Report on Current and Future Biological Research Activities

PREPARED BY: IPHC SECRETARIAT (J. PLANAS, C. DYKSTRA, J. FORSBERG, A. JASONOWICZ, T. LOHER, L. SADORUS, 24 MAY 2020)

PURPOSE

To provide the Scientific Review Board with an update of current progress on research projects conducted and planned within the IPHC's five-year research plan (2017-21).

BACKGROUND

The primary biological research activities at IPHC that follow Commission objectives are identified and described in the proposed Five-Year Research Plan for the period 2017-21. These activities are integrated with stock assessment and the management strategy evaluation processes (Appendix I) and are summarized in five main categories, as follows:

1) Migration and Distribution. Studies are aimed at further understanding reproductive migration and identification of spawning times and locations as well as larval and juvenile dispersal.
2) Reproduction. Studies are aimed at providing information on the sex ratio of the commercial catch and to improve current estimates of maturity.
3) Growth and Physiological Condition. Studies are aimed at describing the role of some of the factors responsible for the observed changes in size-at-age and to provide tools for measuring growth and physiological condition in Pacific halibut.
4) Discard Mortality Rates (DMRs) and Survival. Studies are aimed at providing updated estimates of DMRs in both the longline and the trawl fisheries.
5) Genetics and Genomics. Studies are aimed at describing the genetic structure of the Pacific halibut population and at providing the means to investigate rapid adaptive changes in response to fishery-dependent and fishery-independent influences.

UPDATE ON PROGRESS ON THE MAIN RESEARCH ACTIVITIES

1. Migration and Distribution.

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

   1.1. Larval distribution and connectivity between the Gulf of Alaska and Bering Sea. Knowledge of the dispersal of Pacific halibut larvae and subsequent migration of young juveniles has remained elusive because traditional tagging methods are not effective on these life stages due to the small size of the animals. This larval connectivity project, in cooperation with NOAA EcoFOCI, used two recently developed modeling approaches to estimate dispersal and migration pathways in order to better understand the connectivity of populations both within and between the Gulf of Alaska and Bering Sea. A manuscript of the results has been submitted to the peer-reviewed journal Fisheries Oceanography (Sadorus et al., in review). In brief, to improve current understanding of larval dispersal pathways and migrations of young fish within and between GOA and BS, investigations were conducted to (1) examine pelagic larval dispersal and connectivity between the two

basins using an individual-based biophysical model (IBM), and (2) track movement of fish up to age-6 years using annual age-based distributions and a spatio-temporal modeling approach. IBM results indicate that the Aleutian Islands constrain connectivity between GOA and BS, but that large island passes serve as pathways between these ecosystems. The degree of connectivity between GOA and BS is influenced by spawning location such that up to 50-60% of simulated larvae from the westernmost GOA spawning location arrive in the BS with progressively fewer larvae arriving proportional to distance from spawning grounds further east. There is also a large degree of connectivity between eastern and western GOA and between eastern and western BS. Spatial modeling of 2-6 year old fish shows ontogenetic migration from the inshore settlement areas of eastern BS towards Unimak Pass and GOA by age 4. The pattern of larval dispersal from GOA to BS, and subsequent post-settlement migrations back from BS toward GOA, provides evidence of circular, multiple life-stage, connectivity between these ecosystems, regardless of temperature stanza or year class strength.

1.2. Wire tagging of U32 Pacific halibut. The patterns of movement of Pacific halibut among IPHC Regulatory Areas have important implications for management of the Pacific halibut fishery. The IPHC Secretariat has undertaken a long-term study of the migratory behavior of Pacific halibut through the use of externally visible tags (wire tags) on captured and released fish that must be retrieved and returned by workers in the fishing industry. In 2015, with the goal of gaining additional insight into movement and growth of young Pacific halibut (less than 32 inches [82 cm]; U32), the IPHC began wire-tagging small Pacific halibut encountered on the National Marine Fisheries Service (NMFS) groundfish trawl survey and, beginning in 2016, on the IPHC fishery-independent setline survey (FISS). In 2019, a total of 821 Pacific halibut were tagged and released during the NMFS Gulf of Alaska trawl survey and 885 tags were released during the NMFS Bering Sea survey. Through 2019, a total of 6,536 tags have been released in the NMFS groundfish trawl survey and, to date, 52 tags have been recovered. On the IPHC FISS, a total of 3,112 U32 Pacific halibut had been wire tagged are released and 74 of those have been recovered to date. The wire tagging effort on the FISS was not implemented in 2019 due to work load commitments on the FISS operation. However, 54 U32 Pacific halibut were wire-tagged as part of other research projects in 2019. The points of release and recovery of wire-tagged Pacific halibut are shown in Figure 1 and the distance traveled from the release location for recaptured Pacific halibut from recent U32 wire tagging efforts is shown in Table 1. Wire-tagging efforts on U32 Pacific halibut both on the NMFS groundfish survey and on IPHC's FISS will continue in 2020.

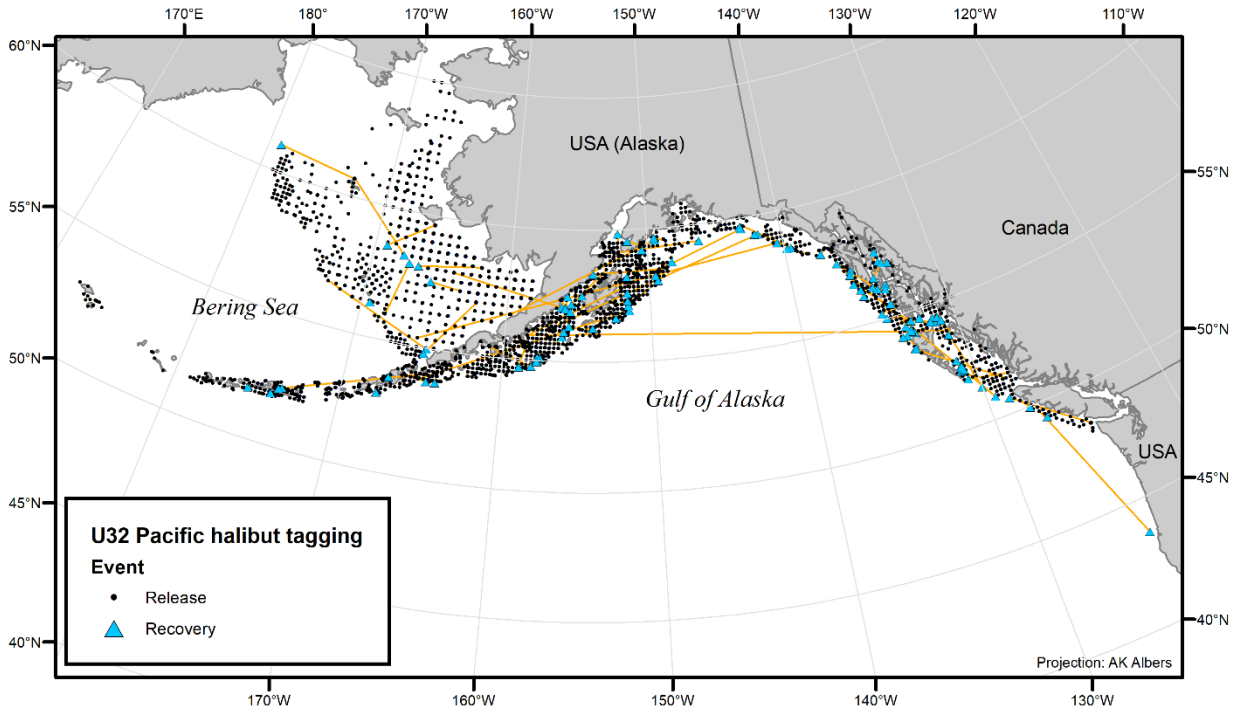

Figure 1. Geographic location of released and recovered wire-tagged fish since 2015. Yellow lines indicate the straight distance between the points of release and recovery of tagged fish.

Table 1. Distance traveled from release location for recaptured Pacific halibut from recent U32 wire tagging efforts.

Distance traveled (nm)	Number recovered	%
0 - 10	45	35%
11 - 50	33	25%
51 - 100	17	13%
101 - 200	17	13%
201 - 300	5	4%
301 - 500	6	5%
501 - 700	4	3%
701 - 900	3	2%

1.3. Electronic archival tagging

Life history and movement of adult Pacific halibut is being investigated by: 1) coastwide archival tagging of U32 Pacific halibut to investigate rearing conditions and dispersal; 2) pop-up satellite tagging in the Norton Sound region to investigate spawning stock structure and connectivity in the Northern Bering Sea.

1.3.1. U32 rearing and migration. Variance in population-level and regional stock productivity can exert strong influences on fishery dynamics and policy choices designed to optimize fishery yield and socioeconomic benefits. Large changes in realized size-at-age of Pacific halibut over the last decade have resulted in considerable declines in fishery yield, but the cause and mechanisms of these changes remain unclear. In particular, competing hypotheses exist regarding relative contributions of food limitation versus thermal forcing as regulators of growth in wild individuals. Additionally, ambiguities regarding the dynamics of pre-recruit migration create uncertainty regarding the degree to which productivity in any given region is ultimately dependent realized growth that was achieved upstream. This project was designed in order to generate data describing thermal rearing conditions of U32 Pacific halibut and relate the thermal history of individuals to individual growth and population-level productivity; and data quantifying ontogenic movement rates with respect to age, sex, and maturation. The former is intended to generate inputs for trophic and stock-productivity models; with a shorter-term objective of producing a validated temperature-$\delta^{18}O$ (i.e., stable oxygen isotope) relationship for Pacific halibut otolith carbonate that can be used to interrogate the IPHC otolith archive in order to examine hypotheses regarding historical relationships between growth and temperature. Migratory data would represent inputs for metapopulation models; with a shorter-term objective of quantifying ages at which migration to winter spawning grounds is initiated in order to compare physiological age-at-maturity to behavioral maturity (i.e., functional contribution to the spawning stock). Both would represent contributions to policy analysis, allowing for increased understanding of the flow of productivity through the system at a variety of spatial and temporal scales. The latter has the potential to refine definitions of functional spawning stock biomass.

This project was initiated in in 2018 with the deployment of 255 fishery-recovery long-term (7 year recording capacity) archival tags from the IPHC's Fishery Independent Setline Survey (FISS) at survey stations spanning Oregon to Unalaska and northward along the easterm Bering Sea shelf Edge, Additionally, 13 Pop-up Archival Transmitting (PAT) tags were deployed in the Aleutian Islands to produce baseline temperature data for a small sample of fish; these tags were programmed to report after two years at liberty in an attempt to produce data from one full summer and two winters for each tagged fish. Deployments in 2019 were designed to focus on rearing areas in the eastern Bering Sea most likely to produce low-temperature otolith carbonate samples that will be required to accurately describe the isotopic relationship in this species. Sixty-three tags were deployed from the NOAA/NMFS Eastern Bering Sea (EBS) trawl survey. Nine tags have been recovered to date: one in 2018 and eight in 2019.

Quantifying the isotopic relationship will require a sufficient number of individuals that have experienced temperatures at liberty spanning at least 3-8° C to allow for at least

(30) temperature-indexed carbonate samples to be produced across range. This number is approximate, representing the mean magnitude of sample sizes associated with published relationships derived from other marine carbonates. The number of samples required will depend upon the amount of variance that is observed in Pacific halibut otolith carbonate at any given temperature; as we have no data describing this relationship, these variance levels are entirely unknown. However, increasing numbers of samples would improve the precision of the derived relationship as well as improving understanding of true variance about the relationship. It is hoped that each full summer at liberty for each tag recovery for which an otolith is obtained will produce one carbonate sample of sufficient volume for analysis; and that winter annuli will need to be pooled among multiple individuals. However, determining this will require preliminary tests comprised of sectioning and milling otoliths in order to determine the sample volumes that can be produced. Identifying a quantifying onset of behavioral maturity requires data from fish that have matured while at liberty, requiring individual time-series data spanning a minimum of three years at liberty.

1.3.2. Northern Bering Sea connectivity. The IPHC Secretariat is collaborating in a research project led by the Norton Sound Economic Development Corporation (NSEDC), with the University of Alaska Fairbanks (UAF) as an additional participant. This project was proposed by NSEDC in response to observations of recent increasing abundance of both Pacific cod and Pacific halibut in the Norton Sound region and a desire to understand the origins of those fish and the degree to which their regional stock component represents local productivity versus being seasonally or ontogenically connected to other regions, including Russian waters. Of particular interest is anecdotal information that suggest that the local population may be composed of two functional spawning components: one that moves seasonally between this region and the continental shelf edge in US waters (e.g. Middle and Pervenets Canyons in Area 4D), and another that may spawn in Russian waters (e.g. Navarin Canyon) that may be derived of individuals that are reared in Russian nurseries. The project has been jointly funded: IPHC previously provided funding for tags during the initial portion of the study (2018, see below but is not allocating any financial resources); NSEDC is providing additional tags and all vessel, travel, and logistical support; UAF is providing funding for a dedicated graduate student through a Rasmuson Fisheries Research Center (RFRC) fellowship. The project focused primarily upon movements of reproductive stock with a desire to identify spawning locations. It also contains a U32 element that is designed to test the hypothesis that climate change is providing increasing opportunity for immature stock (i.e., individuals that will not undertake seasonal spawning migrations) to remain in the region overwinter due to reductions in cold-pool footprint that would otherwise be expected to invoke redistribution. The work will also contribute to our current understanding of the spawning distribution of Pacific halibut and the results provided will assist the IPHC Secretariat in order to define functional spawning units and accurately quantify distribution and variance in spawning stock biomass. The work also has links to larval advection analyses (*see* 1.1, prior) in that the results will help to quantify release location(s) and timing of the region's reproductive output allowing for inference regarding the nursery region(s) upon which the fishable stock ultimately relies.

Field work was initiated in 2018 with the deployment of 44 PAT tags (27 provided by the IPHC and 17 by NSEDC): five fish were tagged east of St. Matthew Island from the Northern Bering (NBS) trawl survey; 24 fish were tagged in Norton Sound; and 15 at St. Matthew Island from the native village of Savoonga. Tags provided by the IPHC were used to tag relatively small fish (i.e. 58-98 cm) and NSEDC tags deployed primarily on larger (89-137 cm) spawning stock. The tags were programmed to release from their host fish and report their location and archived data during three periods: January 2020 (representing the spawning season); summer of 2020 (investigating site fidelity versus emigration); and summer of 2021 (examining longer-term dispersal). A relatively large number of tags (15) reported prematurely, during late summer and autumn of 2019; this represented nearly all (9) of the tags that had been programmed to report in 2021. Winter (January) locations and data were received for four individuals, leaving ten winter-scheduled tags effectively missing. Inspection of mid-January sea ice data indicated that the region was experiencing its most ice-bound season in decades, and we hypothesize that the majority of the tags detached while the fish were under the sea ice. The individuals whose locations were received confirmed their westward movement towards Navarin Canyon, but with migration timing that appears to be approximately one month later than we have observed elsewhere in the EBS. Field deployments are scheduled to continue in 2020 with NSEDC providing an additional 44 tags and all required logistical support. However, consideration associated with COVID-19 may delay those deployments.

The sample sizes employed in the study simply represent the maximum number of tags available given available funds. If acceptable to the NSEDC's Board of Directors, a third year of deployments will occur which would bring the total sample size to approximately 124 fish.

2. Reproduction.

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

2.1. Sex ratio of the commercial landings. The sex ratio of the commercial fishery catch represents an extremely important source of uncertainty in the annual stock assessment (Stewart and Hicks, 2018). The IPHC has generated sex information of the entire set of aged commercial fishery samples collected in 2017 and in 2018 (> 10,000 fin clips per year) using genetic techniques based on the identification of sex-specific single nucleotide polymorphisms (SNPs) (Drinan et al., 2018) using TaqMan qPCR assays conducted at the IPHC's Biological Laboratory. Therefore, for the first time, direct estimates of the sex-ratio at age for the directed commercial fishery have been available for stock assessment. Genetic analyses of commercial samples from 2017 showed that the proportion of females coastwide was high (82%), ranging from 65% to 92% depending on the biological region. Data from the 2018 commercial samples showed almost identical patterns, with females comprising 80% of the coastwide commercial landings (by number). Given that the sex-ratio data constitutes one of the two most important contributors to estimates of both population trend and scale, the inclusion of this information in the 2019 stock assessment resulted in higher spawning biomass. Current

efforts are devoted to provide sex-ratio information from the 2019 commercial landings as additional years of data are likely to further inform selectivity parameters and cumulatively reduce uncertainty in future estimates of stock size.

2.2. Maturity assessment. Recent sensitivity analyses have shown the importance of changes in spawning output due to skip spawning and/or changes in maturity schedules for stock assessment (Stewart and Hicks, 2018). These results highlight the need for a better understanding of factors influencing reproductive biology and success for Pacific halibut. In order to fill existing knowledge gaps related to the reproductive biology of female Pacific halibut, research efforts are devoted to characterize female maturity in this species. Specific objectives of current studies include: 1) accurate description of oocyte developmental stages and their use to classify female maturity stages; 2) comparison of macroscopic (based on field observations) and microscopic (based on histological assessment) maturity stages and revision of maturity criteria; 3) revision of current estimates of female age-at-maturity; and 4) investigation of skip-spawning in females.

Biological samples (gonads, blood, pituitary, otolith, fat content) from female and male Pacific halibut were collected at monthly intervals throughout an entire calendar year, from September 2017 until August 2018, with an equal sample size, consisting of 30 females and 30 males, collected at each month, for a total of 360 fish of each sex. Fish were size-selected to increase the possibility of capturing reproductively competent individuals: > 90 cm in fork length for females and > 70 cm in fork length for males. Throughout the entire annual fish collection period, fish were captured from a single geographic region, the Portlock region in the central Gulf of Alaska, historically known to contain major spawning grounds (St. Pierre, 1984), in order to attempt collecting fish from a single spawning population of Pacific halibut at various stages during their reproductive cycle. Fish were assigned to the four maturity stages (immature, maturing, ripe and resting) based on visual/macroscopic criteria of the gonads that are currently applied in IPHC's FISS for maturity assessment. Photographic images of the gonads were taken in order to validate the visual assignment. Tissue samples collected from the central portion of the ovary were fixed in 10% buffered formalin and sent to a commercial company where they were embedded in paraffin, sectioned and stained with Hematoxylin-Eosin for microscopic staging.

Macroscopic assignment of maturity status of Pacific halibut females collected monthly during an entire calendar year shows that females classified as ripe were only found during the months of January and February (Fig. 1 in IPHC-2019-SRB015-08), an observation that is consistent with the notion that the known spawning season spans between November and March (St. Pierre, 1984). Following the peak spawning period, females classified as resting are primarily found between January and August. Subsequently, females classified as maturing are found from May to January and represent 80 - 100% of all females scored between July and November. These results confirm that the period during which maturity information is collected during the FISS (June – August) is highly informative with regards to maturity. The progression of the number of mature females, reaching close to 100% maturity in August is accompanied with a marked increase in the gonadosomatic index (gonad weight/round weight x 100;

GSI) in females collected between August and January (Fig. 2 in IPHC-2019-SRB015-08), as further demonstrated with the higher GSI values found in females classified as maturing and ripe (Fig. 3 in IPHC-2019-SRB015-08). Changes in GSI are reflective of the increase in gonad size due to the progression of ovarian development, oocyte growth and hydration. Interestingly, the hepatosomatic index (liver weight/round weight x 100; HSI) begins increasing in females in June and peaks in October (Fig. 2 in IPHC-2019-SRB015-08), suggesting that the increase in liver weight is related to an increase in the production of liver lipoproteins that are incorporated into the oocyte throughout its secondary growth phase (see below). Not surprisingly, the highest HSI values are observed in females classified as maturing (Fig. 3 in IPHC-2019-SRB015-08).

Given that one of the objectives of this study was to compare macroscopic versus microscopic gonadal staging, a first necessary step for staging maturity microscopically was to fully describe the progression of oocyte development. Therefore, the IPHC Secretariat has described for the first time the different oocyte stages that are present in the ovary of female Pacific halibut and how these are used to classify females histologically to specific maturity stages. This information is contained in a manuscript that is currently in preparation for submission to a peer-reviewed journal (Fish et al., in preparation). In brief, 8 different oocyte developmental stages have been described, from early primary growth oocytes until preovulatory oocytes, and their size and morphological characteristics established. Maturity classification was established by assigning maturity status to the most advanced oocyte developmental stage present in ovarian tissue sections. Therefore, 7 different microscopic maturity stages were established. Analysis of oocyte size frequency distribution among the seven different maturity stages provided evidence for the group-synchronous and batch spawning reproductive strategy in female Pacific halibut. The results of this study will allow us to establish a comparison of the microscopic/histological and macroscopic/field classification criteria that are currently used to assign the maturity status of females that is used in stock assessment. Furthermore, this study sets the stage for the in-depth study on temporal changes in maturity, as assessed by microscopic observations of ovarian samples collected throughout an entire annual reproductive cycle, that is currently underway.

3. Growth.

Recent stock assessments conducted by the IPHC Secretariat have indicated that the Pacific halibut stock experienced a continuous coastwide decline from the late 1990s until approximately 2012 largely due to a decrease in size-at-age (SAA) (Stewart and Hicks, 2020). Current low values of SAA combined with low recruitment of cohorts spawned at the time of the initial decrease in SAA in the 1990s have contributed to a decrease in exploitable Pacific halibut biomass. Although the decrease in SAA has been hypothesized as being attributed to several potential causes, including environmental effects such as temperature or food availability, as well as ecological or fishery effects, our knowledge on the actual factors that influence SAA of Pacific halibut is still scarce. The IPHC Secretariat initiated studies aimed at elucidating the drivers of somatic growth leading to the decline in SAA by investigating the physiological mechanisms that contribute to growth changes in the Pacific halibut. The two main objectives of these studies were: 1) the identification and validation of

physiological markers for somatic growth; and 2) the use of growth markers for evaluating growth patterns in the Pacific halibut population and the effects of environmental factors on somatic growth. In order to pursue these objectives, the IPHC Secretariat is conducting investigations on the effects of temperature variation on growth performance, as well as on the effects of density, hierarchical dominance and handling stress on growth in juvenile Pacific halibut in captivity. These studies are partially funded by a grant from the North Pacific Research Board to the IPHC (Appendix II).

The results on the effects of temperature on growth physiological indicators are being prepared for publication in a peer-reviewed journal (Planas et al., in preparation). In brief, juvenile Pacific halibut underwent temperature-induced growth manipulation, whereby somatic growth was suppressed by low temperature acclimation and stimulated by temperature-induced compensatory growth. Molecular signatures of growth suppression and growth stimulation were identified by a comparative transcriptomics approach through RNA sequencing of skeletal muscle. Genes whose mRNA expression levels were altered by temperature-induced growth manipulations were identified and the physiological processes taking place in skeletal muscle under growth suppression and stimulation were described based on the functional classification of genes. Of particular interest were genes whose expression changed in response to the two growth manipulations and in the same direction as growth changes, as these genes could potentially represent useful markers for growth in skeletal muscle. In addition to the transcriptomic approach (evaluating changes in mRNA expression levels of genes), a proteomic approach was also used in the same experiments to identify proteins with altered abundance in response to the two temperature-induced growth manipulations. Proteins with altered abundance levels under growth suppression and stimulation were identified and a subset of these corresponded to genes that also showed changes in mRNA expression levels. In summary, an important set of genes and proteins representing potential markers for growth have been identified. Details related to the methodological aspects and results of this study are found in Planas et al. (in preparation). Currently, molecular assays (i.e. RT-qPCR) are being developed to test the validity of the identified molecular markers for growth on skeletal muscle samples from age-matched adult Pacific halibut of different sizes.

In addition to temperature-induced growth manipulations, the IPHC Secretariat is conducting similar studies to identify physiological growth markers that respond to density and stress-induced growth manipulations. On one hand, changes in SAA in Pacific halibut have been hypothesized, among other potential causes, to be the result of changes in population dynamics of the Pacific halibut stock due to a density effect, whereby high population densities would negatively affect growth (Loher, 2013). On the other hand, we hypothesize that stress responses associated with capture and release of discarded Pacific halibut may affect feeding and growth in the wild, therefore, addressing potential growth consequences related to capture and handling stress. Investigations related to the effects of density and stress exposure are currently underway.

4. Discard Mortality Rates (DMRs) and Survival Assessment.

Information on all Pacific halibut removals is integrated by the IPHC Secretariat, providing annual estimates of total mortality from all sources for its stock assessment. Bycatch and wastage of Pacific halibut, as defined by the incidental catch of fish in non-target fisheries and by the mortality that occurs in the directed fishery (i.e. fish discarded for sublegal size or for regulatory reasons), respectively, represent important sources of mortality that can result in significant reductions in exploitable yield in the directed fishery. Given that the incidental mortality from the commercial Pacific halibut fisheries and bycatch fisheries is included as part of the total removals that are accounted for in stock assessment, changes in the estimates of incidental mortality will influence the output of the stock assessment and, consequently, the catch levels of the directed fishery. For this reason, the IPHC Secretariat is conducting two research projects to investigate the effects of capture and release on survival and to improves estimates of DMRs in the directed longline and guided recreational Pacific halibut fisheries:

4.1. Evaluation of the effects of hook release techniques on injury levels and association with the physiological condition of captured Pacific halibut and estimation of discard mortality using remote-sensing techniques in the directed longline fishery. In order to better estimate post-release survival of Pacific halibut caught incidentally in the directed longline fishery, the IPHC Secretariat is conducting investigations to understand the relationship between fish handling practices and fish physical and physiological condition and survival post-capture as assessed by electronic archival tagging. Currently, the IPHC assigns a 3.5% DMR to Pacific halibut released from longlines with only minor injuries and a 16% DMR to the total estimated volume of U32 discards generated by the target fishery. The former was experimentally derived between 1958 and 1961, and the latter is a result of tagging studies in which the baseline DMR was used as a parameter in tag-recovery models that were used to estimate DMRs for fish returned to the water in relatively poorer condition than “minor”. As such, if the 3.5% is mis-specified, the subsequent rates that rest upon that value will be inaccurate, as will be our estimates of total discard mortality within the fishery. The baseline rate was generated from at-sea captive holding studies that reported that observed mortality patterns were, at least in part, due to fluctuating environmental conditions from which the fish could not escape, and for which they attempted to compensate analytically. Ambiguity therefore exists regarding the degree to which the baseline rate is accurate, necessitating additional studies in order to resolve this issue. For this reason, the IPHC Secretariat, with partial funding by a grant from the Saltonstall-Kennedy Grant Program NOAA (Appendix II), conducted studies to evaluate the effects of hook release techniques on injury levels, their association with the physiological condition of captured Pacific halibut and, importantly, generated experimentally-derived estimates of DMR in the directed longline fishery. As part of this study, injury profiles and release viabilities for different release techniques (careful shake, gangion cutting, and hook stripping) have been developed. The results obtained indicate that injury patterns were similar for careful shake and gangion cutting, with most injuries being a small puncture to the cheek, and greater than 70% of the released fish were classified to be in excellent viability. The hook stripper produced more severe physical injuries with significantly greater numbers of fish classified as moderate or poor in viability condition upon release. Blood glucose, lactate, and cortisol levels from all fish released have been determined using specific assays in the Biological Laboratory.

Results are suggestive of a trend towards lower glucose and higher lactate blood levels in fish classified as dead in terms of the release condition. Cortisol levels do not show a significant trend across the release condition categories. Results on glucose, lactate, and cortisol plasma levels in fish according to physical injury code show a fair amount of variation within groups. The relationship of blood glucose, lactate, and cortisol levels to other measured parameters in discarded fish (fat levels, condition index, time out of water, temperature exposure, etc.) are under ongoing investigation. A subset (n=79) of these fish were tagged with acceleration-logging survivorship Pop-up Archival (sPAT) tags. These tags are capable of detecting and recording high-frequency (1Hz) triaxial acceleration for periods of up to 96 days, allowing for the fish to be released into the wild and post-release activity and survival outcomes to be inferred without the need to recapture the fish. The ability of these tags to index mortality outcomes in Pacific halibut has been experimentally validated and the technology used to investigate mortality rates in response to explanatory variables following release from trawls. Here, we used this technology to examine post-release mortality of longline-captured fish released with a minimum of injury in the Gulf of Alaska, after being subjected to capture conditions representative of typical commercial fishing practices. A draft manuscript describing the work and its results has been prepared (Loher et al., in preparation). In summary, the results derive DMR estimates ranging from 4.0-8.7%; but it is worth noting that the time-course of mortalities observed (i.e., delayed by 6 weeks or more) suggest mortality rates considerably lower than prior experiments in which essentially all mortality was indexed within three weeks of fish capture.

Electronic monitoring (EM) systems were proven to be effective at accurately capturing the release method applied to each animal. Footage is now being reviewed to determine the ability of EM systems to provide length estimates of captured fish from the existing footage, and additional in season work on a FISS vessel is proposed.

4.2. Quantification of handling practices and physiological stress in Pacific halibut released in the charter recreational fishery. The IPHC has begun a research project to better characterize the nature of charter recreational fisheries with the ultimate goal of better understanding discard practices relative to that which is employed in the directed longline fishery. This project has received funding from the National Fish and Wildlife Foundation (Appendix II). As an initial step in this project, information from the charter fleet on types of gear and fish handling practices used was collected through stakeholder meetings and on dock interviews with charter captains and operators. Results show that the guided recreational fleet predominantly uses circle hooks (75-100%), followed by jigs. Predominant hook release methods included reversing the hook (54%), or twisting the hook out with a gaff (40%), and the fish were generally handled by supporting both the head and tail (65%), while other common techniques included handling by the operculum (10%) or by the tail alone (10%). This information will inform the design of the experimental test fishing that is expected to take place in 2021 and in which fish condition and stress will be evaluated to identify best practices intended to minimize discard mortality in this fishery. In this context, the IPHC is currently preparing a funding request to NPRB that would provide resources to conduct experimental evaluation of post-release mortality using sPAT tags, similar to the work described in 4.1.

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

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

5.1.1. Determine the genetic structure of the Pacific halibut population in the North-eastern Pacific Ocean. Understanding population structure is imperative for sound management and conservation of natural resources (Hauser, 2008). Pacific halibut in US and Canadian waters are managed by the International Pacific Halibut Commission (IPHC) as a single coastwide unit stock since 2006 (Stewart, 2014). The rationale behind this management approach is based on our current knowledge of the highly migratory nature of Pacific halibut as assessed by tagging studies (Webster *et al.*, 2013) and of past analyses of genetic population structure that failed to demonstrate significant differentiation in the North-eastern Pacific Ocean population of Pacific halibut by allozyme (Grant, 1984) and small-scale microsatellite analyses (Bentzen, 1998; Nielsen *et al.*, 2010). However, more recent studies have reported slight genetic population structure on the basis of genetic analysis conducted with larger sets of microsatellites suggesting that Pacific halibut captured in the Aleutian Islands may be genetically distinct from other areas (Drinan *et al.*, 2016). These findings of subtle genetic structure in the Aleutian Island chain area are attributed to limited movement of adults and exchange of larvae between this area and the rest of the stock due to the presence of oceanographic barriers to larval and adult dispersal (i.e. Amchitka Pass) that could represent barriers to gene flow. Unfortunately, genetic studies suggesting subtle genetic structure (Drinan *et al.*, 2016) were conducted based on a relatively limited set of microsatellite markers and, importantly, using genetic samples collected in the summer (i.e. non-spawning season) that may not be representative of the local spawning population. With the recent collection of winter (i.e. spawning season) genetic samples in the Aleutian Islands by the IPHC in early 2020, a collection of winter samples from 5 different geographic areas across the North-eastern Pacific Ocean (i.e. British Columbia, Central Gulf of Alaska, Bering Sea, Central and Western Aleutian Islands) is now available to re-examine the genetic structure of the Pacific halibut population. Importantly, novel, high-throughput and high-resolution genomics approaches are now available for use, such as low-coverage whole genome resequencing (Therkildsen and Palumbi, 2017; Clucas *et al.*, 2019), in order to describe with unprecedented detail the genetic structure of the Pacific halibut population. The recently sequenced Pacific halibut genome (deposited at DDBJ/ENA/GenBank under the accession JABBIT000000000) will constitute an essential resource for the success of the whole genome resequencing approach. The results from the proposed genomic studies would provide important information on spawning structure and provide management advice regarding the relative justifiability for considering the western Aleutians as a genetically-distinct substock.

Methods

Collected fin clips preserved in ethanol from Pacific halibut during the spawning season (i.e. winter) will be processed for DNA extraction and purification using Qiagen kits. The available samples correspond to the following geographic areas and dates of winter collection: British Columbia (Haida Gwaii; 1998-1999, 2004, 2007), Central Gulf of Alaska (Portlock region; 1998-1999, 2004, 2007, 2018), Bering Sea (Pribilof Canyon; 2004, 2007), Central Aleutian Islands (Adak; 2007, 2020) and Western Aleutian Islands (Attu; 2020). Samples from 50 individuals from each of these collections, totaling 600 individuals, will be processed for genetic analyses. Libraries for low-coverage whole-genome resequencing will be prepared according to published protocols (Clucas *et al.*, 2019) and sequencing will be conducted on a NovaSeq S4, with an output of 2.5B reads (750Gb) per lane.

The software ANGSD (Korneliussen et al. 2014) and ATLAS (Link et al. 2017) will be used detect SNPs through the Pacific halibut genome. ANGSD will also be used to estimate measures of genetic diversity (allele frequencies and heterozygosity) for each sample collection. We expect to identify millions of SNPs taking this approach (Therkildsen and Palumbi 2017; Clucas et al. 2019). Measures of genetic differentiation (F_{ST}) will be estimated among the sample collections to examine levels of divergence between them and test for patterns of isolation by distance. To investigate the possibility of cryptic population structure, clustering methods will be used. The software ngsAdmix (Skotte et al. 2013), will be used to infer the number of genetic clusters across the range of Pacific halibut without making a priori assumptions about sample origin. This program also attempts to estimate the ancestry of individual fish and therefore will be useful in the identification of potential migrants. F_{ST} outlier tests will also be used to scan the genome for SNPs showing signals of divergent selection. These SNPs showing potential signatures of selection may offer more power to resolve population structure in highly migratory marine fish (Grewe et al. 2015; Anderson et al. 2019). Furthermore, SNPs showing signals of selection may functionally relevant and linked to local adaptations. Transcriptomic resources currently under development by the IPHC Secretariat will be very useful in interpreting the functional significance of the many SNPs that we expect to identify in this study.

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

through a length-age key, will allow us to provide information on genetic variability among fish that are at or near their settlement or nursery grounds.

Methods

Fin clips from 150 fish from the eastern Bering Sea and from 150 fish from the Gulf of Alaska, all between 2 and 3 years of age, will be used for DNA extraction and purification using Qiagen kits. For fish of unknown sex, genetic sex will be determined using SNPs to two sex-linked loci previously developed (Drinan *et al.*, 2018) and used to determine the genetic sex of the commercial Pacific halibut captures.

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

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

5.2.1. Genome sequencing. The IPHC Secretariat has recently completed conducting a project aimed at generating a first draft sequence of the Pacific halibut genome, the blueprint for all the genetic characteristics of the species. This study is being conducted in collaboration with the French National Institute for Agricultural Research (INRA, Rennes, France). An initial sequencing effort using genomic DNA from one Pacific halibut female in half an Illumina lane in 2 x 250 pair end mode resulted in a total size of assembled scaffolds of 700 Mb. This non-contiguous genomic sequence was complemented by long read sequencing using the Nanopore technology (i.e. PromethION) combined with Hi-C sequencing for chromosome-scale scaffolding of the genome assembly. Briefly, the Pacific halibut genome has a size of 586 Mb and contains 24 chromosome-size scaffolds covering 98.6% of the complete assembly. Some of the assembly metrics include the sizes of the shorter and longest scaffolds at 11.3 Mb and 32.4 Mb, respectively, and a N50 scaffold length of 25 Mb. The Pacific halibut genome sequence has been submitted to NCBI with submission number SUB7094550 and with accession number JABBIT000000000.

5.2.2. Expressed Sequence Tags. The IPHC Secretariat has completed transcriptome (i.e. RNA) sequencing of a wide variety of tissues (12) in Pacific halibut including white and red skeletal muscle, liver, heart, ovary, testis, head kidney, brain, gill, pituitary, spleen and retina. The functional annotation of these transcriptomes to describe tissue-

specific gene expression complements the genome sequencing efforts and represents a resource that will provide biological insights at a molecular level for ongoing and future IPHC research.

Illumina sequencing resulted in 625 million raw sequence reads from all 12 tissues (Table 2). Sequence reads were assembled using Trinity (v2.6.6) (Haas et al. 2013) into tissue-specific transcriptomes as well as a comprehensive combined assembly. The total size of the assemblies ranges from 28 Mb to 308 Mb and an average of 109,713 (39,638 – 224,625) transcripts were assembled per tissue (Table 2). The size of the combined assembly is 486 Mb and contains assembled 390,243 transcripts (Table 2). BUSCO (v4.0.6) (Benchmarking Universal Single-Copy Orthologs) (Waterhouse et al. 2018). Briefly, BUSCO assigns a quantitative measure of assembly completeness based on matches to a set of known single-copy orthologs. Single-copy orthologs from the Actinopterygii lineage were used for this assessment. Of the 3460 Actinopterygii BUSCO's, 3,341 complete and 146 fragmented were identified in the combined assembly (95.5% completeness) (Figure 2). Protein coding sequences from the assembled transcripts were identified using TransDecoder (v5.5.0) (Haas et al. 2013).

Tissue	Strategy	Raw Reads	Assembled Transcripts	Mean Transcript Length (bp)	Total Size (bp)	N50 (bp)	Coding Sequences
White muscle	2x100 (HiSeq 2500)	15,147,000	39,638	721	28,598,382	1,198	15,082
Liver	2x100 (HiSeq 2500)	10,508,300	40,814	692	28,237,340	1,096	14,525
Ovary	2x100 (HiSeq 2500)	21,651,000	60,084	1,240	74,513,854	2,494	30,371
Testis	2x100 (HiSeq 2500)	19,792,300	87,644	1,015	88,917,698	2,004	37,576
Red muscle	2x100 (HiSeq 2500)	22,398,400	86,561	1,052	91,050,930	2,104	38,502
Heart	2x100 (HiSeq 2500)	20,974,600	70,338	1,146	80,597,106	2,322	33,019
Brain	2x150 (HiSeq X10)	76,065,400	167,141	1,325	221,490,218	2,777	79,623
Gill	2x150 (HiSeq X10)	82,576,000	174,240	1,328	231,330,062	2,669	86,451
Head kidney	2x150 (HiSeq X10)	71,182,800	87,831	1,007	88,448,096	1,925	39,069
Pituitary	2x150 (HiSeq X10)	102,300,100	174,284	1,279	222,970,464	2,652	81,108
Retina	2x150 (HiSeq X10)	104,584,700	224,625	1,375	308,753,198	2,879	104,966
Spleen	2x150 (HiSeq X10)	78,450,500	103,359	931	96,236,002	1,749	45,220
Combined		625,631,100	390,243	1,246	486,241,300	2,659	158,493

Table 2. Summary of sequencing and *de novo* assembly for the transcriptomes of 12 tissues in Pacific halibut.

The Trinotate annotation suite (Bryant et al. 2017) was used to annotate the transcript assembly for each tissue. Trinotate integrates searches of sequence (SwissProt) (The UniProt Consortium 2019) and protein domain (Pfam) (El-Gebali et al. 2018) databases along with signal peptide (SignalP) (Nielsen 2017), transmembrane protein domain (TMHMM) (Krogh et al. 2001), and ribosomal RNA sequence (RNAmmer) (Lagesen et al. 2007) predictions. Additional homology searches were conducted

against sequence databases obtained from UniProt (The UniProt Consortium 2019) for zebrafish (*Danio rerio*) and two additional flatfish species, the tongue sole (*Cynoglossus semilaevis*) and turbot (*Scophthalmus maximus*) resulting in additional transcript annotations (Table 3). Of the transcripts in the combined assembly, 165,718 were successfully annotated, corresponding to a 42.5% annotation rate. Annotation rates for the tissue-specific assemblies ranged from 47.9% to 58.7%. Tissue-specific gene expression profiles were also generated and are being interrogated for tissue-specific expression patterns.

The raw sequence data has been uploaded to NCBI's Sequence Read Archive (Accession numbers SAMN14989915 - SAMN14989926) for public access and a description of this resource is being prepared for publication in a peer-reviewed journal. Current plans regarding this extensive transcriptomic dataset include generating a reference transcriptome for the species and to create a user-friendly, searchable database to be made public in the IPHC website.

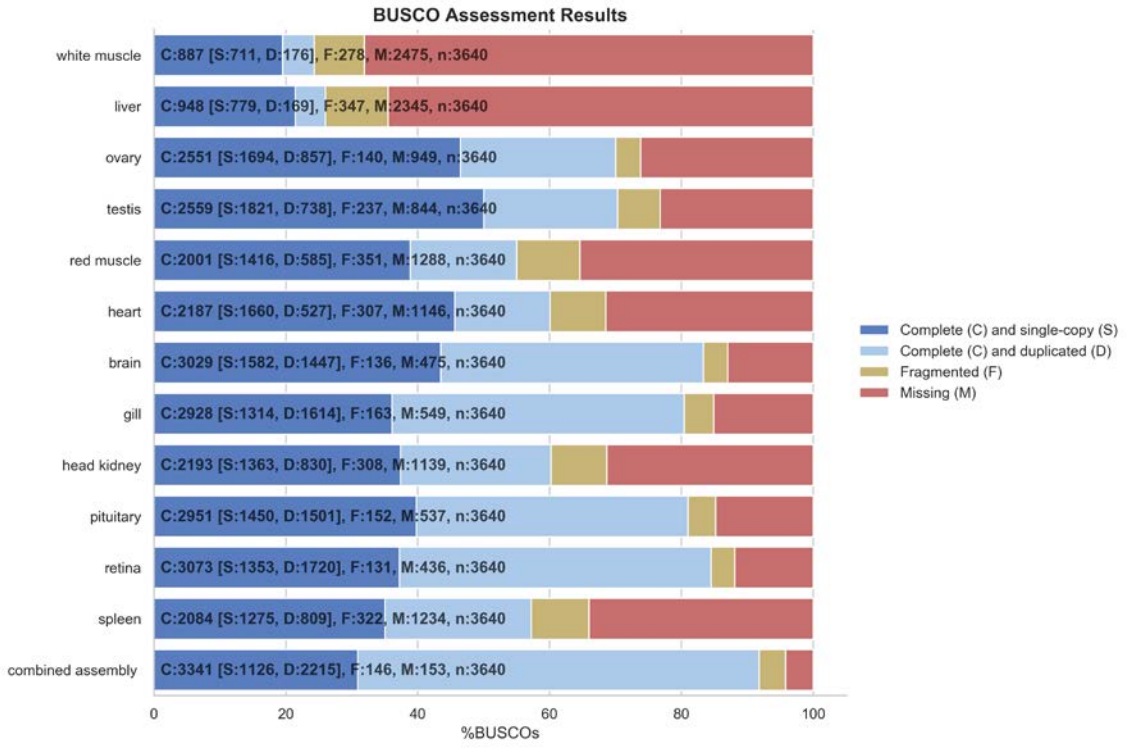

Figure 2. BUSCO assessment results for the transcriptomes of 12 tissues in Pacific halibut.

Tissue	Total Annotated Transcripts	SwissProt	*Danio rerio*	*Cynoglossus semilaevis*	*Scophthalmus maximus*
white muscle	23,117	19,529	20,458	21,278	21,460

liver	22,764	19,268	20,157	20,980	21,169
ovary	35,262	30,708	31,727	32,458	32,820
testis	47,022	39,389	41,249	42,532	43,226
red muscle	47,185	40,157	41,816	43,167	43,704
heart	39,718	34,142	35,326	36,346	36,845
brain	85,032	74,222	76,636	78,403	78,987
gill	94,179	81,943	84,938	86,881	87,506
head kidney	49,797	42,151	44,089	45,555	46,039
pituitary	87,599	76,377	79,236	81,202	81,644
retina	107,623	93,378	97,014	99,306	99,515
spleen	59,339	49,816	52,009	53,963	54,593
combined	165,718	139,284	145,345	149,246	150,900

Table 3. Summary of annotations using various sequence databases for the transcriptomes of 12 tissues in Pacific halibut.

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APPENDIX I

Integration of biological research, stock assessment and harvest strategy policy

Biological research		Stock assessment	Stock assessment MSE
Research areas	**Research outcomes**	**Relevance for stock assessment**	**Inputs to stock assessment and MSE development**
Reproduction	Sex ratio Spawning output Age at maturity	Spawning biomass scale and trend Stock productivity Recruitment variability	Sex ratio Maturity schedule Fecundity
Growth	Identification of growth patterns Environmental effects on growth Growth influence in size-at-age variation	Temporal and spatial variation in growth Yield calculations Effects of ecosystem conditions Effects of fishing	Predicted weight-at-age Mechanisms for changes in weight-at-age
Discard Survival	Bycatch survival estimates Discard mortality rate estimates	Scale and trend in mortality Scale and trend in productivity	Bycatch and discard mortality estimates Variability in bycatch and uncertainty in discard mortality estimates
Migration	Larval distribution Juvenile and adult migratory behavior and distribution	Geographical selectivity Stock distribution	Information for structural choices Recruitment indices Migration pathways and rates Timing of migration
Genetics and Genomics	Genetic structure of the population Sequencing of the Pacific halibut genome	Spatial dynamics Management units	Information for structural choices

APPENDIX II

Summary of awarded research grants

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