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) to identify and assess critical knowledge gaps in the biology of the Pacific halibut;
2) to understand the influence of environmental conditions; and
3) to 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. 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. 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. The proposed work on DMRs is aimed at providing updated estimates of DMRs in both the longline and the trawl fisheries. 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. 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 CONTINUING AND NEW BIOLOGICAL PROJECTS

For 2017, seven new projects were approved that cover specific research needs related to key aspects of the biology of the Pacific halibut: Reproduction (Projects 674.11, 650.21), Migration (Projects 650.21, 675.11, 672.11), Growth (Project 673.14), Viability Assessment and Survival Post-Capture (Projects 672.11, 672.13) and Genetics (Project 673.13) (Appendix I).

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 June 2017 on IPHC's fishery-independent setline survey and will continue on a monthly basis through the summer of 2018 on chartered vessels (please see below for a full description).

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 females prior to the spawning season in order to identify potential spawning areas in Regulatory Area 4B. A total of 14 females were successfully tagged in July 2017 at the Bower's Ridge area, with 12 PAT tags programmed to emit the following summer and 2 PAT tags programmed to emit the following winter.

Project 675.11 ("*Tail pattern recognition analysis in Pacific halibut*") consisted in a pilot study that proposed to identify individual fish by ways of photographic recognition of tail patterns to complement migratory studies. With full involvement by an IPHC intern, 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.

Project 672.12 ("*Condition Factors for Tagged U32 Fish*") proposed 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 is currently underway on IPHC's fishery-independent setline survey.

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 studies 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.

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 will take place in late summer-early fall 2017 during two trips of a chartered vessel and various hook release methods will be alternated randomly at each skate (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.

Furthermore, eight continuing projects were approved, including two projects dealing with sex identification (621.15, 621.16), 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 (650.18, 650.20, 670.11) 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 indicated below:

Project 621.15 ("*Voluntary at-sea sex marking*") proposed using a method for physically marking sex by the commercial fleet that was to be recorded by IPHC's port samplers. As continuation of the initial effort in 2016 in Regulatory Area 2B, where 10 R/Vs participated in the study allowing for the collection of 325 fin clip samples, a voluntary coast wide sex marking effort was proposed for 2017. Samples on sex-marked trips are still being collected at the present time (please see below for a full description).

Project 621.16 ("*Development of genetic sexing techniques*") proposed identifying molecular markers for sex in order to provide a genetic validation of the physical marking of sex (Project 621.15). Three single nucleotide polymorphisms (SPNs) have been identified to be associated with sex and molecular assays have been developed for two of the identified SNPs. These assays have an accuracy of 97.5%, as reported in a report that is being prepared for submission to a peer-reviewed journal (Drinan et al., 2017). Analysis of the 325 sex-marked samples collected in 2016 resulted in a sex-marking efficiency of 79%. Samples collected in 2017 will be analyzed with the developed methods (please see below for a full description).

Projects 642.00 ("*Assessment of mercury and other contaminants*") and **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 have 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 juvenile or sublegal Pacific halibut in order to further understand coastwide migratory patterns of Pacific halibut. As of mid-summer of 2017, a total of 1,219 Pacific halibut were tagged on the NMFS trawl survey (566 in the Gulf of Alaska and 653 from the Bering Sea) and 1,292 on the IPHC's fishery-independent setline survey.

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.

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 processing

plants at the ports, 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 in commercial 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 proved to be successful, genetic sex assays could then be used as a validation tool to determine the sex marking accuracy. In 2016, a developed sex marking protocol, involving identifying females by cuts in the dorsal fin and males by a cut in the operculum, was implemented 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. In parallel, work in collaboration with geneticists at the University of Washington resulted in the identification of three single nucleotide polymorphisms (SNPs) that were associated with sex (Drinan et al., 2017a). Molecular assays were developed for two of the three SNPs and it was determined that each of the two molecular assays had an accuracy of 97.5% when using samples originating from fish whose sex was morphologically identified. The identification of the sex markers and the development and application success of the derived molecular assays have been described in a manuscript that has been submitted for publication in a peer-reviewed scientific journal (Drinan et al., 2017b). The two molecular assays were applied to identify the genetic sex in DNA samples from a total of 325 fish that were marked at sea in 2016 in British Columbia. By comparing the sex-related marking and genetic sex identification for each of these fish, we have determined that the efficiency of sex marking at sea is 79%. In 2017, the sex marking project is requesting voluntary participation coastwide from the commercial fleet. To date, approximately 47 vessels from the Alaska fleet have participated in the project and the number of participating vessels from British Columbia is still undetermined. In total, approximately 591 samples from 50 marked trips have been collected coastwide to date and more samples are expected to be collected before the end of the fishing season.

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, female maturity schedule is used to estimate spawning stock biomass. Currently used estimates of maturity-at-age indicate that the age at which 50% of female Pacific halibut are sexually mature is 11.6 years in 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 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 will be captured from the Portlock region in the central Gulf of Alaska and a variety of samples will be collected for physiological analyses of reproductive parameters throughout an entire 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 will be 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 will also be 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) will be collected from the caudal vein and 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.

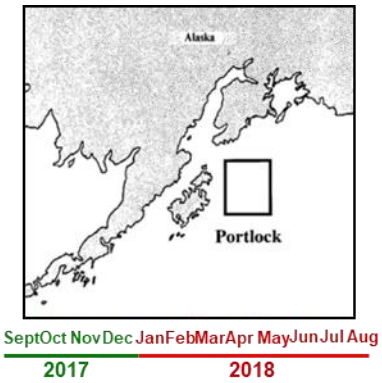

Figure 1. Pacific halibut monthly sampling schedule and location.

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 NPRB-funded study 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. 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).

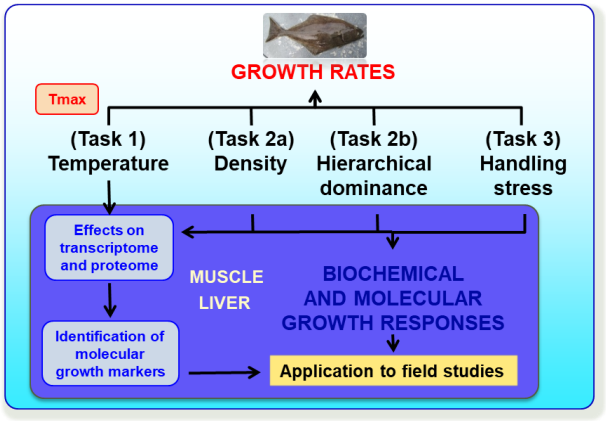

Figure 2. Diagram of the objectives of the NPRB project with indication of the different tasks.

2.1 Investigations on the effects of **temperature** variation on growth potential (Objective 1) will describe the thermal conditions leading to maximal growth and the temperature-induced molecular and biochemical differences between juvenile Pacific halibut growing at different rates. the proposed experiments will specifically describe molecular and biochemical features of skeletal muscle performance under different growth rates. The results from these studies will provide insight into the possible effects of growth patterns on physiological mechanisms underlying swimming performance in relation to anti-predator behavior, given that in some species high growth has been linked with decreased swimming performance.

In order to investigate temperature-dependent growth over a wide range of temperatures to capture the temperature variation that juvenile Pacific halibut may experience throughout its distribution range, juvenile Pacific halibut (age 0, 5-7 cm length, N = 75) will be individually tagged (PIT tags) and acclimated at 10°C for 4 weeks. After the acclimation period, fish will be divided into 5 groups (N=15 per group) and reared at 2°C, 5°C, 10°C, 15°C and 20°C in triplicate tanks (N=5 per tank) for 6 weeks. After 2 weeks at each of these temperatures, fish will be measured for weight and length (time 0) and growth monitored every 2 weeks, (at 4 and 6 weeks from the beginning of the temperature experiment). During the experiment fish will be fed ad-libitum daily rations. Growth parameterization will allow for calculation of the temperature at which growth is maximal (T_{max}). At the end of the experiment (week 6), fish will be sacrificed by an overdose of anesthetic (MS-222), and muscle and liver samples will be 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.

In order to describe the molecular and biochemical features of high growth under temperature-induced growth compensation, individually tagged juvenile Pacific halibut (age 0, 5-7 cm length, N = 60) after a period of acclimation at 10°C will be divided into two groups and reared at 2°C (N = 30and 10°C (N = 30) for 8 weeks, being fed ad-libitum daily rations. After the 8-week temperature regime, 10 fish from each group will be removed from the tanks and sampled as described below and will provide information on temperature effects on growth. Subsequently, half of the fish reared at 2°C will then be acclimated to 10°C for an additional eight weeks of growth in order to induce compensatory growth (temperature compensation effects). Each temperature treatment will be conducted with 10 fish in each of two experimental replicate tanks. Fish will be measured at 2-week intervals to determine the temperature-dependent growth potential. However, because the fish will be individually tagged, we will also be able to characterize the amount and size-based pattern of individual growth rate variation. At the end of the experiment, fish will be measured, sacrificed by an overdose of anesthetic (MS-222), muscle and liver samples will be excised and fish will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N_2 and stored at -80°C for biochemical and protein analyses. A preliminary study evaluating the effects of temperature on growth in juvenile Pacific halibut was recently completed. 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.

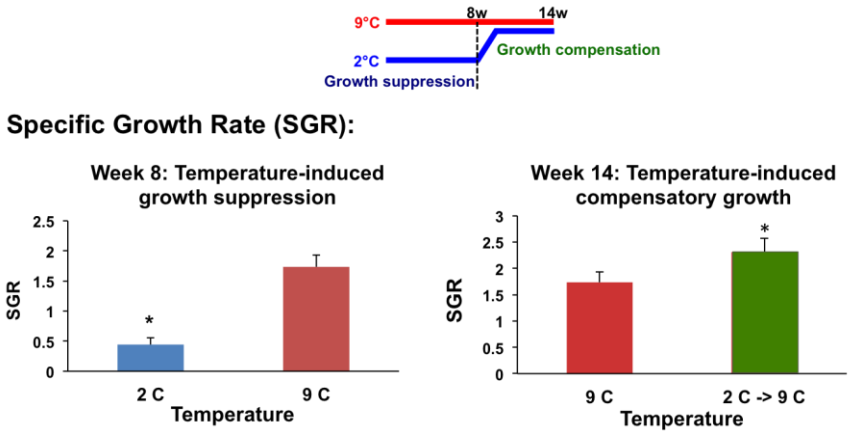

Figure 3. Effects of temperature manipulation on specific growth rate in juvenile Pacific halibut.

2.2. Investigations on the effects of **density** on growth (Objective 2) will assess the potential importance of density-dependence for growth as reflected in the association of growth rates to stock sizes observed by Clark and Hare (2002), with growth being negatively related to biomass or abundance. In order to accomplish this, individually tagged juvenile Pacific halibut (age 0, 5-7 cm length; N = 30) will be reared at 10°C at three different densities (1, 4 and 10 fish/tank) for 12 weeks, being fed limited daily rations at 1% growth/day in order to mimic the effects of density-dependent competition in the wild. Two experimental replicate tanks will be used per density treatment. Fish will be measured at 2-week intervals, with daily rations adjusted based on increasing fish sizes. At the end of the experiment, fish will be measured and blood samples will be drawn from the caudal vein with the use of heparinized syringes and needles. Fish will be sacrificed by an overdose of anesthetic (MS-222), muscle and liver samples will be excised and the rest of the body will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N_2 and stored at -80°C for biochemical and protein analyses. Blood samples will be centrifuged at 1,500 x g for 30 min at room temperature and plasma will be separated and stored at -80°C until assayed for metabolites (e.g. glucose, free fatty acids) and stress hormones (e.g. cortisol, catecholamines).

2.3. Investigations on the effects of **dominance** hierarchies on growth potential (Objective 2) will examine the impacts that behavioral interactions and social dominance structures

have on growth and on the expression of growth marker genes in juvenile Pacific halibut. The proposed work addresses the issue that size-based interactions may impact foraging opportunities in the wild, based on studies demonstrating that the feeding behavior of Pacific halibut may be influenced by size, with larger fish feeding first and growing faster than smaller fish. Individually tagged juvenile Pacific halibut (age 0, 5-7 cm length; N = 20) will be reared in pairs (2 fish/tank) at 10°C for 12 weeks in 10 experimental replicate tanks, being fed ad-libitum daily rations. Subordinate and dominant fish will be identified by directly observing feeding responsiveness and by recording their PIT tag IDs. At the end of the experiment, fish will be measured and blood samples will be drawn from the caudal vein with the use of heparinized syringes and needles. Fish will be sacrificed by an overdose of anesthetic (MS-222), muscle and liver samples will be excised and the rest of the body will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N_2 and stored at -80°C for biochemical and protein analyses. Blood samples will be centrifuged at 1,500 x g for 30 min at room temperature and plasma will be separated and stored at -80°C until assayed for metabolites (e.g. glucose, free fatty acids) and stress hormones (e.g. cortisol, catecholamines).

2.4. Investigations on the effects of **stress** manipulations on growth potential (Objective 3) will examine the effects of experimentally-induced handling stress on growth, blood stress indicators and gene expression in halibut with the goal of identifying biochemical and genetic markers of fish undergoing post-handling stress. The rationale behind these studies is to determine whether there can be lingering effects from the stresses associated with capture and release of Pacific halibut that affect feeding and growth in the wild. In order to understand the effects of stress on growth, individually tagged juvenile Pacific halibut (age 0, 5-7 cm length; N = 45) will be reared at 10°C at a density of 5 fish per tank for a total of 4 weeks, being fed ad-libitum daily rations except during the stress manipulation period. Fish will be subjected or not (control group) to two different stress manipulations: a) air exposure for 5 min, b) air exposure for 10 min once a week for duration of the 4-week experimental period. Three experimental replicate tanks will be used per stress treatment. Fish will be measured only at the termination of the experiment in order to avoid additional handling stress and feeding disturbance. At the end of the experiment, blood samples will be drawn from the caudal vein with the use of heparinized syringes and needles and fish will be sacrificed by an overdose of anesthetic (MS-222). Muscle and liver samples will be excised and the rest of the body will be frozen for compositional analyses. Muscle and liver tissue samples will be preserved for molecular analyses in RNAlater and stored at -20°C until analysis. In addition, muscle and liver tissue samples will be frozen in liquid N_2 and stored at -80°C for biochemical and protein analyses. Blood samples will be centrifuged at 1,500 x g for 30 min at room temperature and plasma will be separated and stored at -80°C until assayed for metabolites (e.g. glucose, free fatty acids) and stress hormones (e.g. cortisol, catecholamines).

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 have proposed 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 will be 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). 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 proposed involves evaluating the effects of different release techniques on injury levels and associated physiological condition levels using the large (16/0) circle hooks used in the Pacific halibut longline fishery.

Fish capture. One vessel chartered to operate in Alaskan waters (within IPHC´s Regulatory Area 3B) will be used for the study. The fishing location will be 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, discard fish are considered to be all fish under 84 cm in length. The vessel will operate 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. Average line soaking times used in the commercial fleet will be adopted. Two fishing trips consisting of 6 fishing days per trip will be targeted. On each day, 3 hauls of 8 standard skates (i.e., 100 hooks) each will be targeted for a total of 288 skates of gear. Vessel will need to have a secondary roller with automatic hook-removal setup inboard of the outboard roller. Based on IPHC's survey data from 2016 in Regulatory Area 3B and the proposed effort, we estimate to catch a total of 1,864 fish, with 1,229 fish at or under 84 cm and 635 fish over 84 cm in length.

Hook release techniques. Pacific halibut will be released from the hook using three different careful release methods as well as by the use of automated hook-stripping devices (i.e. hook stripper), yielding a total of four (4) treatments. The careful release methods used will be: careful shaking, hook straightening, and gangion cutting (approved under IPHC regulation and described in detail in Kaimmer and Trumble, 1998). Hook release with the use of automated hook-stripping devices will also be evaluated given that, although this is not an accepted hook release method, it occurs nevertheless whenever fish fail to be manually unhooked. 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 is important. In order to evenly distribute the release treatments throughout the course of the experiment, release methods will be randomly assigned by skate, within each set of gear, so that each haul will consist of two skates of each release method.

Hook injury assessment. All landed fish corresponding to each of the hook release techniques or treatments will be measured for length and weight, examined to record the extent of the hook injury, sampled for blood and their physiological condition will be assessed. We will follow 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). Only fish that are 84 cm or less in length will be tagged.

Blood determinations. After assessing injury levels of Pacific halibut released using each of the four above-mentioned treatments, a blood sample (approximately 1-2 ml) will be taken for 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 will be recorded with the use of dataloggers (Star Oddi DST centi-TD), as well as ambient temperature, light intensity on deck and sea state (Beaufort scale).

Assessment of physiological condition. The physiological condition of each selected fish from each of the four 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 will calculate 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 is 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 will be 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 will be 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 selected fish (84 cm or less) from each of the release techniques that have associated injury level and physiological condition will be tagged using wire tags, as previously described (Forsberg et al., 2016). In brief, wire tags are inserted between the opercular bones of the eyed side of the fish and the two ends of the tag are twisted together around the operculum. The use of wire tags will allow for the long-term assessment of survival in the ocean; however, it is worth-noting that we do not expect to recover enough wire tags within the study's stated period to formally estimate rates associated with various survival covariates, and that estimates of survival rates using this approach are confounded by natural mortality and unreported recaptures. A total of ~300 fish will be tagged per treatment.

Biotelemetric monitoring of released fish with the use of satellite-transmitting archival tags. Pacific halibut identified to be in excellent condition (e.g., minor injury category) will also be tagged with sPAT archival tags equipped with accelerometers (Wildlife Computers) in order to evaluate post-release mortality. Only the excellent viability category will be studied because 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, assuming a "known" excellent-fish survival rate. Tagged fish will not be released in the presence of whales. In total, 80 Pacific halibut under 84 cm in length will be tagged with

sPATs programmed to detach and report after 150 days at liberty. Although this exceeds the 60-day survival period currently being used to study trawl DMR, current data indicate that shorter period survivorship can be accurately calculated using longer time-series data. The longer recording period will allow us to conduct standard DMR analysis while expanding the scope of the work to gain greater insight into time-course to recovery or normal behavior or delayed mortality in individuals whose records exceed 60 days. No field data currently exist with respect to these aspects of post-release physiology. Tags will be randomly distributed among individuals in the excellent category and the number of tags used (80) will allow us to be able to estimate survival with a confidence level of 95% and a margin of error of 8%. Sex of all tagged individuals will be determined using established ultrasonic techniques. As a visual summary, the workflow of activities between fish handling practices, fish physiological condition and survival as assessed by tagging is shown in Figure 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 method will be 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 proposed work involves 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 will be evaluated for viability and subsequent survival for the four release methods implemented. Third, evaluation of EM data whereby reviewers will record 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).

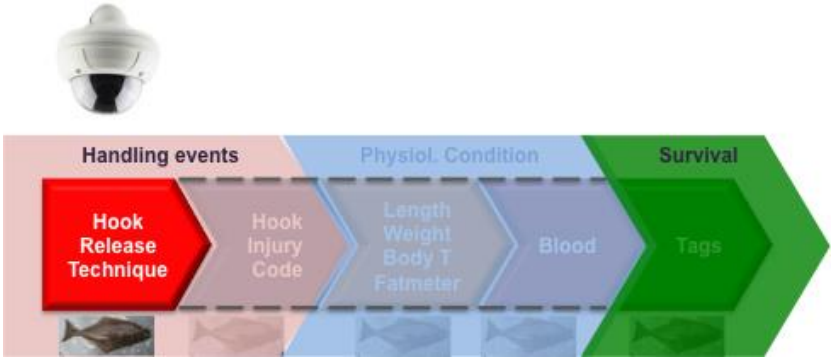

Figure 5. Schematic diagram of the workflow of activities in Task 3.

3.4. Development of **non-invasive methods** for measuring the levels of physiological factors indicative of stress and physiological disturbance. The proposed work will involve a controlled experiment to explore the use of mucus cortisol concentration as a stress indicator in Pacific halibut with the potential for use in evaluating probability of survival in bycatch and sublegal size fish. Unlike plasma samples, mucus samples can be collected in a relatively non-invasive fashion, thereby decreasing the likelihood of the sampling procedure influencing the stress response. In addition, mucus sampling can be conducted quickly and easily in field settings.

Fish capture. One vessel chartered to operate in Alaskan waters (within IPHC´s Regulatory Area 3A) will be used for fish capture. During the Fall of 2017, 16 – 24 adult Pacific halibut will be caught by jigging natural and artificial baits on the seafloor near Seward, AK. Only adult halibut between 20 and 31 inches will be brought onboard and kept for use in the experiment. This size range has been selected both to minimize the potential for variations in cortisol response in study subjects due to size and as representative of fish of commercially-sublegal size. Once on board, fish will immediately be placed in onboard holding tanks for transfer to the UAF Seward Marine Center (Seward, AK) where all experimental work will be conducted. During holding, 50% of the water in the tanks will be replaced twice every hour to maintain dissolved oxygen concentrations and water temperature at levels resembling sea surface conditions.

Animal housing and care. Fish will be housed in 6 ft. x 3 ft. circular tanks (approximate filled volume = 580 US gallons) at the UAF Seward Marine Center (Seward, AK). Fish will be randomly assigned to tanks, and no more than 3 fish will occupy each tank. Water temperature and dissolved oxygen level will be kept constant and waste will be removed using an open flow through seawater system that will draw water from Resurrection Bay. Photoperiod will be standardized on a 12:12 light:dark regime. During the entire course of the experiment, the fish will be fed a fishmeal-based pellet diet once daily at a rate of 1 kg feed/kg fish. In order to allow increased cortisol levels caused by the capture, transport, and acclimation to the experimental housing to return to baseline levels, the fish will be left undisturbed (except for feeding) for a period of no less than 30 days. Fish will also be left undisturbed (except for feeding) between experiment subcomponents.

Magnitude and rate of cortisol absorption and elimination in mucus. Captive halibut will be randomly divided into three groups. Individuals from two of the groups will receive intraperitoneal injections of different doses of cortisol (0.1 μg/g of fish and 0.01 μg/g of fish). Individuals from the third group will act as a control, receiving intraperitoneal injections of sterile phosphate buffered saline. Blood and mucus will be sampled from three parallel fish in the three groups at 0, 0.5, 2, 5, 24, 36, 48, and 72 hours after injection. In order to reduce handling stress, the individuals exposed to cortisol or control injections for 72 hours will be housed in the same tank and injected first. In the same fashion, the 48, 36, 24, 5, 2, 0.5, and 0 hour groups will be housed in separate tanks, each of which will be injected at successive pertinent times. Blood and mucus sampling for plasma and mucus cortisol levels will occur at the same time for all fish. For each tissue and treatment group, changes in cortisol concentration over time will be examined

using repeated measures analysis of variance. Mann-Whitney U tests will be used to compare of the magnitudes of maximum cortisol levels between tissues and treatment groups, and Pearson's linear regression will be used to correlate cortisol values between tissues. Plasma and mucus cortisol values from the control group will be used to ensure the validity of results from both these experimental studies and the field studies described in 3.1.

Stress induction experiments. Adrenocorticotrophic hormone (ACTH) is secreted rapidly in response to stress and acts on the interrenal gland to stimulate the release of cortisol. In order to examine cortisol rates of increase in plasma and mucus in response to ACTH administration, captive Pacific halibut will be randomly divided into three groups. Individuals from two of the groups will receive intraperitoneal injections of 1ml of Ringers solution containing 0.5 μM or 5 μM ACTH. Individuals from the third group will act as a control, receiving intraperitoneal injections of 1ml of Ringers solution. Blood and mucus will be sampled from three parallel fish in the three groups at 0, 0.5, 2, 5, 24, 36, 48, and 72 hours after injection. In order to reduce handling stress, the individuals exposed to ACTH or control injections for 72 hours will be housed in the same tank and injected first. In the same fashion, the 48, 36, 24, 5, 2, 0.5, and 0 hour groups will be housed in separate tanks, each of which will be injected at successive pertinent times. Blood and mucus sampling for plasma and mucus cortisol levels will occur at the same time for all fish. For each tissue and treatment group, changes in cortisol concentration over time will be examined using repeated measures analysis of variance, and Mann-Whitney U tests will be used to compare of the magnitudes of maximum cortisol levels between tissues and treatment groups. Pearson's linear regression will be used to correlate cortisol values between tissues. Post-injection plasma concentrations of ACTH will not be measured in this study. Plasma and mucus cortisol values from the control group will be used to ensure the validity of results from both these experimental studies and the field studies described in 3.1.

Blood and mucus sampling and cortisol extraction and analysis. Blood samples (approximately 1-2 ml) will be collected from the caudal vein and used to separate the plasma component. Samples of skin mucus (approximately 1-2 ml) will be collected by gently scraping the side of the fish with a cotton swab or small plastic rod and diluted with phosphate buffered saline (1:2) prior to analysis. Plasma and cortisol levels will be measured by enzyme linked immunoabsorbent assay.

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APPENDICES

Appendix I: Summary of new and continuing research projects approved for 2017

Appendix II: Summary of external research projects awarded for funding for 2017

APPENDIX I

Summary of new and continuing research projects approved for FY2017

Project #	Project Name	Priority	Budget (US$)	Principal Investigator	Management implications
New Projects					
674.11	Full characterization of the annual reproductive cycle	High	91,098	Planas	Maturity assessment
650.21	Investigation of Pacific halibut dispersal on Bowers Ridge	High-Medium	124,527	Loher	Spawning areas
675.11	Tail pattern recognition analysis in Pacific halibut	High	2,370	Dykstra	Adult distribution
672.12	Condition Factors for Tagged U32 Fish	High	13,000	Dykstra	DMR estimates
673.14	Identification and validation of markers for growth	High	27,900	Planas	Changes in biomass/ size-at-age
672.13	Discard mortality rates and injury classification profile by release method	High-Medium	16,123	Dykstra	DMR estimates
673.13	Sequencing the Pacific halibut genome	High	22,500	Planas	Population estimate
Continuing Projects					
621.15	Voluntary at-sea sex marking	High	18,120	Loher	Stock spawning biomass
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
650.18	Archival tags: tag attachment protocols	High	2,800	Loher	Adult distribution
650.20	Investigation of Pacific halibut dispersal on the 4D Edge	High	5,500	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
	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 for 2017

Project #	Grant agency	Project name	Partners	Total/ (IPHC) Budget ($US)	IPHC Staff	Management implications	Submission status
1	**S-K NOAA**	Improving discard mortality rate estimates in the Pacific halibut by integrating handling practices, physiological condition and post-release survival	Alaska Pacific University	$286,121/ ($223,220)	Planas (lead PI), Dykstra, Loher, Stewart, Hicks,	Bycatch estimates	Awarded 09/01/2017 – 08/31/2019
2	**NPRB**	Somatic growth processes in the Pacific halibut (*Hippoglossus stenolepis*) and their response to temperature, density and stress manipulation effects	AFSC-NOAA-Newport	$230,127/ ($131,891)	Planas (lead PI)	Changes in biomass/ size-at-age	Awarded (NPRB 1704) 09/01/2017 – 08/31/2019
Total amount awarded to IPHC ($US)				**$355,111**			