, a blood sample was taken from each fish for hematocrit determinations and for extracting the plasma. The levels of stress and physiological disturbance indicators (e.g., cortisol and catecholamines as endocrine indicators of stress responses, lactate and glucose as biochemical indicators of catabolic responses to stress, sodium, potassium ions and osmolarity as biochemical indicators of cellular disturbance; and pH) will be measured in plasma samples.

Monitoring of environmental conditions. In addition to recording the time elapsed between hook removal and return of tagged fish back into the ocean, sea bottom temperature was recorded with the use of dataloggers (Vemco Minilog-II), as well as ambient temperature, fish temperature, and sea state (Beaufort scale).

Assessment of physiological condition. The physiological condition of each selected fish from each of the three release techniques with associated injury levels will be determined in two different ways. First, we will calculate two different condition factor indices (i.e. Fulton's K, relative K) that express differently the relationship between length and weight and that have been recently used to evaluate the condition of landed Pacific halibut (Briones Ortiz, 2017). Second, we measured the energy (fat) levels by using a microwave-based device (Distell Fish Fatmeter, model 692, Distell, West Lothian, Scotland) that is applied directly onto the skin of the fish allowing energy determinations in the musculature without the need to sample tissues. This was a direct, non-invasive and harmless measure of energy levels that can be taken from live fish and that has also been recently used at IPHC to measure fish condition and shown to correlate well with relative K condition index as well as with the hepatosomatic index (Briones Ortiz, 2017). Surface body temperature was recorded with the use of a hand-held infrared thermometer.

3.2. Investigations on the effects of fish handling methods and associated injury level and physiological condition on **post-release survival**. In order to evaluate the survival of discarded fish, two types of tagging approaches were used: 1) mark-and-recapture of released fish with wire tags and 2) biotelemetric monitoring of released fish with the use of satellite-transmitting electronic archival tags equipped with accelerometers.

Mark and recapture of released fish with wire tags. All fish of 84 cm in length or less were assessed for injury levels, tagged using wire tags (as previously described by Forsberg et al., 2016) and released. In brief, wire tags were inserted between the opercular bones of the eyed side of the fish and the two ends of the tag were twisted together around the operculum. The use of wire tags has the potential to allow for the long-term assessment of survival in the ocean; however, we do not expect to recover enough wire tags from this study to formally estimate rates associated with various survival covariates, and that estimates of survival rates using this approach are confounded by natural mortality and variable reporting rates. Releases conducted during this study should be viewed as a foundation upon which additional releases might be added in the future.

Biotelemetric monitoring of released fish with the use of satellite-transmitting archival tags. A subset of captured Pacific halibut identified to be in excellent condition (e.g., minor injury category) were tagged with sPAT archival tags equipped with accelerometers (Wildlife Computers "survivorship PAT" tag, or sPAT) in order to evaluate post-release mortality rate, time elapsed between capture and inferred mortality, and post-release dispersal. Only the excellent viability category was studied because the cost of deploying sPAT tags (~ $4000 US per tag) prevents large sample sizes and restricts the scope of such studies. The excellent category was chosen as it represents the vast majority of targeted-fishery discards and, hence, the bulk of assumed mortality. Additionally, uncertainty regarding the survivorship of halibut that are discarded in excellent condition has the greatest impact upon current estimates of survivorship in the remaining viability categories. This is because the latter estimates have been derived by comparing tag recovery rates from fish tagged within these categories to the rate of recovery of tags from excellent fish, where the expected tag return rate for fish in excellent condition was modelled on the basis of assumed rates of

natural mortality, fishing mortality, and tag reporting rates. In the current study, Pacific halibut were tagged with sPATs programmed to detach and report after 96 days at liberty. Although this exceeds the 60-day survival period recently used to study trawl DMRs (Rose et al., in preparation), shorter-period survivorship can be accurately calculated using longer time-series data if desired. The longer recording period will allow us to conduct DMR analysis that is comparable to that referenced in the trawl study while expanding the scope of the work to gain greater insight into the possibility of delayed mortality, as well as time-course to recovery or normal behavior, in individuals whose records exceed 60 days. A total of 79 Pacific halibut ranging from 53-81 cm FL were tagged from 20 October to 2 November, 2017. Sex of the fish was unknown at time of tagging, but fin clips were collected so that genetic sex can be determined *post* hoc via using molecular techniques (Drinan et al., 2018). One tag released from its host fish immediately likely due to a tethering failure and one tag failed to produce sufficient satellite uplinks to determine its location or download any archived acceleration data. As such, data from 77 tags are available for analysis. Times at liberty ranged from 43-96 days and straight-line displacement between tagging and reporting locations ranged from 0-1,042 km. Seven tags detached from their host fish prematurely, after periods ranging from 43-95 days, and therefore may have represented post-release mortalities. However, inspection of the acceleration data from these tags indicates that four of the fish were active through tag release (i.e., up to and including the last recording period prior to the tag reaching to sea surface) and the tags therefore most likely shed from live individuals. As such, excellent-condition longline DMR of U32 Pacific halibut in this experiment is estimated to be 4%.

Figure 4. Schematic diagram of the workflow of activities in Tasks 1 and 2.

3.3. Application of **electronic monitoring** (EM). In this project, a profile of injuries associated with different release methods is being developed, while at the same time quantifying the accuracy of EM in enumerating release methods, and fish conditions (Figure 5). Both of these aspects will be necessary to transform EM imagery into useable/actionable data. The work involved three different aspects. First, installation of an EM System involving a standard 3-camera EM system (Archipelago Marine Research Ltd). Second, the development of an injury profile by release method whereby Pacific halibut caught on fixed gear were evaluated for viability and subsequent survival for the three release methods implemented. Third, evaluation of EM data whereby reviewers recorded the release method and condition of released fish. This data set will be compared to those collected by personnel at sea as part of their tagging efforts (equivalent to the human observer data).

4. 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 influencing that distribution. There are a number of projects currently taking place that address migration and distribution at various stages of Pacific halibut development.

4.1. Overall larval distribution, differences in distribution related to environmental factors, and modelling the magnitude of connectivity between the Gulf of Alaska and Bering Sea populations through larval drift. The major Gulf of Alaska currents (primarily the Alaska Coastal Current) flow westward and eventually through Aleutian Island passes into the Bering Sea (Stabeno et al. 2002). Unimak Pass is the easternmost conduit between the two basins and is also the only direct linkage between the Gulf of Alaska and Bering Sea continental shelves. This work involves using data collected by NOAA during their ichthyoplankton surveys in the Gulf of Alaska and Bering Sea from 1972-2015, and examines the connectivity of the two Pacific halibut sub-populations through larval flow via Unimak Pass. The data include standardized catch (# organisms/m^2) for each tow and a subsample of larval lengths. The standardized catch is used as a proxy for abundance when comparing over time and space. All organisms caught during these surveys are sampled and enumerated, but the surveys are designed to target species other than Pacific halibut such as pollock, salmon, and cod. As a result, annual station location design has fluctuated somewhat to target these various species, creating a mismatch in geographic scope over time.

While basic descriptions of larval distribution overall can be accomplished with the standardized data, comparisons over time and space using multi-year subsets are more difficult. As a solution, the IPHC-developed spatial model was utilized to help analyze these subsets. Comparisons within the model include averages of density and distribution between warm (2001-2005) and cold (2007-2013) stanzas and differences between the ocean basins. While density estimates of larvae were slightly different (i.e. higher in warm years than in cold years), there was also high variability and the estimates were well within the confidence intervals, thus it was concluded that there was no difference. However, the model did detect local differences in densities, specifically higher densities in warm years compared to cold around Unimak Pass, Shelikof Strait, and north of Kodiak Island, and lower densities further west in the Bering Sea.

The next step, in collaboration with researchers at NOAA/EcoFOCI is to use a NOAA-created larval transport model to examine differences in currents during different climatic regimes (e.g. warm vs cold) and the resulting differences in larval advection. Of particular interest is the differences in magnitude of larval transport through Unimak Pass, the likelihood that larvae will be transported onto the Bering Sea shelf vs westward toward Russia, and how far east in the Gulf of Alaska a Pacific halibut larva can originate and still make it through Unimak Pass prior to settlement. The advection modeling portion of this project is tentatively scheduled for Fall 2018.

Also of interest is the catch weighted mean length by month, the developmental stages encountered in each basin for each month, and whether timing of development might

differ between basins. Laboratory growth studies (e.g. Liu et al. 1994, McFarlane et al. 1991) will be used to develop length proxies for age in order to analyze this component.

4.2. Migration studies targeting U32 Pacific halibut. Migration of O32 Pacific halibut has been the focus of numerous studies over the years, but less is known about the migration habits of U32 fish. Research in this category is designed to study the migration of the **post-settlement component of the population and includes** investigations of the dispersal of individuals not yet recruited to the commercial exploitation (U32). This category contains a mixture of both immature (juvenile) and mature individuals considering that a portion of the female Pacific halibut stock matures at lengths >32" FL while male maturity is currently understood to be largely complete at much smaller sizes. As such, the maturity ogive for male Pacific halibut falls entirely within the U32 category and for females occurs largely within O32.

Wire tagging of U32 Pacific halibut. NMFS trawl surveys tend to catch Pacific halibut ranging in size from about 20-100 cm FL, with the majority of the catch in the lower end of the range. The IPHC deploys a sea sampler aboard one of these vessels for each survey specifically to carry out biological sampling of Pacific halibut. The surveys include the Bering Sea annually, the Gulf of Alaska biennially, and the Aleutian Islands biennially. A total of 50% of the Pacific halibut caught on the IPHC-staffed vessel are randomly selected for the wire tagging, and all U32 fish in that sample that are viable according to NMFS observer criteria for trawls, are tagged and released. Beginning in 2017, NMFS also agreed to tag Pacific halibut on the vessel in the Bering Sea survey that did not have an IPHC sampler aboard. The tagging there is more opportunistic due to other demands on their time, but the goal is the same as on the IPHC-staffed vessel. From 2015 through 2017, a total of 4,040 tags were released from all trawl surveys combined: 2,204 in the Gulf of Alaska, 1,666 in the Bering Sea, and 170 in the Aleutian Islands. A total of 24 tags have been recovered thus far. The project is expected to continue for the next several years.

In addition to wire tagging on the trawl surveys, U32 Pacific halibut caught during the IPHC setline surveys are also tagged in areas where otolith sampling is less than 100% (Forsberg 2018). In these areas, U32 Pacific halibut are selected randomly for tagging at area-specific rates with the goal of tagging 500 U32 fish per Regulatory Area. This tagging project began in 2016 on a pilot basis and coastwide in 2017, and was designed to complement the trawl tagging effort. The U32 Pacific halibut caught during the setline surveys tend to have fork lengths near the upper end of the U32 size range. To date, 2,096 Pacific halibut have been tagged and released. Of those, 11 have been recovered. All current wire tagging efforts are intended as ongoing and decadal-scale efforts that will provide a general understanding of dispersal patterns (*sensu* Hilborn et al. 1995) and insight regarding the possibility that long-term changes in dispersal may occur at coastwide or basin-specific scales.

Electronic archival tagging. This study is scheduled to begin in summer 2018, and is expected to provide novel information regarding ontogenic and seasonal dispersal of U32 halibut. With respect to the ontogenic dispersal, it is generally understood that Pacific halibut conduct contranatant migrations to the south and east (Hilborn et al.

1995) and mark-recovery data can provide an indication of the total magnitude of dispersal during an individual's period at liberty. However, conventional tag data provide no information regarding annual redistribution when periods at liberty are in excess of one year. Dispersal-at-age is an important function whose form and magnitude must be specified in spatially-explicit population models that allow for migration among areas. Electronic archival tags allow for daily light-based geopositioning to be conducted, thereby allowing dispersal to be estimated on annual and sub-annual scales. Additionally, recorded depths allow for seasonal migration to be quantified and associated with age and sex. Adult Pacific halibut are known to undertake cyclic onshore-offshore migrations that correspond to the species' annual spawning cycle (Loher 2011) and that the nature of these migrations relative to commercial fishing periods can influence area-specific realized exploitation rates relative to those that are estimated assuming that the stock is non-migratory during the course of each fishing season (Leaman et al. 2002). It is currently unknown at what age Pacific halibut begin to undertake such migrations and whether the initiation, timing, and frequency of seasonal migration might vary according to sex.

4.3. Migration research on O32 Pacific halibut. Studies designed to examine migration have focused upon quantifying seasonal migratory periods and the potential for seasonal fisheries interception, identification of spawning sites, and describing seasonal and interannual dispersal within the Bering Sea and between the Bering Sea and Aleutian Islands (BSAI) region and the Gulf of Alaska. This work has employed PAT tags and has been conducted incrementally given that the high cost of these tags prevents large-scale, coastwide deployments. To date, approximately 400 PAT tags have been deployed to study O32 migration, with more than half of those tags deployed in the BSAI. These deployments have expanded our understanding of the geographic extent of halibut spawning at the southern end of the range (Loher and Blood 2009) as well as on the Bering Sea shelf edge and in the Aleutian Islands (Seitz et al. 2011); have indicated apparent basin-scale segregation of spawning stock (Loher and Clark 2010, Seitz et al. 2017); provided observational data that are consistent with genetic results (Drinan et al. 2016) in suggesting relative isolation of Pacific halibut in the western Aleutian Islands; and confirmed mixing of stock between the Salish Sea and outer coastal population (Loher and Soderlund in review). Tag deployments on Bowers Ridge in 2017 (**Project 650.21**, above) were conducted in this context and future deployments are anticipated that will fill additional geographic gaps in this program; in particular, in northern California (Area 2A), Bering Sea coastal waters (4E), and the far northern Bering Sea shelf edge (4D).

ADDRESSING PARTICULAR REQUESTS RESULTING FROM SRB11

A diagram representing the integration of biological research activities conducted in the five main research areas at IPHC with stock assessment and harvest policy was initially presented at the AM094 and is shown in Appendix III.

In addition to reviewing current and planned research activities conducted by the Biological and Ecosystem Science Branch, the IPHC Secretariat would like to seek guidance from the SRB and engage in discussion regarding the following topics:

- Linking current work on migration, growth, and physiological condition of Pacific halibut to spatial and temporal changes in productivity of the stock.
- Gaps in our knowledge regarding our understanding of (1) spawning site contributions to nursery/settlement areas in relation to year-class and recruit survival and strength and (2) the relationship between nursery/settlement origin and adult distribution and abundance over temporal and spatial scales.
- Application of genetic approaches to address management-relevant questions on population structure, distribution, etc.

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APPENDICES

Appendix I: Summary of new and continuing research projects approved for FY2018

Appendix II: Summary of external research projects awarded for funding

Appendix III: Integration of biological research, stock assessment and harvest policy

APPENDIX I

Summary of new and continuing research projects approved for FY2018

Project #	Project Name	Priority	Budget (US$)	Principal Investigator	Management implications
New Projects					
673.15	Influence of thermal history on growth	High	136,004	Loher	Changes in biomass/size-at-age
650.22	Larval connectivity	High	20,000	Sadorus	Larval distribution
Continuing Projects					
621.16	Development of genetic sexing techniques	High	146,107	Loher	Sex composition of catch
642.00	Assessment of Mercury and other contaminants	Medium	8,400	Dykstra	Environmental effects
650.21	Investigation of Pacific halibut dispersal on Bowers Ridge	High-Medium	124,527	Loher	Spawning areas
661.11	*Ichthyophonus* Incidence Monitoring	Medium	8,055	Dykstra	Environmental effects
669.11	At-sea Collection of Pacific Halibut Weight to Reevaluate Conversion Factors	High	1,500	Soderlund	Length-weight relationship
670.11	Wire tagging of Pacific halibut on NMFS trawl and setline surveys	High	12,000	Forsberg	Juvenile and adult distribution
672.12	Condition Factors for Tagged U32 Fish	High	13,000	Dykstra	DMR estimates
673.13	Sequencing the Pacific halibut genome	High	22,500	Planas	Population estimate
673.14	Identification and validation of markers for growth	High	27,900	Planas	Changes in biomass/ size-at-age
673.13	Sequencing the Pacific halibut genome	High	22,500	Planas	Population estimate
674.11	Full characterization of the annual reproductive cycle	High	123,988	Planas	Maturity assessment
675.11	Tail pattern recognition analysis in Pacific halibut	High	2,370	Dykstra	Adult distribution
	Total - New Projects		**297,518**		
	Total - Continuing Projects		**202,482**		
	Overall Total (all projects)		**500,000**		

APPENDIX II

Summary of external research projects awarded for funding

Project #	Grant agency	Project name	Partners	IPHC Budget ($US)	PI	Management implications	Grant period
1	**S-K NOAA**	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival (Award No. NA17NMF4270240)	Alaska Pacific University, Anchorage, AK	$286,121	Planas (lead PI) Dykstra Loher Stewart Hicks	Bycatch estimates	September 2017 – August 2019
2	**NPRB**	Somatic growth processes in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress manipulation effects (Award No. 1704)	AFSC-NOAA-Newport, OR	$131,891	Planas (lead PI) Rudy Loher	Changes in biomass/size-at-age	September 2017 – August 2019
			Total awarded ($)	**$418,012**			

APPENDIX III

Integration of biological research, stock assessment and harvest policy

Research areas	Research outcomes	Relevance for stock assessment	Inputs to reduce stock assessment uncertainty	MSE development	Inputs to inform MSE development	MSE goals
Reproduction	Sex ratio Spawning output Age at maturity	Spawning biomass scale and trend Stock productivity Recruitment variability	Sex ratio Maturity schedule Fecundity	Operating Model Management Procedures	Sex ratio Maturity schedule Fecundity	Biological sustainability
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	Operating Model Management Procedures	Predicted weight-at-age Mechanisms for changes in weight-at-age	Biological sustainability
Discard Survival	Bycatch survival estimates Discard mortality rate estimates	Scale and trend in mortality Scale and trend in productivity	Bycatch and discard mortality estimates	Operating Model Management Procedures	Bycatch and discard mortality estimates Variability in bycatch and uncertainty in discard mortality estimates	Minimize bycatch mortality Minimize discard mortality
Migration	Juvenile and adult migratory behavior and distribution Larval distribution	Stock distribution Geographical selectivity	Information for structural choices Recruitment indices	Operating Model Management Procedures	Information for structural choices Migration pathways and rates Timing of migration	Biological sustainability Preserve biocomplexity
Genetics and Genomics	Genetic structure of the population Sequencing of the Pacific halibut genome	Spatial dynamics Management units	Information for structural choices	Operating Model Management Procedures	Information for structural choices	Biological sustainability Preserve biocomplexity